# Clinical Pharmacology in Psychiatry

## Bridging the Experimental-Therapeutic Gap

Edited by

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# CLINICAL PHARMACOLOGY IN PSYCHIATRY

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# Preface

At the International Meetings on Clinical Pharmacology in Psychiatry (IMCPP), the first held in Chicago 1979, the second in Tromsø 1980 and now the third in Odense (Hindsgavl), experimental pharmacologists, clinical pharmacologists and clinicians have met for in-depth discussion of various fields of psychopharmacology. Particular aims of these meetings have been to increase the mutual understanding of research strategies and methodological problems in these fields, and to bridge the gap between experimental findings and clinical applications of new knowledge.

The 3rd IMCPP, held at Hindsgavl Castle in the western corner of the island Fyn, thus brought together experts from a wide range of fields and from many countries. Nonetheless, a high degree of mutual understanding of principles and problems was achieved, and the 18-20 hours daylight in Denmark in June permitted long and stimulating discussions. The papers presented at the meeting and published in this book illustrate the continuity from experimental research to clinical utilization of psychotropic drugs.

Odense, Irvine, Tromsø,
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# Abbreviations

| | |
|---|---|
| AAG | $\alpha_1$-acid glycoprotein (= orosomucoid) |
| ACTH | corticotrophin |
| AMP | adenosine monophosphate |
| APS | Adverse Drug Reaction Probability Scale |
| ATC | Anatomical-Therapeutic-Chemical (classification system) |
| ATP | adenosine triphosphate |
| AUC | area under the curve |
| cAMP | cyclic AMP |
| β-CCE | 3-carboethoxy-β-carboline |
| CNS | central nervous system |
| CPRS | Comprehensive Psychiatric Rating Scale |
| c.p.s. | cycles per second |
| CSA | (Northern Ireland) Central Services Agency |
| CSF | cerebrospinal fluid |
| CVS | cardiovascular system |
| DA | dopamine |
| DDAVP | desmopressin acetate |
| DDD | defined daily dose |
| DHA | dihydroalprenolol |
| DHSS | (UK) Department of Health and Social Services |
| dopa | dihydroxyphenylalanine |
| DSM-III | Diagnostic and Statistical Manual of Mental Disorders - 3rd edition |
| DST | dexamethasone suppression test |
| ECG | electrocardiogram |
| ECS | electroconvulsive shock |
| ECT | electroconvulsive (shock) therapy |
| ED | equilibrium dialysis |
| EEG | electroencephalogram |
| EPhMRA | European Pharmaceutical Market Research Association |
| EPS | extrapyramidal side-effects |
| GABA | γ-aminobutyric acid |
| GABA-T | GABA transaminase |
| GAD | glutamic acid decarboxylase |
| GAS | Global Assessment of Severity |
| GFR | glomerular filtration rate |
| GMP | guanosine monophosphate |
| GTP | guanosine triphosphate |
| HBE | His bundle electrocardiography |
| HDRS | Hamilton Depression Rating Scale |
| 5-HIAA | 5-hydroxyindole acetic acid |
| 5-HT | serotonin |
| 5-HTP | 5-hydroxytrytophan |
| HVA | homovanillic acid |
| ICD | International Classification of Disease (WHO) |
| ICU | Intensive Care Unit |
| i.c.v. | intraventricularly |
| IDF | imipramine displacing factor |
| IMCPP | International Meeting on Clinical Pharmacology in Psychiatry |

| | |
|---|---|
| i.n. | intranasal |
| Li | lithium (salt) |
| LVET | left ventricular ejection time |
| MAO | monoamine oxidase |
| MAOI | MAO inhibitor |
| 5-MeODMT | 5-methoxy-*N*,*N*-dimethyltryptamine |
| MHPG | 3-methoxy-4-hydroxy-phenylglycol |
| NDA | (US Food and Drug Administration) New Drug Application |
| NE | norepinephrine |
| NHS | (UK) National Health Service |
| PCPA | *p*-chlorophenylalanine |
| PDLP | phenylalanine-D-lysine-phenylalanine |
| PEP | pre-ejection period |
| QNB | quinuclidinyl benzilate |
| REM | rapid eye movement (sleep) |
| RIA | radioimmunoassay |
| SADS | Schedule for Affective Disorders and Schizophrenia |
| SGOT | serum glutamate-oxaloacetate transaminase |
| SLi | serum lithium |
| TCA | tricyclic anti-depressant(s) |
| UF | ultrafiltration |
| VMA | vanillylmandelic acid |
| WHO | World Health Organization |

# Section One

# Utilization of Psychotropic Drugs: Geographical and Cultural Determinants and Relationship to Morbidity

# Utilization of psychotropic drugs in Sweden and the other Nordic countries

Ulf Bergman[1], Ingegerd Agenäs[2] and Monica Dahlström[2]

## INTRODUCTION

Marked geographical differences have been found in the utilization of important drugs, e.g. antidiabetics, antihypertensives and psychotropics, both within and between several European countries (Bergman *et al.*, 1975, 1979b; Grimsson *et al.*, 1977; Baksaas, 1978). As there are no data available to suggest correspondingly large differences in the disease patterns between these countries, geographical variations are of great potential interest to public health.

Inappropriate use of drugs is a matter of concern to public health authorities, pharmacologists, doctors and patients (Sjöqvist, 1975). Drug utilization studies are a great help in detecting discrepancies between prescribing practices and acceptable pharmacotherapeutic principles. The studies give additional knowledge that can be used in rational planning and follow-ups of drug information programs. They comprise studies of the use of drugs in different geographical areas, prescribing patterns of physicians and patients' drug-taking behavior.

In agreement with the suggestions made by the WHO Symposium in 1969 (World Health Organization, 1970), statistics on drug utilization have been published regularly in Sweden since the mid-1970s (Svensk Läkemedelsstatistik, 1980-1982). Similarly the Nordic Council on Medicines has decided to publish statistics on medicines regularly. Through these sources, sales figures of psychotropic drugs in the five Nordic countries between 1975 and 1980 have been made available for this paper (Nordic Council on Medicines, 1979, 1982). These data will be discussed in relation to Swedish national prescription data.

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In a cross-national interview study of the use of certain psychotropic drugs, differences in use were found between a number of European countries (Balter *et al.*, 1974). This finding and the fact that there are no strict guidelines for the prescribing of many psychotropic drugs make it important to study their utilization and to follow its development with time.

## MATERIALS AND METHODS

### Type of Data Available

Denmark, Finland, Iceland, Norway and Sweden constitute the five Nordic countries with 22 million inhabitants, ranging from 0.225 million in Iceland to 8.3 million in Sweden.

Drug utilization can be studied using wholesale statistics available from drug manufacturers or national drug control agencies and data available through prescriptions retrieved from pharmacies inside and outside health institutions.

Drug utilization at the patient level can be studied by interviews, recording of the drug purchases of the individual patient and monitoring of drug concentrations.

If not otherwise stated, wholesale data, which are available in all Nordic countries, are used in this paper. These data should not be regarded as synonymous with the real intake of drugs in the populations studied, but they reflect fairly well the attitude among physicians to the use of psychotropic drugs.

Data on drug use in hospitals are available only in Sweden.

A continuous Swedish nationwide prescription survey gives information about, for example, the age and sex of the patient and the number of prescribed daily doses for each drug. The survey is a 1-in-288 representative sample of all prescriptions filled at Swedish pharmacies (Kristoferson and Wessling, 1977).

Since 1978 the Diagnosis and Therapy Survey (Agenäs *et al.*, 1980) has given information about the diagnoses behind drug prescribing (International Classification of Disease: World Health Organization, 1965). The information is obtained from a running random sample of physicians participating for one week each.

### Unit of Comparison

In 1981, four companies furnished the Swedish drug market with about 70 diazepam preparations: oral, rectal and parenteral dosage forms in various strengths and pack sizes and at different prices. The difficulty of getting an accurate estimate of the overall utilization of diazepam is thus obvious. It is even more complicated to compare the utilization of similar drugs, e.g. diazepam versus oxazepam or nitrazepam.

To permit convenient comparisons of the utilization of similar drugs, so-called defined daily doses (DDD) were established for each drug (Baksaas-Aasen *et al.*, 1975; Lunde *et al.*, 1979). The DDD was chosen according to what was recommended to be the average maintenance dose of the particular drug on its assumed major indication. The DDD shall be regarded as a technical unit of measurement and comparison.

In Sweden the overall utilization of diazepam (DDD = 10 mg) was 32 million DDDs in 1981. The corresponding number for nitrazepam (DDD = 5 mg) was 74 million DDDs. The number of DDDs per 1000 inhabitants per day should give a rough estimate of the number of subjects who might have used the drug in a certain area, e.g. 10.5 DDDs of diazepam per 1000 inhabitants per day compared to 24.3 DDDs of nitrazepam (Svensk Läkemedelsstatistik, 1982).

The sum of all drugs within a therapeutic group represents an estimate of the 'therapeutic intensity' in the population (e.g. 45.9 DDDs of benzodiazepines per 1000 inhabitants per day in Sweden in 1981).

For drugs used in various doses for different purposes (e.g. neuroleptics) and for drugs used in short-term treatment (e.g. antibiotics, minor analgesics), there is no relevance in morbidity calculations of the DDD per 1000 inhabitants per day estimate. Furthermore, the estimate does not take into account differences in the frequency of combination therapy.

### Classification

A prerequisite for comparisons of drug utilization between geographical areas and over time is a common classification of drugs. The comparisons of wholesale data between the five Nordic countries were based on the Anatomical-Therapeutic-Chemical Classification system (ATC code) - a five-level code which is a development of the three-level EPhMRA/IPMRG code (Nordic Council on Medicines, 1979). On its fifth level, the ATC code identifies single chemical substances. (As an example, all plain diazepam preparations are given the code N 05 B A 01.)

## RESULTS AND DISCUSSION

The pattern and extent of psychotropic drug use were different in the five Nordic countries in the period 1975 through 1980 (figure 1). The publication of the data initially available in four countries (Denmark excluded) has obviously also had some impact on the overall sales in the preceding years (Grimsson *et al.*, 1977). *Hypnotics, sedatives* (N 05 C) and *tranquilizers* (N 05 B) dominated the sales in all countries but the trends in use were quite different (figure 1). In Denmark, with the highest use of hypnotics

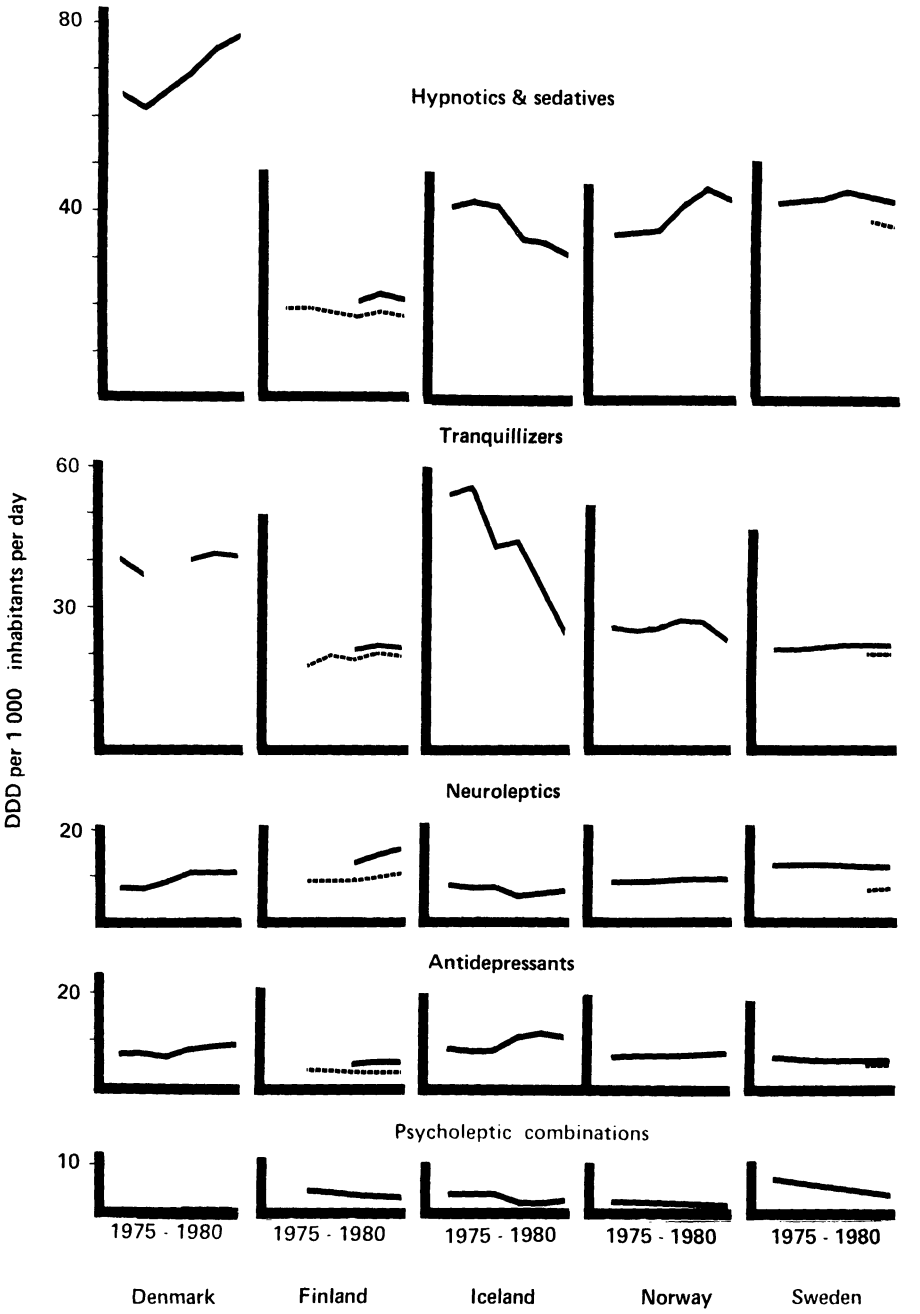


Figure 1. Trends in sales of psychotropic drugs in the Nordic countries. From the top: hypnotics and sedatives

(N 05 C), tranquilizers (N 05 B), neuroleptics (N 05 A), antidepressants (N 06 A + C) and psycholeptic combinations (A 03 C) (see text). Amitriptyline combined with chlordiazepoxide or perfenazine (N 06 C) were only available in Finland, where it made up 31% of the total outpatient antidepressant use in 1980 and was included here in the figure for antidepressants. Psychostimulants (N 06 B), below 1 DDD per 1000 inhabitants per day in all countries, are not included. The area between the dotted and solid lines indicates hospital sales in Finland (estimates) and in Sweden (Nordic Council on Medicines, 1979, 1982).

and sedatives in 1975, a gradual increase was seen during the study period, whereas the use of these drugs and of tranquilizers decreased in Iceland.

In Iceland, a 20% reduction in the use of hypnotics, sedatives and tranquilizers was found between 1976 and 1978, partly depending on the removal of the 10 mg tablets of diazepam from the market and partly as an effect of feedback to doctors about their prescribing habits (Olafsson *et al.*, 1980), an effect that seems to have persisted through 1980.

In Norway the use of tranquilizers during 1975 through 1977 was 25% higher than that in Finland and Sweden. In 1980 the use was almost at the same level in all three countries due to a new regulation allowing prescriptions for tranquilizers to be dispensed only once and by removing some drugs from the Norwegian market, e.g. most of the barbiturates and methaqualone (Halvorsen and Jøldal, 1981).

In Finland and Sweden, the use of hypnotics, sedatives and tranquilizers was very stable in this six-year period. The sales of hypnotics and sedatives, however, were much lower in Finland than in any other Nordic country. The use of benzodiazepines (N 05 BA and N 05 CD) (figure 2) varied between 73% in Denmark and Sweden and almost 100% in Iceland of the total use of hypnotics/sedatives and tranquilizers in 1980. Diazepam and nitrazepam dominated the use in all five countries in that year.

Barbiturates were still in use in four countries (not in Iceland) with a general downward trend. The use in Denmark (about 22 DDD per 1000 inhabitants per day) was threefold that in Sweden. In 1980 Finland and Norway had the lowest use of barbiturates, 4 and 1 DDD per 1000 inhabitants per day, respectively.

Neuroleptics (N 05 A) are a group of drugs creating special problems in the interpretation of results obtained by the DDD methodology. Neuroleptic drugs are used for several indications besides psychosis and, therefore, in different dosages and modes of administration and in combination with other drugs. The DDDs for neuroleptics are based on the treatment of psychosis (the assumed major indication). A much lower dose is given for minor

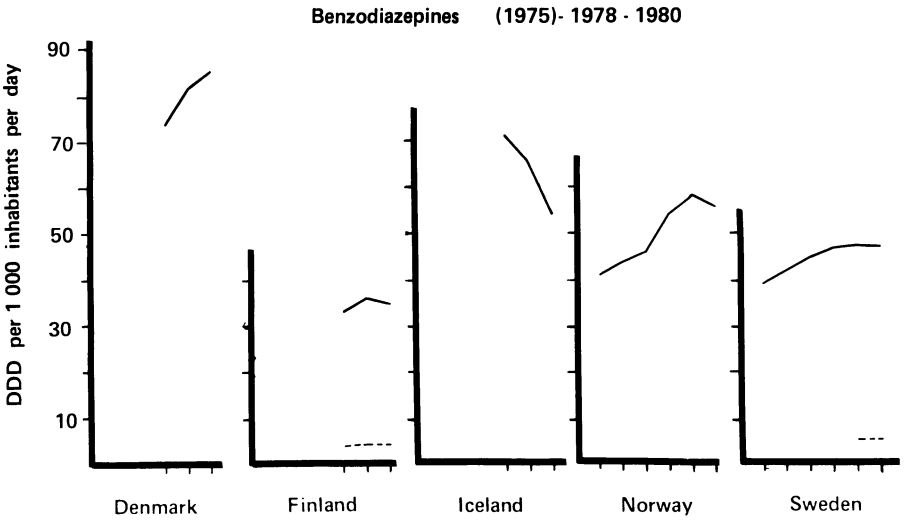


**Figure 2.** Trends in sales of benzodiazepines (N 05 BA, N 05 CD) in the Nordic countries: Denmark, Finland, Iceland, Norway and Sweden in (1975)-1978-1980. The area between the dotted and base lines indicates hospital sales (Nordic Council on Medicines, 1979, 1982).

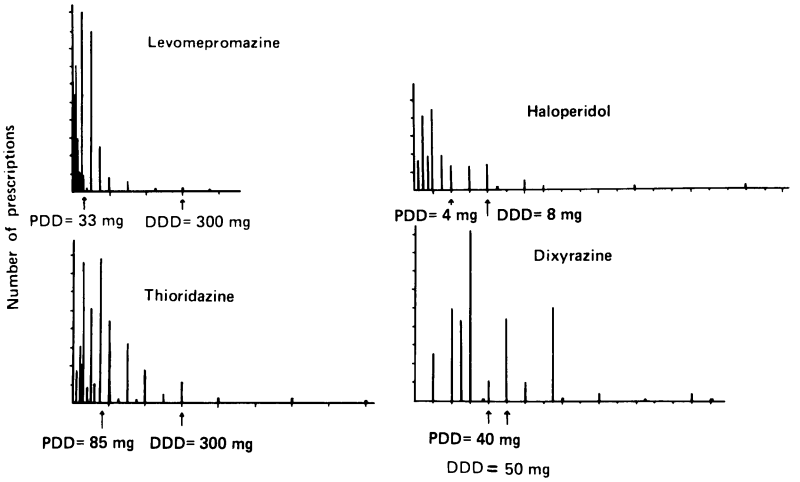


**Figure 3.** Variability in the prescribed daily doses of certain neuroleptic drugs (N 05 A) in Sweden in 1980 - data from the nationwide prescription survey. As indicated by arrows the average prescribed daily dose (PDD) is lower than the defined daily dose (DDD). For levomepromazine PDD/DDD = 0.11, for thioridazine 0.28, for haloperidol 0.50, and for dixyrazine 0.80.

psychiatric disturbances (figure 3). In Sweden, these drugs have also been recommended to be used as hypnotics and minor tranquilizers (Edgren and Roos, 1970).

At the time of publication of the *Nordic Statistics on Medicines*, no national data on the indications for drug prescribing were available in Sweden or in the other Nordic countries. In a study at a multi-doctor district health center in Sweden, minor psychiatric symptoms, such as sleep disturbances, were found to be common reasons for the prescribing of neuroleptics (Bergman *et al.*, 1979a). The Diagnosis and Therapy Survey (Agenäs *et al.*, 1980) shows that minor psychiatric symptoms were also common reasons for the prescribing of neuroleptics to outpatients in the period 1978 through 1981 (tables 1 and 2). This finding would explain the high use of neuroleptics in Sweden compared with the other Nordic countries with the exception of Finland (see below). Interestingly, hospital consumption represented as much as about 40% of the total use of neuroleptics, a fact which suggests that psychosis may still be the major indication for the overall use of neuroleptics as assumed when establishing DDDs for neuroleptics.

In the first Nordic publication, Finnish hospital data were missing (Nordic Council on Medicines, 1979). In 1978 through 1980 estimated hospital data indicate that Finland had the highest utilization of neuroleptic drugs compared with all the other Nordic countries. No information on indications for drug prescribing in Finland is officially available. However, if neuroleptic drugs were commonly prescribed in minor psychiatric disorders in Finland as well, it could explain the relatively low sales of other psychotropics there compared to other Nordic countries (figure 1).

There was a wide variation in the use of *lithium* (N 05 AX, DDD = 24 mmol) between the five Nordic countries in the period 1978 through 1980 (Nordic Council on Medicines, 1982; Bergman, 1981). Denmark and Sweden were the highest users, their consumption being about five times that of Iceland, which was the lowest user (about 1.3 and 0.3 DDD per 1000 inhabitants per day, respectively). Norway and Finland had an intermediate use of lithium (0.5-0.6 DDD per 1000 inhabitants per day). Since 1976-1977 there has been a decline in the use of lithium in Sweden, possibly related to a report suggesting chronic renal lesions following long-term treatment with lithium (Hestbech *et al.*, 1977). Interestingly, the pattern of use in individual counties in Sweden varied greatly. In a few counties a low and stable level of about the same height as in Finland and Norway was seen during the period 1975 through 1980. In one county there was a steep increase above the Swedish mean. The three counties with the highest use in the period 1975 through 1977 showed marked decrease in the period 1978 through 1980. Recently, a group of experts from the Nordic countries recommended general principles for long-term lithium therapy (Amdisen *et al.*, 1980). It will thus be of great interest

Table 1. Most commonly stated indications for prescribing psychotropic drugs in Sweden (data from the Diagnosis and Therapy Survey in 1978-1981 (Agenäs *et al.*, 1980)).

| **Neuroleptics** (N 05 A)($n$ = 2057) | | **Tranquilizers** (N 05 B)($n$ = 4529) | |
|---|---|---|---|
| Psychoneurosis | 41% | Psychoneurosis | 60% |
| Psychosis | 22% | Disturbance of sleep | 18% |
| Disturbance of sleep | 11% | Depression | 4% |
| Alcoholism | 5% | Psychosis | 2% |
| Depression | 5% | Other indications | 16% |
| Other indications | 16% | | |
| **Hypnotics and sedatives** (N 05 C) ($n$ = 3489) | | **Antidepressants** (N 06 A) ($n$ = 1288) | |
| Disturbance of sleep | 73% | Depression | 49% |
| Psychoneurosis | 9% | Psychoneurosis | 24% |
| Depression | 2% | Psychosis | 13% |
| Psychosis | 2% | Disturbance of sleep | 4% |
| Other indications | 14% | Other indications | 10% |

Table 2. Most commonly stated indications for prescribing certain neuroleptic drugs (N 05 A) in Sweden (data from the Diagnosis and Therapy Survey in 1978-1981 (Agenäs *et al.*, 1980)).

| **Thioridazine** ($n$ = 275) | | **Levomepromazine** ($n$ = 296) | |
|---|---|---|---|
| Psychoneurosis | 46% | Disturbance of sleep | 46% |
| Psychosis | 27% | Psychoneurosis | 19% |
| Depression | 6% | Psychosis | 16% |
| Disturbance of sleep | 5% | Alcoholism | 3% |
| Alcoholism | 1% | Depression | 2% |
| Other indications | 15% | Other indications | 14% |
| **Dixyrazine** ($n$ = 339) | | **Haloperidol** ($n$ = 137) | |
| Psychoneurosis | 57% | Psychosis | 51% |
| Alcoholism | 14% | Psychoneurosis | 30% |
| Disturbance of sleep | 11% | Disturbance of sleep | 2% |
| Depression | 3% | Depression | 2% |
| Psychosis | 2% | Other indications | 15% |
| Other indications | 13% | | |

to follow the future trends in the use of lithium in the Nordic countries, and also to relate the use to the frequency of adverse reactions (Bergman *et al.*, 1978).

*Antidepressants* (N 06 A + C) also showed differences in the pattern and extent of use between the five countries (figure 1). Amitriptyline was the most common antidepressant drug in all the Nordic countries. Coinciding with the decrease in the use of hypnotics, sedatives and tranquilizers in Iceland, there was an increase of almost 40% in the use of antidepressants, which became twofold that in Finland. A gradual increase in the use of antidepressants is also seen in Denmark and Norway. Sulpiride, classified as a neuroleptic, is available only in Finland and only for the indication of depression. If this drug is added to the antidepressant group, the Finnish use of antidepressants approaches the same level as that in Sweden (Nordic Council on Medicines, 1982).

In 1973, a study at a district health center in Sweden showed that the non-specific psychiatric diagnoses depressio mentis and psychoneurosis were the principal indications for the prescribing of antidepressants (Bergman *et al.*, 1979a). Manic-depressive psychosis made up about 5% of the total number of antidepressants prescribed. Thus, as earlier shown in the Australian general practice morbidity and prescribing survey (Royal Australian College of General Practitioners, 1976) and now also by national Swedish data (table 1), antidepressants were used for rather non-specific and less well documented psychiatric disturbances.

Controlled clinical trials have shown that the mean effective dose of amitriptyline and nortriptyline in depressed hospitalized patients is around 150 mg per day (50 mg t.i.d.) (Sjöqvist, 1975), which is the dose used in comparisons with new antidepressant drugs. In 1980 the mean dosages prescribed in Sweden for amitriptyline and nortriptyline were 61 mg and 62 mg, respectively. The dosages corresponded to those found in two earlier Swedish studies (Boethius and Sjöqvist, 1978; Bergman *et al.*, 1979a) - all far below the effective dose (150 mg) suggested for treatment of depressive disorders (Asberg, 1976) and in agreement with earlier English data from general practice (Johnson, 1974). In newly depressed patients receiving tricyclic antidepressants, he found that only one-fourth of the patients were prescribed more than 75 mg per day, and the prescribed daily dose was even lower at subsequent consultations. Out of the 73 general practitioners questioned about what maximum dose of tricyclic drugs they would use, only 21 replied 100 mg or more as daily dose in general practice. The almost universal reason given for not using higher doses was the problem of side-effects.

*Gastrointestinal antispasmodics and anticholinergics in combination with psychoactive substances* (psycholeptic combinations) (A 03 C) were included in the section of psychotropic drugs in the first issue of *Nordic Statistics on Medicines* because

it had been claimed that considerable use of so-called hidden psychotropics existed (Hemminki, 1974). The sales of psycholeptic combinations were small and decreasing in all the Nordic countries (figure 1). Data on analgesic compounds with psycholeptics were, however, not included in the first issue. In 1980, these compounds made up a low proportion of total analgesics. The lowest sales were found in Finland with most compounds on the market (Nordic Council on Medicines, 1982).

In contrast to many other countries, hidden psychotropics were widely used in Spain in 1980 (Laporte *et al.*, 1981). Nowadays, such use does not seem to be a major problem in the Nordic countries. The need for having these compounds on the market can, however, be questioned, particularly as some countries, e.g. Iceland and Norway, do not have them.

## The Pattern of Age and Sex Distribution

A uniform finding in studies of drug consumption is that the use of drugs increases with age and that women are prescribed more drugs than men in almost all age groups. These facts are most pronounced in the groups hypnotics, sedatives and tranquilizers (Westerholm *et al.*, 1978). Thus, differences in age and sex distribution between populations have to be taken into account in comparisons of drug consumption.

The proportion of elderly in Sweden is higher than that in any other Nordic country. However, age and sex distribution alone does not explain the differences in drug use between the Nordic countries.

## Indications for Prescribing Psychotropic Drugs

Psychoneurosis and sleep disturbances were the most common reasons for the prescribing of psychotropic drugs in Sweden (figure 4). There was a considerable overlapping in the types of drugs prescribed for the various indications (figure 4) and consequently these drugs are prescribed for a variety of reasons (table 1). Within the therapeutic subgroups the individual drugs also have their diagnostic profiles. The major reason for prescribing the neuroleptic drug levomepromazine was sleep disturbances, while psychoneurosis was the main indication stated for the prescribing of thioridazine and dixyrazine, and psychosis the main one for the prescribing of haloperidol (table 2). National characteristics in the diagnostic profiles of psychotropic drug prescribing may be a major explanation for the striking differences between the Nordic countries.

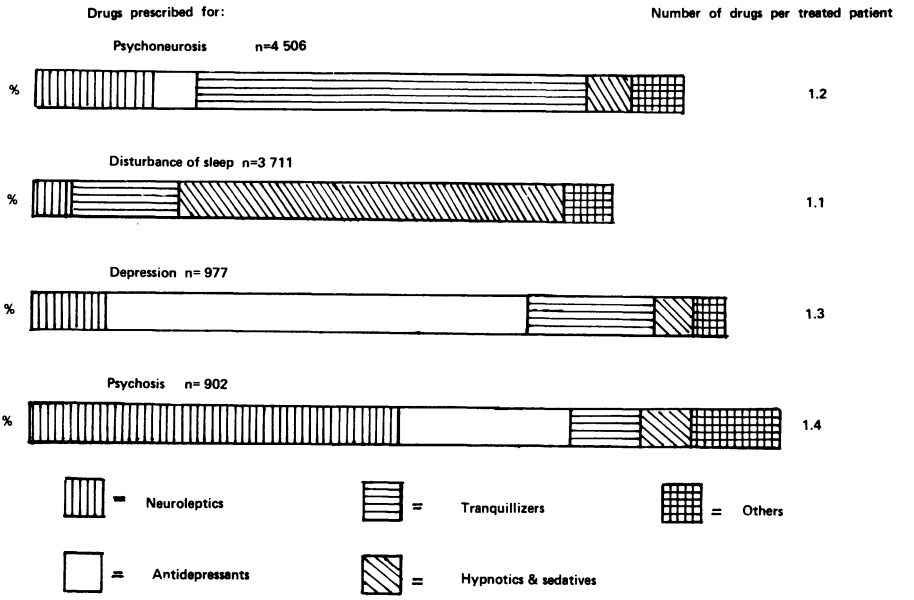


**Figure 4.** Drugs prescribed for the main psychiatric disorders in outpatients in Sweden from 1978 through 1981 according to the Diagnosis and Therapy Survey (Agenäs *et al.*, 1980). As more than one drug was prescribed on average, the sum is above 100%.

## Hospital Drug Utilization

By using a uniform methodology in studies of drug utilization in hospitals, marked differences in the use of hypnotics, sedatives and tranquilizers were found between similar hospital wards in Czechoslovakia, Norway, Spain and Sweden (Stika *et al.*, 1981; Bjørndal, 1982; Laporte *et al.*, 1981; Westerholm, 1974; Bergman *et al.*, 1980). Deliveries of drugs were calculated in number of DDDs per bed-day (Westerholm, 1974; Bergman *et al.*, 1980). This estimate, validated in Sweden and Norway, provides a clinically relevant measure of drug utilization in hospitals (Bergman *et al.*, 1980; Bjørndal, 1982).

The Norwegian study revealed a close correlation ($r$ = 0.93) between general and hospital utilization of psychotropic drugs in different parts of Norway (Bjørndal, 1982). Withdrawal of benzodiazepines in normal doses is a clinical problem (Petursson and Lader, 1981). These facts are in agreement with the findings in a Swedish study showing that long-term sleeping medication was most commonly introduced during stays in general medical wards (Bolander *et al.*, 1982).

## CONCLUDING REMARKS

In the light of the large geographical variations in psychotropic drug use demonstrated here, problems related to long-term treatment with these drugs may differ between the Nordic countries. An example of such a problem is the balance between the risks of dependence on certain hypnotics, sedatives and tranquilizers and the risk of tardive dyskinesia when using low doses of neuroleptics in minor psychiatric disorders (table 2, figure 3). With available data on drug utilization, such evaluations can be specifically directed to certain geographical areas with vastly different patterns of drug use.

In view of the extensive use of psychotropic drugs reported in many countries (Balter *et al.*, 1974), it is obvious that these drugs are an important part of medical practice. Their use, like the use of alcohol, is related to cultural, social, educational, economic and political conditions. Despite the great socio-economic similarities between the Nordic countries and the close contacts between them, we were able to demonstrate marked differences in the overall use of psychotropic drugs. It is important to study the reasons for the differences, particularly the attitude among physicians to these drugs.

Drug utilization data constitute a valuable feedback instrument in the planning and evaluation of drug information programs, in detecting and identifying areas requiring further investigations and in post-marketing drug evaluations. The DDD methodology, once introduced, is an inexpensive indicator of drug utilization and a suitable basis for therapeutic audit.

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# Patterns of use of psychotropic drugs in Spain in an international perspective

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## INTRODUCTION

The increase in the consumption of psychotherapeutic drugs, and particularly of sedatives and hypnotics, has led to increasing concern about the overprescribing and abuse of these drugs by the public. The study of drug consumption, and eventually the identification of particular areas of misuse, soon leads to a search for medical, social or cultural factors which can influence drug utilization. Drug utilization has been defined by the World Health Organization (1977) as 'the marketing, distribution, prescription and use of drugs in a society, with special emphasis on the resulting medical, social and economic consequences'. Five questions arise when the utilization of sedatives and hypnotics is to be studied: (1) which are the characteristics of the milieu which may have some influence on the consumption of these drugs; (2) which are the characteristics of the supply of these drugs; (3) which are the most consumed drugs; (4) how are these drugs prescribed, dispensed and consumed; and (5) which (if any) are the effects of the consumption of these drugs.

## THE MILIEU

It is well known that the characteristics of the health system have a relevant influence on the consumption of pharmaceuticals. The Spanish Social Security System covers 31 million inhabitants, out of a total population of 37 million inhabitants. National health costs amounted to around $4800 million in 1980, of which $1008 million (21%) were spent in pharmaceuticals. In 1980 the

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Social Security System expenditure on drugs accounted for 83% of total drug expenditure in Spain. The organization of outpatient services of medical assistance has a decisive influence on drug abuse and misuse. A recent survey made in Barcelona (Gabinet d'Assessoria i Promoció de la Salut, 1979) indicated that more than 30% of the users of general medical services go to the outpatient clinic only to obtain prescriptions. The same survey showed that 78% of these people get a prescription (mean of 1.35 prescriptions per person). According to general practitioners, only 31% of their total prescriptions have a clear therapeutic indication.

Frequently, national drug consumptions are compared in terms of US dollars per head, but it is not the same thing to spend, say, $25 per head each year in West Germany as it is in Spain; gross national products per capita income are very different in these two countries. If drug consumption is expressed in terms of per capita income, this results in more valid comparisons (Laporte, 1981). As table 1 shows, Nordic countries tend to spend less on drugs than the other European countries included in this analysis ($p<0.01$).

Table 1. Per capita drug expenditure related to per capita income in some European countries (1977).

| | (1) Per capita drug expenditure ($, 1977)* | (2) Income per capita ($, 1977)† | (1) as a percentage of (2) |
|---|---|---|---|
| Denmark | 42.4 | 8295 | 0.51 |
| Finland | 52.6 | 5652 | 0.93 |
| Norway | 49.7 | 7179 | 0.69 |
| Sweden | 52.6 | 8266 | 0.63 |
| France | 62.1 | 6304 | 0.99 |
| Greece | 34.1 | 2795 | 1.22 |
| Italy | 37.9 | 2730§ | 1.39 |
| West Germany | 65.7 | 7328 | 0.90 |
| Spain | 33.2 | 2925 | 1.14 |

*Data from the Nordic Council on Medicines (1979) and Gisbert (1981).

†Data from *Statistical Yearbook* (Department of International Economic and Social Affairs Statistical Office, 1979).

§1976.

## THE SUPPLY OF PHARMACEUTICALS, AND PARTICULARLY OF PSYCHOTROPIC DRUGS

During the last 10-15 years, various groups of health professionals, health authorities in some countries and the WHO have been increasingly concerned with the fact that drugs are not used to their full potential in terms of efficacy, safety or economy (Lunde, 1980a). There are wide geographical variations in overall drug therapy profiles (for review, see Baksaas and Lunde, 1981). While the Nordic countries have a supply of drugs ranging from 2000 to 3000 marketed pharmaceutical specialities, in countries like West Germany, France, Italy and Spain the number of marketed products ranges from 10 000 to 30 000. These variations from one country to another (see table 2) also add to the complex question - what is really rational and optimal drug therapy? As Lunde (1980b) has pointed out, it has never been proven that an infinite number of drugs provides any greater benefits for public health than a more reasonable product offering. On the contrary, a large number of drugs may result in confusion at all levels of the therapeutic chain, and represent a waste of manpower and money.

In Spain 2477 active drugs are marketed; there are 8003 trade names (pharmaceutical specialities) and 17 778 pharmaceutical forms. One distinctive characteristic between the pharmaceutical markets in Nordic and other European countries is that while in the former there is a limited number of fixed-dose combinations, in the latter fixed-dose combinations make up a high proportion of the overall supply. In Spain this proportion is 55.7%, in France it is 58.8%, in Italy 59.2%, in the USA 45.9%, and in the UK 43.1% (Laporte, 1975). Of all these countries, at least in Spain a high percentage of marketed products do not fulfill generally accepted criteria of efficacy and safety (Erill *et al.*, 1973).

With respect to psychotherapeutic drugs, in Spain there are 61 active drugs marketed and classified as 'psycholeptics' (group N 05 in the so-called anatomical classification of the European Pharmaceutical Market Research Association, EPhMRA). The Spanish official drug catalog includes 410 pharmaceutical specialities in the group N 05; however, 346 additional pharmaceutical specialities containing sedative and hypnotic drugs are classified in other groups. This is due to the high proportion of fixed-dose combinations, which are classified according to their main therapeutic indication.

In a previous paper (Laporte *et al.*, 1981) we have described the marketing and consumption of sedatives and hypnotics in Spain: 15 barbiturates, 20 benzodiazepines, 10 sedatives and hypnotics of other classes, 8 tricyclic antidepressants, 2 tetracyclic antidepressants, 5 MAOI antidepressants, 4 antidepressants of other classes, 15 phenothiazines, and 8 antipsychotic drugs of other classes are on the market in our country. All these drugs are marketed in the form of more than 800 pharmaceutical specialities.

**Table 2.** Consumption of nine groups of medicines in five European countries (percentages of total drug budget) in 1973 *

| Group | West Germany | France | Italy | Spain | United Kingdom |
|---|---|---|---|---|---|
| Analgesics | 5.0 | 5.5 | 4.4 | 3.3 | 5.1 |
| Antibiotics and chemotherapeutic agents | 4.1 | 8.5 | 10.8 | 27.3 | 13.5 |
| Anti-inflammatory drugs | 6.3 | 4.0 | 4.0 | 3.7 | 7.5 |
| Cardiovascular drugs | 18.2 | 14.6 | 8.1 | 5.2 | 11.0 |
| Dermatological products | 5.9 | 4.6 | 4.7 | 4.3 | 1.5 |
| Hormones | 2.9 | 4.4 | 8.4 | 4.3 | 7.2 |
| Psychopharmacological agents | 5.0 | 7.0 | 1.9 | 4.5 | 7.9 |
| Respiratory system drugs | 6.7 | 7.2 | 6.2 | 4.1 | 6.4 |
| Vitamins | 3.4 | 3.0 | 5.1 | 5.4 | 2.2 |
| Total | 57.5 | 58.8 | 53.6 | 62.1 | 62.3 |

*Data from Gisbert (1981).

While in Spain there are 20 benzodiazepines on the market, the number of these drugs marketed in other countries is smaller: 16 in Italy (1979); 11 in Germany (1978); 9 in Switzerland (1978); 8 in the UK (1978); 7 in Finland (1976), in the USA (1978) and in Norway (1976); 5 in Sweden (1979); and 3 in Israel (1976) (Bellantuono *et al.*, 1980). The regulations which allow the marketing of this high number of active drugs have been reviewed by Erill (1974). The last modification of the law regulating the requirements for marketing drugs in Spain, made in March 1970 (Ministerio de la Gobernación, 1970), required, for the first time, pharmacokinetic data (although it is not clearly stated that they should be obtained in human studies) and reports from clinical trials in the applications submitted for the marketing of new drugs. It is not surprising, therefore, that the need for more complete and updated marketing regulations is felt strongly.

## THE CONSUMPTION OF PSYCHOTROPIC DRUGS

It has been suggested that the consumption of psychotropic drugs in Spain is much lower than in other European or American countries (Bellantuono *et al.*, 1980; Balter *et al.*, 1974). The outcome of any survey on psychotherapeutic drug use obviously depends on which drugs are included. Differences in drug classification can lead to very different conclusions about the character, extent and appropriateness of drug prescribing and use. A classification scheme based on chemical structure or pharmacological action tends to group drugs with very different clinical uses and with little relation to clinical practice; however, it can be useful as a consumption denominator when adverse effects of drugs are evaluated, or when public health decisions have to be taken. On the other hand, a classification based on clinical applications in medicine would be most appropriate when the use of psychotherapeutic drugs in the treatment of psychic symptoms associated with anxiety and depression is to be investigated. Table 3 shows the distribution of pharmaceutical specialities containing sedatives and hypnotics, according to their sales figures in 1980. As this table shows, 20 out of 33 pharmaceutical specialities with sales figures higher than half-a-million units are not classified as tranquilizers, hypnotics or sedatives in classifications frequently used to compare consumption of drugs from one country to another. It is clear from these figures that very different results can be obtained depending on the criteria used to include or exclude the pharmaceutical specialities marketed in one country.

Comparisons of drug consumption from one country to another or from one period to another in the same country can be done in terms of expenditure per capita. Although these data can be useful in the investigation of the health budget of a group of countries (see table 2), the drug bill does not permit comparisons

**Table 3.** Hidden sedatives and hypnotics - pharmaceutical specialities containing sedative and hypnotic drugs, according to their sales volumes and to their classification.*

| Units sold in 1980 | Number of pharmaceutical specialities | | |
|---|---|---|---|
| | Classified in group N 05 | Classified in other groups | Total |
| More than 1 million | 4 | 8 | 12 |
| 500 000-999 999 | 9 | 12 | 21 |
| 100 000-499 999 | 52 | 44 | 96 |
| 50 000-99 999 | 33 | 30 | 63 |
| 10 000-49 999 | 90 | 68 | 158 |
| 5000-9999 | 7 | 6 | 13 |
| Less than 1000 | 188 | 154 | 342 |
| Total | 410 | 346 | 756 |

*_Catálogo de Especialidades Farmacéuticas_, 1981(Consejo General de Colegios Oficiales de Farmacéuticos, 1981).

of drug utilization between different countries or within one country in different periods of time. A more suitable unit of comparison has been defined by the Drug Utilization Research Group (Lunde *et al.*, 1979). This unit is the 'defined daily dose' (DDD), which is the mean daily dose used for the main indication of each drug. The DDD is an amount of an active substance. The number of DDDs consumed is usually given per 1000 inhabitants per day, and this provides a gross estimation of the number of patients being treated with each drug (Bergman *et al.*, 1983). Table 4 lists the DDDs of the most consumed psychotropic drugs marketed in Spain. In order to define the consumption of sedatives and hypnotics in our country better, and in order not to overlook hidden sedatives and hypnotics (see table 3), active substances, whether as single drugs or fixed-dose combinations, have been taken into account to make up the final figures of DDDs consumed.

Figure 1 shows the consumption of barbiturates, benzodiazepines and other sedatives and hypnotics in 1980 in Spain. The total consumption of tranquilizers, sedatives and hypnotics in Spain is not very different from that in other European countries (Laporte *et al.*, 1981), as other authors had suggested (Bellantuono *et al.*, 1980; Balter *et al.*, 1974). However, the consumption of benzodiazepines is lower in Spain (Laporte *et al.*, 1981) than in Northern Ireland (King *et al.*, 1980), and in the majority of Nordic countries (Grimsson *et al.*, 1979).

In Spain, among single drugs, barbiturates account only for 9.8% of total consumption. As a whole, two-thirds of the total consumption of sedative/hypnotic drugs is made up of medicines which are fixed-dose combinations of two or more drugs. For most

Table 4. Some of the principal psychotropic drugs and their defined daily doses (mg)

| Barbiturates | DDD | Benzodiazepines | DDD |
|---|---|---|---|
| Allobarbital | 100 | Chlordiazepoxide | 30[a]-50[b] |
| Amobarbital | 100 | Clonazepam | 8[a]-2[b] |
| Aprobarbital | 100 | Clorazepate potass. | 20 |
| Butalbital | 250 | Cloxazolam | 8 |
| Heptabarbital | 200 | Diazepam | 10 |
| Hexobarbital | 250 | Flunitrazepam | 2 |
| Phenobarbital | 100 | Flurazepam | 30 |
| Secobarbital | 100 | Lorazepam | 2.5 |
| | | Medazepam | 20 |
| | | Nitrazepam | 5 |
| | | Oxazepam | 50 |
| | | Oxazolam | 50 |
| **Antidepressants** | **DDD** | **Antipsychotics** | **DDD** |
| Tricyclic | | Phenothiazines | |
| Amitriptyline | 75 | Acepromazine | 100[a]-50[b] |
| Clomipramine | 100 | Chlorpromazine | 300[a]-100[b] |
| Doxepin | 100 | Fluphenazine | 10[a]-1[b] |
| Imipramine | 100 | Levomepromazine | 300[a]-100[b] |
| Nortriptyline | 75[a]-30[b] | Perphenazine | 30[a]-10[b] |
| Trimipramine | 150 | Perphenazine enant. | 7[b] |
| | | Pipotiazine | 5 |
| | | Propericiazine | 50[a]-20[b] |
| Tetracyclic | | Thioproperazine | 75[a]-20[b] |
| Maprotiline | 100 | Thioridazine | 300 |
| | | Trifluoperazine | 20[a]-8[b] |
| | | Others | |
| MAOI | | | |
| Nialamide | 100 | Haloperidol | 8 |
| Tranylcypromine | 10 | Trifluperidol | 2 |
| | | Tiotixene | 30 |
| | | Lithium carbonate | 2400 |

[a]Oral route.
[b]Parenteral route.

of these drugs the main indication is not anxiety, insomnia or nervousness, but pain, and less frequently digestive symptoms, cardiovascular conditions and non-specific problems of old age. A high consumption of these hidden sedatives and hypnotics has also been noted in other countries, where barbiturates are marketed in

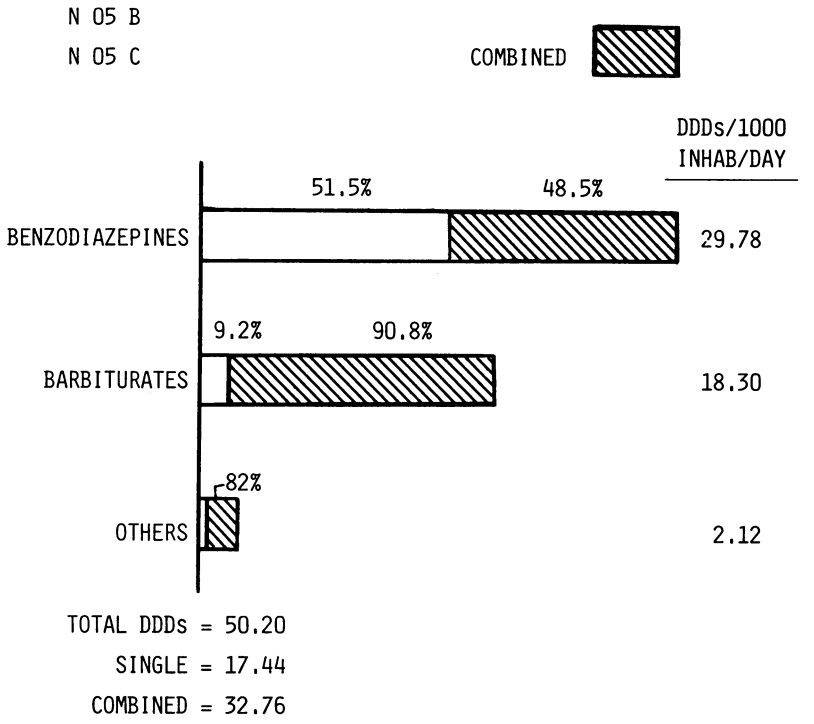


**Figure 1.** Consumption of tranquilizers, hypnotics and sedatives in 1980 in Spain.

fixed-dose combinations with analgesics-antipyretics. Thus, in Czechoslovakia 16.9 DDDs per 1000 inhabitants per day of single-sedative hypnotics were consumed in 1977, while 22.3 DDDs per 1000 inhabitants per day of combinations containing barbiturates were consumed in the same period (Stika and Vinař, 1980). On the other hand, in Northern Ireland the consumption of fixed-dose combinations containing barbiturates only amounted to 12% of total barbiturate consumption in 1979 (King *et al.*, 1980). In Finland 110.4 DDDs per 1000 inhabitants per day of the 10 most sold psychotropic substances were consumed in 1977, of which 36.6 DDDs per 1000 inhabitants per day (33%) were hidden psychotropic drugs. In Norway these figures were respectively 121.0 DDDs per 1000 inhabitants per day and 11.3 DDDs per 1000 inhabitants per day (9%). In Sweden the proportion of hidden psychotropic drugs among the 10 most sold of these products was 16% (Hemminki, 1981). Attitudes of doctors, pharmacists and the public in relation to the consumption of drugs are influenced by drug regulations, and this may explain, at least partly, the striking differences in the

consumption of hidden sedative-hypnotics from one country to another. As table 5 shows, fixed-dose combinations account for 91% of the barbiturate consumption. In particular, a pharmaceutical speciality containing propyphenazone, caffeine and 50 mg of butalbital (Optalidon®) is most popular in Spain. Almost 17 tons of butalbital were sold in 1980 in the form of Optalidon®.

Table 5. Consumption of barbiturates in Spain (1980)* (defined daily doses per 1000 inhabitants per day).

| Barbiturate | Single | Combined | Total |
|---|---|---|---|
| Butalbital | - | 6.3 | 6.3 |
| Phenobarbital | 1.3 | 4.7 | 6.0 |
| Amobarbital | 0.2 | 2.5 | 2.7 |
| Allobarbital | - | 2.1 | 2.1 |
| Others | 0.2 | 1.0 | 1.2 |
| Total | 1.7 | 16.6 | 18.3 |
| (%) | (9.3) | (90.7) | (100) |

*Data from Laporte *et al.* (1981).

## HOW PSYCHOTROPIC DRUGS ARE PRESCRIBED, DISPENSED AND CONSUMED

A survey made in Barcelona in 1980 showed that 8% of all prescriptions written by doctors in the outpatient clinics of the Social Security System are of medicines which contain some psychotropic drug. No differences in the prevalence of prescriptions of psychotherapeutic drugs were found among elderly people, as compared to younger people (Mas *et al.*, in press). Another survey, in which 395 elderly people were interviewed at home, showed that 21.3% of them were taking some psychotropic drug regularly, the prevalence of use of these drugs being higher in women than in men (Mas *et al.*, in press). Another survey, in which 232 people over 55 years old and living in the community were interviewed, yielded similar results about the prevalence of use of psychotropic drugs. More interestingly, many people (22 out of 232) were taking additive doses of the same drug contained in different marketed pharmaceutical specialities. The most frequently repeated drugs were phenylbutazone, barbiturates, corticosteroids, diazepam and codeine, most of them known as common or serious causes of adverse reactions in the elderly (Mas *et al.*, 1981).

A recent survey in which the prescription of sedative/hypnotic drugs in all the hospitals of the Spanish Social Security System was studied (Laporte *et al.*, 1981), showed that the consumption of these drugs amounted to 287 DDDs per 1000 bed-days, and that the pattern of use of the different groups of these drugs

in hospitals was approximately the same as in the community: benzodiazepines accounted for 53% of total consumption, and barbiturates accounted for 43%.

With the aim of determining which were the drugs most frequently found at home (regularly consumed or merely hoarded), a survey was made in 538 randomly chosen homes in five different socio-geographical areas of Catalonia (Martín *et al.*, 1980). The mean number of medicines found was 21.7 ± 9.7. Medicines containing tranquilizers were found in 78% of visited homes; in their majority they were not regularly consumed by people living in these homes, but they had been merely hoarded. Optalidon®, one of the most popular fixed-dose combinations used as analgesic (containing 50 mg of butalbital per tablet), was found in 60% of visited homes, and it was consumed as an analgesic with varying frequencies.

We feel that these data are relevant when the prevention of acute drug overdoses is considered (see below). Optalidon® is the most frequent offending drug among cases of acute drug overdoses admitted to the Hospital de Sant Pau in Barcelona (Camí et al., 1980). As butalbital is an intermediate-acting barbiturate, overdosage by this drug is potentially more serious than overdosage by long-acting preparations (Harvey, 1980; Berman *et al.*, 1956). In Spain Optalidon® is not included in the official list of over-the-counter medicines (Ministerio de Trabajo, Sanidad y Seguridad Social, 1981). However, a recent survey shows that it can be readily obtained in pharmacies without a medical prescription (see table 6), as compared to Luminal®.

Table 6. Results of a survey on the dispensation of two medicines containing barbiturates in 60 pharmacies in Barcelona (1982)

| | Number of pharmacies visited | Dispensed without any prescription | Prescription required |
|---|---|---|---|
| Luminal® was asked for | 30 | 0 | 30 |
| Optalidon® was asked for | 30 | 30 | 0 |

## LOOKING FOR CLINICAL AND EPIDEMIOLOGICAL END-POINTS OF BARBITURATE MISUSE: SEVERE ACUTE OVERDOSE IN A UNIVERSITY HOSPITAL

In order to know the severity, in terms of morbidity and mortality, of acute overdoses caused by different drugs, an analysis was made of all the 91 cases admitted to the Intensive Care Unit (ICU) of the Hospital de Sant Pau in Barcelona between 1974 and

1980 (Frati, 1982). As during this period only two patients with an acute overdose died in this hospital before their admission to the ICU (one before admission to the emergency room and the other in the emergency room), this sample can be considered as representative of the more severe cases of acute overdoses. Optalidon® was the most commonly implicated medicine in this series (see table 7).

The barbiturates with short half-life and high lipid solubility are more toxic than the more polar, long-acting compounds, such as phenobarbital, and poisoning with the short-acting agents is more dangerous. Among 39 deaths by barbiturate poisoning reported by Poklis and Gantner (1981), only three were related to phenobarbital and two to barbital, the rest being related to amobarbital, butalbital, pentobarbital, secobarbital and other short-acting barbiturates. In our series, out of 25 cases of acute overdose by butalbital, 19 presented with deep coma on admission, and six with superficial coma, whereas among the six cases related to phenobarbital, only two presented with deep coma ($p<0.05$). Respiratory complications were also more common among patients with butalbital intoxication: nine suffered bronchoaspiration and pneumonitis while admitted to the ICU, whereas only one case of pneumonitis was recorded among patients with a phenobarbital overdose. Out of the 91 patients with acute drug overdose admitted to the ICU, five died; in two of these cases the implicated drug was butalbital.

Table 7. Medicines most commonly implicated in acute overdoses among 91 patients with acute overdose admitted to the Intensive Care Unit of the Hospital de Sant Pau (Barcelona) during the period 1974-1980

| Medicine | *n* |
|---|---|
| Optalidon® (butalbital + propyphenazone + caffeine) | 25 |
| Nobitrol® (amitriptyline + medazepam) | 7 |
| Luminal® (phenobarbital) | 6 |
| Meleril® (thioridazine) | 6 |
| Valium® (diazepam) | 4 |
| Tranxilium® (dipotassium clorazepate) | 4 |
| Tofranil® (imipramine) | 3 |
| Dormodor® (flurazepam) | 3 |

## SUMMARY AND CONCLUSIONS

Drug utilization statistics provide one of the tools needed for the understanding of the role that drugs, prescribed or unpre-

scribed, play in a society, and for adjusting that to the real needs of the society. In recent years, some astonishing differences have arisen between drug markets in different countries, largely as a result of variations in drug regulation policy. The use of psychotropic drugs, and particularly that of sedatives and hypnotics, shows wide geographical variations. The high number of drugs and the high proportion of fixed-dose combinations marketed in many countries make comparisons of drug use more difficult, and confuse the real indications for the use of drugs at all the levels of the therapeutic chain (prescriber, dispenser and user). Some scientifically unsound cultural attitudes tend to appear when the number of drugs is not restricted to an optimal level. As an example, the ease of obtaining intermediate-acting barbiturates, sold for indications other than the treatment of psychic symptoms, is illustrated in this paper with figures of consumption and by some medical consequences in the field of drug overdosage and poisoning.

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# Heavy drug use among the elderly: prescription surveys in Manitoba

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## INTRODUCTION

Infirmity and disease have always been identified with old age, but it is only within the last generation that some relief from the discomforts and disabilities of aging has really been possible. Appropriate drug therapy can bring substantial benefits to older patients, but benefits must be carefully weighed against risks. The hazards of adverse drug effects are markedly increased with aging. This is in part due to changes in drug disposition and drug effect with age, but is more likely related to the number of drugs used by the elderly, drugs which are often prescribed simultaneously and chronically.

Thus, drug use in itself must be considered as an important risk factor. The elderly use more drugs than the young (Boethius, 1977; Skoll *et al.*, 1979), and the number of drugs prescribed increases with the variety of illnesses being treated. Polypharmacy is a real danger for the elderly, and each drug prescribed adds measurably to the risk of adverse drug effects. Hurwitz (1969) demonstrated this for hospitalized patients, and more recently, Williamson and Chopin (1980) found that the prevalence of adverse reactions increased markedly with the number of drugs prescribed for a series of geriatric patients requiring admission to hospital.

Studies on changes in disposition and drug effect must be undertaken to identify drugs of risk to older patients, but patients at risk must also be identified, namely those elderly patients who receive multiple prescription drugs for long periods with the attendant danger of adverse reactions. There is a need for information concerning the number and type of drugs used by heavy drug users among the elderly, and it was the purpose of these studies to provide such observations.

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## METHODS

Manitoba's Pharmacare plan offers partial reimbursement to individuals or families whose annual prescription drug costs are more than $50.00. Each application for Pharmacare benefits provides specific information as to the type, amount and cost of all prescribed drugs, as well as the route of administration and duration of therapy. These variables were related to the age and sex of the claimants by examining random samples of applications for the years 1975 and 1978.

The sample for 1975 consisted of 429 applications from claimants aged 65 and older whose individual drug costs were more than $50.00 in that year. In addition, the applications of 387 claimants aged 50 to 64 were reviewed as a control sample to determine age-related differences in prescription frequencies. Although the numbers of claims reviewed in 1975 and 1978 were approximately equal (Aoki *et al.*, 1982), the application of a correcting factor for inflation reduced the number of 1978 applications surveyed for the purposes of this study to 365 for those aged 65 and older and 380 for those aged 50 to 64. Since the mean cost per Pharmacare claimant rose from $132.39 to $171.34 between 1975 and 1978, only these individuals who spent more than $64.70 on prescription drugs in 1978 are included in the comparisons made in this publication.

The data were analyzed to relate the number and types of drugs to the age and sex of the claimants. Differences between means were tested by analysis of variance, and differences between frequencies by chi-square analysis. Values $p<0.05$ were taken to indicate statistically significant differences for a two-tailed test.

## RESULTS

The population of Manitoba is just over a million, and in 1978 there were 117 000 Manitobans older than 65 years of age. In that year, 24% of this population spent more than $64.70 on prescription drugs, and are characterized as heavy drug users for this study.

### Sample Characteristics

The two samples are compared in table 1. In both years, more women than men were represented, especially among claimants aged 50 to 64. More drugs were prescribed for women than men in both samples, but the difference was not statistically significant for the older claimants in either year. There was no difference in the number of drugs prescribed between younger and older claimants.

Table 1. Sample characteristics *

| | 50 to 64 years | | 65 years and older | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| 1975 | | | | |
| Number in group | 160 | 227 | 201 | 228 |
| Number of drugs/year | 8.5 ± 0.4 | 10.3 ± 0.4 | 8.6 ± 0.4 | 9.8 ± 0.4 |
| Cost in dollars/year | 209 ± 13 | 221 ± 10 | 188 ± 8 | 170 ± 6 |
| Number of doctors/year | 2.3 ± 0.1 | 2.6 ± 0.1 | 2.1 ± 0.1 | 2.2 ± 0.1 |
| 1978 | | | | |
| Number in group | 163 | 217 | 163 | 202 |
| Number of drugs/year | 7.1 ± 0.4 | 8.7 ± 0.4 | 8.2 ± 0.4 | 8.9 ± 0.4 |
| Cost in dollars/year | 199 ± 10 | 200 ± 15 | 183 ± 11 | 187 ± 9 |
| Number of doctors/year | 2.1 ± 0.1 | 2.3 ± 0.1 | 2.0 ± 0.1 | 2.0 ± 0.1 |

*Mean and standard error (S.E.M.) are shown for each group.

The proportion of drugs prescribed as combination products was quite constant over all groups in both years, and ranged from 38% to 45% of the annual total. Similarly, the proportion of drugs prescribed chronically, that is, for more than 120 days annually, was also consistent, ranging from 36% to 41% of the number of drugs prescribed. Women tended to receive more drugs chronically and more drugs as combination products, but no statistically significant differences were found.

## Prescription Frequencies

Drugs prescribed for claimants were classified into categories containing one or more drugs with similar pharmacological properties. The most commonly prescribed categories for claimants aged 65 or older are shown in table 2, which ranks the percentage of older claimants receiving prescriptions for drugs in these categories in 1975 and 1978. There were relatively few differences in prescription frequency apparent over the interval, but the substantial decline in the use of phenobarbital and of barbiturates other than phenobarbital should be noted.

Psychotherapeutic agents were very commonly prescribed in both years, and occupied seven of the first 25 places in our survey out of a total of 154 categories. For these older Manitobans, benzodiazepines were second only to thiazide-type diuretics in prescription frequency.

In 1975, the five categories most commonly prescribed chronically for older claimants were thiazide-type diuretics, benzodiazepines, digoxin, α-methyldopa and barbiturates other than phenobarbital. By 1978, salicylates had displaced other barbiturates from this ranking.

**Table 2.** Most commonly prescribed drug categories for claimants aged 65 years and older*

| | 1975 ($n$ = 429) | | 1978 ($n$ = 365) | |
|---|---|---|---|---|
| 1 | Thiazide-type diuretics | 55.9 | Thiazide-type diuretics | 53.2 |
| 2 | Benzodiazepines | 47.1 | Benzodiazepines | 41.9 |
| 3 | Salicylates | 31.2 | Salicylates | 28.5 |
| 4 | Sympathomimetics | 25.9 | Sympathomimetics | 24.7 |
| 5 | Other barbiturates† | 25.6 | Digoxin | 24.1 |
| 6 | Codeine | 24.9 | Codeine | 22.7 |
| 7 | Digoxin | 24.2 | Furosemide | 17.5 |
| 8 | Tetracyclines | 20.5 | Tetracyclines | 17.3 |
| 9 | Phenobarbital† | 20.0 | α-Methyldopa | 17.3 |
| 10 | Anticholinergics† | 19.8 | β-Blocking drugs | 14.8 |
| 11 | Sulfonamides† | 17.7 | Phenobarbital | 14.0 |
| 12 | Furosemide | 15.6 | Other barbiturates | 13.7 |
| 13 | Theophylline | 15.4 | Anticholinergics | 13.7 |
| 14 | α-Methyldopa | 14.5 | Tricyclic antidepressants | 13.7 |
| 15 | Profen-type drugs | 14.5 | Theophylline | 13.2 |
| 16 | Tricyclic antidepressants | 13.8 | Potassium supplements | 12.9 |
| 17 | Ampicillin | 13.5 | Ampicillin | 12.6 |
| 18 | Potassium supplements | 13.5 | Triamterene | 12.3 |
| 19 | Phenylbutazone† | 13.3 | Profen-type drugs | 10.7 |
| 20 | Systemic corticosteroids† | 13.1 | Indomethacin | 10.4 |
| 21 | β-Blocking drugs | 12.1 | Phenothiazines | 9.9 |
| 22 | Phenothiazines | 12.1 | Co-trimoxazole | 9.3 |
| 23 | Propoxyphene | 10.0 | Sulfonamides | 8.5 |
| 24 | Triamterene | 9.8 | Systemic corticosteroids | 8.2 |
| 25 | Indomethacin | 8.9 | Phenylbutazone | 7.7 |

*Percentage of claimants aged 65 and older who received prescriptions for drugs from these categories in 1975 and 1978.

†Designates significant difference in prescription frequency between 1975 and 1978 ($p < 0.05$).

## Prescription of Psychotherapeutic Drugs

In table 3 the prescription of the major psychotherapeutic drug categories is detailed. Other drugs may alter neurological or psychological function in the elderly, but the drug categories listed in table 3 clearly have their major effects on the central nervous system and are prescribed for these effects. It is in this context that these agents are grouped together as psychotherapeutic drugs.

Sedative-hypnotic drugs were prescribed for many heavy drug users. The benzodiazepines were frequently prescribed, and more than half the prescriptions in both years were considered chronic

Table 3. Most commonly prescribed psychotherapeutic categories*

| | | 50 to 64 years | | 65 years and older | |
|---|---|---|---|---|---|
| | | Men | Women | Men | Women |
| Benzodiazepines | 1975† | 49.4 (31.3) | 64.8 (46.3) | 39.3 (25.4) | 53.9 (35.1) |
| | 1978 | 34.4 (19.0) | 55.3 (31.8) | 37.4 (16.6) | 45.5 (25.7) |
| Phenobarbital | 1975 | 18.1 (9.4) | 18.9 (7.5) | 17.9 (10.4) | 21.9 (10.5) |
| | 1978 | 11.0 (4.9) | 12.4 (4.1) | 10.4 (4.9) | 16.8 (6.4) |
| Other barbiturates | 1975 | 18.1 (9.4) | 25.6 (15.0) | 22.4 (14.4) | 28.5 (17.1) |
| | 1978 | 6.7 (3.1) | 13.8 (7.8) | 10.4 (4.9) | 16.3 (10.9) |
| Codeine | 1975 | 23.8 (4.4) | 33.5 (8.4) | 26.4 (3.0) | 23.7 (3.9) |
| | 1978 | 21.5 (4.3) | 25.8 (6.9) | 20.8 (1.8) | 24.3 (8.4) |
| Propoxyphene | 1975 | 7.5 (1.3) | 16.3 (5.3) | 7.0 (2.0) | 12.7 (3.9) |
| | 1978 | 1.2 (0.0) | 5.5 (2.3) | 5.5 (1.2) | 7.4 (2.5) |
| Tricyclic antidepressants | 1975†§ | 17.5 (6.3) | 24.2 (15.9) | 9.5 (7.0) | 17.5 (13.6) |
| | 1978 | 11.7 (8.6) | 15.2 (9.7) | 11.7 (8.0) | 15.3 (10.4) |
| Phenothiazines | 1975†§ | 8.1 (4.4) | 23.8 (16.7) | 10.4 (6.5) | 13.6 (8.8) |
| | 1978 | 7.4 (4.3) | 13.8 (8.8) | 4.9 (1.8) | 13.9 (7.4) |

*Open figures indicate the percentage of claimants in each group who received prescriptions for drugs from these categories, and figures in parentheses indicate the percentage in each group who received prescriptions for more than 120 days.

†Designates differences related to age at $p < 0.05$ (50 to 64 yr vs. 65 yr and older).

§Designates differences related to sex at $p < 0.05$ (all men vs. all women).

because the drug was prescribed for more than 120 days during the year. More women than men used benzodiazepines in 1978, but the age-related difference observed for the 1975 survey was not apparent in 1978.

Phenobarbital is included in a variety of combination products in Canada, usually with anticholinergic drugs, and this accounts for most of the use recorded in our surveys. There were no differences in prescription frequency related to age or sex in either year, and prescriptions for phenobarbital decreased between 1975 and 1978 for all groups.

The other barbiturates marketed in Canada are available only as single drugs or barbiturate combinations and are prescribed solely as sedative-hypnotics. More women than men received prescriptions for these drugs in both years, but there were no age-related differences. Use of sedative-hypnotic barbiturates fell

for all groups over the three-year interval, and indeed decreased more than phenobarbital use.

Several salicylate-codeine combination products are popular in Canada, and prescriptions for these agents accounted for most of the codeine given to these heavy drug users. Similarly, most of the propoxyphene prescribed was contained in various salicylate-propoxyphene combinations. As well, the only minor sedative-hypnotic to be prescribed to any extent, meprobamate, was used as a salicylate-codeine-meprobamate combination. (It was prescribed for 4.7% of older claimants in 1975 and 3.8% in 1978.) The prescription of codeine was high and not different between groups in both years, and although the use of propoxyphene declined between 1975 and 1978, the decrease was statistically significant only for the younger claimants.

Tricyclic antidepressants were prescribed for many claimants in both 1975 and 1978, as were the phenothiazines, and the prescription frequencies for these categories did not change over the interval. More of both these categories were prescribed for younger claimants than for older in 1975, and more for women than for men. Only the sex-related difference in phenothiazine prescription held in 1978.

### Multiple Psychotherapeutic Prescriptions

Many of these heavy drug users received more than one drug with major effects on the central nervous system. As table 4 indicates, over 65% of the claimants aged 65 and older received at least one such psychotherapeutic drug in either year, and over 40% were given such drugs chronically.

Concurrent use of psychotherapeutic drugs was considered probable if two or more such drugs were prescribed chronically. In 1975, 48% of the women and 33% of the men over the age of 65 met this criterion, and in 1978, 34% of the older women and 18% of the older men had concurrent psychotherapeutic prescriptions. Similar patterns were observed for the younger claimants, and both the sex-related difference in concurrent prescriptions and the decrease over the interval were statistically significant for older and younger claimants.

## DISCUSSION

Several studies have examined the use of prescription drugs by the elderly and the World Health Organization (1981) estimates that, where the proportion of elderly in the population approaches 20%, they account for over 50% of the total drug consumption. In Canada, a study in our neighboring province found that the 11% of Saskatchewan's population aged 65 and older received 28% of all prescriptions written in 1976 (Skoll *et al.*, 1979). In that year,

Table 4. Multiple psychotherapeutic prescriptions*

| Number of prescriptions | | 50 to 64 years | | 65 years and older | |
|---|---|---|---|---|---|
| | | Men | Women | Men | Women |
| One | 1975 | 23.8 (15.6) | 22.9 (13.7) | 33.3 (19.9) | 25.0 (12.7) |
| | 1978 | 25.8 (13.5) | 34.6 (17.1) | 39.3 (18.4) | 26.7 (14.9) |
| Two | 1975 | 23.8 (17.5) | 25.1 (17.2) | 19.9 (13.4) | 25.4 (19.3) |
| | 1978 | 14.1 (11.7) | 17.1 (10.1) | 13.5 (9.2) | 22.3 (16.3) |
| Three | 1975 | 11.9 (10.6) | 12.8 (11.0) | 11.9 (11.4) | 20.6 (17.1) |
| | 1978 | 8.0 (6.7) | 11.5 (8.8) | 9.8 (4.9) | 14.4 (9.9) |
| Four | 1975 | 5.0 (5.0) | 11.0 (11.0) | 7.0 (5.0) | 7.9 (7.5) |
| | 1978 | 4.3 (2.5) | 9.2 (6.9) | 4.9 (3.7) | 5.0 (4.0) |
| Five | 1975 | 7.9 (6.6) | 7.9 (6.6) | 2.5 (2.0) | 3.9 (3.1) |
| | 1978 | 1.2 (1.2) | 1.8 (1.8) | 0.6 (0.0) | 3.0 (2.5) |
| Six or more | 1975 | 4.4 (3.8) | 7.0 (7.0) | 1.0 (1.0) | 0.9 (0.9) |
| | 1978 | 1.2 (1.2) | 2.8 (2.8) | 0.6 (0.6) | 1.5 (1.5) |

*Open figures indicate the percentage of claimants in each group who received that number of psychotherapeutic drugs, and figures in parentheses indicate the percentage in each group who received that number for more than 120 days.
A prescription for a psychotherapeutic drug specified a drug from one of the following categories: phenothiazines, other major tranquilizers, tricyclic antidepressants, MAO inhibitors, lithium, benzodiazepines, chloral hydrate and other minor sedative-hypnotics, levodopa, codeine, other narcotic analgesics, propoxyphene, phenobarbital, other barbiturates, hydantoins, and other anticonvulsants.

77% of those over 65 received at least one prescription drug, and the average number prescribed for the elderly in that province was 4.1.

Our surveys in 1975 and 1978 have attempted to define the drugs prescribed for that portion of the older population that could be characterized as heavy drug users. Although a $50 annual expenditure for prescription drugs provides a rather arbitrary limit, we feel that it reasonably encompasses all the heavy drug users in Manitoba. This study indicates that 24% of all Manitobans aged 65 or older in 1978 met this criterion, and actually had a mean expenditure of $185 for an average 8.6 prescription drugs that year. It should be noted that these surveys did not include hospitalized or institutionalized elderly who might be predicted to be heavy drug users.

Although there were more women than men represented in the random samples from both years, it was only for the claimants aged 50 to 64 that this sex-related difference was statistically significant. For the claimants aged 65 and older, the female:male

distribution approximated the female:male distribution of Manitoba's over 65 population (1.24:1 in 1978). Similarly, women received more drugs than men in both 1975 and 1978, but the difference was statistically significant only for the younger groups.

This study has focused on the use of psychotherapeutic drugs by older heavy drug users, and the widespread prescription of these drugs to the elderly was predictable. A study in Boston found that 20% of all patients admitted to general medical and surgical wards there had used psychotropic agents in the three months prior to hospitalization, and that 'use of these drugs was higher in middle and older-aged groups, and higher in women than among men' (Greenblatt *et al.*, 1975). Similarly, a study of American adults showed differences in psychotherapeutic drug use related to sex and age, such that 28% of the men and 35% of the women over 60 years of age had used such drugs in the preceding year (Parry *et al.*, 1973).

As might be expected, the prescription of psychotherapeutic drugs was much higher in our surveys of heavy drug users. Approximately two-thirds of the claimants aged 65 and older had been given psychotherapeutic agents in both 1975 and 1978. Although the percentage of men and women who had received at least one such prescription was not different in either year, there were more women than men who got more than one of these drugs, and more women who used psychotherapeutic drugs chronically.

It was not unexpected that prescriptions for benzodiazepines made up the bulk of this drug use, but the amount of sedative-hypnotic barbiturate prescribed for this population is surprising. By 1975, barbiturates were well recognized as being obsolete and potentially dangerous drugs, especially for the elderly (Koch-Weser and Greenblatt, 1974). Thus, although their prescription frequency decreased for 1975 to 1978, it is difficult to understand why these barbiturates were still being used by a substantial proportion of elderly Manitobans. If new prescriptions were not being written, it is likely that this use represents a continuation of prescriptions originally given to these heavy drug users some years earlier. In this way aging patients, possibly assisted by aging physicians, may tend to accumulate drugs.

Our surveys indicate that antidepressants and antipsychotic agents were often given to these heavy drug users and, in most instances, prescribed chronically. There may be more justification for the prescription of these drugs for the elderly than for the prescription of barbiturates. However, what Fries (1980) refers to as 'the loss of the ability to maintain homeostasis' makes these medications more hazardous for the elderly because of their prominent cardiovascular and anticholinergic effects.

It is not clear why the age-related differences in prescription frequency of the tricyclic antidepressants and phenothiazines recorded in 1975 were not present in 1978. Indeed, there were no age-related differences in prescription frequency for any of the major psychotherapeutic categories in 1978, suggesting further

that the patterns of psychotherapeutic drug use shown by these Manitobans had actually been established before they reached old age.

Codeine was widely prescribed for these elderly Manitobans and its use is emphasized because the prescription of codeine and other narcotic analgesics adds to the burden of drugs borne by geriatric patients. The demonstration in our study of the substantial number of older heavy drug users who receive multiple and concurrent psychotherapeutic drugs is particularly worrisome. Deterioration of intellectual and emotional function are the major adverse effects associated with the use of these agents in the elderly, and the study of Williamson and Chopin (1980) demonstrates that drugs acting on the central nervous system are the most likely to result in adverse effects leading to hospital admission for geriatric patients.

These surveys provide a database by which we can examine the magnitude of this problem in Canada, and through which we may confirm that elderly patients at risk may be identified by the amount and type of drugs prescribed for them.

## SUMMARY AND CONCLUSIONS

Claims submitted under Manitoba's Pharmacare plan are being sampled at three-year intervals to provide information on elderly Manitobans who spend more than $50 annually on prescription drugs. In 1975 and 1978, the claims of individuals aged 65 and older were compared with those of claimants aged 50 to 64. In both years, there were more younger women than men who spent more than $50 on drugs, and the younger women received more drugs than the younger men. In contrast, the sex distribution of older claimants approximated that of the province's over-65 population (55% females), and the difference in number of drugs received was not statistically significant. The most frequently prescribed drug categories for the elderly claimants were thiazide-type diuretics, benzodiazepines, salicylates, sympathomimetics, digoxin and codeine.

Benzodiazepines, codeine, phenobarbital, other barbiturates, tricyclic antidepressants and phenothiazines were the most commonly prescribed psychotherapeutic categories for the elderly claimants. There were few differences in prescription frequency between 1975 and 1978, but there were substantial decreases in the prescription of phenobarbital and other barbiturates. More women than men aged 65 and older received prescriptions for benzodiazepines, phenothiazines and barbiturates other than phenobarbital in both years. Over two-thirds of the elderly claimants received prescriptions for at least one psychotherapeutic drug in both years, and concurrent prescriptions of these agents was relatively common.

Drugs prescribed for the elderly tend to accumulate like the maladies for which they are prescribed, and each drug adds to the risk of adverse drug effects. Psychotherapeutic drugs are no exception to this rule and, indeed, are often associated with the most devastating consequences.

These surveys have demonstrated that a substantial proportion of Manitoba's elderly population are heavy drug users, and that much of that drug use consists of psychotherapeutic agents. Our information on the hazards posed by this use is incomplete, but enough is known to make us promote the appropriate and considerate prescription of psychotherapeutic drugs as an essential goal for those who care for the elderly.

**Acknowledgements**

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# To what extent are inter- and intra-regional differences in psychotropic drug use explained by demographic and socio-economic factors?

David J. King[1] and Kathryn Griffiths[2]

## INTRODUCTION

Geographical comparisons made between psychotropic drug usage levels in different countries (Grimsson *et al.*, 1979) or particular regions (Westerholm, 1979; Elmes *et al.*, 1976a) have not usually taken into consideration the varying proportions of inhabitants that constitute the 'at risk' population, or any differences in their living conditions, and without reference to these characteristics of the people receiving drug treatment it is difficult to judge the clinical relevance of any disparities found. Furthermore, since comparable and reliable psychiatric morbidity data are particularly difficult to obtain, regional differences in psychotropic drug prescribing are probably more usefully related to objective demographic and socio-economic measures in the first instance. A Central Mental Health Records Scheme, based on returns from all psychiatric hospitals and units in Northern Ireland, has been in operation since 1960. Although these data are incomplete in some respects, it is hoped to make some comparisons with the drug utilization data in a subsequent study.

Drug prescribing studies in Northern Ireland benefit from a number of advantages, which are as follows. (i) Data on general practice prescribing can be supplied by the Northern Ireland Central Services Agency (CSA) which has been processing over 95% of the National Health Service (NHS) prescriptions in the province through a computerized pricing system since 1966. (ii) There is no hospital prescribing for outpatients, and tentative estimates

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of hospital prescribing for inpatients indicate that this only accounts for between 5 and 10% of total NHS psychotropic prescribing, and therefore the CSA data represent virtually all the psychotropic drugs prescribed in the province. (iii) With the exception of the Belfast area, the Department of Health and Social Services (DHSS) districts in Northern Ireland correspond to groups of district council areas for which census and more recent housing condition data are available. The DHSS districts can also be related to the travel to work areas or aggregations of employment service office areas for unemployment statistics. (iv) Our department has collaborated for a number of years in the WHO 'Drug Utilization Research Group', which has coordinated the work of a number of European countries which have agreed on a common system of drug classification and quantification. The classification system adopted is the so-called anatomical classification of the European Pharmaceutical Market Research Association (EPhMRA) and the unit of measurement, the defined daily dose (DDD), is used as a technical unit of measurement and of comparison, which can be applied to drug data from any source (Lunde *et al.*, 1979).

## METHODS

General practice prescribing data were obtained from the Northern Ireland CSA for all practices for a one-month period (October) of each year until 1970 but thereafter a three-month sample (April to June) was provided. This was then collated and converted to a measure of drug consumption per 1000 of the population per day using DDDs provided by the Nordic Council on Medicines (1979b), and expressed as DDD per 1000 inhabitants per day for each drug, as described previously (Elmes *et al.*, 1976b; McDevitt and McMeekin, 1979). The term 'tranquilizer' includes all benzodiazepines (excluding clonazepam) normally prescribed for daytime use, together with meprobamate, hydroxyzine and benzoctamine. 'Hypnotic' is synonymous with 'hypnotics and sedatives' in the EPhMRA classification. Barbiturate and non-barbiturate hypnotics are considered separately. Antidepressants include all tricyclics and monoamine oxidase inhibitors; L-tryptophan, viloxazine, mianserin and tofenacin were included from 1977 onwards and nomifensine from 1978. The term 'major tranquilizer' has been avoided and 'neuroleptic' used instead to avoid confusion. The European data were provided from sales figures, but since the Northern Ireland prescribing data cover 90-95% of all NHS psychotropic drug use in the province, the two sets of data were considered to be comparable.

The general practice psychotropic drug prescribing data for each of Northern Ireland's 17 health districts were then analyzed in detail for the year 1978. The drug usage level 'dependent' variables were mapped and their degree of association with 11 'independent' socio-economic variables examined. These demo-

graphic or socio-economic measures were chosen on the basis of factors which had previously been linked with high drug utilization or psychiatric morbidity, social indicators related to these reported factors, and variables suggested by the geographical pattern of Northern Ireland psychotropic drug utilization as being suitable for investigation. Details of these variables and their sources have been published elsewhere (King *et al.*, 1982). The district council areas for which these data were available corresponded closely to the DHSS health districts except for Belfast, which was treated as one area for some of the official statistics. The city data could not be subdivided according to the three health districts which were therefore treated as tied ranks for some of the socio-economic variables.

The degree of association between psychotropic drug utilization and each socio-economic variable for the 17 districts was found by calculating zero-order Spearman rank correlation coefficients. A multivariate analysis was performed on the psychotropic drug 'dependent' variables of tranquilizers, hypnotics, neuroleptics, antidepressants and psychostimulants with respect to six demographic and social indicators - the five that appear to be most highly correlated with drug utilization at the zero order, plus list size per doctor to standardize workload. Fifth-order Spearman partial correlation coefficients were calculated between the prescribing levels and each of the 'independent' variables in turn (population density, overcrowding, the proportion of females aged 45-59 in the population, the percentage of people over the age of 65, the unemployment rate, and list size per doctor) by keeping the effects of the remaining five socio-economic variables constant. The utilization figures were then transformed into logarithms and subjected to multiple regression analyses against population density, overcrowding, females aged 45-59, persons aged over 65 years, and list size per doctor. This procedure was repeated using unemployment rather than overcrowding as the fifth 'independent' socio-economic variable.

## RESULTS

The overall pattern of psychotropic drug prescribing in Northern Ireland from 1966 to 1980, and the comparisons with Great Britain and other European countries, have been reported in detail elsewhere (King *et al.*, 1982). Total psychotropic drug consumption reached a peak of 88 DDD per 1000 inhabitants per day, i.e. approximately 8.8% of the total population, or 12.5% of the adult population (over 15 years), in 1975. The subsequent decline has been due to the changing pattern of benzodiazepine use since this has represented an increasingly large proportion of the total psychotropic drugs prescribed, so that in 1980 they constituted 98% of all tranquilizer, 78% of all hypnotic and 75% of all psychotropic drugs prescribed. Thus both total tranquilizer and

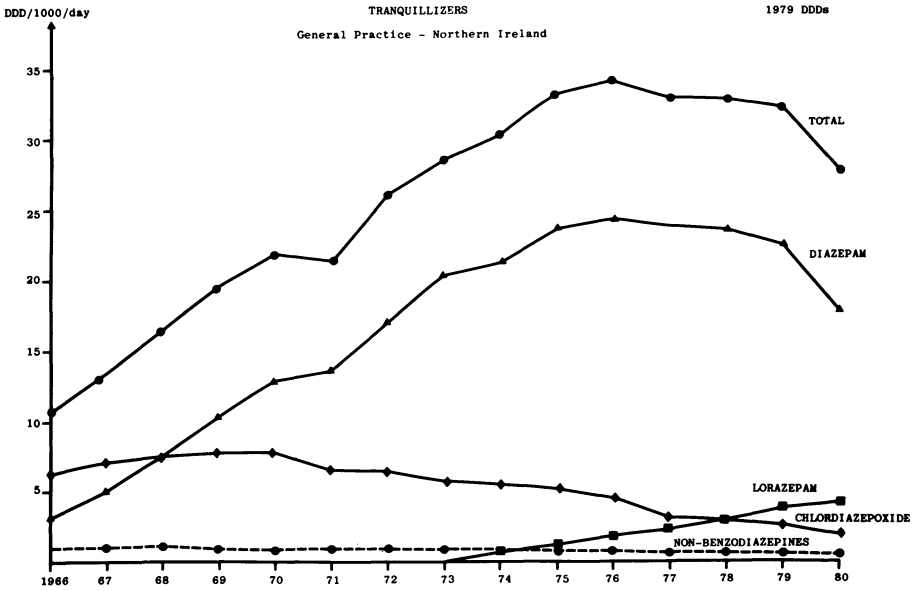


**Figure 1.** Psychotropic drug prescribing levels in Northern Ireland 1966-80. Defined daily doses per 1000 per day for diazepam, lorazepam, chlordiazepozide, non-benzodiazepines and total tranquilizers.

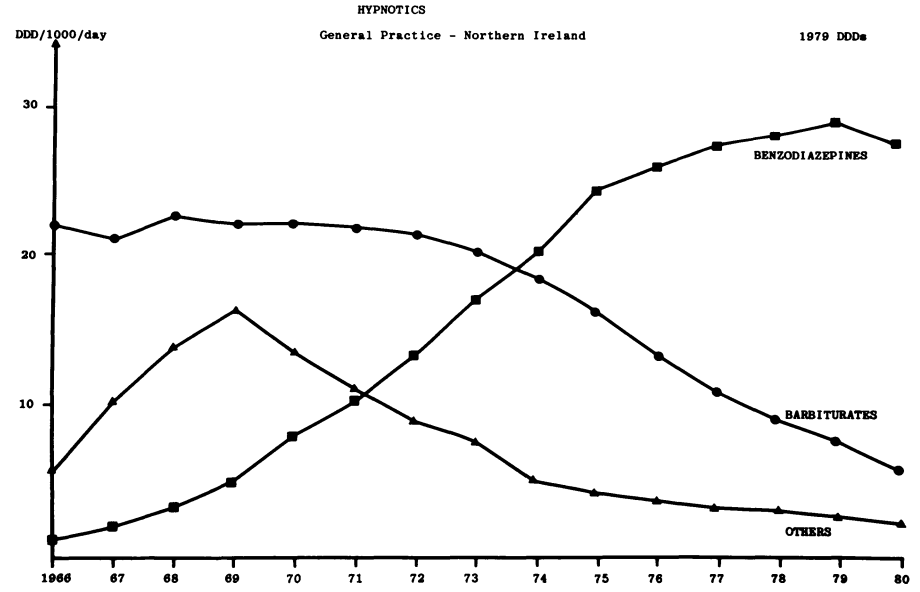


**Figure 2.** Psychotropic drug prescribing levels in Northern Ireland 1966-80. Defined daily doses per 1000 per day for barbiturate and benzodiazepine hypnotics.

diazepam prescribing showed a similar pattern to the total psychotropic drug use, reaching a peak a year later (1976) and after a three-year plateau began to decline after 1979 (figure 1). Earlier decreases were evident in psychostimulant and barbiturate hypnotic use. The latter has been falling steadily since 1968 (figure 2). The main effect of the 1975-77 CURB campaign on this trend was to accelerate the decline in the quantity of barbiturates prescribed but not in the number of barbiturate prescriptions written (King *et al.*, 1980). The successful reduction in barbiturate prescribing has probably been due to the ready availability of alternative hypnotics, for although non-barbiturate exceeded barbiturate hypnotic prescribing in 1972, and benzodiazepine exceeded barbiturate hypnotics two years later (figure 2), total hypnotic drug use did not show any reduction until 1979.

The remaining drug groups have continued to show steady but more gradual increases over the period of study. Antidepressant prescribing doubled between 1966 and 1975 (King *et al.*, 1977) and neuroleptic use also almost doubled from 1.5 to 2.9 DDD per 1000 per day between 1966 and 1980 (figure 3). It is evident that

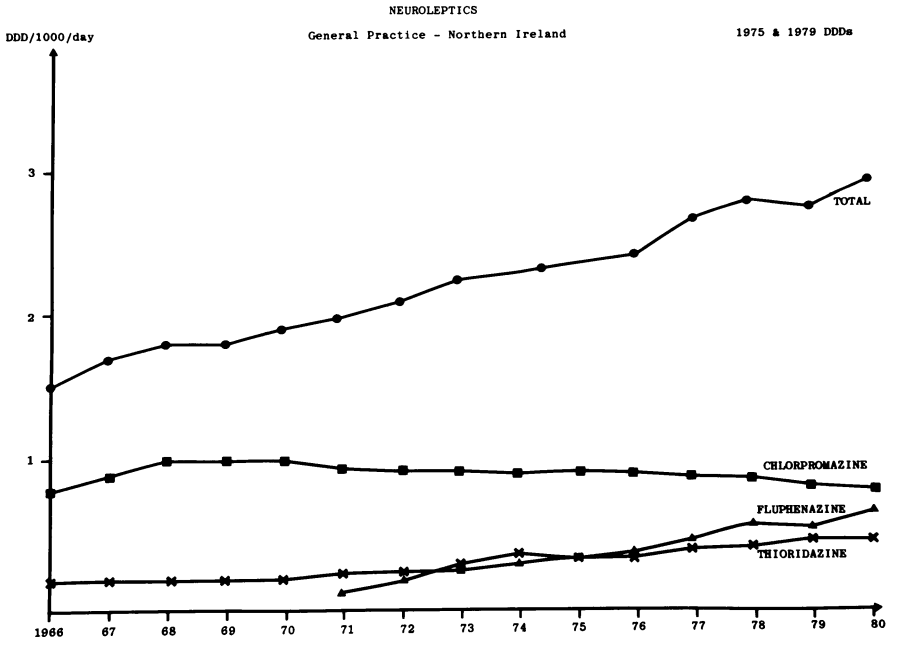


**Figure 3.** Psychotropic drug prescribing levels in Northern Ireland 1966-80. Defined daily doses per 1000 per day for chlorpromazine, thioridazine, fluphenazine and total neuroleptics.

general practitioners have been making increasing use of depot neuroleptic preparations while chlorpromazine prescribing has remained remarkably static.

The inter-regional comparisons of the use of the largest psychotropic drug group, benzodiazepine tranquilizers, are shown in figure 4. Clearly for the decade after 1966 the rise in tranquilizer use in Northern Ireland was more rapid than elsewhere, but the decline since 1976 means that the utilization here is now less than in Iceland and Denmark but still higher than in Norway, Sweden, Finland and Czechoslovakia. Although it has been speculated that various local factors such as the introduction of a monitoring scheme for benzodiazepines in Finland (Idänpään-Heikkilä, 1977), or the widening of the advertised indications for tranquilizer use (Hemminki *et al.*, 1981) may account for the fluctuations in consumption, the wide differences between Iceland on the one hand and Czechoslovakia on the other are difficult to

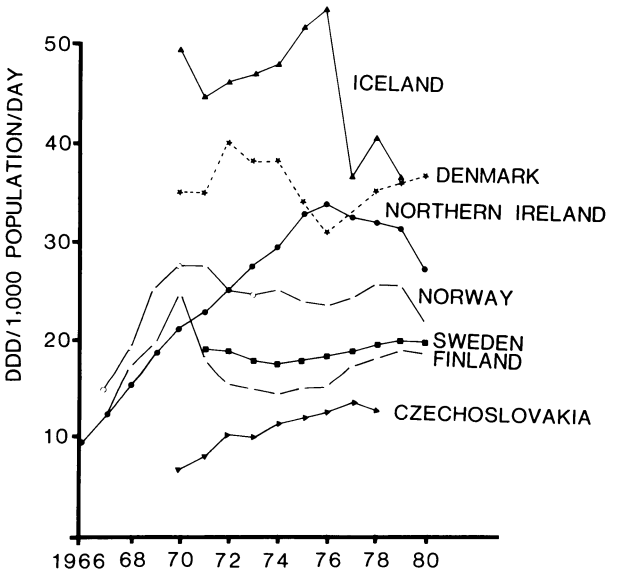


**Figure 4.** Benzodiazepine tranquilizer use in Czechoslovakia, Finland, Iceland, Norway, Sweden, Denmark and Northern Ireland for 1966-80, in defined daily doses per 1000 per day. (Figures for Iceland for 1976 and 1977 estimated from total benzodiazepines (Olafsson *et al.*, 1980) and tranquilizers (Nordic Council on Medicines, 1979a.) From King *et al.* (1982). Psychotropic drug use in Northern Ireland 1966-80. *Psychol. Med.* (in press). Reproduced by permission of Cambridge University Press.

explain. Differences in clinical indications for using these drugs seem to be unlikely since they have been predominantly prescribed for women and for persons in the 45-59 year old age range in all regions studied (Balter *et al.*, 1974; Skegg *et al.*, 1977; Böethius and Westerholm, 1977; Anderson, 1980; Grímsson and Olafsson, 1981). Our inter-regional analysis was designed to see to what extent differences in demographic structure or socio-economic circumstances explained variations in psychotropic drug use.

When the psychotropic drug utilization rates were mapped out for 1978, high tranquilizer and hypnotic prescribing appeared to be associated with predominantly urban districts whereas neuroleptic and antidepressant use seemed to be greater in the rural areas. The striking contrast between these two patterns of prescribing is shown for tranquilizers and neuroleptics in figures 5 and 6. The eastern border area of Newry and Mourne ranked highly in each type of psychotropic drug usage but other country areas adjacent to the Republic of Ireland tended to have low tranquil-

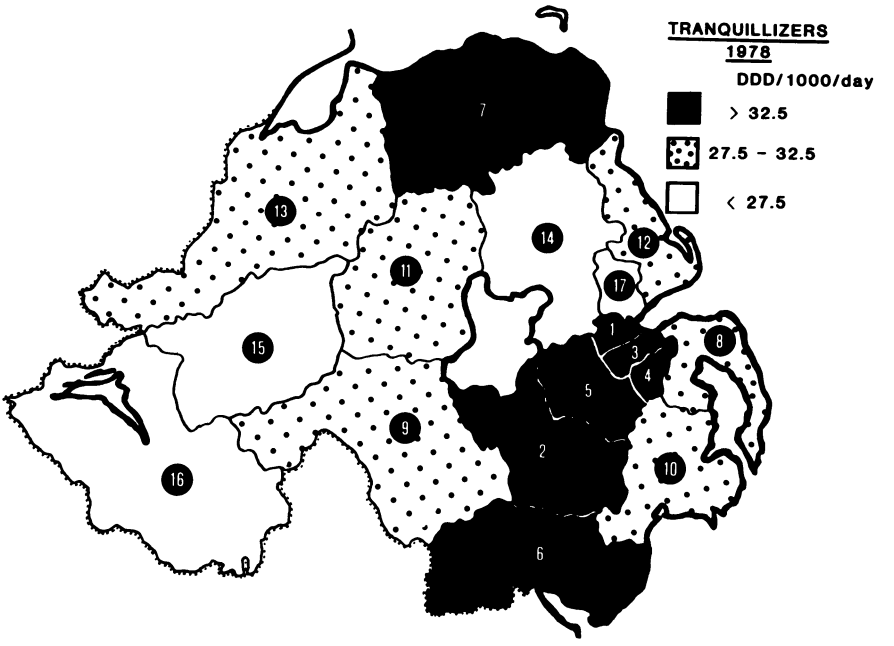


**Figure 5.** Seventeen health districts in Northern Ireland ranked according to high, intermediate and low prescribing of tranquilizers in 1978. From King *et al.* (1982). Psychotropic drug use in Northern Ireland 1966-80. *Psychol. Med.* (in press). Reproduced by permission of Cambridge University Press.

izer, hypnotic and psychostimulant utilization rates with slightly higher antidepressant prescribing and above-average neuroleptic use.

The zero-order Spearman rank correlation coefficients showed that antidepressant and neuroleptic utilization rates were significantly inter-related although neither correlated well with the other types of psychotropic drug usage. High antidepressant prescribing was associated with a low benzodiazepine proportion of tranquilizer and hypnotic use ($r_s$ = - 0.52) and a high barbiturate proportion of hypnotic prescribing tended to be linked with high psychostimulant and antidepressant utilization ($r_s$ = 0.51). Some of the 'independent' socio-economic variables were also strongly inter-related, the most striking association being between unemployment and overcrowding ($r_s$ = 0.87). Both poor housing conditions, measured in terms of overcrowding (the proportion of people living at more than 1.5 persons per room), and high unemployment existed in rural areas with low population density ($r_s$ = 0.68 and $r_s$ = 0.69 respectively).

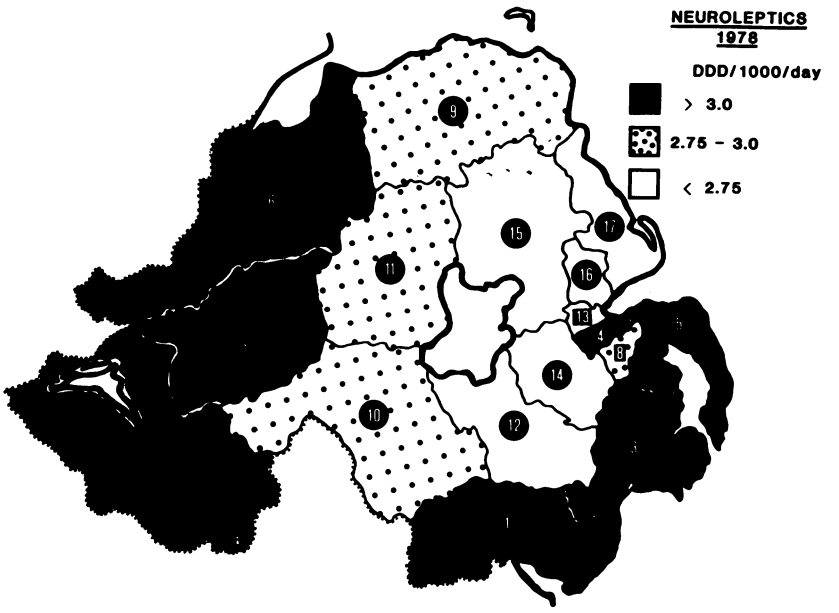


**Figure 6.** Seventeen health districts in Northern Ireland ranked according to high, intermediate and low prescribing of neuroleptics in 1978. From King *et al.* (1982). Psychotropic drug use in Northern Ireland 1966-80. *Psychol. Med.* (in press). Reproduced by permission of Cambridge University Press.

The main psychotropic drug group and socio-economic variable zero-order correlation coefficients are shown in table 1. These indicated a significant correlation between population density and tranquilizer but not neuroleptic prescribing. Neuroleptic prescribing correlated most highly with overcrowding and unemployment, whereas hypnotic prescribing was most significantly correlated with females aged 45-59 and persons over 65 years. The fifth-order multivariate analyses are shown in table 2. These led to the loss of significance for a number of the zero-order correlations but the emergence of population density as significantly correlated with neuroleptic prescribing. Age above 65 years was significantly correlated with both hypnotic and neuroleptic prescribing. The exclusion of overcrowding or unemployment did not account for the 'disappearance' of their significance and the emergence of population density as the most relevant variable in neuroleptic prescribing. There were no significant correlations between list size per doctor and any of the psychotropic drug prescribing levels, nor between estimated population movement and psychotropic drug prescribing.

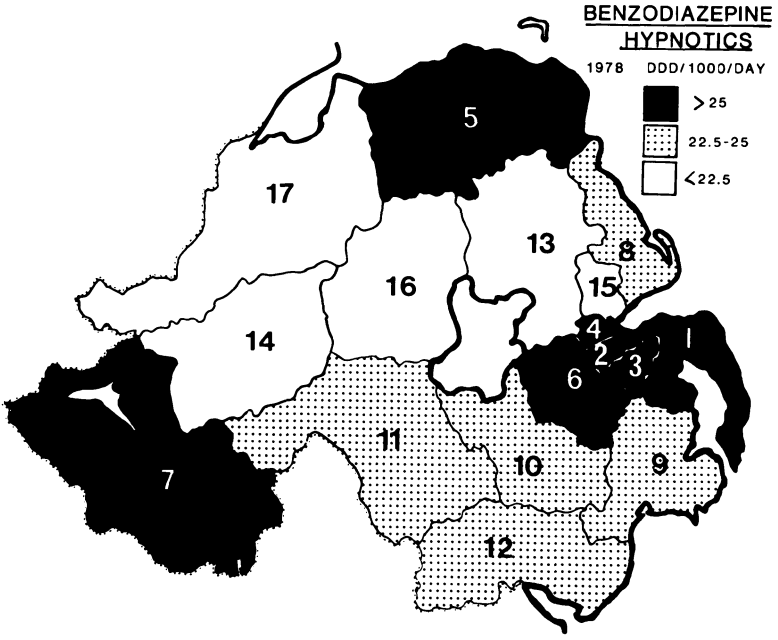


**Figure 7.** Seventeen health districts in Northern Ireland ranked according to high, intermediate and low prescribing of benzodiazepine hypnotics in 1978.

Table 1. Zero-order Spearman correlation coefficients*

| Prescribing level | | Population density | Overcrowding | Females aged 45-59 | Persons aged 65+ | Unemployment | List size per doctor |
|---|---|---|---|---|---|---|---|
| 1 | Tranquilizers | 0.56† | - 0.14 | 0.47 | 0.43 | - 0.19 | - 0.10 |
| 2 | Benzodiazepine hypnotics | 0.50 | - 0.49§ | 0.74† | 0.85† | - 0.44 | 0.10 |
| 3 | Barbiturate hypnotics | 0.33 | - 0.08 | 0.29 | 0.44 | - 0.13 | - 0.10 |
| 4 | Hypnotics | 0.48§ | - 0.28 | 0.57† | 0.73† | - 0.25 | - 0.03 |
| 5 | Neuroleptics | - 0.25 | 0.60† | - 0.41 | 0.25 | 0.59† | - 0.30 |
| 6 | Antidepressants | - 0.32 | 0.37 | - 0.29 | 0.28 | 0.46 | - 0.41 |
| 7 | Psychostimulants | 0.42 | - 0.20 | 0.32 | 0.32 | - 0.14 | 0.06 |

* From King et al. (1982). Psychotropic drug use in Northern Ireland 1966-80. Psychol.Med. (in press). Reproduced by permission of Cambridge University Press.
† $p<0.02$
§ $p<0.05$

The findings of the multiple regression analyses including unemployment are summarized in table 3. Here it can be seen that although population density is once again the most relevant socio-economic variable associated with tranquilizer prescribing, it only accounted for 31.7% of the variance, and the total variance explained was only 32.5%, which did not reach statistical significance. A significant proportion (75%; $p<0.01$) of neuroleptic prescribing, however, was accounted for by overcrowding, the proportion of elderly (persons over 65 years), females aged 45-59 years, and population density, in that order. An even greater proportion of hypnotic (82%; $p<0.01$), particularly benzodiazepine hypnotic (95.1%; $p<0.01$), but not barbiturate hypnotic (38.8%; n.s.), prescribing was accounted for by the socio-economic variables. For all hypnotic groups the proportion of elderly was the most important explanatory variable. Substituting unemployment for overcrowding did not materially alter the results. The geographical pattern of benzodiazepine hypnotic prescribing is shown in figure 7.

Table 2. Fifth-order partial correlation coefficients*

| Prescribing level | | Population density | Overcrowding | Females aged 45-59 | Persons aged 65+ | Unemployment | List size per doctor |
|---|---|---|---|---|---|---|---|
| 1 | Tranquilizers | 0.42 | 0.27 | 0.11 | 0.21 | 0.06 | - 0.19 |
| 2 | Benzodiazepine hypnotics | 0.08 | - 0.13 | 0.12 | 0.76† | - 0.04 | 0.07 |
| 3 | Barbiturate hypnotics | 0.36 | 0.03 | - 0.19 | 0.40 | - 0.10 | - 0.20 |
| 4 | Hypnotics | 0.38 | - 0.03 | - 0.08 | 0.60§ | 0.05 | - 0.16 |
| 5 | Neuroleptics | 0.60§ | - 0.08 | - 0.53 | 0.64§ | 0.15 | - 0.40 |
| 6 | Antidepressants | 0.23 | - 0.30 | - 0.34 | 0.49 | 0.22 | - 0.45 |
| 7 | Psychostimulants | 0.44 | - 0.18 | - 0.24 | 0.34 | 0.10 | - 0.08 |

* From King et al. (1982). Psychotropic drug use in Northern Ireland 1966-80. Psychol.Med. (in press). Reproduced by permission of Cambridge University Press.
† $p<0.02$
§ $p<0.05$

## DISCUSSION

The recent decline in Northern Ireland tranquilizer prescribing may reflect an effect of the 'Systematic review of benzodiazepines' in 1980 (Committee on the Review of Medicines, 1980) and the associated media publicity at that time about the dangers of benzodiazepine dependence. It certainly suggests that major campaigns such as was mounted for barbiturates are unnecessary to influence prescribing (King *et al.*, 1980). Clearly the severe rioting from 1969, which peaked in 1972, bears no direct relationship to tranquilizer prescribing in the province as a whole, although who can say that those civil disturbances did not have prolonged or delayed effects?

It seems likely that broad trends in psychotropic drug use follow certain world-wide changes in prescribing fashions and patient demands. For instance, in the United States the number of

Table 3. Order of independent variables (in parentheses) in the regression equation*, and the percent cumulative variance explained

| | Tranquilizers | Benzodiazepine hypnotics | Barbiturate hypnotics | Hypnotics | Neuroleptics | Antidepressants | Psychostimulants |
|---|---|---|---|---|---|---|---|
| Population density | (1) 31.7 | (3) 92.7 | (5) 38.8 | (4) 78.7 | (4) 75.0 | (3) 33.0 | (4) 21.9 |
| Overcrowding | (3) 32.3 | (4) 94.6 | (3) 33.7 | (5) 82.0 | (1) 42.6 | (1) 12.2 | (3) 17.6 |
| Females aged 45-59 | (4) 32.5 | (2) 92.3 | (4) 34.0 | (2) 76.4 | (3) 71.6 | (5) 40.3 | (1) 14.7 |
| Persons aged 65+ | (2) 32.0 | (1) 81.9 | (1) 28.3 | (1) 70.6 | (2) 69.8 | (2) 21.9 | (5) 27.2 |
| List size per doctor | (5) 32.5† | (5) 95.1 | (2) 32.0 | (3) 78.0 | (5) 75.1 | (4) 40.0 | (2) 16.8 |
| *F* | 1.44 | 43.04** | 1.39 | 10.00§ | 6.65§ | 1.49 | 0.82 |
| Unemployment | (2) 32.2 | (5) 94.4 | (3) 35.8 | (4) 79.7 | (1) 36.9 | (1) 11.0 | (2) 16.8 |

* From King et al. (1982). Psychotropic drug use in Northern Ireland 1966-80. Psychol.Med. (in press). Reproduced by permission of Cambridge University Press.

† No more variance accounted for than in 4.

§ $p < 0.01$

** $p < 0.001$

tranquilizer prescriptions reached a peak in 1973, and the total quantity prescribed peaked in 1978 (Rickels, 1981). There remain marked inter- and intra-regional differences, however, which are not easily accounted for and raise the question as to whether some regions are over-treating or others under-treating psychiatric illness.

In our intra-regional study of the 1978 psychotropic drug data, the most striking differences appeared at first sight to be due to the urban/rural divide (see figures 5 and 6). Total psychotropic prescribing in Belfast was approximately 50% higher than in country districts. This may partly reflect less readily available services in rural districts, and, indeed, the district average number of rural practitioner fund units paid to doctors for patients living more than three miles from the surgery was inversely (but non-significantly) correlated with psychotropic drug prescribing. The higher use of tranquilizers and hypnotics in urban areas was expected and was consistent with previous findings in Northern Ireland (Elmes *et al.*, 1976a), Iceland (Olafsson, 1981) and the higher urban 'anxiety' consultation rates reported by the Royal College of General Practitioners (1979) in Great Britain. On the other hand, the higher use of neuroleptics in rural areas had not been predicted.

The statistical comparisons yielded different answers for different drug subgroups. An association between tranquilizer and hypnotic prescribing and population density was confirmed in the zero-order Spearman rank correlation coefficients. However, when the separate effects of the different demographic and socio-economic variables were controlled in the multivariate analysis, it emerged that, while neuroleptic prescribing was significantly correlated with population density, tranquilizer and hypnotic prescribing were not (table 2). Moreover both hypnotic and neuroleptic prescribing were also significantly correlated with the proportion of elderly persons (aged over 65 years). In fact the two most potent explanatory variables identified by the multiple regression analyses were the relative proportions of elderly persons (over 65 years) and females aged 45-59 years, which alone accounted for 92.3% of the variance in benzodiazepine hypnotic prescribing, and also contributed substantially to the variance in use of most of the other psychotropic drug groups. The variables included in the multiple regression analysis accounted for 75.1% of neuroleptic prescribing but only 32.5% of tranquilizer prescribing. Combining the results of these two statistical exercises it must be concluded, therefore, that as far as Northern Ireland is concerned, the prescribing of benzodiazepine hypnotics is a function of the proportion of elderly and women aged 45-59 years; neuroleptic prescribing is largely a function of factors associated with rural areas (overcrowding and unemployment) together with the proportion of elderly in the community; but that neither tranquilizer, antidepressant, barbiturate hypnotic nor

psychostimulant prescribing were satisfactorily explained by any of the variables we studied.

Nevertheless, the success of demographic and socio-economic factors in explaining benzodiazepine hypnotic and neuroleptic prescribing suggests that further extensions or refinements of this approach should be able to account for the variations in prescribing in the remaining psychotropic drug groups. Other factors which we were unable to include in this first study, such as marital status and social class, may have been responsible for some of the unexplained variance and should be included in future studies. In addition, data on alcohol consumption and, of course, morbidity should greatly improve the degree of explanation reached for intra-regional psychotropic drug prescribing differences. Some of the differences in psychiatric morbidity data in turn might also be expected to be subjected to demographic and socio-economic determinants (Hollingshead and Redlich, 1958; Langer and Michael, 1963; Shepherd *et al.*, 1966; Factor and Waldron, 1973). Thus these variables must be taken into account before valid conclusions about any inter- or intra-regional differences in either psychotropic drug utilization or psychiatric morbidity data can be drawn, or any inferences made about relative over-treatment or under-treatment of psychiatric illness in particular regions.

**SUMMARY**

A study of psychotropic drug prescribing derived from the computerized pricing data in Northern Ireland from 1966 showed that the use of these drugs reached a peak in 1975, when about 12.5% of the adult population were estimated to have been receiving them, and declined in the following five years. The rate of increase in benzodiazepine tranquilizer utilization was greater than in other European countries, but the level was generally lower than in Iceland and Denmark. The influence of 11 demographic and socio-economic variables was studied in an intra-regional analysis of the 1978 data for the 17 health districts in the province, using multivariate and multiple regression statistics. In benzodiazepine hypnotic prescribing 92.3% of the variance was accounted for by the proportion of elderly (over 65 years) and women aged 45-59 years; neuroleptic prescribing was largely a function of factors associated with rural areas (overcrowding and unemployment) and the proportion of elderly; but neither tranquilizer, antidepressant, barbiturate hypnotic nor psychostimulant prescribing were satisfactorily explained by these variables.

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# Section Two

# Methodology of Clinical Trials in Psychopharmacology

# Methodology of clinical trials: current issues

Bernard J. Carroll[1]

## INTRODUCTION

One of the most sobering problems in the clinical pharmacology of depression is that, although we have had antidepressant drugs available for the past 25 years, we still do not know who responds specifically to them. As several authors in this volume have observed, the tricyclic antidepressant drugs are widely prescribed, mostly by non-psychiatrist physicians. Much of this widespread prescribing of tricyclic antidepressants is appropriate: hospital first-admission rates are declining and untreated severely depressed patients are rarely seen any more. On the other hand, we must strongly suspect that many simply unhappy or dysphoric patients are given these drugs unnecessarily and with predictable consequences in terms of morbidity from side-effects, mortality from overdosage, economic waste, and irrational, unproductive clinical management.

As the new generation of antidepressant drugs enlarges, the need to document *specificity of drug effect* in particular types of depressed patients grows correspondingly. Even for the tricyclic antidepressants there are no generally accepted criteria to select 'depressed' patients for drug treatment. Roland Kuhn originally emphasized that patients with the syndrome of 'vital depression' were the most likely to respond to imipramine (Kuhn, 1957). This diagnostic category corresponds to the syndrome 'endogenous depression' or 'melancholia' in other European, British and American settings. Yet we all know that in practice the diagnostic distinction between melancholic and non-endogenous depression is regarded as a difficult decision for many individual patients. As a result, many patients are 'given the benefit of the doubt' and receive tricyclic antidepressants on an empirical basis. Such a practice

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is not good medicine, any more than is the indiscriminate prescribing of antibiotics for non-bacterial respiratory infections. Even in cases where the depression is clearly recognized as non-endogenous there are no firm guidelines established for the use or avoidance of antidepressant drugs.

This problem of nosology affects us not only from a public health perspective and in our clinical practice but also in our scientific work of identifying effective new drugs and understanding their pharmacokinetics. As one example, it seems likely that a relationship between plasma imipramine concentration and clinical effect obtains for endogenous but not for non-endogenous depressed patients (Gram *et al.*, 1977). Good studies of this question with other antidepressants are difficult to find.

Clearly, our elegant studies of the chemistry, metabolism, pharmacokinetics and pharmacodynamics of antidepressant drugs need to be supplemented by equivalent rigor in the design of our clinical trials. Some of the more important aspects of this area are briefly summarized below.

## DESCRIPTION OF THE PATIENTS

No report of a clinical trial is adequate unless the reader can judge from it the extent to which the results can be generalized. The *clinical setting* is well known to affect the selection and referral of depressed patients (Paykel *et al.*, 1970). Some research units deal mainly with atypical, chronic or refractory patients but this circumstance is rarely apparent in their published reports.

The group of patients studied needs to be clearly described in the following ways in addition to the usual demographic features of age and sex:

(a) inpatient or outpatient;
(b) acute or chronic depression;
(c) first episode or recurrent;
(d) untreated or refractory;
(e) delusional or non-delusional;
(f) unipolar or bipolar;
(g) associated physical disorders;
(h) associated psychiatric disorders (e.g. personality disorder);
(i) overall level of social and occupational functioning (e.g. the Global Assessment of Severity (GAS) rating from the Schedule for Affective Disorders and Schizophrenia (SADS) (Spitzer *et al.*, 1977));
(j) severity of depressive symptoms, preferably with both observer ratings and self-ratings.

## DIAGNOSTIC CRITERIA

Explicit, operationally defined diagnostic criteria are required for ease of communication from one clinical group to another. This is the primary purpose of such criteria. At the same time, it must be understood that *all* sets of diagnostic criteria are arbitrary conventions. So far, no one set of criteria is proven superior to others in terms of validity.

For this reason, it is an advantage if patients entered in clinical trials can be characterized simultaneously according to several sets of criteria - ICD, Washington University (St Louis or Feighner criteria), Research Diagnostic Criteria, DSM III, Newcastle Diagnostic Index I and II, and so forth. This will allow the respective performances of the sets of criteria to be compared and partly validated against response to treatment. Such a process can contribute to the eventual selection of some criteria in preference to others.

### Misuse of Diagnostic Criteria

Considering the current state of nosologic problems in depression, the selection of patients for a clinical trial is an important task that should be carried out by the most experienced clinicians available. All sets of diagnostic criteria require a thorough knowledge of clinical phenomenology. The most appropriate way to use diagnostic criteria is to confirm that patients selected by the experienced clinicians have the specified number of relevant clinical features as part of the current episode of depression. On the other hand, since many psychiatric symptoms and signs are non-specific, it is *not* appropriate to use a set of diagnostic criteria in stand-alone fashion as a simple checklist (Carpenter *et al.*, 1980; Carroll *et al.*, 1980). The criteria should be regarded as necessary but not in themselves sufficient for the diagnosis, which must first be made by standard clinical assessment. Those patients who also meet the diagnostic criteria can then be entered into the clinical trial.

It is especially deplorable to entrust the diagnostic assessments to research assistants or medical students who are not well trained in clinical psychiatry. In some centers such raters are trained to administer a structured interview from which items on the diagnostic criteria sets are checked off, sometimes by computer programs. However, raters of this kind are not necessarily skilled in identifying complex or subtle phenomenologic features and they lack the experience of interpreting what they see in a clinical context. In particular, they lack the ability to discriminate a syndromal depressive illness from a collection of dysphoric complaints. A related issue is the use of symptomatic volunteers in clinical trials. These subjects are recruited

either from newspaper advertisements or from checklist screening in general practice settings. While they might satisfy the nominal diagnostic criteria for depression, few of them would be likely candidates for antidepressant drug treatment when their overall clinical status is considered.

Finally, since most clinical trials now include a 7-10 day placebo washout period, a requirement for entry should be that the patient still meets the *diagnostic criteria at that time*. In many current trials the only requirement is that the patient meet a predetermined severity criterion after the placebo washout period. The problem with that approach is that many non-depressed patients will score significantly on any depression rating scale. Hamilton and others have long cautioned that such scales cannot be used for diagnostic purposes (Hamilton, 1960; Carroll *et al.*, 1973).

## DOCUMENTING THE RELIABILITY OF CLINICAL ASSESSMENTS

In our laboratory work we have come to expect that quality control measures, estimates of laboratory error, between-assay variance and so forth will be routinely documented. By now, the same principles can be expected for the clinical measures of diagnosis and severity. Any clinical research unit today should be able to publish estimates of diagnostic reliability such as kappa coefficients. Similarly, for ratings of severity among members of the group the intra-class correlation coefficients and coefficients of variation should be stated. In the author's unit the kappa coefficient for the diagnosis of melancholia among the senior clinicians is 0.80. The intra-class correlation coefficient for Hamilton Rating Scale measures by multiple raters for the same subjects is 0.74, while the coefficient of variation of these ratings is 10%. This last figure is comparable to the coefficient of variation of many laboratory assays.

## NEED FOR PLACEBO CONTROL

Despite some opinions to the contrary, there still are cogent reasons for insisting on the inclusion of a placebo control in the evaluation of new antidepressant drugs. These reasons will remain at least until the current problems of identifying specific subtypes of depression that respond to drugs are resolved.

From a statistical point of view the danger of using only an active drug control group is that very large numbers of patients are needed to avoid Type II errors. The new drug may not be statistically inferior to the standard antidepressant drug but at the same time it may not be statistically superior to placebo. Obviously, unless a placebo control group is included this information will never be obtained.

The finding of a new drug to be equivalent in efficacy to a standard drug is especially likely to occur when both drugs are tested in patients with only a broad, non-specific diagnostic entity such as 'major depressive disorder' in DSM III. This category is so heterogeneous that it will accommodate large numbers of depressed patients whom experienced clinicians would never elect to treat with drugs in the first place. Those who object most strongly to the use of a placebo control on ethical grounds are those who use antidepressant drugs selectively in more typical unipolar and bipolar melancholic patients.

In other centers, however, such typical patients are in the minority. Certainly, in the United States pharmaceutical firms target their new products mostly at the large market of milder, ambulatory, non-melancholic dysphoric patients. It is not difficult to see the potential danger of this approach. A new drug may be tested in the population of non-specific depressed patients, compared only with a standard tricyclic drug, found to be not inferior to the standard drug, and then approved for clinical use as an 'antidepressant'. Under these circumstances, there is no guarantee that the new drug will be effective for severely depressed melancholic patients. The ethical responsibility of the investigator extends, in other words, beyond the patients in his particular study to future patients who may be given the drug with the expectation derived from his trial that it will be effective for them. For this reason the Food and Drug Administration in the United States continues to require a placebo control in the testing of new antidepressants.

Another reason for a placebo control is a check on the ability of the investigators. To be qualified as a unit for the testing of new drugs each group of investigators needs to establish their credentials. This means they must be able to show that in their clinical program the referral, selection, diagnostic and treatment practices are adequate to demonstrate the efficacy of a standard drug over placebo.

## USE OF LABORATORY MARKERS

Several promising laboratory tests are now receiving widespread evaluation as markers for the diagnosis of subtypes of depression. Some of these tests may also be found to have prognostic utility or to aid in the selection of specific antidepressant drugs. As in the case of clinical diagnostic criteria discussed above, the use of laboratory diagnostic tests requires informed judgement, common sense and knowledge of conditional probability theory on the part of the psychiatrist (Carroll, 1981, 1982a).

The potential contribution of laboratory diagnostic tests to clinical trials of antidepressant drugs is considerable. For example, both the dexamethasone suppression test (DST) and the

sleep EEG are abnormal in patients with melancholic depression rather than in those with simple major depressive disorder. As in other areas of medicine, such laboratory tests can provide a degree of objectivity in the diagnosis and selection of patients that is impossible to achieve with clinical features alone.

In a multicenter trial of a new drug, for example, the investigators in different units will agree to use similar selection procedures and standardized clinical diagnostic criteria. At the same time, we are all aware that the standardization of these clinical procedures among units is not always uniform, despite the best efforts of all concerned. Thus, if a group in one center finds an abnormal DST rate of 80% in their patients, while the rate in another center is only 20% - even though both units claim to be using the same clinical diagnostic criteria - then we can tell that the two centers are not studying comparable groups of patients. The laboratory tests can then serve as a measure of the consistency of selection and diagnostic practice among the participating centers in a clinical trial. Further, the treatment results in the overall study can be analyzed by stratifying the total sample according to the laboratory test results and this analysis may provide very useful information.

The ultimate validity of tests like the DST is still being assessed by many clinical research groups. In time, it is likely that new definitions of depressive subtypes will be developed that give weight to the laboratory test results along with the classical clinical features (Carroll, 1982b). Even before this point is reached by consensus in the field, however, the applications of such tests for the purposes described above in clinical trials can already be adopted.

## CONCLUSIONS

The need to identify specific drug-responsive types of depressed patients is a serious problem for all concerned with the clinical pharmacology of antidepressant drugs. Diagnostic precision with standardized clinical criteria and new laboratory tests will help to improve the current situation and should lead eventually to a more effective nosology of depression. The generalizability of a clinical trial is of critical importance for other centers to assess and this can only be done if adequate, comprehensive descriptions of the patients are provided, along with documented reliability and consistency estimates for the clinical assessment procedures. For the time being, placebo controls are still necessary in the development of new antidepressant drugs and individual units need to establish that in their own clinical setting a standard drug can be distinguished from a placebo.

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# Practical aspects of the utilization of double-blind trials using adjusted doses of psychotropic drugs

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## INTRODUCTION

Controlled clinical trials usually compare a fixed dose of a drug *x* with either a placebo or a fixed dose of a reference drug. In the first case, the question to be answered is: 'Is *x* an efficient drug?' In the second case: 'Is *x* more (or equally, or less) efficient than the reference drug?'

It is clear that bias can interfere in such comparisons, particularly the choice of the dosages. Moreover, the conditions under which the drugs are prescribed differ completely from the usual therapeutic attitude.

Even if these studies are sometimes sufficient to satisfy the drug registering authorities, it remains to answer various practical questions: How should the drug be prescribed to patients? What is the 'ideal' dosage? Is it a collective one or an individual one? And, in the second case, what is the best way to reach it? What are the equi-active dosages of two drugs? Could one compare the therapeutic margins of two drugs? At different dosages, what are the percentages of occurrence of various effects, either positive or negative?

Before looking at the possible solutions to these questions, one could try to define the 'proper dose of a drug'. It has been said to be that amount of drug which is 'enough but not too much', that is enough to produce the optimum therapeutic effect with the smallest possible amount of the drug.

The definition of 'optimum' is difficult, but it clearly refers to the ratio between risks and benefits: where is the limit between an acceptable side-effect and an unacceptable one? 'The least possible amount of the drug' is a very often forgotten concept and its appreciation, necessary from economical and ecologi-

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cal points of view, is not devoid of ethical problems: when an effective and safe dosage is found for an individual patient, are we allowed to decrease the dosage until the disappearance of the effect?

## POSSIBLE ADAPTATIONS OF CLASSICAL CONTROLLED TRIALS

### Adjustment of Dosage According to Simple Characteristics of the Subjects

Usually the dosage is predetermined and cannot be changed. In case of unbearable side-effects, the drug is stopped and the result is 'negative'.

Just a few published studies consider adapting the dosage according to weight or to body surface.

### Adjustment of Dosage According to the Plasma Level of the Drug

If one has to compare lithium with a new drug expected to be an alternative preventive treatment of bipolar patients, everybody would agree that the dosage of patients receiving lithium has to be adapted according to the plasma level. One could predict that it will be the case for other drugs in the near (?) future.

### Adjustment of Dosage According to Biological Criteria

This is supposed to be related to the therapeutic effect, but it is not yet true for psychotropic drugs. The discovery of biological markers sensitive to treatment could change this situation.

### Classical Trials Comparing Different Dosages of the Same Drug (or of Two Drugs)

The comparison of two drugs at three different dosages is certainly a minimum to define the dose-effect relationship of the two drugs. The number of patients necessary to undertake such a comparison is generally unachievable. Some intensive designs with multiple crossover in the same patients have been proposed but necessitate both very stable disease and short-acting drugs.

### Trials with Systematic Variations of Dosage in the Same Patients

For example, the dosage is increased every week or every second day. Of course, it is difficult to increase the dosage if important side-effects occur or if the symptoms (or the disease) disappear.

## CLINICAL TRIALS WITH INDIVIDUAL ADJUSTMENT OF DOSAGE DURING THE TRIAL ACCORDING TO A DECISION TABLE

### Difficulties Related to the Decision Table

This decision table could be prepared according to side-effects, according to efficacy, or according to side-effects and efficacy. In this last case, a schematic representation is given in table 1. The decisions to take if the situation corresponds to 1, 2, 6, 7 and 9 are easy and are respectively increase, increase, decrease, stop and decrease. In fact, this is true only if the activity increases with the dosage; of course, inverted U shaped curves (described e.g. with nortriptyline) would necessitate other decisions.

Table 1.

| | | Efficacy | | |
|---|---|---|---|---|
| | | 0 | + | ++ |
| Side-effects | 0 | ↑ 1 | ↑ 2 | ? 3 |
| | + | ? 4 | ? 5 | ↓ 6 |
| | ++ | Stop 7 | ? 8 | ↓ 9 |

What to decide if the situation corresponds to 4, 5 and 8 depends on the severity (and risks) of the side-effects and on the benefit expected with the drug. In position 3, some doctors would consider it unethical to decrease the dosage and others would consider it unethical to continue the same dosage. If one accepts that the proper dose of a drug corresponds to the least possible quantity of drug, the decision - during a trial - must be to decrease the dosage.

The latency of effects - either therapeutic or adverse - could make the use of the decision table more difficult. For example, with tricyclic antidepressant drugs, the lack of efficacy during the first or even the second week must not be taken into consideration.

The greatest difficulty is related to the multicenter character of these trials: it implies a standardization. Even if all the psychiatrists or doctors involved agree with the decision table, it is very improbable that they would always take the same decision in front of a patient.

### Difficulties Related to the Frequency of Evaluations

Have these evaluations to be performed at regular intervals? It would not be realistic, especially if side-effects occur. Differ-

ent patients do not react in the same way: some will wait for some days to complain (either of a side-effect or of a lack of activity), some will phone immediately to their psychiatrist.

The ideal frequency of evaluations would have to consider the half-lives of the drugs and one could imagine the difficulties in double-blind conditions in comparing two drugs with different half-lives.

Theoretically it would be better if the clinical evaluations were dependent of validated scales: for example, increase dosage if the Hamilton Anxiety Rating Scale did not decrease at least 5 points. Practically, the decisions have to be taken quickly, especially with outpatients and the use of such rating scales is not realistic to adjust dosage from one consultation to another.

### Analysis of the Results

When two drugs are compared in these conditions, the only acceptable analysis is the comparison between the percentages of 'good results' with drug *A* and with drug *B*. Of course, it is absolutely necessary to define clearly, *before the study*, what is a 'good result'.

Two possible sources of bias must be avoided:

(1) The non-equivalent unitary dosages of *A* and *B*. Whatever decision table is used, one could think that the habit of the prescribers favors a certain dosage (e.g. 3 tablets per day). It would be necessary in order to confirm this hypothesis to compare the same drug at two different unitary dosages (e.g. diazepam 5 mg vs diazepam 10 mg).

(2) The criteria of adjustment which could be more favorable for one drug than for the other.

Besides the main analysis, it is of interest for future prescribers to know the results obtained with the different dosage for the same drug, but this analysis must remain descriptive.

## A PRACTICAL EXAMPLE: ALPRAZOLAM VS DIAZEPAM

The details of this trial will be published elsewhere. The protocol was certainly not ideal: it was a 'compromise' resulting from discussions with the 12 participating psychiatrists. It is certainly not presented as a model to follow.

Patients were defined as chronic anxious outpatients with a minimal score on the Hamilton Anxiety Rating Scale and excluding clear depressive states.

On the basis of animal studies and preliminary human studies, we decided to compare identical capsules containing either alprazolam (0.25 mg) or diazepam (5 mg). Allocation of treatment was decided by randomization (random number table) and the whole study was performed under double-blind conditions.

The psychiatrists knew that each capsule contained either 5 mg of diazepam or an (expectedly) equi-active dose of another benzodiazepine. The initial dosage was a free decision according to the habits of the patient (and of the psychiatrist) and to the severity of anxiety. An initial dosage between 2 and 6 capsules per day was 'recommended'.

The dosage could be changed at the occasions of weekly consultations or consecutively by phone calls from the patient.

The schematic decision table shown in table 2 was proposed (and theoretically accepted by the 12 participants). In the absence of side-effects, the dosage had to be increased up to optimum efficacy. In presence of minor side-effects, the choice is more difficult and for 'the central case' we had to accept a free decision from the prescriber, which had to take into consideration efficacy and side-effects in the case of this particular patient. In the case of significant side-effects the decrease of dosage is imperative.

Table 2.

| | | Anxiety | | |
|---|---|---|---|---|
| | | Unchanged | Decreased | Suppressed |
| Side-effects | None | Increase dosage | Increase dosage (at a lesser degree) | Unchanged |
| | Minor | Increase dosage (at a lesser degree) | 'Your decision' | Decrease dosage |
| | Serious | Stop (drop out) | Decrease dosage | Decrease dosage |

The primary evaluations were performed at the beginning of the study and at its end (28 days):

(a) Hamilton Anxiety Rating Scale;
(b) global score of anxiety (on 1 to 10 scale);
(c) checklist of possible side-effects;
(d) global score of side-effects (on a 1 to 10 scale).

The results could be summarized as follows: 270 patients were included but only 222 observations were accepted (rejection before breaking the code); 77 men and 145 women, 107 received alprazolam and 115 diazepam. The initial dosages were the same in the two groups (4.24 vs 2.29 capsules with variations from 1 to 12!).

At the end of the trial, the mean numbers of daily capsules

were significantly different ($p<0.05$): alprazolam 6.45, diazepam 5.50 (corresponding respectively to 1.61 and 27.5 mg).

The percentages of 'good results' were not significantly different. For the final evaluations, alprazolam was superior to diazepam according to global evaluation of efficacy and of side-effects but identical when considering the Hamilton Anxiety Rating Scale. Finally, for the same efficacy, alprazolam appears to be less sedative than diazepam.

### Possible Improvements for Future Trials

Is there a way to control the bias related to the variation of the number of capsules used and its psychological effects upon the investigator and the patient? When an evaluation is made, it should be as independent as possible from the number of capsules received by the patient at that time. For that reason, it would be desirable to keep the number of capsules constant during the trial and to modify only the unitary dosage of the capsules. This could be accomplished daily by the pharmacist for studies with inpatients.

It would be interesting to consider the different results of each patient at the times of the consultations or of the changes of dosage but (semi-) quantitative evaluations of efficacy and side-effects would be necessary. It would allow one to approach the 'individual therapeutic margin'.

Better training of the participating psychiatrists would be necessary (one full day was clearly insufficient).

## CONCLUSIONS

During the study of a (new) drug, comparative clinical trials with realistic (naturalistic) adjustment of dosage must be performed. However, the ideal design of such studies is not yet sufficiently defined and a symposium on this subject would certainly be welcome.

In the field of psychotropic drugs, the adjustment of dosage depends mainly on side effects, especially for neuroleptics and for classical antidepressants. It can be used for antianxiety drugs, for hypnotics and also for neuroleptics when a sedative effect is wanted. For these drugs, such trials will certainly not replace classical ones but they will help to increase our knowledge and to improve the therapeutic use of these drugs.

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# Improving reliability and validity of adverse drug reaction assessment in psychopharmacology

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## INTRODUCTION

The assessment of adverse drug reactions* presents few unique methodologic issues not shared with determination of drug efficacy or trial methodology in general. For example, sample size is always an important consideration. However, drug trial protocols, drug trial methodology and published reports illustrate that assessment of drug efficacy (benefit) receives detailed attention but the counterpart assessment of risk is with few exceptions given relatively cursory attention. Such a relative imbalance is difficult to defend and far from ideal. Perhaps this imbalance reflects the lesser intrinsic appeal of looking for problems rather than therapeutic benefits. Such assessment is also difficult and hence time-consuming and expensive to do properly. *A priori*, a full knowledge of benefit and risk is needed. Since the additional margin of benefit of many newer neuropharmacologic agents is relatively small, optimal therapy with most will be determined by the burden of acute and chronic toxicity. The assessment of adverse reactions due to psychotropic drugs includes three methodologic components of particular importance: detection of a potential drug-related event; establishment of the probability of a causal relationship; and evaluation of the severity or clinical importance of the event in relation to the therapeutic benefits.

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*An **adverse drug reaction** is any noxious, unintended and undesired effect of a drug which is observed at doses usually administered in man. This definition excludes cases of drug overdose, drug abuse or therapeutic errors.

## DETECTING AND DISTINGUISHING DRUG-RELATED FROM NON-DRUG EVENTS

### Signal-to-Noise Ratio

The probability of discovering an adverse reaction (or any event) depends upon: the actual or perceived relative frequency of the drug-induced event to the spontaneously occurring event in the patient population under study and to the non-patient control population; the sensitivity, validity and reliability of the method; the frequency and duration of observations for the event (intensity of observation); and to a lesser extent the mechanism of the drug-induced reaction (Feinstein, 1974).

The manifestations of adverse events are usually not unique and hence what is caused by the drug must be distinguished from other possible etiologies. Accordingly, the detection of adverse events depends on the relative characteristics and magnitudes of two factors, the frequency of the adverse event occurrence and its clinical importance, and the concurrent spontaneous baseline occurrence of the event in the absence of drug. Background noise is contributed by the symptoms and signs of primary illness. Such basal noise increases population variance and occasionally generates a signal that is identical and detectable as a possible drug-elicited signal (Jick, 1977). When a drug-induced event is frequent, it is usually recognized quickly in clinical trials. In contrast, when the drug-induced illness is less common, large prospective investigations of a cohort of patients receiving the drug and/or retrospective case-control studies are needed (Jick, 1977). If both drug-related and spontaneous events are rare or very common, no method exists for detection of drug-induced events (Jick, 1977). For example, detection of drug-induced depression in depressive illness may be difficult or impossible. In practice, since the frequency of drug-related and disease-related events is usually not known, selecting the ideal method for detecting specific reactions may be difficult. We usually have only a general idea of the true natural history of disease and treatment outcome in a specific group of patients and of other factors that may increase baseline variance, e.g. alcohol use, smoking, drug abuse, other drug use and non-compliance (Dirks and Kinsman, 1982). Large inter-individual variations in steady-state drug concentrations and variations in patient sensitivity to drug effects may also introduce further variations in risk. The detection of adverse reactions in such circumstances will be extremely difficult even with large patient samples; however, the use of within-subject design with multiple crossovers, sophisticated statistical techniques such as discriminant analysis and intensive patient monitoring can be powerful procedures to maximize the likelihood of detecting within-subject and group adverse drug events (Raskin, 1982). Such techniques, however, can only be

effective for as long as the trial is conducted; hence adverse reactions which develop insidiously or long after exposure could be missed.

Normal subjects are often used for pharmacokinetic and interaction studies. Since these subjects usually receive few drugs, a baseline free of spontaneously occurring adverse events could be determined and used as an absolute control. For infrequent events such a procedure has little advantage; however, for events that are common and which can be confused with symptoms of disease (e.g. depression, anxiety, sleep disorders), normal subjects may be suitable.

**Required Sample Size**

Clinical trials are usually short-term studies conducted in a few hundred or thousand patients before marketing a drug. Such limited exposure of patients to drug limits detection in the pre-marketing phase to those adverse events that are acute and common. The release of the new antipsychotic drug clozapine is an example (Anderman and Griffith, 1977; Idänpään-Heikkilä *et al.*, 1977; Idänpäan-Heikkilä and Palva, 1977). Clozapine was introduced in Finland in 1975 when about 200 subjects had been studied in that country and 900 elsewhere. Within the first six months of post-marketing drug use, about 3200 patients received the drug and 17 cases of serious hematologic reactions (agranulocytosis 10; neutropenia 7) were reported to the Finnish National Drug Monitoring Center. Because of these reactions, the drug was withdrawn from the market. The estimated frequency of developing agranulocytosis or severe granulocytopenia during clozapine treatment was 5.3 per 1000 (Anderman and Griffith, 1977; Idänpään-Heikkilä *et al.*, 1977; Idänpään-Heikkilä and Palva, 1977). This example illustrates the importance of post-marketing monitoring of any new drug, irrespective of the safety shown in clinical trials. It also indicates the role of physicians in detecting the serious toxicity of newly introduced drugs by voluntarily reporting adverse reactions to national drug monitoring centers. An incidental aspect of this example is that the observed frequency was between 8.8 and 21 times greater than observed in other countries and with other neuroleptics (0.47 per 1000). A pharmacogenetic determinant was suspected.

No available method for detecting adverse reactions could have predicted such events, only the administration of the drug to a sufficient number of subjects resulted in the discovery.

For dose-related adverse reactions, detection also depends on the administered dose of the drug. The sample sizes required for detecting three different adverse events (hepatitis, nausea, somnolence) which occur with varying probability depending on the dose of a hypothetical drug are shown in table 1. The assumptions

made are that the adverse events are independent events; when giving 50 mg of the drug, the true probability of any one subject developing the adverse event is given for hepatitis ($p$ = 0.001), nausea ($p$ = 0.004) and somnolence ($p$ = 0.005); the probabilities of the events increase linearly, and the chance to detect the event is 90% (power 1 - $\beta$ = 0.90).

Table 1. Number of subjects ($N$) needed to detect adverse events

| Dose (mg) | Adverse events | | | | | |
|---|---|---|---|---|---|---|
| | Hepatitis | | Nausea | | Somnolence | |
| | $p$ | $N$ | $p$ | $N$ | $p$ | $N$ |
| 50 | 0.001 | 2301 | 0.004 | 574 | 0.005 | 459 |
| 100 | 0.002 | 1150 | 0.008 | 286 | 0.01 | 229 |
| 200 | 0.004 | 574 | 0.016 | 142 | 0.02 | 113 |
| 400 | 0.008 | 286 | 0.032 | 70 | 0.04 | 56 |

Power = 90%
$p$ = probability of event (power 1 - $\beta$ = 0.90)

To have a 90% chance of detecting one case of hepatitis induced by such a drug when 400 mg are given, a minimum of 286 patients exposed to the drug are required (table 1). This example illustrates why some adverse events are only detected when a large number of subjects have received the drug and why even with an actual frequency of 5.3 per 1000, the failure to observe the hematologic adverse events with clozapine after 200 patients is not surprising. Detection with greater assurance (e.g. power 1 - $\beta$ = 0.95, 0.99) will require larger numbers of patients. Similarly, if patient monitoring is not intense enough to detect *all* drug-related events, more patients will be required.

Animal studies are helpful predictors of dose-related toxicity that must be looked for in humans. Observations in animals can be translated into anticipation and intensive monitoring for the possible reaction in the human. In contrast, dose-independent adverse events (e.g. drug allergy) are confined to individuals or patient subsets with particular genetic or immunologic characteristics. Therefore, these reactions are rarely predictable from toxicity studies in animals. If the reactions are infrequent, then large numbers of susceptible patients must receive the drug.

Since the absolute frequency of a reaction as well as the relationship to the dose are unknown, it is impossible to predict *a priori* the actual number of subjects required to detect a specific adverse reaction.

**Current Monitoring Methods**

Methods for collecting information on adverse reactions in clinical trials include unstructured and structured interviews, physical examination and laboratory tests. The procedures most commonly used are the open-ended unstructured interview, designed to eliminate suggestion of reactions to the patient, and a standardized list-of-symptoms checklist (Petrie and Levine, 1978). With the unstructured inquiry, the subject is asked at regular intervals about appearance of any symptoms or changes in body function. This method is of unknown reliability because of variable awareness in subjects of such symptoms, the difficulty in relating symptoms to the drug, and variation in patient motivation, affect, memory or judgement due to disease (e.g. dementia). Such unstructured inquiry may result in under-reporting or, in some situations, even over-reporting. For example, the use of a list of symptoms in neurotics was more likely to lead to the conclusion that a drug induced more side-effects (Downing *et al.*, 1970). Such simple questionnaires or observational methods may suffice for frequent and clinically important adverse reactions. However, the sensitivity, validity and reliability of such methods is unknown; hence, they are inadequate from a methodologic point of view.

In an attempt to standardize the assessments of adverse reactions in clinical trials, adverse drug reaction assessment scales, consisting of checklists of symptoms and signs, have been developed. However, the application of these scales is problematic because of inadequate operational definitions of terms and severity ratings, inclusion of a wide variety of symptoms and signs, and complex formats (Petrie and Levine, 1978). Examples of these procedures for assessing psychotropic drugs are the Treatment Emergent Symptoms Scales (Vinař, 1971; Guy, 1976). Guy's scale includes 33 symptoms, laboratory items and syndromes and has been widely used. A 'treatment emergent symptom' is defined as follows: the symptom was not present at pre-treatment, or if present was aggravated during the course of medication; and/or the symptom required some form of action or intervention as a consequence of its occurrence. The scale provides an eight-point action scale, the severity of the adverse reactions are classed as mild, moderate and severe, and the relationship between the drug and the adverse event is also recorded (none, remote, possible, probable, definite), but the definitions are vague and confusing. Many features of this scale have been utilized in transmuted form in many drug trials. The main difficulties of Guy's scale are the undefined or non-parallel items, the confusion that raters experience with the scale and the difficulty in data analysis (Petrie and Levine, 1978). In addition, the sensitivity, validity and reliability for different patient and racial populations is not established. The under-representation of females in pre-marketing

trials suggests that adverse reaction information in that population is particularly sparse (Kinney *et al.*, 1981).

## DETERMINATION OF CAUSALITY

The *causal association* between a drug and adverse events has usually been classified as definite, probable, possible or doubtful as follows:

(a) *definite* - a reaction which (i) follows a reasonable temporal sequence after administration of the drug, or in which the drug level has been established in body fluids or tissues; (ii) follows a known response pattern to the suspected drug; and (iii) is confirmed by improvement on dechallenge and by reappearance on rechallenge;

(b) *probable* - a reaction which (i) follows a reasonable temporal sequence after drug administration; (ii) follows a known response pattern; (iii) is confirmed on dechallenge but not on rechallenge; and (iv) cannot be explained by the known characteristics of the patient's disease;

(c) *possible* - a reaction which (i) follows a temporal sequence; (ii) may or may not follow a known response pattern; and (iii) could be explained by the known characteristics of the patient's clinical state; and

(d) *doubtful* - the event is more likely related to other factors than the suspected drug (Naranjo *et al.*, 1981).

The use of such standard definitions of probability of a causal relation generates wide variability in assessment (Karch and Lasagna, 1975; Koch-Weser *et al.*, 1977; Blanc *et al.*, 1979; Naranjo *et al.*, 1981). The suspected drug is usually confounded with other causes and often the adverse clinical event is not distinguishable from manifestations of the disease. Recently, the assessment of causality of adverse reactions has been systematized by using operational definitions such as those reported by Kramer *et al.* (1979) and those by Naranjo *et al.* (1981). The method proposed by Kramer *et al.* (1979) is a long and detailed questionnaire, which provides valid and reliable assessments. A simpler method, the Adverse Drug Reaction Probability Scale (APS) is also valid and reliable in a variety of clinical situations (Naranjo *et al.*, 1981). The APS is a 10-item questionnaire which systematically analyzes and scores the various components that must be assessed to establish a causal association between drug(s) and adverse events. The probability of the adverse reaction is given by the total score. The scores obtained with the APS are highly correlated with those obtained using the method of Kramer *et al.* (Busto *et al.*, 1982). Because reliable and valid assessment of the probability of a causal relation between drug and event is desirable, such techniques should be used.

## ASSESSMENT OF SEVERITY

The *severity* of adverse reactions is usually classified as mild, moderate, severe or lethal as follows:

(a) *mild* - no antidote, therapy or prolongation of hospitalization is necessary;
(b) *moderate* - requires a change in drug therapy although not necessarily drug discontinuation; it may prolong hospitalization and requires specific treatment;
(c) *severe* - potentially life-threatening; requires discontinuation of drug and specific treatment; and
(d) *lethal* - directly or indirectly contributes to the death of the patient.

The validity, reliability and appropriateness of the definitions and application of this categorical approach are unknown. The classification lacks flexibility in the sense that many reactions are not uniquely classifiable, e.g. tardive dyskinesia. Attempts for systematizing the assessment of the drug benefit/risk ratio have been reported (Tallarida *et al.*, 1979). However, more refinement to assess the balance between the severity of the underlying disease and the adverse event are required. The severity or impact of the reaction must always be tested against benefit from the therapy. No interpretation of a 'severe' reaction is possible without information of expected and achieved improvement and the natural history of the disease. Similarly, the exclusion of 'mild' adverse events from reporting is not justified since the assumption is implicitly made that benefit is constant and substantial.

## CONCLUSIONS

The methodologic principles for detection and evaluation of adverse reactions are theoretically well established, but are not fully operational. Some improvement would occur if more attention and scientific rigor were directed to assessing risk by ensuring that studies are conducted by trained clinical pharmacologists; incorporating an appropriate balance of spontaneous and elicited data gathering in all trials; inclusion of APS or similar methods to standardize the determination of causality; intense review of case report data during early trials with a high index of suspicion for possible adverse reactions; reporting of all clinical event data; use of newer data analysis techniques; including intense phase IV studies for all newly marketed drugs.

Research is needed to develop primary validated and reliable instruments for adverse reactions assessment; to develop standardized operational methods to rate severity and compare it to benefit; to explore the feasibility of using normal subjects to estab-

lish the absolute range and severity of adverse events; and to develop trial monitoring techniques that will ensure that all organ systems in man receive equal attention in adverse event monitoring irrespective of drug class under investigation, and that intensity of monitoring in any trial can be characterized.

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# Clinical evaluation of the cardiac effects of new psychotropic drugs

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## INTRODUCTION

This paper briefly describes some methods for investigating the cardiac effects of psychotropic drugs and describes some studies with the newer antidepressant drugs - nomifensine and zimelidine.

Over recent years there appears to have been a growing interest in the cardiotoxicity of psychotropic drugs. Nevertheless, there is still great controversy in this area.

A great variety of symptoms have been reported after the ingestion of an overdose of a tricyclic compound. Typically these include disorientation, ataxia, vomiting, coma, convulsions, ECG changes and dysrythmias. Since some of the antidepressants show an affinity for myocardial tissue, it is not surprising that a large proportion of individuals experiencing overdoses of these drugs show signs of cardiotoxicity. Even at therapeutic dose levels there is compelling evidence that changes occur in cardiac parameters.

Using surface ECG recordings and in other studies using His bundle electrocardiography, we have shown that intracardiac conduction was prolonged in patients taking therapeutic doses of tricyclic antidepressants. Possible changes in cardiac function occurring at therapeutic dose levels are highly relevant to the treatment of depressed people with pre-existing cardiac problems.

Following tricyclic antidepressant overdose, the effects produced by the cardiotoxicity of these drugs can be most dangerous and may be difficult to correct.

A frequent enquiry from clinicians responsible for the care of people who have recently suffered a myocardial infarction and are still receiving intensive inpatient care is whether it is safe

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to prescribe an antidepressant drug when depressive disorder is prominent. What are the criteria? What drug should be prescribed?

At the Second International Meeting on Clinical Pharmacology in Psychiatry, Tromsø, 1980, we reviewed some of the clinical studies in this area (Burrows *et al.*, 1981b); a more detailed paper, including some animal studies, has also been reported (Burrows *et al.*, 1981a).

When applied to the study of cardiotoxicity of antidepressants, animal models may appear to measure effects dissimilar from those seen in man and may be totally divorced from clinical problems. No single animal method of testing for cardiotoxicity has become accepted, and a great variety of experimental approaches have been used by different workers. As a result, conflicting results and viewpoints occur, and comparisons between different research groups are almost impossible.

The same can be said of clinical studies in man; no single method is universally accepted, and a great variety of methods have been used (Glassman *et al.*, 1981).

## TRICYCLIC ANTIDEPRESSANTS

The pharmacological activity of the older tricyclic compounds includes a number of actions: anticholinergic, norepinephrine re-uptake blockade, 5-HT re-uptake blockade, and quinidine-like action.

The quinidine-like actions are thought to be a direct action on the myocardium, probably due to the local cocaine-like anesthetic activity of these compounds. Quinidine, a class 1 anti-arrhythmic agent, acts by affecting the action potential. Quinidine decreases the rate of rise of the action potential, thereby prolonging the effective refractory period. It delays conduction, as shown by prolongation of the QRS, QT and PR intervals on the electrocardiogram.

Quinidine also depresses contractility thought to be brought about by interfering with the fast initial inward current of sodium ions.

Tricyclic antidepressant drugs have been the pharmacological treatment of choice for most depressed patients in general and psychiatric practice. In recent years, psychiatrists have tended to use larger doses of these drugs, often giving them in a single nightly dose.

Because of this tendency, and because of the fact that the diagnosis of depression is being made more frequently in patients with ischemic heart disease (with tricyclic antidepressants being widely used in rehabilitation), an awareness of the pharmacology of the drugs, particularly their cardiac effects, is essential.

Tricyclic antidepressant pharmacological activity includes an anticholinergic action which is apparent at low drug concen-

trations. The tricyclic drugs block the re-uptake of norepinephrine and so raise the levels of circulating catecholamines. At higher concentrations, myocardial contractility and heart rate are depressed. Metabolic acidosis and respiratory depression, which may occur especially in unconscious patients, may also affect the cardiac state (Burgess and Turner, 1981).

In therapeutic doses of less than 200 mg per day in people without heart disease there is usually no need for concern, but the findings that therapeutic (and not only toxic) doses may cause significant prolongation of distal atrioventricular conduction indicate that these drugs should be used cautiously in people with known heart disease. This would apply particularly to the elderly or to young children.

Caution is necessary, but for the moderately to severely depressed patient with persistent symptoms, the benefits of antidepressants should not be withheld since the risks are small.

Cardiotoxicity is a major feature of tricyclic antidepressant overdosage where sinus tachycardia, conduction defects, supraventricular tachycardia, ST and T wave abnormalities, ventricular arrhythmias, profound bradycardia and finally asystole may be observed.

Before the tricyclic antidepressants are used, cardiological assessment should be made, starting with a clinical history and followed by a physical examination and an ECG. Chest X-ray and exercise testing may be considered. Patients with heart failure and/or angina may be made worse by the increase in heart rate produced by tricyclic drugs. If the ECG is abnormal and shows evidence of bundle branch block, the use of tricyclic drugs should be reconsidered, since they may lead to complete heart block and cardiac syncope. Dosage should be increased gradually and patients seen at regular intervals (Burrows *et al.*, 1976).

These cardiovascular problems have stimulated the pharmaceutical industry to produce other antidepressants - ones that act more quickly with few subjective side-effects, have fewer cardiovascular effects (both at therapeutic levels and in overdosage) than the tricyclic group, and do not have the interactional problems of the monoamine oxidase inhibitors. Before discussing two newer drugs, the methods used clinically to study cardiotoxicity will be briefly described.

## METHODS FOR CLINICAL EVALUATION OF CARDIAC EFFECTS

### Electrocardiogram

Obviously the electrocardiogram (ECG) is the most readily available method for the clinician. It would seem that the first report of tricyclic antidepressant ECG changes in man occurred 21 years ago in Scandinavia (Kristjansen, 1961), with descriptions of

ST-T changes and hypotension occurring following exercise, in depressed patients who had been prescribed imipramine.

Unfortunately, most of the numerous individual case reports of ECG abnormalities during tricyclic antidepressant therapy have been of patients receiving other multidrug administrations. The few studies of ECG changes occurring during pure tricyclic antidepressant therapy were reported elsewhere (Burrows *et al.*, 1981a).

The general pattern of ECG which emerges from these studies is of ST-T wave changes including ST deviation, increases in PR and QRS width, bundle branch block and sinus tachycardia, ventricular arrhythmias, profound bradycardia, supraventricular tachycardia and asystole. These changes appear reversible in patients without pre-existing heart disease when the drugs are withdrawn. Only a few studies have also monitored plasma levels along with the ECG changes and they have been reviewed elsewhere (Burrows *et al.*, 1981a; Burgess and Turner, 1981).

The results have been conflicting. Some studies have shown that there is an increase in ECG changes in patients with the higher plasma levels while others have shown no correlation between the plasma level and the extent of the QRS widening. The range of plasma levels, following overdose, overlap with those found in patients receiving chronic oral administration for therapeutic reasons, thus making the use of plasma levels alone an unreliable index of tricyclic overdosage.

Nevertheless, the most reliable and readily available clinical index of tricyclic overdosage is prolongation of the QRS width by 100 ms or more. QRS widening, arrhythmia and increased total plasma tricyclic antidepressant levels indicate major cardiotoxicity.

## METHODS USED TO ASSESS THE EFFECT OF TRICYCLIC ANTIDEPRESSANTS ON THE HEART

### Systolic Time Intervals

This is a non-invasive method for studying left ventricular function. The left ventricular pre-ejection period (PEP) is compared to the left ventricular ejection time (LVET). Simultaneous recording of ECG external carotid pulse and either phonocardiogram or apex cardiogram are needed to measure these intervals.

The PEP is measured from the onset of ventricular systole (beginning of first heart sound or from the apex cardiogram) to the onset of the carotid pulse. The LVET is measured from the onset of the carotid pulse to the carotid incisura. The PEP/LVET ratio is increased with impairment of left ventricular function.

The normal value of the PEP/LVET ratio is 0.345 ± 0.036 (S.D.). This ratio is inversely correlated with cardiac stroke volume. It is increased with heart disease and heart failure. The ratio is decreased with digitalis as a result of a positive inotropic effect.

### Radioisotope Scanning

Images of the cardiac cavity may be obtained by intravenous injections of radionuclides, technetium-99 ($^{99}Tc_m$) albumin and photographing under ECG control.

This non-invasive method may be used to measure both left ventricular function and regional dysfunction.

### Left Ventricular Systolic Ejection Fraction

This involves the angiographic estimate of the left ventricular ejection fraction. The left ventricular end-diastolic and end-systolic volumes are determined by angiography. The difference between the two volumes is divided by the end-diastolic volume giving the ejection fraction. The upper limit of normal ventricular end-diastolic volume is 99 ml.

The cardiac output may be measured by recording the time course of change in radioactivity, by radioscopic scanning following i.v. injection of the labeled radionuclide. This is an application of the indicator-dilution principle.

The use of this technique to study psychotropic drug effects is only just beginning.

### His' Bundle Electrocardiography

The relationship between the His' bundle electrocardiography (HBE) and the standard ECG is shown in figure 1. This invasive technique involves cardiac catheterization facilities and professional personnel. Following local anesthesia, electrode catheters are percutaneously placed into a femoral vein, advanced under fluoroscopic and ECG control into the right ventricular cavity and slowly withdrawn to the region of the right tricuspid valve.

Tricyclic antidepressants have been shown in both therapeutic and toxic doses to prolong the H-V interval significantly. The effect on proximal or A-V conduction is variable.

We have suggested that prolongation of the H-V interval with some of these drugs may give a clue to the increased incidence of sudden deaths that have been reported in 'cardiac patients' taking tricyclic antidepressants.

### Echocardiography

Echocardiography is perhaps the major diagnostic cardiological innovation in the past decade. It provides diagnostic information regarding structure and function in qualitative and quantitative form. This is as good as that obtained from invasive studies and,

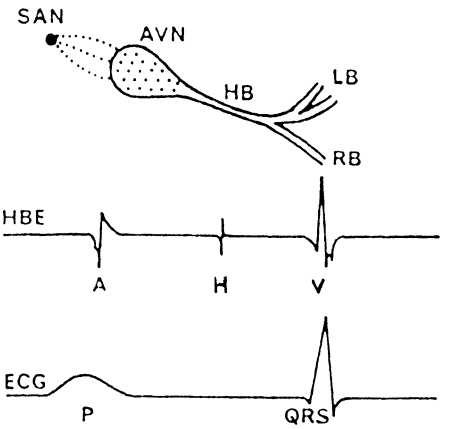


**Figure 1.** His' bundle electrogram (HBE) with the standard ECG. An anatomical model of the conduction system at the top of the figure is orientated to show the sites of origin of the electrical waves. The A-H and H-V intervals represent proximal and distal intracardiac conduction respectively.

in some instances, better. The availability of ultrasound equipment is rapidly becoming widespread and, therefore, familiarity with the technical equipment is necessary for both general practitioners and specialists.

The examination is performed with the patient recumbent in a semi-left lateral position. A transducer is coupled to the chest wall. A number of ultrasound positions may be used. The more common are in the 3, 4 and 5 interfaces at the left sternal edge. The echo beam is swept from the aortic root to the apex of the heart.

Two-dimensional echocardiography is becoming increasingly available and provides another useful technique for the assessment of global and segmental myocardial function. Studies have been carried out before and after tricyclic antidepressant drug administration.

## NOMIFENSINE

### Cardiological Effects

The structure of nomifensine is distinct from the tri- or tetracyclic antidepressants, monoamine oxidase inhibitors, or any of the other existing groups of psychotropic agents. It is a deriva-

tive of tetrahydroisoquinoline. Double-blind controlled studies of nomifensine against placebo, imipramine, amitriptyline, nortriptyline and chlomipramine have shown it to be an effective antidepressant.

Studies in animals showed nomifensine to have slight and transient effects on the cardiovascular system. More recent studies on isolated guinea-pig atria, in the anesthetized rat, and on the right ventricular papillary muscle of the cat confirm that the effects of nomifensine in animals are less pronounced than those of other antidepressants.

Nomifensine did not cause significant changes in heart rate, cardiac output, mean arterial pressure, pulmonary artery pressure, or end-diastolic left ventricular pressure. There were no electrocardiographic (ECG) changes. Non-invasive techniques of high-speed surface ECG and the measurement of systolic time intervals have shown that nomifensine does not appear to have a significant effect on the heart.

Reports of overdosage also indicate that nomifensine does not cause the serious cardiac arrhythmias which are present with tricyclic overdoses.

We investigated the effects of nomifensine on the CVS system. Preliminary results were reported at the 2nd IMCPP (Burrows *et al.*, 1981b).

Our study consisted of 10 patients suffering from primary unipolar depressive illness of moderate severity (Hamilton rating scores > 17). There was no clinical evidence of cardiac disease in any of the subjects.

A one-week drug-free period was allowed before patients were assigned to a graded dosage regimen, leading to a final daily dose of 100 or 200 mg nomifensine after seven days. There were five patients in both the 100 and 200 mg groups.

A full standard ECG was recorded prior to the commencement of nomifensine therapy and after three weeks of therapy. The parameters measured were heart rate, PR, QRS, corrected QT intervals, and repolarization changes. A His' bundle electrogram was also recorded prior to nomifensine and after three weeks of treatment. From this, values for A-H and H-V intervals could be obtained.

To check for possible side-effects of the drug, routine biochemical measurements were performed at the end of the pretreatment 'washout' period and after three weeks of therapy. These included hematocrit, hemoglobin, white blood cell count/differential, red blood cell count, alkaline phosphatase, bilirubin, SGOT, blood urea nitrogen, and urinalysis.

Besides routine clinical evaluation, the severity of the depression was assessed by the use of the Hamilton depression rating scale on days 0, 8, 15 and 22 of the study. Side-effects were also rated using a 12-point scale as described by Burrows *et al.* (1972).

### Blood Sampling and Analysis

Samples (20 ml) of venous blood were collected on days 0, 8, 15 and 22. These samples were collected into cold lithium heparin tubes, immediately centrifuged at 4°C and the plasma separated, then stored frozen (- 20°C) in the dark until analysis. This method of collection and storage was found to be necessary in order to prevent significant degradation of the nomifensine samples. The analysis gave unconjugated nomifensine and total nomifensine (unconjugated plus an acid-labile *N*-glucuronide conjugate).

### Results

Treatment with nomifensine was associated with an increase in heart rate in seven of the 10 patients (table 1), but this increase was not statistically significant ($p>0.05$; Wilcoxon signed rank test). One patient showed an increase in PR interval of 0.01 s while on nomifensine.

The A-H interval, reflecting the time taken for the cardiac impulse to pass through the atrioventricular node, was within normal limits for the 10 patients (table 2). Nomifensine caused no significant change in the A-H interval

### Interventricular Conduction

The H-V interval, representing the time taken for the cardiac impulse to travel through the His' bundle and bundle branches to the Purkinje fibers, was within normal limits for the 10 patients (table 2). Nomifensine caused no significant change in the H-V interval ($p>0.05$; Wilcoxon signed rank test). Nomifensine also had no effect on the QRS width.

### Corrected QT Interval and Repolarization Changes

Nomifensine caused no significant change in the corrected QT interval ($p>0.05$; Wilcoxon signed rank test). There was no evidence of ST or T wave changes in the ECG.

### Blood Pressure, Blood Chemistry and Urinalysis

Supine and standing blood pressure measurements were performed in all 10 patients on days 0 and 22. Nomifensine caused no significant change in supine and standing systolic and diastolic blood pressure ($p>0.05$; Wilcoxon signed rank test). No consistent changes in the various hematological and biochemical factors monitored were observed.

Table 1. Effect of nomifensine on heart rate and PR interval in the ECG

| Patient no. | Age (years) | Sex | Dose (mg per day) | Heart rate (beats/min) | | PR interval (s) | |
|---|---|---|---|---|---|---|---|
| | | | | Before nomifensine | Day 22 | Before nomifensine | Day 22 |
| 1 | 34 | F | 100 | 81 | 76 | 0.18 | 0.18 |
| 2 | 62 | M | 100 | 92 | 100 | 0.20 | 0.20 |
| 3 | 18 | F | 100 | 84 | 95 | 0.18 | 0.19 |
| 4 | 54 | M | 100 | 63 | 60 | 0.16 | 0.16 |
| 5 | 40 | M | 100 | 86 | 92 | 0.18 | 0.18 |
| 6 | 31 | F | 200 | 67 | 57 | 0.18 | 0.18 |
| 7 | 33 | F | 200 | 67 | 77 | 0.17 | 0.17 |
| 8 | 38 | F | 200 | 89 | 99 | 0.20 | 0.20 |
| 9 | 59 | F | 200 | 65 | 92 | 0.18 | 0.18 |
| 10 | 63 | F | 200 | 82 | 86 | 0.20 | 0.20 |

Table 2. Effect of nomifensine on A-H and H-V intervals in the His' bundle electrogram*

| Patient | A-H interval (ms) | | H-V interval (ms) | |
|---|---|---|---|---|
| | Before nomifensine | Day 22 | Before nomifensine | Day 22 |
| 1 | 80 | 85 | 48 | 50 |
| 2 | 100 | 100 | 60 | 60 |
| 3 | 104 | 100 | 50 | 55 |
| 4 | 70 | 70 | 40 | 40 |
| 5 | 85 | 80 | 45 | 45 |
| 6 | 70 | 70 | 45 | 45 |
| 7 | 70 | 80 | 40 | 40 |
| 8 | 110 | 95 | 45 | 50 |
| 9 | 80 | 90 | 45 | 45 |
| 10 | 95 | 75 | 60 | 60 |

* Normal values: A-H = 92 ± 38 ms; H-V = 43 ± 12 ms.

### Nomifensine Plasma Concentrations

Plasma nomifensine concentrations were determined in all 10 patients (mean values are shown in table 3). These results reveal that the concentrations for day 22 of the trial were significantly lower than those of days 8 and 15. Day 22 samples were collected 12-15 h after the previous nightly dose of nomifensine, because a drug-free period at this time was required before the second His' bundle electrogram could be performed. The samples from days 8 and 15 were collected 2 h after the previous dose.

Table 3. Mean unconjugated and total nomifensine plasma concentrations in 10 patients receiving two dosage regimens (mean ± S.D.)

| Dose | Unconjugated plasma concentrations ($\mu g\ l^{-1}$) | | | Total plasma concentrations ($\mu g\ l^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Day 8 | Day 15 | Day 22 | Day 8 | Day 15 | Day 22 |
| 100($n$ = 5) | 15± 8 | 13± 3 | 9± 3 | 289±143 | 246± 73 | 185± 68 |
| 200($n$ = 5) | 36±36 | 35±26 | 19±15 | 753±618 | 590±422 | 370±272 |

No significant correlation between unconjugated or total plasma concentrations of nomifensine at day 22 and blood pressure (supine and standing), heart rate, PR, H-V, QRS, or corrected QT intervals was observed ($p>0.05$; Spearman rank order correlation coefficient). A significant negative correlation was found between unconjugated concentration (day 22) and A-H interval ($r_s$ = - 0.62; $p>0.05$) and between total concentration (day 22) and A-H interval ($r_s$ = 0.69; $p>0.05$).

### Relationship Between Clinical Response and Plasma Concentrations

No significant correlation between unconjugated or total plasma concentrations of nomifensine at days 8, 15, or 22 and Hamilton ratings were found ($p>0.05$; Spearman rank order correlation coefficient).

### Side-Effects

No severe adverse side-effects of nomifensine therapy were noted.

## Discussion

Nomifensine has shown little influence on the cardiovascular system of the experimental animal. In the present study, 10 endogenously depressed patients, free of previous cardiovascular disease, revealed that nomifensine at a dose of up to 200 mg per day had no significant effect on blood pressure and heart rate, and the ECGs were unremarkable.

The present study revealed no intracardiac conduction defects with nomifensine therapy.

A significant negative correlation between the A-H interval and both unconjugated and total plasma concentrations at day 22 was observed. As nomifensine therapy did not cause a significant change in the A-H interval, the clinical relevance of the correlation remains to be elucidated in a larger patient population. None of the other cardiological parameters examined were found to correlate with nomifensine plasma concentrations.

The present study also showed no significant correlation between plasma concentrations and clinical response. This observation may not be generally applicable as only a small patient population was investigated. Further pharmacokinetic studies have shown the half-life of nomifensine to be 2-4 h, indicating that there should be a wide variation of plasma concentration within one day.

The cardiological data from this and previous studies suggest that, because of its relative lack of cardiotoxicity, nomifensine may be of value in the treatment of depressive syndromes, particularly those patients in whom the quinidine-like tricyclic antidepressants may not be desirable because of pre-existing disease of the cardiac system.

We previously reported (Vohra *et al.*, 1978) a 43-year-old woman with a bipolar affective disorder who took an overdose of nomifensine while receiving treatment for depression. She consumed 3.5 g nomifensine, 20 mg nitrazepam, and 200 mg chlorpromazine. On admission, approximately 4 h later, she was alert but her speech was slurred. She remained alert and conscious throughout her hospital stay. Her blood pressure on admission was 95/65 mmHg and pulse 90/min; ECG showed sinus rhythm and minor flattening of T waves. Throughout her hospital stay her ECG remained normal and the QRS width did not exceed 0.8 s. The corrected QT interval was also normal throughout. Plasma levels of nomifensine were measured every 4 h from 17 h after ingestion.

Montgomery *et al.* (1978) have also reported an overdose (1.5 g). Although their patient showed sinus tachycardia and ours did not, neither patient had any cardiovascular, ECG, or neurological abnormality.

These findings suggest that nomifensine is relatively free from cardiovascular side-effects. This drug may therefore have an advantage in the treatment of depression in cardiac patients,

particularly those in whom quinidine or quinidine-like drugs may not be desirable because of pre-existing intracardiac conduction defects (Burrows *et al.*, 1978; Dumovic *et al.*, 1979; McIntyre *et al.*, 1980).

## ZIMELIDINE

Zimelidine is a selective serotonin uptake inhibitor with a monocyclic structure. Very few cardiovascular studies have been reported.

We have just completed a double-blind trial, to compare zimelidine and placebo in a group of 28 endogenously depressed patients. Selection for the study was based on clinical judgement and the Roth and Gurney scale. Patients with other psychiatric conditions, concomitant physical illness or evidence of drug dependence were excluded from the trial. After a five-day washout period, patients who fulfilled the criteria were assigned to one of the two treatment groups. Patients were matched for age, sex and initial severity of depression. The efficacy of the treatment was determined using the Hamilton rating scale for depression, clinical global rating and Zung self-rating scale, administered on days 0, 4, 7, 10, 14, 21, 28, 35 and 42. Side-effects and plasma concentrations were determined on the same schedule. A sequential analysis of the results showed zimelidine to be significantly better than placebo ($p>0.05$).

### Cardiovascular Effects

There were no significant differences in blood pressure or pulse between the two groups. Two patients (one zimelidine and one placebo) developed persistent orthostatic hypotension. In three other patients, all in the zimelidine group, orthostatic hypotension was observed in the first two weeks of treatment but did not persist. One patient on zimelidine was withdrawn from treatment because of an adverse reaction, which included intermittent hypertensive episodes.

Some changes in the ECG were noted in both groups, but none were of the severity that required cessation of therapy. In the zimelidine treated group ST-T wave changes (three patients), ventricular extrasystoles (one patient) and sinus bradycardia (one patient) were recorded. ST-T wave changes were present in two patients before treatment and were unaltered throughout the trial period. Individual and mean (± standard deviation) PR, QRS, ST intervals and heart rate for the zimelidine treated patients are presented in table 4. These parameters were not significantly different after three or six weeks of zimelidine compared to baseline measures ($p>0.05$; Kruskal-Wallis analysis of variance for

Table 4. ECG parameters for patients on zimelidine*

| Patient no.† | Baseline | | | | Week 3 | | | | Week 6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PR | QRS | ST | HR§ | PR | QRS | ST | HR§ | PR | QRS | ST | HR§ |
| 2 | 0.18 | 0.10 | 0.34 | 69 | 0.18 | 0.10 | 0.28 | 70 | 0.14 | 0.10 | 0.30 | 72 |
| 8 | 0.14 | 0.10 | 0.26 | 84 | 0.18 | 0.10 | 0.56 | 71 | 0.16 | 0.10 | 0.38 | 72 |
| 10 | 0.14 | 0.08 | 0.32 | 65 | 0.18 | 0.10 | 0.32 | 78 | - | - | - | - |
| 12 | 0.18 | 0.08 | 0.30 | 76 | - | - | - | - | 0.18 | 0.08 | 0.28 | 77 |
| 13 | 0.18 | 0.10 | 0.30 | 74 | 0.18 | 0.10 | 0.36 | 76 | 0.18 | 0.12 | 0.30 | 69 |
| 18 | 0.14 | 0.12 | 0.32 | 56 | 0.14 | 0.12 | 0.30 | 57 | 0.14 | 0.10 | 0.30 | 56 |
| 20 | 0.16 | 0.10 | 0.28 | 66 | 0.16 | 0.08 | 0.28 | 90 | 0.18 | 0.10 | 0.32 | 62 |
| 23 | 0.16 | 0.10 | 0.36 | 54 | 0.20 | 0.08 | 0.36 | 54 | 0.18 | 0.08 | 0.36 | 53 |
| 28 | - | - | - | 75 | - | - | - | 52 | - | - | - | 55 |
| Mean | 0.16 | 0.098 | 0.31 | 68.7 | 0.17 | 0.097 | 0.35 | 68.5 | 0.17 | 0.097 | 0.32 | 64.5 |
| ± S.D. | 0.02 | 0.013 | 0.03 | 9.7 | 0.02 | 0.014 | 0.10 | 13.3 | 0.02 | 0.014 | 0.04 | 9.2 |

* Normal ranges (s): PR, 0.12-0.20; QRS, 0.08-0.12; ST, 0.27-0.35

† ECGs were not taken for patients 6, 16 and 28

§ HR = heart Rate

repeated measures), and are not significantly different from normal values. In the placebo group, sinus tachycardia (two patients) and ventricular extrasystoles (one patient) were recorded.

There were no significant correlations between plasma zimelidine or norzimelidine concentrations and clinical response (as judged by the Hamilton scores or the amelioration scores) at weeks 2, 3, 4, 5 or 6 ($p>0.05$; Spearman rank order correlations). Similarly, there were no significant correlations between zimelidine or norzimelidine concentrations and side-effects (or corrected side-effects scores; obtained by subtracting the baseline values from side-effects scores at weeks 2, 3, 4, 5 and 6) ($p>0.05$; Spearman rank order correlations).

The present study did not confirm the finding that patients with high plasma norzimelidine concentrations (above 1000 nmol $l^{-1}$) show poor response to treatment (Montgomery *et al.*, 1981). Although one patient with high plasma norzimelidine levels did not respond to zimelidine therapy, two others showed a significant improvement. The present study could not confirm the finding that patients with high norzimelidine concentrations (above 1000 nmol $l^{-1}$) had a higher incidence of anticholinergic side-effects (Montgomery *et al.*, 1981), or a higher incidence of sleep disturbance. The incidence of anticholinergic side-effects and sleep disturbance was no different between the three patients with norzimelidine concentrations greater than 1000 nmol $l^{-1}$ and those with lower concentrations.

Plasma zimelidine concentrations at week 6 were found to be significantly negatively correlated with ST interval ($r = -0.8827$, $p>0.01$; Spearman rank order correlation), and significantly positively correlated with heart rate ($r = 0.7714$, $p>0.05$; Spearman rank order correlation). These parameters, however, did not show a significant correlation or a similar trend after three weeks of zimelidine treatment: zimelidine concentrations and ST interval, $r = 0.1273$, $p>0.05$, Spearman rank order correlation; zimelidine concentrations and heart rate, $r = -0.4286$, $p>0.05$, Spearman rank order correlation. As all cardiovascular parameters are within the normal range and not significantly altered by zimelidine therapy, the above correlations are not clinically significant.

Zimelidine concentrations were not significantly correlated with other cardiovascular parameters, and norzimelidine concentrations were not significantly correlated with any cardiovascular parameters at weeks 3 or 6 ($p>0.05$; Spearman rank order correlations).

It should be emphasized that patients with cardiac conditions who have organic psychiatric illnesses should be capable of being treated with appropriate psychiatric drugs. In general the cardiovascular side-effects of psychiatric drugs given in therapeutic doses are minimal. Self-poisoning will always be a problem.

The techniques that are currently available for evaluating the side-actions of psychotropic drugs are really the major investigation tools that are currently available for assessing myocardial function and intracardiac conduction.

It is important to stress that the *resting values* have limited application whereas measurements taken under physical and emotional stress are more relevant to the 'real life' situation. This is the area of future development.

In a recent study (Veith *et al.*, 1982), 24 depressed patients with heart disease were treated with antidepressants. The tricyclic antidepressants doxepin and imipramine had no effect on left ventricular ejection fraction at rest or during maximal exercise as measured by radionuclide ventriculograms obtained before and after treatment. These researchers under-scored the need for reappraisal of the cardiovascular risks of tricyclic antidepressants. They also suggest that in the absence of severe impairment of myocardial performance depressed patients with pre-existing heart disease could be effectively treated, without an adverse effect on ventricular rhythm or hemodynamic function. Obviously, if the depression is severe enough active treatment with antidepressants is warranted.

Perhaps initially lower doses of the drugs are required and there would be some value, possibly, in monitoring both cardiovascular parameters and plasma antidepressant levels.

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# The implications of left ventricular performance for tricyclic antidepressant drug treatment

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## INTRODUCTION

Early animal work with the tricyclic antidepressants indicated that these drugs are cardiotoxic and, more specifically, that they adversely affect cardiac contractility. This has been a consistent finding across a large number of clinical studies looking at a variety of tricyclic drugs in a variety of test animals (Kaumann *et al.*, 1965; Laddu and Somani, 1969; Langslet *et al.*, 1971). However, as is so commonly the problem with such data, these studies implied, but in no way could definitively establish, what these drugs would do in humans. An uncertainty remained because of the difficulty in interpreting concentration and metabolic differences between man and various experimental animals. During the 1960s a number of investigators reported cases of tricyclic overdose where myocardial failure was a major clinical problem (Laddu and Somani, 1969; Sigg *et al.*, 1963). These reports cite the animal data as evidence to explain the clinical symptomatology of these patients and then concluded that the clinical symptomatology of these patients was *prima facie* evidence for the existence of a direct negative inotropic effect from tricyclic drugs. Unfortunately, these reports did not include direct measurements of left ventricular performance. In fact, the first study to examine direct measurements of left ventricular performance was published in 1974 (Thorstrand, 1974), almost 20 years after the tricyclics were first introduced.

## EARLIER CLINICAL STUDIES

When the cardiovascular effects of tricyclic antidepressants are reviewed, the discussion is almost always limited to the effects

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of the tricyclic antidepressant drugs on the electrical activity of the heart. This is because the electrocardiogram (ECG) is such a readily available tool and the ECG measures electrical activity. The mechanical activity, or pumping function of the heart, is more difficult to evaluate. Only since the mid-1970s have investigators begun to look at the effects of tricyclic drugs on the mechanical function of the heart. Muller, Burckhardt and Raeder were among the first to attempt a systematic investigation of the effect of tricyclic drugs on left ventricular performance (Muller and Burckhardt, 1974; Burckhardt *et al.*, 1978). Over the course of several years they studied a group of 66 depressed patients receiving an assortment of tricyclic and tetracyclic antidepressant drugs. Although it was difficult to ascertain if there were any differences between the drugs examined, there was a clear overall negative inotropic effect from the tricyclic drugs. A few years later, Taylor and Braithwaite examined eight depressed patients before and after receiving nortriptyline and reached the same conclusion (Taylor and Braithwaite, 1978); the tricyclic drug showed an adverse effect on cardiac contractility. Several other investigators have subsequently reached similar conclusions. Burgess *et al.* (1979) studied a normal group of volunteers given a single dose of amitriptyline, and Camp *et al.* (1979) reported on a series of 12 hyperactive children treated with 5 mg $kg^{-1}$ of imipramine hydrochloride. In both cases there was evidence of a negative inotropic effect of the tricyclic antidepressant.

A perplexing contradiction to this apparent unanimity of opinion came from the original overdose study by Thorstrand (1974) in Scandinavia. About the same time that Muller published his first studies of contractility during therapeutic treatments with tricyclics, Thorstrand published a study of 10 patients with tricyclic antidepressant overdose, comparing them to patients with barbiturate overdose. Using direct cardiac catheterization techniques Thorstrand found no evidence of impairment of the heart's mechanical performance. Even when the patients were comatose, Thorstrand found evidence of high, not low, cardiac output.

The studies of Muller, Burckhardt, Camp, Taylor and Burgess all used systolic time intervals as a measure of left ventricular function. The systolic time interval is an indirect measure of ventricular performance that is dependent, in part, on the QRS duration. Because QRS lengthening is a characteristic induced by the tricyclic drugs, we were concerned that these drugs might spuriously alter the systolic time interval so that it would no longer be an accurate measurement of left ventricular performance in the presence of these compounds. In order to examine this possibility, we studied the effects of both imipramine and desipramine on left ventricular performance in a group of depressed patients using both echocardiographic fractional shortening and systolic time interval measures (Giardina *et al.*, 1982). Consistent with the earlier studies, both drugs altered the systolic time

interval by increasing the pre-ejection period and consequently pre-ejection period/left ventricular ejection time ratio. However, as we had postulated, the echocardiographic fractional shortening remained unchanged with either drug. Recently, Langou *et al.* (1980) have published additional overdose data that also would argue against the conclusion implied by systolic time interval measurements.

## RADIONUCLIDE ANGIOGRAPHY

The paucity of data involving the effects of tricyclic drugs on the mechanical action of the heart is not accidental. The most reliable method for measuring left ventricular performance involves the direct catheterization of the heart. These techniques, although accurate, are not without risk and hard to justify in a patient without overt heart disease, where the only purpose of catheterization is to examine drug effects. As a result, it has been traditional to measure drug effects with indirect measures of left ventricular performance such as systolic time intervals, echocardiography, or ballistocardiograms. These measures are all complex and, while they are not invasive, they are subject to a number of inherent pitfalls. For that reason the recent development of radionuclide angiography represented a significant advance in both clinical cardiology and clinical pharmacology. It supplies a reliable measure of left ventricular performance that is non-invasive and reliable, even in patients with serious heart disease. As a result it can readily be used to study drug effects on left ventricular function.

In 1981, Giardina *et al.* first published data using radionuclide angiography to examine the effects of tricyclic drugs on left ventricular performance (Giardina *et al.*, 1981). The primary focus of the study was the anti-arrhythmic activity of tricyclics in cardiac patients who were not depressed. They studied 10 patients treated with imipramine and 12 with nortriptyline and found no effect of either drug on left ventricular performance. This further confirmed our original suspicion that the systolic time interval data were systematically flawed. Recently, Veith *et al.* (1982) published data on 17 depressed patients, eight treated with imipramine and nine with doxepin, studied both before and after drug and in both the resting and exercise condition. They also found no evidence that tricyclics impair left ventricular performance. However, they cautioned that only limited conclusions should be drawn from their data because they only studied a very limited number of patients who had a significant degree of left ventricular impairment as a baseline condition. They suggested that those patients with severe pre-existing left ventricular impairment may be more vulnerable to any negative inotropic effect that a drug might possess. We have recently ex-

amined a group of patients selected because they had pre-existing left ventricular impairment and found no evidence of any further impairment in left ventricular function while on tricyclics even in this significantly impaired group (Glassman *et al.*, in press).

## PHARMACOKINETICS AND LEFT VENTRICULAR PERFORMANCE

However, it became apparent that, even though tricyclic drugs do not adversely affect left ventricular performance, it does not follow that it is either safe or, in any sense, ordinary to administer these drugs to those depressed patients with compromised ventricular performance. The collection of a group of depressed patients with impaired left ventricular performance also allowed an examination of drug clearance in these patients. In spite of the large inter-individual variability in metabolism characteristic of these compounds (Glassman *et al.*, in press), the plasma concentrations of imipramine and its demethylated metabolite were dramatically higher in those patients with impaired left ventricular function than would be predicted on the basis of oral dose given. In this group an average oral dose of less than 3.5 mg $kg^{-1}$ resulted in a mean plasma level of 388 ng $ml^{-1}$ of imipramine and its demethylated metabolite (imipramine + desipramine). In a previous study of 60 depressed patients essentially free of heart disease, this same oral dose of imipramine produced a mean imipramine + desipramine steady-state plasma concentration of 200 ng $ml^{-1}$. This represents a highly significant statistical difference ($p<0.001$) even though the magnitude of the difference in plasma concentrations is understated because three of the patients with impaired left ventricular function stopped the drug before they reached steady state. In addition to the difference in total steady-state plasma concentrations of the drug, impaired left ventricular function would seem to alter the normal distribution of imipramine and its metabolites. In these patients, both imipramine and 2-OH-imipramine are far more common than one ordinarily would expect. In a large number of patients without cardiac disease, Cooper reported that desipramine is present in higher concentration in plasma than its parent compound, and Potter has found desipramine and 2-OH-desipramine to be present in higher concentrations (Cooper and Prien, personal communication; Potter *et al.*, 1982). In patients with left ventricular failure the opposite was true; imipramine concentrations were almost twice those of its demethylated metabolite (see figure 1). Similarly it has been shown that in depressed patients free from cardiac disease, 2-OH-imipramine is readily demethylated to 2-OH-desipramine and the concentrations of the demethylated metabolite usually are four or five times those of 2-OH-imipramine. However, in the depressed patient with left ventricular impairment the concentrations of 2-OH-imipramine were essentially equal to those of the

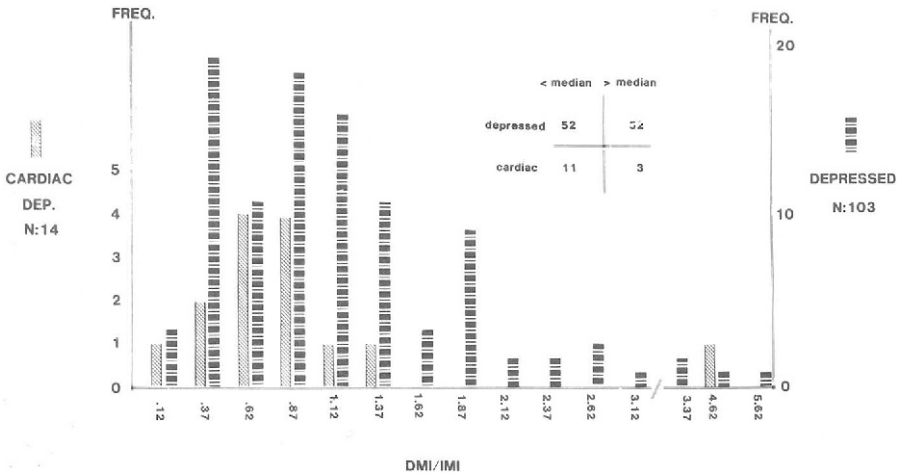


**Figure 1.** The frequency distribution of the ratio desipramine/imipramine (DMI/IMI) in two groups of depressed patients. The dark bars represent the ratios of 104 depressed patients free of cardiovascular disease, treated with 150 mg per day of imipramine. The lighter, hatched bars represent the ratios of 15 depressed patients with impaired left ventricular function. The chi-square measures the distribution of these ratios above and below the median ratio for the entire sample in the cardiac and non-cardiac patients. The difference is significant at the level $p<0.05$.

hydroxylated demethylated compound. The pattern of alterations in metabolism suggest that they result from inadequate demethylation. This would not be surprising given that demethylation is a flow-dependent process and these patients would be expected to have reduced hepatic blood flow secondary to their impaired left ventricular function. However, because these patients all have significant left ventricular impairment, the vast majority of them are simultaneously receiving cardiovascular medication. Thus, one cannot say unequivocally whether the observed differences in metabolism between this group and control populations are because of decreased hepatic blood flow, decreased renal blood flow or the cardiovascular drugs to which they are usually exposed. The reason for this difference in metabolism remains to be clarified.

## ORTHOSTATIC HYPOTENSION

Although it is in no way obvious that it is causally connected with the metabolic differences just discussed, patients with impaired left ventricular function have a dramatic increase in the

frequency of severe orthostatic hypotension. Among the 15 imipramine treated patients with clinical evidence of impaired left ventricular performance, seven developed orthostatic hypotension so severe that they fell and the drug had to be discontinued. Two additional patients had their drug discontinued at the end of the protocol period because of measured drop in blood pressure averaging 33 mmHg. Although they did not fall, these drops in measured pressure were considered precarious in this population. This intolerance to imipramine is a dramatic increase over any rate previously reported.

There is an interesting question if the falls experienced by this group of patients were due entirely to the orthostatic drop in systolic pressure or if these patients may also have a preexisting vulnerability to postural changes in blood pressure. Ordinarily, brain blood flow is maintained at a constant rate independent of systemic blood pressure, when systemic pressure is between 70 and 180 mmHg. If, however, the systemic blood pressure is above or below that range, then brain blood flow is directly related to systemic pressure. There is now evidence that tricyclic antidepressants decrease the range of brain blood flow independence (Preskorn *et al.*, 1982). It may be that heart failure in some way further restricts this independence. If so, it is possible that patients with heart failure treated with tricyclics are at risk to fall because small changes in systemic pressure will affect brain blood flow.

In order to examine further the relationship between orthostatic hypotension and cardiovascular disease, we reviewed data collected from 39 consecutive admissions to the research unit having both a diagnosis of major affective disorder and heart disease. The cardiovascular disease varied in type and severity although the more severe grades of functional impairment were likely to occur in those patients with impaired left ventricular performance because we recruited patients with left ventricular impairment regardless of severity. Depressed patients with functional impairment related to angina were not actively recruited and were undoubtedly under-represented in this population.

We identified those patients who fell and had the drug stopped (eight patients) and those patients who had the drug stopped or dose decreased because of orthostatic hypotension but did not fall (three patients). Thus, 11 of our 39 cardiac depressives had trouble due to orthostatic hypotension. The most striking observation is that 11 of the 25 males given imipramine had orthostatic trouble but none of the 14 female patients experienced serious difficulty. This difference is significant at the $p < 0.003$ level. In addition, clinical evidence of impaired left ventricular performance (a large heart on X-ray and/or a history of congestive heart failure), the number of cardiovascular medications, or the degree of functional impairment as measured by the New York Heart Association classification, were also significant

variables and were equally associated with the likelihood of developing orthostatic 'trouble'. Interestingly, neither age, pre-drug orthostatic change, nor plasma concentration of imipramine or its demethylated metabolite predicted that a patient would have orthostatic difficulties when treated with imipramine.

## SUMMARY AND CONCLUSION

For two decades there has been an impression that tricyclic drugs adversely affect left ventricular performance. This initially arose out of animal studies and was subsequently supported by a series of systolic time interval studies. Though it is easily understood how that impression gained wide acceptance, the impression is nonetheless erroneous. The overdose data of Thorstrand (1974) and Langou *et al.* (1980) and the radionuclide studies of Giardina *et al.* (1981), Veith *et al.* (1982) and our own recent radionuclide studies at usual therapeutic levels, leave little reason to believe that tricyclic antidepressants, in any usual situation, have an adverse effect on left ventricular performance.

Ironically, although it was this concern about the effects of tricyclic antidepressants on left ventricular performance that prompted us to study patients with ventricular impairment, it turns out that the clinical problem in treating these patients probably results from the effect of impaired left ventricular performance on their metabolism. It is now clear that, in depressed patients with impaired left ventricular performance, the clearance of the drug is altered and the chances of orthostatic hypotension with serious consequences is vastly increased - certainly this is true in depressed males. It would seem that the occurrence of an apparently simple adverse event, i.e. falling secondary to orthostatic hypotension, is related to the patient's cardiac condition - impaired left ventricular performance - sex and, perhaps, even his psychiatric diagnosis. It is intriguing to note that the data of Giardina *et al.* (1981) obtained in cardiac patients without depression showed no evidence of orthostatic hypotension compared to the severe orthostatic hypotension in our depressed, cardiac population. Although it remains to be clarified why this high rate of orthostatic hypotension occurs and whether it occurs with tricyclics other than imipramine, it is clear that the treatment of depressed patients with impaired left ventricular performance is a complex and, at least with imipramine, a potentially dangerous undertaking.

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# Registration of antidepressant drugs - views of a regulatory agency

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## INTRODUCTION

The prime objective of a national drug policy is to provide the community with reasonably priced, safe and efficacious drugs of high pharmaceutical quality. To achieve this objective, a number of regulatory activities have to be carried out constantly, among which are: inspection of pharmaceutical plants and laboratories; analytical and biological control; assessment of clinical trial protocols and safety documentation; assessment of new drug applications (NDAs); post-marketing surveillance, including drug promotion activities; drug consumption surveys; drug prescribing patterns; and adverse reaction reporting. Society expects its drug regulatory agency to take actions promptly whenever a drug hazard is identified. Similarly, it expects the introduction of new valuable drugs with the minimum of delay caused by the regulatory handling of the application.

## REGISTRATION

To be licensed, a new drug must prove efficacy beyond doubt and its side-effects must not be disproportional to its intended effect. Controlled clinical trials are mandatory for most drugs in order to provide acceptable data for claims of efficacy. Ideally, safety data should be collected similarly but, for long-term risk assessment, data from open studies usually suffice. More importantly, risk assessment studies should be guided by results of prior appropriate animal pharmacology and toxicology studies as well as by human pharmacokinetic and pharmacodynamic data indicating possible risks in patients with, for example, hepatic or renal failure, low-capacity drug metabolism, or treatment with other drugs subject to possible interactions. The data

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submitted to prove efficacy and safety must be scientifically valid and should meet the standards of contemporary first-line research. As progress is made, obsolete drugs should be phased out by industry and little action should be needed from regulatory agencies. However, commercial interests prevail and, hence, regulatory agencies must also act in this area. It is important to note that actions taken in order to remove a drug from the market should similarly rest on scientifically valid grounds, whether related to efficacy or safety problems.

Applying these criteria to the Swedish drug market, presently around 2500 pharmaceutical specialities are registered, representing about 900 substances and a variety of dosage forms and different strengths. Psychotropic drugs constitute less than 10% of the total number of drugs. There are 24 neuroleptics, 11 antidepressants, seven benzodiazepines, six barbiturates and less than 20 combination products available. Partly as a result of regulatory activities, the number and use of barbiturates and combination products have been falling drastically over the last few years, the centrally acting anorectic drugs being removed from the market almost two years ago.

Swedish drug regulatory control is in practice executed as a joint activity between professionals in the regulatory body and external experts. The necessity of keeping a qualified staff within the divisions of pharmacy, animal pharmacology and toxicology, pharmacotherapeutics and clinical research in order to respond to the goals of the health program and ongoing progress in the field is recognized by recruiting staff on scientific merit and allotting budgeted time for research and clinical activities. Recognizing this as extraordinary in comparison to the situation in most other regulatory agencies, the fact remains that efficient drug control calls for quality in order to meet the demands of society and the challenge by industry. To make up the gap, it is mandatory that experts in university positions contribute to the decision making. There will never exist a regulatory body staffed to cope fully with the wide range of activities expected from it. There are also good reasons to question the cost/benefit of striving in that direction. The participation of external experts in regulatory work is, thus, vital and the overall quality of drug use will depend on it. This view must be recognized and leading clinicians and scientists insisting on improvement in drug utilization can serve the cause by taking part in regulatory activities.

With regard to new drugs, true innovative drugs have become increasingly sparse. The majority of the recent introductions by pharmaceutical companies have been improvements to existing compounds yielding compounds with, for example, improved bioavailability, sustained duration of action, less dependence on metabolism, improved receptor selectivity with consequences also for side-effects. Some of these new drugs have indeed provided means for better pharmacotherapy; others seem to indicate doubtful gains

of prolonged efficacy and improved compliance. The case has been made many times that innovative research is extremely expensive and risky. Hence, to some extent, the industry must play it safe. Indeed, Lasagna (1982) has recently voiced the possibility that innovative research might be arrested altogether due to non-profitability. Are all really new drugs to be orphan drugs?

It is not my task to look into the future. Yet, the scope and format of pharmaceutical research relates strongly to drug regulatory activities. Indeed, it is important to both parties to engage in constructive cost/benefit analyses of current animal toxicology testing, methodology of clinical trials, post-marketing surveillance schemes, etc. It would be extremely helpful to determine in advance reasonable levels of security as to, for example, impurities in extraction of biosynthetic polypeptides, animal toxicology studies required for new classes of compounds, as well as the strategy for clinical trials. In this way, planning will be less time-consuming, operational procedures streamlined, and regulatory work optimized. Recent international attempts to seek more exchange of information between regulatory authorities and to standardize the control procedures are encouraging.

## ANTIDEPRESSANT DRUGS

To illustrate this point more concretely, it seems appropriate to discuss some problems that we have experienced in reviewing clinical data for antidepressant drugs. Over the last five years our regulatory agency has received six NDAs for this class of drug. So far only two drugs have been cleared; namely, lofepramine, essentially a prodrug of desipramine and accordingly assessed more by pharmacokinetic standards than by clinical ones, and zimelidine, a selective 5-HT uptake inhibitor. Doxepine was rejected primarily, owing to a poor submission failing to prove efficacy.

The submissions for the antidepressants raised a number of methodological problems, some of which were shared with drugs of different therapeutic classes. Two different types of antidepressants were distinguished, one claiming to possess a novel mechanism of action and one showing a higher degree of receptor selectivity than available tricyclic compounds, with possible consequences for clinical efficacy as well as side-effects.

In either case the main problem related to proof of efficacy (table 1). For most of the drugs, a number of small, unrelated clinical trials were submitted in which the new drug had been compared on a fixed-dose basis with standard doses of amitriptyline, imipramine, etc., in 20-50 patients during 4-6 weeks trials, many of which were open. The selection of patients and dropouts was too often poorly accounted for. In some studies, patients with endogenous depression were included together with patients with other types of depression. The selection of dose was infrequently supported by appropriate studies, either by re-uptake inhibition

Table 1. Problems in clinical documentation

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|---|
| **Efficacy** |
| Lack of appropriate animal models |
| Inadequate extent of human pharmacology studies including dose-finding studies and plasma concentration-effect relations |
| Poor definition of patient inclusion and exclusion criteria |
| Unsatisfactory accounts of dropouts |
| Lack of calculations of appropriate sample size and, hence, frequently too small patient materials |
| Inadequate or lacking description of how representative the study population is |
| Too many uncontrolled studies |
| Too many fixed-dose comparative studies |
| Lack of predefined criteria for effect |
| **Safety** |
| Poor registration and follow-up of adverse effects |
| Too few controlled studies of adequate duration |

models or pharmacological studies with regard to biological markers or possible side-effects, e.g. sedation or anticholinergic effects. In fact, the lack of stringent explorative phase I studies was distressing. Likewise, the design of many clinical studies was poor.

## RECOMMENDATIONS

Information about the state of affairs was passed on to our Board of Drugs, the advisory board on licensing of new drugs. It was decided to form a small task force with the mandate to work out guidelines for clinical documentation of antidepressant drugs. That work is still ongoing but I would like to share with you some suggestions made by the task force (table 2). Thus, it was felt that the diagnostic criteria must be stringent and acknowledged and validated rating scales should be employed. Early clinical studies with new antidepressant drugs should be done in patients with primary major depression (endogenous depression, melancholia) in order to prove the antidepressant properties of the drug. The patient material should be well characterized as to age, sex, duration and severity of disease, prior or ongoing treatment, hospitalization or outpatients, etc. It is important to include elderly patients in the trial program since this patient category will be a target group for therapy upon release of the drug.

For inclusion, the severity of disease in terms of rating scores should be specified. For therapeutic effect, the required reduction in rating scores should be stated prior to commencing

**Table 2.** Recommendations for clinical trials

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|---|
| Stringent diagnostic criteria utilizing acknowledged and validated rating scales |
| Adequate characterization of patient material with regard to other factors such as age, sex, duration and severity of disease, prior or ongoing treatment, hospitalization or outpatients, etc. |
| Ensure that the study program is representative of clinical therapy, e.g. inclusion of old patients |
| Inclusion of statistical analysis of patient numbers to meet the objective of the study |
| Use of washout periods prior to study treatment to reduce placebo response |
| Predefined criteria for effect recordings |
| Need to establish *early* efficacy of a new compound necessitating placebo-controlled studies, plasma concentration-effect studies and/or large controlled comparative studies |

the study. To reduce the placebo response, a preceding washout period should be included.

The number of patients needed to make a study worthwhile is truly important to consider. It is our experience that the numbers often are inadequate to meet the objectives of the study. Too frequently the studies include no statistical analysis of power justifying the design of the study. It must be borne in mind that 30-40% of the patients may respond to a placebo and that tricyclic antidepressants may have a therapeutic effect in 60-75% of the patients, all depending on the severity of disease. With this setting, handbooks in statistics show that in order to prove a new agent's efficacy of the mentioned order of magnitude in comparison to placebo, a study of more than 50 patients on either agent must be undertaken to assure significance. Conversely, it is evident that using too small patient populations and comparing the efficacy of two drugs with one another may result in no difference, and a Type II error.

For antidepressant drugs, small fixed-dose studies have dominated the scene during recent years. In addition to the reduced possibility of identifying a difference in efficacy between drugs, such a design may introduce non-comparability with regard to side-effects. Thus, when a drug is used in a non-equipotent dose, this might not be revealed in a small study whereas a low dose might well be associated with a low incidence of side-effects. The overall result will inevitably read: 'This drug is as efficacious but gives fewer side-effects.'

With the developments in this field, there are reasons to demand proofs of efficacy as well as comparative studies of incidence of side-effects. To achieve this, multicenter trials or

multiple independent trials with identical study protocols seem to be unavoidable. Theoretically, the effect of a new drug may be established initially in placebo-controlled clinical trials or by demonstration of a plasma concentration-effect relationship. Current discussions in our country bring attention to the problem of conducting conventional placebo-controlled trials in depressed patients. It has been suggested that such studies may replace plasma concentration-effect studies or the use of long washout periods in comparative studies. It has also been suggested that placebo techniques should only be used in patients resistant to available antidepressant treatment or in withdrawal studies. Apparently, the ethical problems are handled differently, judging from the fact that placebo-controlled studies are undertaken in the US. From the scientific point of view, placebo studies provide excellent means for assessment of efficacy and side-effects of a new therapeutic agent.

Controlled comparative studies are needed to provide a firm basis for judging a new drug's merits against established therapy. As has been pointed out, it is mandatory that such studies are planned to yield the information required, again pointing to large studies in well-characterized patients. In advocating such an approach, it is realized that clinics must cooperate rather than act on an individual basis. Such collaboration would hopefully eliminate some trials of non-innovative drugs, since resources would be directed towards developing areas, thereby also guiding pharmaceutical company research. Concentration is likely to yield more interest in and impact on the trial protocol by the participants. Today, too many trials seem to be carried out to collect a desired overall number of patients, whereas fewer but larger and better designed trials would advance scientific knowledge as well as meeting regulatory requirements.

It should however be understood that with this type of study the results may not be representative of the patient population, and this may be a sacrifice in exchange of trial quality. Thus, subsequent studies will have to face the fact that the overall patient population with depressive disorders likely to benefit from drug treatment may well present a spectrum of characteristics and responses with consequences for the trial protocols. Yet, to provide a solid basis of knowledge for such studies to follow seems to be highly recommendable and necessary for regulatory decision. It is also pertinent to point to this development in other areas, such as cardiovascular research. From a regulatory point of view, it is desirable that the professionals in the field address the problem of how clinical trials should best be conducted.

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# Phase-4 studies in psychopharmacology - new antidepressant drugs

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## INTRODUCTION

The well known 'phase system' used in evaluation of new drugs consists of phases 1, 2 and 3 which are performed before marketing and phase 4 which is carried out after marketing. The pre-marketing phases (1, 2 and 3) aim at describing intended and unintended drug effects in well defined, but rather small, groups of subjects or patients. In contrast, the phase-4 study aims at describing the events following the use of the drug in a larger, more heterogeneous and less well defined population. This system is generally accepted and used in all countries having a modern drug regulatory system, and has generally served its purpose well.

The relatively rigid but variable framework of phases 1, 2 and 3 should be considered when defining the content of phase-4 studies and the research methods to be used. This problem can best be examined by studying the limitations of the phase-3 investigations. The phase-3 investigations cover a limited number of patients; the duration of treatment is often short; and the indications for the treatment are strict and often not commensurate with real life. Phase-3 studies are increasingly often carried out in a special research regimen by particularly interested researchers, but still the designs of many studies are not always comparable and many trials suffer from obvious methodological deficiencies. All these factors point to the necessity of further studies of the new drug after marketing.

Several problems are to be solved in the phase-4 study. Focus should be not only on safety problems and side-effects but also on further registration of therapeutic effect. New indications for use of the compound should be kept in mind. Clinical pharmacological investigation should be continued and the need for

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therapeutic drug level monitoring should be thoroughly examined (Hvidberg, 1980; Morselli, 1981). These studies should end with a sort of cost/benefit analysis of the new therapy relative to existing therapy.

## NEW ANTIDEPRESSANTS AND PHASE-4 STUDIES

During the past few years several new compounds (e.g. iprindole, viloxazine, mianserin and nomifensine) have been marketed in several countries as effective antidepressants. These drugs have already been widely used and are probably also accepted in clinical practice.

Since these drugs are relatively devoid of effects on the monoamine re-uptake mechanisms, their introduction has created doubt about the validity of the amine hypothesis of depression and mechanism of action of tricyclic antidepressants (Zis and Goodwin, 1979). However, their clinical antidepressant effect has been questioned (Brogden *et al.*, 1978, 1979; Zis and Goodwin, 1979; Hollister, 1981), and as discussed by Strandberg (1983) these difficulties in proving the therapeutic effect are associated with the design of the clinical studies. The many methodological problems and especially the criteria for patient selection make it difficult to conclude that these new drugs really are antidepressants.

It is generally accepted that response to tricyclic antidepressants has been scientifically established only in endogenously depressed patients (Kiloh *et al.*, 1962; Greenblatt *et al.*, 1964; Morris and Beck, 1974; Bielski and Friedel, 1976). The therapeutic effects reported in the published studies on new antidepressants have often been insufficiently demonstrated, and the possible differences from conventional tricyclic antidepressants have not been sufficiently examined. This may, at least partly, be because the studies deal with heterogeneous patient populations. The criteria for diagnostic classification of patients are often poorly defined and not always given, and endogenously depressed patients are not always separated from patients with other depressive states. A conclusion concerning the therapeutic effect in different patient populations is, therefore, impossible.

Perusal of the literature on phase-3 studies on new antidepressants thus points to a need for re-examination of their therapeutic effects in the phase-4 studies. In addition, phase-4 studies should yield extensive information with regard to unintended effects, in particular serious adverse reactions that occur infrequently and therefore have not been detected in the pre-marketing studies. Several new antidepressants have been introduced with claims of fewer cardiovascular side-effects, and such claims ought to be challenged in the phase-4 studies. Depend-

ing on the particular aim of the study, phase-4 studies may concentrate either on collection of limited information in a large patient population, or on more intensive studies in smaller patient populations.

Studies of the latter type should be carried out in comparison with standard drugs, ideally in randomized parallel groups, but this is often not feasible. The usual blinding procedure can often be eliminated, while the number of patients and duration of observation period should be realistic. An alternative to parallel groups may be the prospective comparison of sequential treatment series with new antidepressants alternating with established antidepressants. The inherent weakness of this design is that the patient population may change over time, and therefore it is important to include treatment periods with standard drug at regular intervals. This design has been used by us in studies on antidepressant treatment in the elderly and has also been adopted by other groups (von Zeersen and Cording-Tömmel, 1981; Cording-Tömmel, 1982).

## DESIGN OF PHASE-4 STUDIES ON ANTIDEPRESSANTS - AN EXAMPLE

Psychiatrists generally believe that antidepressant treatment of elderly patients (>60 years) is troublesome and in many cases contraindicated (Burrows *et al.*, 1983) in relation to the expected increase in undesired effects, in particular cardiovascular reactions. Many clinicians, therefore, prefer electroconvulsive treatment in elderly endogenously depressed patients.

Until recently, our knowledge about the cardiovascular effects of tricyclic and other antidepressants in the elderly has been rather limited, and the practical value of plasma concentration monitoring has not been studied systematically in this age group.

At the Clinical Psychopharmacology Research Unit at Odense University, we have therefore established a standard research program for treatment of elderly endogenously depressed patients. This program has been designed in accordance with earlier plasma level/effect studies in which heed was given to the methodological problems in question (Gram *et al.*, 1981). To date, studies with imipramine, nortriptyline and mianserin (recently marketed in Denmark) have been carried out.

### Methods and Patients

Patients over 60 years of age with endogenous depression are examined in a prospective research program that includes the following:

(1) Open plasma level monitored treatment, one week on

placebo followed by at least five weeks on active treatment.

(2) Patients classified as endogenously depressed on a diagnostic scale (Newcastle Diagnostic Index: Gurney *et al.*, 1972; Kragh-Sørensen *et al.*, 1976; Bech *et al.*, 1980) and scoring more than 17 points on the Hamilton Depression Rating Scale (HDRS: Hamilton, 1967) are included. During active treatment, weekly registration of therapeutic effect (HDRS) and side-effects (Asberg *et al.*, 1971) are carried out.

(3) Orthostatic blood pressure reaction (lying and 1-6 min standing) and standard electrocardiogram (ECG) are recorded weekly. Systolic time intervals and 24 h ECT monitoring are carried out in the placebo week and in the second and third weeks of active treatment (Thayssen *et al.*, 1981; Møller *et al.*, 1983).

In each of the completed studies, 14-18 patients were started on placebo and 11-15 patients were started on active treatment (table 1).

Only oxazepam for sedation and already instituted treatment for heart disease (e.g. diuretics, digoxin) were allowed. Some patients had mild cardiac disease, but all patients were cardiovascularly well compensated.

Imipramine and nortriptyline were given twice daily at 8 a.m. and 8 p.m., and the dose was adjusted to give therapeutic plasma concentrations (imipramine + desipramine >200 $\mu g\ l^{-1}$; nortriptyline 60-150 $\mu g\ l^{-1}$).

In the mianserin study, a fixed-dose regimen was used. The first 11 patients were treated with 60 mg daily and 20 mg daily was given to the last four patients. The dose was given in a single dose at 8 p.m.

## Results and Discussion

The primary aim of these studies has been to examine adverse reactions; in particular, the cardiovascular effects of the three antidepressants and possible pharmacokinetic differences between them.

The therapeutic effect was examined concurrently and evaluated on the basis of comparison between the consecutive studies. As shown in table 1, the different compounds were given to similar patient populations and in almost identical settings.

With this background, it is interesting that distinct and clinically relevant differences between the three drugs were demonstrated. In the following, a brief outline of the pharmacodynamic, pharmacokinetic and therapeutic differences will be given (for further details, see Thayssen *et al.*, 1981; Bjerre *et al.*, 1981; Kragh-Sørensen *et al.*, 1981; Møller *et al.*, 1983).

Table 1. Imipramine, nortriptyline and mianserin treatment in elderly (⩾ 60 years) endogenously depressed patients: clinical data

| Drugs | Patients initially included | Excluded during placebo | Sex | Patients given active treatment | |
|---|---|---|---|---|---|
| | | | | Age (mean and range) | HDRS at the end of placebo period (mean ± S.D.) |
| Imipramine | 15 | 4 | 3M, 8F | 73 (63-83) | 25 ± 4 ($N$ = 11) |
| Nortriptyline | 14 | 2 | 5M, 7F | 70 (60-78) | 23 ± 4 ($N$ = 12) |
| Mianserin | 18 | 3 | 4M, 11F | 68 (60-86) | 25 ± 5 ($N$ = 15) |

## Cardiovascular Effects

Both imipramine and mianserin caused significant orthostatic blood pressure drop, whereas nortriptyline caused only a slight and clinically insignificant drop (figure 1) (Thayssen *et al.*, 1981; Kragh-Sørensen *et al.*, 1981; Møller *et al.*, 1983). The effect on orthostatic blood pressure was very pronounced during treatment with imipramine and in two patients it was associated with fall and fracture of the collum femoris. The pronounced drop in orthostatic blood pressure during imipramine treatment necessitated cautious dosage which, in turn, meant that several patients did not reach therapeutic plasma concentrations within the five-week treatment period. The orthostatic blood pressure reaction after mianserin did not give rise to similar pronounced problems, perhaps due to a compensatory increase in heart rate not seen during imipramine treatment (figure 1).

Some patients with hypotensive reactions both in the imipramine and the mianserin groups were subsequently treated with nortriptyline in therapeutic plasma concentrations (60-150 μg $l^{-1}$) without any noticeable effect on orthostatic blood pressure.

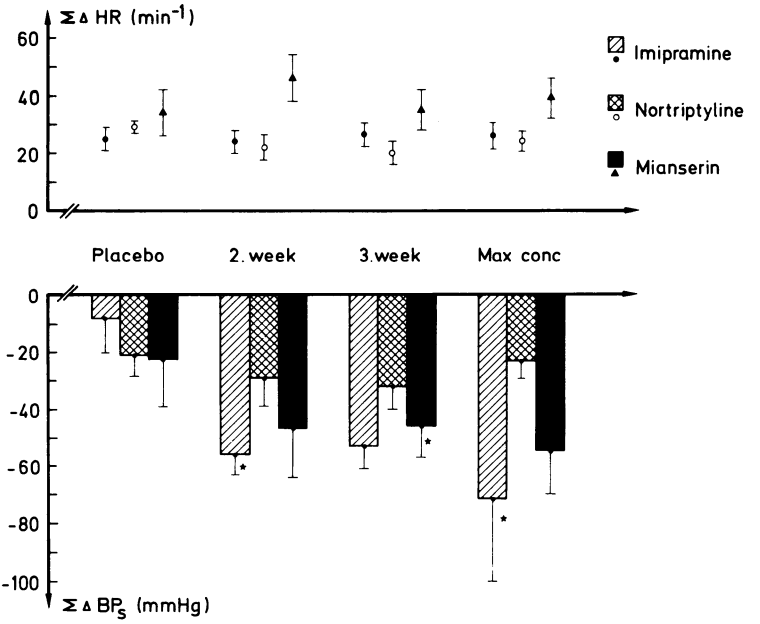


**Figure 1.** The cumulative changes in heart rate (HR) and systolic blood pressure ($BP_S$) from supine position to 1, 3 and 5 min in the standing position during treatment with imipramine ($N$=10), nortriptyline ($N$=10) and mianserin ($N$=10). Values are given as mean ± S.E.M. *$p$<0.05 for differences between placebo and active treatment.

Cardiac ventricular performance evaluated by systolic time interval measurement showed changes in the ratio pre-ejection period/left ventricular ejection time (PEP/LVET) indicating impairment of the left ventricular performance during nortriptyline and mianserin treatment, but not during imipramine treatment, probably due to the relatively low plasma levels in our imipramine patients (*vide supra*).

It has been suggested (Glassman *et al.*, 1983) that the changes seen in PEP and PEP/LVET during antidepressant therapy may be induced by alteration in intraventricular conduction. However, as no prolongation of the electrocardiographic intervals was found in our study, the significant changes in PEP/LVET ratio most likely reflect impairment of left ventricular function. Another possible mechanism is that the antidepressants cause vein dilatation ($\alpha_1$ adrenoceptor blockade) resulting in reduced cardiac preload and increased PEP/LVET ratio. In any case, the changes in PEP/LVET ratio were modest and not associated with signs of cardiac decompensation.

The 24 h ECG monitoring showed that nortriptyline increases the supine heart rate, whereas neither imipramine, nortriptyline nor mianserin induced changes in the cardiac conduction time or arrhythmias.

From these results it can be concluded that during plasma level controlled therapy in elderly patients, imipramine, nortriptyline and mianserin have modest effects on myocardial contractility, no significant influence on the cardiac conductance, and no arrhythmogenic effect. The clinically most important cardiovascular effect is the orthostatic hypotension, which is pronounced with imipramine, moderate with mianserin and slight or insignificant with nortriptyline.

**Pharmacokinetics**

The imipramine treatment in the elderly was complicated by dose-dependent kinetics resulting in disproportional rise in desipramine concentrations with increasing dose of imipramine (Bjerre *et al.*, 1981). Proportionality between dose and steady-state plasma levels was found with nortriptyline (Kragh-Sørensen and Larsen, 1980; Bjerre *et al.*, 1981) and with mianserin (unpublished data). Dose adjustment in order to obtain therapeutic drug levels was thus more difficult with imipramine than with nortriptyline and mianserin.

Interaction between tricyclic antidepressants and neuroleptics is a well known phenomenon (Gram, 1977). In these series of investigations, this interaction was observed for all three compounds at relatively low perphenazine doses given late in the treatment period (fourth to eighth week) (Bjerre *et al.*, 1981). The interaction between perphenazine and mianserin resulted in a

rise in the plasma concentration of both mianserin and the main metabolite desmethylmianserin when perphenazine was added to the treatment (figure 2).

Steady-state plasma levels of imipramine and nortriptyline on conventional doses have been shown to be higher in elderly than younger patients (Gram *et al.*, 1977; Nies *et al.*, 1977; Kragh-Sørensen and Larsen, 1980). The mianserin dose given in this investigation was 30-60 mg per day and corresponded to plasma concentrations of 25-70 μg $l^{-1}$. This plasma level range is the same as that found in younger patients (Coppen *et al.*, 1976; Montgomery *et al.*, 1978; Perry *et al.*, 1978; Russell *et al.*, 1978).

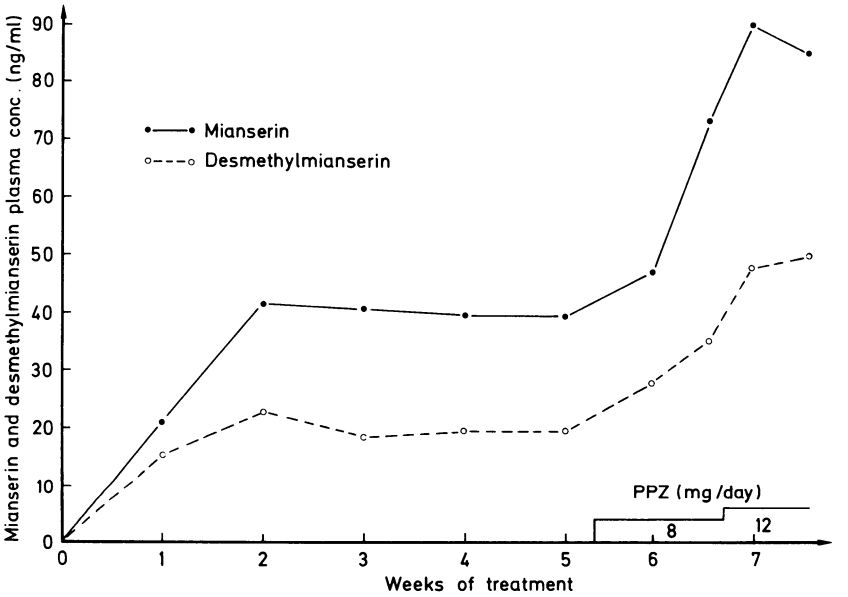


**Figure 2.** Drug interaction between mianserin and perphenazine (PPZ) in a hospitalized 72-year-old male patient.

## Therapeutic Outcome

The analysis of the data concerning the therapeutic effect was particularly interesting in relation to the results with mianserin, although mianserin was selected primarily because it was marketed as an antidepressant having no or few cardiovascular effects.

As shown in table 1, the patient materials in the three studies were comparable in terms of selection criteria, placebo

Table 2. Imipramine, nortriptyline and mianserin treatment in elderly (⩾60 years) endogenously depressed patients: therapeutic outcome (responders = sum of HDRS ⩽7 points)

| Drug and no. of patients (*N*)* | Responders within 5 weeks | Responders 5-8 weeks | Total no. of Responders |
|---|---|---|---|
| Imipramine (*N* = 9) | 2 (22%) | 5 (56%) | 7 (78%) |
| Nortriptyline (*N* = 11) | 7 (64%) | 2 (18%) | 9 (82%) |
| Mianserin (*N* = 12) | 1† (8%) | 0 (-) | 0 (-) |

* Dropouts before week 4 not included
† Depressive relapse in week 6

dropouts, initial severity (HDRS), age and sex; 15 patients entered the active treatment with mianserin.

Surprisingly, it was found that nine patients on mianserin treatment did not respond at any time to the treatment (table 2). Four of these patients deteriorated to such an extent that electroconvulsive therapy was required. The other five patients subsequently responded on treatment with nortriptyline. In six patients mianserin had some therapeutic effect according to HDRS. Two of these patients were followed on continued mianserin treatment, but within 5-8 weeks their condition worsened and nortriptyline treatment was instituted. In two other patients, mianserin was discontinued in the fifth week due to poor effect. In two patients (partial responders on HDRS) the mianserin treatment was stopped because of severe drowsiness after week 5, and both could be discharged from hospital without specific antidepressive treatment, and with few or no symptoms of depression.

Assuming that our design permits comparison of the results from the three studies, it can be seen (table 2) that the therapeutic effect after eight weeks in both the imipramine and nortriptyline groups was clearly superior ($\sim$ 80% recovery). This recovery rate is comparable with the therapeutic effect seen in younger patient populations (Glassman, 1981).

The poor results after five weeks' treatment for the group of patients treated with imipramine was due to the difficulties in adjusting the dose to yield therapeutic plasma concentrations because of orthostatic hypotension and dose-dependent kinetics.

A possible explanation for the unexpected poor therapeutic result with mianserin could be that elderly patients with endogenous depression generally react poorly to antidepressive drug treatment. However, nine of the mianserin patients responded satisfactorily on subsequent nortriptyline treatment.

Other reasons for inconsistency in therapeutic effect between mianserin and imipramine and nortriptyline could be disparity in sex, age, severity of disease, polarity of depression, diagnostic selection, etc., but these factors were not disparate in our investigation, and possible differences in exclusion criteria, particularly in the placebo washout period, were unlikely (table 1).

In only one study (Montgomery *et al.*, 1978) has therapeutic plasma concentration ranges of mianserin been suggested (15-70 $\mu g\ l^{-1}$). In our study, the 11 patients who completed four weeks on active mianserin treatment all had steady-state plasma concentrations lying within this range (25-60 $\mu g\ l^{-1}$). All patients showed stable steady-state concentrations after two weeks' treatment (cf. figure 2). We have, therefore, no pharmacokinetic or clinical explanation of the poor therapeutic effect of mianserin. The dosage chosen and the plasma concentration achieved are the same as those used in phase-3 studies (Brogden *et al.*, 1978).

It seems unlikely that mianserin, in contrast to imipramine and nortriptyline, should be particularly ineffective in elderly patients.

## Conclusion

The described design of this prospective phase-4 study in elderly endogenously depressed patients fulfills all requirements for clinical psychopharmacological research on antidepressants with regard to selection and stratification of patient material and measurement of pharmacokinetic variables (Gram *et al.*, 1981). In a phase-4 study, procedures involving randomization and control therapy usually are not feasible.

Because of the negative therapeutic findings in the mianserin study, the design of phase-4 studies and research on therapeutic drug monitoring becomes pertinent. In particular, it is essential to establish the validity of phase-4 studies carried out as sequential single-drug studies without use of control therapy. Using a design quite similar to ours, von Zeersen and Cording-Tömmel (1981) and Cording-Tömmel (1982) have also questioned the antidepressant effect of mianserin in a younger patient population suffering from severe endogenous depression.

We cannot conclude from these results that mianserin does not have antidepressant properties. However, we can question the value of previously published phase-3 investigations, by far the greatest part of which did not satisfy requirements for controlled clinical trials with antidepressants (Brogden *et al.*, 1978; Zis and Goodwin, 1979; Strandberg, 1983). Therefore, the logical consequence of this study and the study from the Munich group (von Zeersen and Cording-Tömmel, 1981; Cording-Tömmel, 1982) must be that further controlled studies with mianserin should be carried out. The many methodological problems not sufficiently dealt with

in many recent studies (phases 2 and 3) on new antidepressant drugs indeed have the perspective that inactive compounds possibly can be marketed as antidepressants.

From a theoretical point of view these results, if replicable, will question the value of many new theories concerning the mechanism of action of antidepressants that were proposed after the introduction of these new compounds, and perhaps bring some new support to the monoamine hypotheses. Also, the validity of quantitative electroencephalographic measurements (Itil *et al.*, 1972) to predict the antidepressant efficacy in patients accordingly can be questioned. Indeed, all new theories need clinical justification before being accepted.

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# Section Three

# Clinical Pharmacokinetics of Neuroleptics

# Overview: measuring plasma concentrations of psychotherapeutic drugs

Leo E. Hollister[1]

## INTRODUCTION

Determining plasma concentrations of psychotherapeutic drugs is still relatively new, most of the studies having been done in the past 10-15 years. The idea of such monitoring is not new; when bromides were widely used in psychiatry and neurology, monitoring serum concentrations was recommended although seldom done. Technological advances have made routine monitoring possible. The only question that remains is when it is worth doing, assuming that the laboratories doing such work provide accurate determinations at a reasonable cost.

As experience with measuring plasma concentrations of psychotherapeutic drugs is of variable duration, I shall consider each group in terms of the length of the past experience.

## LITHIUM

This ion has a relatively narrow therapeutic margin and fairly well defined range of serum concentrations of 0.9-1.4 mEq $l^{-1}$. The lower limits of concentration for its prophylactic use are still being investigated but may be as low as 0.5 mEq $l^{-1}$. Lithium is an unusual therapeutic agent, however, as it is distributed in body water for the most part, undergoes no protein binding, is not metabolized and is virtually totally excreted unchanged. Thus, the proven value of measuring serum lithium concentrations may be difficult to extend to drugs with high volumes of distribution, high protein binding, varying degrees of lipid solubility, extensive metabolism, and sometimes active metabolites.

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The convention is to measure lithium levels as close to 12 h after the last dose as possible (Amdisen, 1977). Such measurements should not be done until the patient has been on a given dose of lithium for about five days, when steady-state concentrations are reached. Ordinarily, measurements are made at intervals of one week during the first month of treatment but as infrequently as once every month or two during maintenance treatment. Measurements should be repeated shortly after another drug has been added to the therapeutic program (diuretics and non-steroidal anti-inflammatory agents increase levels) or whenever some intercurrent illness appears. One should pay heed to toxic symptoms even if plasma concentrations are reported to be in the therapeutic range.

Abundant experience has indicated that good clinical results are sometimes obtained in some patients treated with 'subtherapeutic' concentrations of lithium while toxicity has occurred when serum levels were within the therapeutic range. Accordingly, such determinations can be used only as guides to dosing and should not take precedence over clinical observations.

## TRICYCLIC ANTIDEPRESSANTS

Despite extensive study, controversy still exists as to whether a range of therapeutic plasma concentrations can be defined. Part of the problem is due to the heterogeneity of depressions, for the specific antidepressant effect of tricyclics is largely limited to depressions characterized as 'endogenous', a minority of all depressions.

Another difficulty is that some of these drugs produce active metabolites, often in greater abundance than the parent drug, that have either the same or somewhat different pharmacological actions. Failure to take into consideration significant amounts of demethylated or hydroxylated metabolites may confound the issue.

Some ranges of reported therapeutic plasma concentrations for various tricyclics are shown in table 1. Greatest agreement centers around nortriptyline. Recent work indicated that the 10-hydroxy metabolite of nortriptyline has a significant pharmacological activity and this metabolite is usually more abundant than the parent drug. Yet virtually all studies attempting to relate plasma concentrations to clinical effects of this drug have ignored this metabolite.

Evidence is highly suggestive, although far from conclusive, that too low a concentration of tricyclic antidepressant may be associated with a suboptimal clinical response, while concentrations that are very high may be associated with serious side-effects (mental confusion or cardiac disturbances). Such a relationship seems to be entirely logical. The issue to be settled is whether the present guides can be translated into improved clinical practice.

Table 1. Proposed therapeutic ranges for antidepressants ($\mu g\ l^{-1}$)

| | |
|---|---|
| Nortriptyline | 50-150 (Kragh-Sorensen *et al.*, 1976) |
| | 200 (poorer response) (Montgomery *et al.*, 1978) |
| Imipramine | 45 imipramine/75 desipramine (Reisby *et al.*, 1977) |
| | 180 total (Glassman *et al.*, 1977) |
| Amitriptyline | 60-230 total (Vandel *et al.*, 1978) |
| | 200 total (Kupfer *et al.*, 1977) |
| Desipramine | up to 145 (Amsterdam *et al.*, 1979) |
| Protriptyline | 70-170 (Biggs and Ziegler, 1977) |
| Clomipramine | 240-700 desmethyl clomipramine (Della Corte *et al.*, 1979) |
| | 100-250 clomipramine (Stern *et al.*, 1980) |
| Maprotiline | 200-300 (Pinder *et al.*, 1977) |

When might it be appropriate to monitor plasma concentrations of tricyclics? The following are a few suggestions.

(1) The most important indication by far is when a patient has received what could be an adequate dose and has failed to respond. A low plasma concentration might be construed as some abnormality of the drug kinetics in that patient or, more likely, failure to take medication as prescribed. Advising patients with low plasma levels to take their medication exactly as prescribed often results in an increase in plasma concentration, even with no change in dose. On the other hand, if the plasma concentration is in the therapeutic range, but not excessively high, one might feel encouraged to increase the dose so long as clinical toxicity was not evident.

(2) Higher than usual doses of tricyclics merit measurement of plasma concentrations. Doses of 300 mg per day or more of amitriptyline and imipramine seem to show altered kinetics with a non-linear increase in plasma concentrations. Monitoring might help to avoid problems with toxicity and would demonstrate a degree of prudence.

(3) When treating very old or very young patients one might wish to check plasma concentrations to keep them low. The reason for doing so is that protein binding of these highly protein-bound drugs is impaired at the extremes of age. Thus, more drug is present in the unbound, or pharmacologically active, form. The usual interpretation of plasma concentrations will not hold under these circumstances, so that one may wish to keep them at about 50% of what one might wish in a robust patient in the middle years of life.

(4) The presence of intercurrent illness may be a reason to lower plasma concentrations to the lower limits of potential therapeutic efficacy, as drug kinetics may be altered in complex ways during illness.

(5) In cases of drug overdose, plasma concentrations may be particularly useful late in the course when making a decision about when to relax vigilance. A plasma concentration that has returned to the therapeutic range, as well as clinical evidence of remission, would make one feel secure in removing the patient from intensive care. Such determinations would not be especially useful in the course of intoxication, for the level of drug has relatively little bearing on the subsequent clinical course of the intoxication; that is best judged by the prevailing clinical appearance of the patient.

## NEUROLEPTICS

Neuroleptics have been used longer than tricyclics in clinical practice, but fewer investigators have tried to define a range of therapeutic plasma concentrations. Some of the limitations on these data are due to technical problems. Chlorpromazine has many potentially active metabolites so that it is really uncertain that simply measuring levels of the parent drug is adequate. The same is true for thioridazine. Some piperazinylphenothiazines, such as fluphenazine, produce only very low steady-state levels, so close to the level of sensitivity of the measuring technique that chances for error are large. Thiothixene has two isomers, one of which contains most of the therapeutic activity. These are somewhat difficult to separate using techniques other than mass spectrometry. Further, steady-state concentrations of this drug also tend to be very low. The relatively sparse data are summarized in table 2.

Haloperidol has emerged as a very popular neuroleptic. It has the advantage that its only active metabolite is present only in inconsequential amounts. Consequently, a great deal of interest has focused on trying to delineate its range of therapeutic plasma concentrations. As can be seen in table 2, neither the lower nor the upper limits have been clearly defined. One might assume that a concentration of 8-10 $\mu g\ l^{-1}$ might represent a suitable lower limit for such a range.

In summary, the data are too sparse and contain too many possible sources of error to accept claims about a range of therapeutic plasma concentrations for neuroleptic drugs. Laboratories can provide numbers, at least for some of these drugs, but their meaning is unclear.

Table 2. Proposed therapeutic ranges for neuroleptics ($\mu g\ l^{-1}$)

| | |
|---|---|
| Chlorpromazine | 50-300 adults (Rivera-Calimlin *et al.*, 1976) |
| | 40-80 children (Rivera-Calimlin *et al.*, 1979) |
| Thioridazine | 740 total (Cohen *et al.*, 1980) |
| Butaperazine | 400-300 (plasma) (Garver *et al.*, 1977) |
| | 20-60 (erythrocyte) (Garver *et al.*, 1977) |
| Haloperidol | 8-18 (Magliozzi *et al.*, 1981) |

## BENZODIAZEPINE ANXIOLYTICS

The pharmacokinetics of almost every one of these drugs has been extensively described. Yet no investigator has proposed that measurement of plasma concentrations be adopted in clinical practice. A very early study suggested that minimal plasma concentrations of diazepam for clinical efficacy should be in the region of 400 $\mu g\ l^{-1}$ for total diazepam and its active metabolite, nordiazepam (Dasberg *et al.*, 1974). Patients being treated with the drug have shown, however, a very wide range of plasma concentrations, often seemingly doing well at concentrations less than 400 $\mu g\ l^{-1}$.

As the margin of safety with benzodiazepines is very large, it seems unlikely that clinicians will change their present habits of adjusting doses on the basis of clinical response rather than on the basis of plasma concentrations. Still, monitoring of plasma concentrations might detect poor compliance with the treatment program, or possible abuse of the drug.

## CONCLUSIONS

The present practice of monitoring serum lithium concentrations is well established, valuable and inexpensive. Arguments for the cost-efficacy for measuring plasma concentrations of other psychotherapeutic drugs are far from persuasive. Particular situations may arise in which such monitoring could be useful. One must remember that not all laboratories provide accurate estimates and that such information might be more of a hindrance to good medical practice than a help.

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# Possible role of hydroxymetabolites in the action of neuroleptics

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## INTRODUCTION

A major cause of the rapid growth of clinical pharmacokinetics as a discipline was the observation that the same dose of a drug may give very different plasma drug levels in individual patients. Pharmacokinetic variation could thus explain part of the individual variation in drug response, and therapeutic monitoring of plasma drug levels has since become a useful tool in the management of treatment with various drugs.

Further development of chemical techniques has made possible the measurement of plasma levels of drug metabolites together with the parent compound. From this it has been found that the ratio between the plasma concentrations of a metabolite and the parent drug may show large variations between individual patients.

Active drug metabolites may have a pharmacodynamic profile and toxicity different from that of the parent drug, and therefore it may be expected that the contribution from an active metabolite to the effects of a drug may be different between individual patients. Monitoring of plasma levels of active metabolites in addition to the levels of the parent compound should, at least in theory, provide additional information which may be useful in the management of individual patients.

## METABOLITES OF NEUROLEPTIC DRUGS

### Plasma Levels

Oral administration of neuroleptics often results in an especially large range of plasma drug levels (Dahl, 1979, 1981). As discussed

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earlier (Dahl, 1979), it seems likely that for the phenothiazines inter-individual variation in the extent of pre-systemic metabolism may be a major reason for the observed variation in the plasma drug levels. There have been relatively few studies comprising plasma level measurement of neuroleptic drug metabolites, but those which have been published have reported a 7- to 20-fold variation in the plasma concentration ratios of metabolite to parent drug at steady state within small groups of patients (Dahl, 1982).

Chemical and pharmacological problems concerning the large number of different phenothiazine drug metabolites which may be formed have previously received much attention (Kaul *et al.*, 1974). As discussed recently (Dahl, 1982), the number of phenothiazine drug metabolites which are formed in such amounts that they may be expected to contribute to the effects of the drug in man is, however, usually much lower than the total number of identified or postulated metabolites. An example of this is given in figure 1, which shows the formulas of the metabolites of levomepromazine which have been identified in urine (Dahl and Garle, 1977; Johnsen and Dahl, 1982) and in plasma (Dahl and Garle, 1977; Dahl *et al.*, 1982c) from psychiatric patients, after repeated oral doses of the drug. As shown in figure 1, at least 10 different levomepromazine metabolites are formed in man. The five metabolites which are formed by a single phase I reaction were, however, found in much higher concentrations than the other metabolites in the urine, and only these five metabolites could be identified in the plasma.

Another study demonstrated that the steady-state blood levels of two of these metabolites were above the concentrations of unmetabolized levomepromazine in four out of five patients (Dahl *et al.*, 1982a). The three other levomepromazine metabolites which have been identified in plasma, namely 3-hydroxy, 7-hydroxy and *O*-desmethyl levomepromazine (figure 1), have not yet been assayed in plasma or whole blood from patients. It was found, however, that each patient had about the same plasma concentration of 3-hydroxy levomepromazine as that of 7-hydroxy levomepromazine, at steady state (Dahl *et al.*, 1982c). This demonstrates that the pattern of aromatic hydroxylation of levomepromazine in man is different from that of the congener chlorpromazine, which appears to be hydroxylated only in the 7-position.

## Molecular Structure and Biological Activity of Chlorpromazine Metabolites

7-Hydroxy chlorpromazine has been demonstrated to be clinically active in a crossover trial against chlorpromazine, in five patients (Kleinman *et al.*, 1980). As shown in figure 2, the

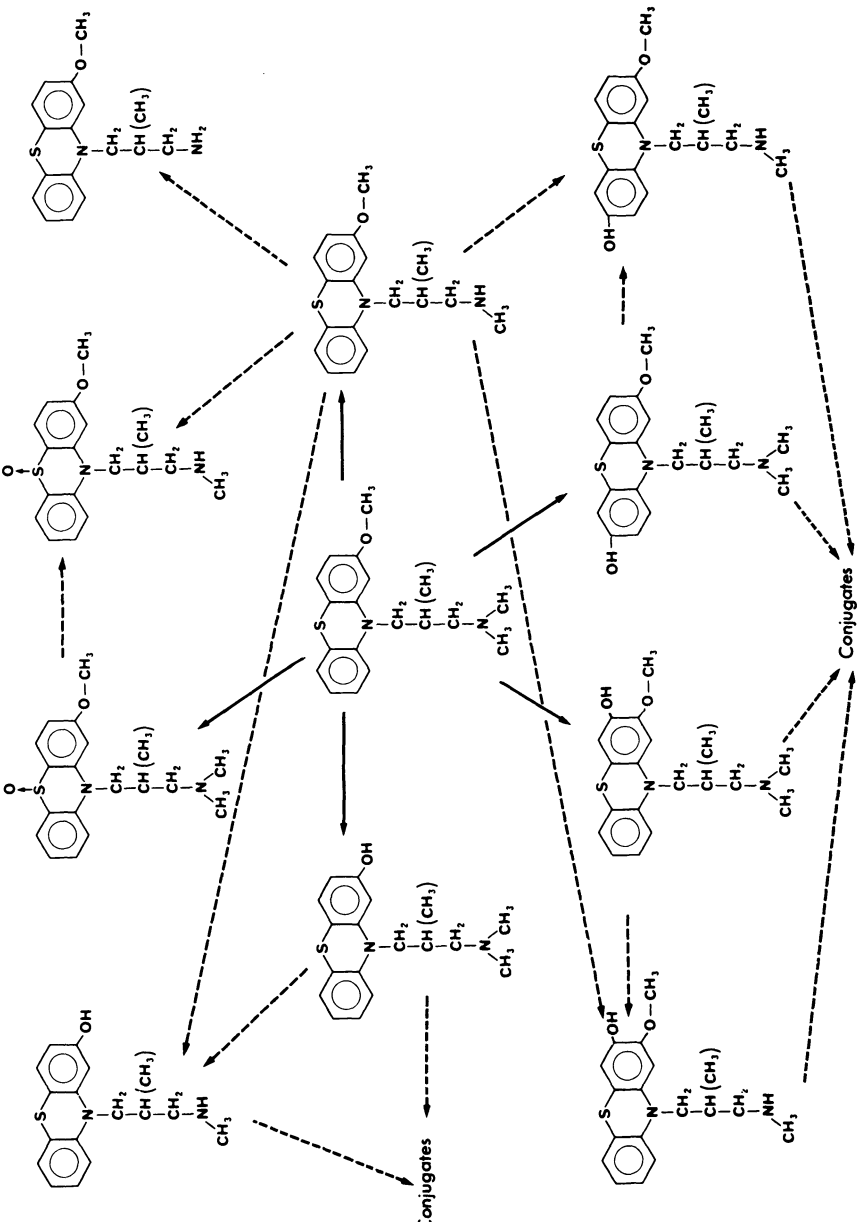


**Figure 1.** Metabolic scheme of levomepromazine in man. Arrows drawn using solid lines point to the five metabolites which have been identified in the plasma; the other metabolites have been identified in urine only.

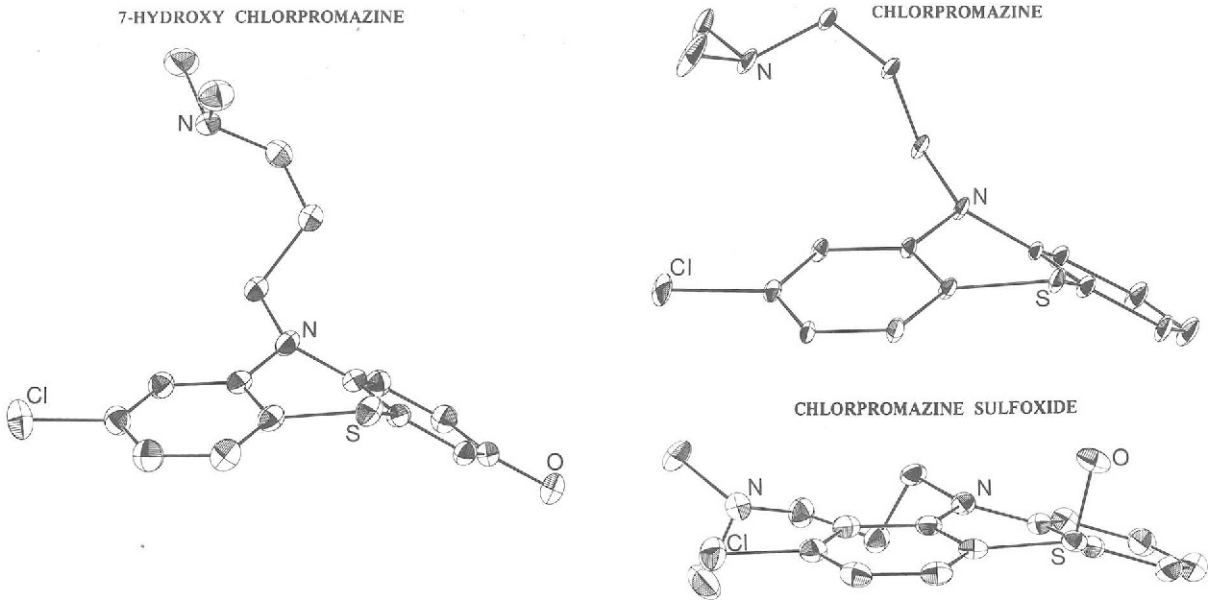


**Figure 2.** Molecular structures of chlorpromazine and two of its metabolites in the solid state. Computerized drawings were made by the ORTEP program (Johnson, 1971), from previously published atomic coordinates for chlorpromazine (McDowell, 1968), 7-hydroxy chlorpromazine (McDowell, 1977) and chlorpromazine sulfoxide (Dahl *et al.*, 1982b). Hydrogen atoms are not drawn. Carbon and other atoms, the latter of which are marked by their chemical symbol, are represented by ellipsoids. The size and orientation of the ellipsoids indicate the magnitude and directions of the thermal vibration of the atoms in the molecule.

**Table 1.** Biological activity of chlorpromazine and some of its metabolites (+, active; -, inactive). Relative potencies of metabolites, compared to chlorpromazine, are given in parentheses

| Test system | CPZ | 7-OH CPZ | *N*-DCPZ | *N*-DDCPZ | CPZNO | CPZSO | Reference |
|---|---|---|---|---|---|---|---|
| EEG, isolated perfused rat brain | + | + (0.50) | | | | - | Krieglstein *et al.* (1980) |
| Inhibition of MAO activity, human brain | + | + (0.95) | + (1.06) | + (1.01) | | - (0.03) | Roth *et al.* (1979) |
| Inotropic effect, isolated rat or guinea-pig atrium | + (negative inotropic) | + (positive inotropic) | | | | | Dahl and Refsum (1976), Temma *et al.* (1977) |
| Plasma prolactin, male rats | + | + (0.85) | | | | - | Meltzer *et al.* (1977) |
| Amphetamine-induced stereotyped behavior, rats | + | + | + | - | + | - | Lal and Sourkes (1972) |
| Various pharmacological tests, mice | + | + | | | | | Manian *et al.* (1965) |

CPZ, chlorpromazine; 7-OH CPZ, 7-hydroxy chlorpromazine; *N*-DCPZ, *N*-monodesmethyl chlorpromazine; *N*-DDCPZ, *N*-didesmethyl chlorpromazine; CPZNO, chlorpromazine N-oxide; CPZSO, chlorpromazine sulfoxide.

solid-state molecular structure of 7-hydroxy chlorpromazine resembles that of chlorpromazine itself, while chlorpromazine sulfoxide has another conformation of the side chain in the solid state. As discussed earlier (Dahl *et al.*, 1982b), it is possible that the different molecular conformations of the two metabolites in the solid state may have biological significance.

The biological activities of 7-hydroxy chlorpromazine and chlorpromazine sulfoxide have been examined in various systems, as summarized in table 1. Figure 3, which is reproduced from one of these studies, represents a typical example of the results. Chlorpromazine caused a significant increase in the percentage of delta waves in isolated perfused rat brain, and the same effect, although of a smaller magnitude, was observed for 7-hydroxy chlorpromazine (figure 3). Chlorpromazine sulfoxide, in the same concentrations, had no significant effect in this preparation.

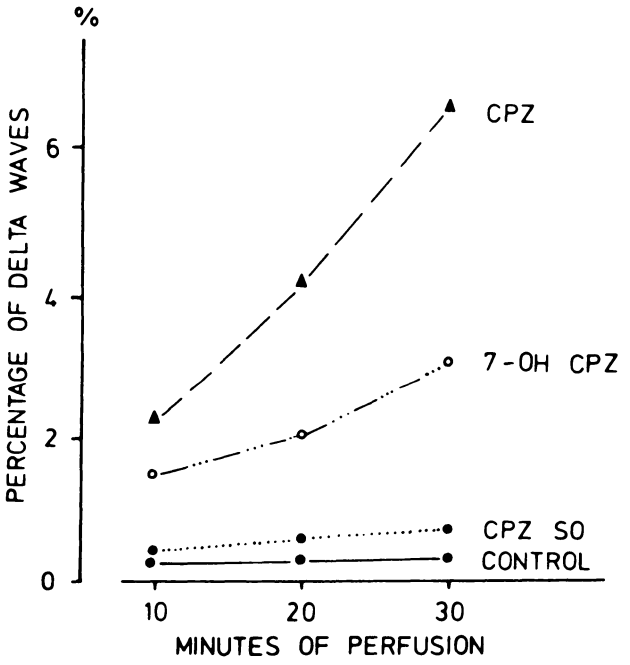


**Figure 3.** EEG effects of chlorpromazine (CPZ), 7-hydroxy chlorpromazine (7-OH CPZ) and chlorpromazine sulfoxide (CPZSO) in isolated perfused rat brain: increase of delta activity. Reproduced from Krieglstein *et al.* (1980), with kind permission from Pergamon Press Ltd.

The positive inotropic effect of 7-hydroxy chlorpromazine in isolated guinea-pig atrium, which is mentioned in table 1, is an example of a pharmacological effect of a drug metabolite which is qualitatively different from that of the parent compound. Chlorpromazine itself is generally 'cardiodepressive', and a negative inotropic effect of chlorpromazine has been demonstrated in isolated rat atria (Dahl and Refsum, 1976).

## BINDING AFFINITIES OF NEUROLEPTIC DRUG METABOLITES TO DOPAMINERGIC AND ALPHA-ADRENERGIC RECEPTORS IN THE BRAIN

The binding affinities to three different receptor types in rat brain have been examined for the major metabolites of chlorpromazine and levomepromazine in man (P.A. Hals and S.G. Dahl, unpublished results). Preliminary data from this study are given in table 2, together with the results of a previous study by Dahl and Hall (1981).

Reference samples of 3-hydroxy levomepromazine, 7-hydroxy levomepromazine and *O*-desmethyl levomepromazine were synthesized from levomepromazine in our laboratories. Reference samples of the other levomepromazine metabolites and of the chlorpromazine metabolites used in the receptor binding assays were generously donated by Rhône-Poulenc Industries, France.

*N*-Monodesmethyl levomepromazine was the most potent levomepromazine derivative both in dopaminergic ($DA_2$) and in $alpha_1$-adrenergic receptor binding (table 2). 3-Hydroxy levomepromazine was relatively active, compared to levomepromazine itself, in dopaminergic ($DA_2$) receptor binding but not in $alpha_1$-adrenergic binding. As may be noted from table 2, the 3-hydroxy derivative of both drugs was 3-4 times more active than the corresponding 7-hydroxy derivative, in dopaminergic ($DA_2$) receptor binding.

It appears from table 2 that *N*-monodesmethyl chlorpromazine is nearly twice as active as chlorpromazine in $alpha_2$-adrenergic binding. It should be emphasized, however, that the results from the $alpha_2$-adrenergic binding experiments which are given in table 2 are preliminary, and have to be confirmed by a larger number of experiments.

7-Hydroxy fluphenazine has been identified as a metabolite of fluphenazine in the dog (Dreyfuss and Cohen, 1971), but conclusive data identifying this compound as a metabolite of fluphenazine in man have not yet been published. 7-Hydroxy fluphenazine has relatively low affinity in dopaminergic ($DA_2$) receptor binding, compared to fluphenazine itself, but had 41% of the potency of the parent drug in $alpha_1$-adrenergic binding in rat cortex (table 3).

Table 2. Receptor binding affinities in rat brain: Potencies* of major metabolites† of chlorpromazine and levomepromazine, relative to that of the parent drug

| Compound | Relative potencies (parent drug=1.00) Receptor type, Brain region, Ligand | | |
|---|---|---|---|
| | Dopaminergic($DA_2$) Striatum $^3$H-Spiroperidol | $\alpha_1$-Adrenergic Cortex $^3$H-WB4101 | $\alpha_2$-Adrenergic Cortex $^3$H-Para-amino-clonidine |
| Levomepromazine (LM) | 1.00 | 1.00 | 1.00 |
| 7-Hydroxy LM | 0.17 | 0.08 | 0.22 |
| 3-Hydroxy LM | 0.51 | 0.04 | 0.22 |
| *O*-Desmethyl LM | 0.04 | 0.03 | 0.05 |
| *N*-Monodesmethyl LM | 0.71 | 0.62 | 0.24 |
| LM sulfoxide | 0.05 | 0.19 | 0.03 |
| Chlorpromazine (CPZ) | 1.00 | 1.00 | 1.00 |
| 7-Hydroxy CPZ | 0.53 | 0.31 | 0.19 |
| 3-Hydroxy CPZ | 2.04 | 0.34 | 0.63 |
| *N*-Monodesmethyl CPZ | 0.37 | 0.52 | 1.92 |
| CPZ sulfoxide | 0.02 | 0.06 | 0.05 |

*The potency ratios were obtained by comparing the $IC_{50}$'s of the metabolites to that of the parent compound (P.A. Hals and S.G. Dahl, unpublished results; Dahl and Hall, 1981).
†Although the 3-hydroxy derivative has been identified as a major metabolite in man for levomepromazine but not for chlorpromazine, 3-hydroxy chlorpromazine was included in this study for comparison.

Table 3. Potencies* of 7-hydroxy fluphenazine relative to fluphenazine in receptor binding

| Compound | Relative potency | | |
|---|---|---|---|
| | Dopaminergic ($DA_2$) $^3$H-Spiroperidol Sheep caudate | Dopaminergic ($DA_2$) $^3$H-Spiroperidol Calf caudate | $\alpha_1$-Adrenergic $^3$H-WB4101 Rat cortex |
| Fluphenazine | 1.00 | 1.00 | 1.00 |
| 7-Hydroxy fluphenazine | 0.014† | 0.15§ | 0.41† |

*Relative potencies were obtained by the comparison of the $IC_{50}$ of 7-hydroxy fluphenazine to that of fluphenazine.
†Data from Bylund (1981).
§Data from Wiles (1981).

Loxapine is a relatively new neuroleptic of the same chemical group (dibenzoxazepine) as clozapine. Loxapine has two major metabolites in man, both of which attain higher steady-state plasma levels than the parent drug after oral doses of loxapine: 8-hydroxy loxapine and 8-hydroxy-*N*-desmethyl loxapine (Cooper and Kelly, 1979). These metabolites have 13% and 6%, respectively, of the potencies of loxapine in $DA_2$ receptor binding in rat striatum (Coupet and Rauh, 1979).

## *IN VIVO* TEST OF NEUROLEPTIC POTENCY OF PHENOTHIAZINE DRUG METABOLITES

A substantial body of evidence has suggested that neuroleptic drugs exert their clinical effects mainly through antagonism of $DA_2$ receptors in the brain (Snyder, 1981). *In vitro* assessment of $DA_2$ receptor binding affinity is generally not sufficient to predict the clinical potency of a potential neuroleptic, since this also depends on its distribution in the brain and other pharmacokinetic variables. Inhibition of apomorphine-induced climbing in mice is a behavioral test, based on blockade of a DA receptor mediated event, which may be used as an *in vivo* test of neuroleptic potency (Worms *et al.*, 1982).

The potencies of the compounds which are included in tables 2 and 3 have been examined in this test (E. Morel, S.G.Dahl and K.G. Lloyd, unpublished results), using male albino mice weighing 23-25 g. Reference samples of fluphenazine and 7-hydroxy fluphenazine were generously donated by E.R. Squibb and Sons, Inc., New Jersey, USA. The results are summarized in table 4, in terms of relative potencies of the metabolites compared to the parent compound.

As may be noted from table 4, the data from the climbing test are generally in agreement with the results of the receptor binding studies (tables 2 and 3). 3-Hydroxy levomepromazine was slightly more potent than the parent compound, and levomepromazine sulfoxide was virtually inactive, in the climbing test. As discussed by Pinder (1983), the $alpha_1$-adrenergic binding affinities of antidepressant drugs are correlated with their sedative effects. Levomepromazine is known as a neuroleptic with very pronounced sedative effects, and as mentioned by Bergman (1983), in a recent Swedish survey 46% of the levomepromazine prescriptions were for sleep disturbances. It is interesting to note, therefore, that *N*-monodesmethyl levomepromazine had both relatively high potency in $alpha_1$-adrenergic receptor binding (table 2) and a strong muscle relaxant or sedative effect in mice, as judged by the grip test which was performed immediately after the climbing test.

It may also be noted that the relative potencies of 7-hydroxy chlorpromazine, 3-hydroxy chlorpromazine and 7-hydroxy fluphena-

Table 4. Inhibition of apomorphine-induced climbing in mice: potencies* of metabolites relative to the parent drug

| Compound | Relative potency |
|---|---|
| Levomepromazine (LM) | 1.0 |
| 7-Hydroxy LM | <0.5 |
| 3-Hydroxy LM | 1.4 |
| *O*-Desmethyl LM | <0.5 |
| *N*-Monodesmethyl LM | - † |
| LM sulfoxide | <0.05 |
| Chlorpromazine (CPZ) | 1.0 |
| 7-Hydroxy CPZ | 0.7 |
| 3-Hydroxy CPZ | 3.1 |
| *N*-Monodesmethyl CPZ | 0.6 |
| CPZ sulfoxide | <0.05 |
| Fluphenazine (FPZ) | 1.0 |
| 7-Hydroxy FPZ | 0.12 |

*Relative potencies were obtained by the comparison of the $ED_{50}$'s of the metabolites to that of the parent drug (E. Morel, S.G. Dahl and K.G. Lloyd, unpublished results).
†Pronounced muscle relaxant activity.

zine were of similar orders of magnitude in $DA_2$ receptor binding in rat brain (tables 2 and 3) and in inhibition of apomorphine-induced climbing in mice (table 4).

Together with the available relevant pharmacokinetic data, the results of these studies could indicate that 3-hydroxy levomepromazine may contribute to the neuroleptic effects of levomepromazine in man while *N*-monodesmethyl levomepromazine may contribute significantly to the sedative effects of this drug. Further, they could indicate that both 7-hydroxy chlorpromazine and *N*-monodesmethyl chlorpromazine may possibly contribute to the neuroleptic effects of chlorpromazine. Although 3-OH CPZ is relatively more potent in both binding and behavioral tests, it is not found in measurable quantities in man (see below). It is still an open question whether significant amounts of 7-hydroxy fluphenazine are formed in man during fluphenazine treatment. This metabolite appears to have low pharmacological activity, and probably does not contribute to the effects of fluphenazine in man.

## COMMENTS

Compared to the substantial amount of information concerning its pharmacology and toxicology that is required before a new drug is

introduced on the market, the available information about the possible effects of identified drug metabolites is, in most cases, minimal.

As mentioned in a recent review (Dahl, 1982), a relationship between plasma drug levels and clinical effects and certain side-effects have now been demonstrated, more or less convincingly, for six different neuroleptic drugs. The presence of relatively high plasma levels of some neuroleptic drug metabolites in some patients raises the following questions which should be solved for each drug:

(1) Which compounds (administered drug, metabolites) may contribute to the therapeutic effects?
(2) Which compounds may contribute to the known side-effects?
(3) Which compounds should be assayed in plasma by therapeutic monitoring and in studies of plasma level-effect relationships?

Problems (1) and (2) can only be resolved by studying the effects of each known metabolite separately. Once this is known, the answer to question (3) is, of course, also given.

Although metabolic hydroxylation of other psychotropic drugs often yields active metabolites (see, for example, Bertilsson *et al.*, 1983), one cannot generally assume that this is the case, as demonstrated by the results given in the present article. Also within a related chemical class of compounds such as the phenothiazines, hydroxylation in the corresponding position on the molecule may lead to more or less potent metabolites, as shown in tables 2, 3 and 4 for the 7-hydroxy derivatives of chlorpromazine, levomepromazine and fluphenazine.

The pattern of enzymatic hydroxylation in man may also be different for compounds belonging to the same chemical class. The appearance of relatively high concentrations of 7-hydroxy chlorpromazine in plasma after chlorpromazine treatment does not necessarily imply that all other phenothiazine drugs are converted to significant amounts of 7-hydroxy metabolites. The formation of comparatively large amounts of 3-hydroxy levomepromazine, but not of 3-hydroxy chlorpromazine, after treatment with these drugs, is another example of the same principle.

It should be underlined that inference of the possible impact of a derivative of a drug on the effects of the drug in man cannot be made only from information about the biological activity of the compound. It must also have been demonstrated that the compound in question really is formed in man in more than trace amounts, after therapeutic doses of the drug. The literature contains several examples of authors suggesting that 3-hydroxy chlorpromazine and 7,8-dihydroxy chlorpromazine, which are both pharmacologically active, may contribute to the effects and toxicity of chlorpromazine. There is, however, no evidence that significant amounts of these compounds are formed in man.

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# Radioreceptor assay for measurement of anticholinergic drugs in serum

Joseph T. Coyle[1] and Larry E. Tune[1]

## INTRODUCTION

Extrapyramidal side-effects (EPS) including Parkinsonian symptoms, dystonic reactions and akathisia frequently complicate use of neuroleptic drugs (Ayd, 1961; Donlon and Stenson, 1976). These iatrogenic symptoms, which are both stigmatizing as well as uncomfortable, deter many patients from compliance with neuroleptic treatment (Van Putten, 1974). Thus, the management of EPS must be considered an essential part in the effective treatment of psychotic disorders with neuroleptics. Reduction in neuroleptic dosage is usually not the primary strategy in controlling these side-effects because EPS correlate poorly with neuroleptic dose or therapeutic response (Alpert *et al.*, 1978). Rather, co-treatment with the classical anti-Parkinsonian drugs, all of which block muscarinic receptors with the exception of amantadine, is the accepted approach for reducing EPS. Nevertheless, many patients persist in suffering from EPS in spite of treatment with recommended doses of anticholinergics (DiMascio, 1971). Clinicians generally avoid high doses of anticholinergic drugs to treat refractory EPS because of concerns about the confusional states and impaired peripheral parasympathetic function including dry mouth, blurred vision, constipation and urinary retention that can occur with toxic doses of anticholinergic drugs. In light of the marked variation in the disposition of psychotropic medications, we have undertaken studies to clarify the relationship between serum levels of anticholinergics measured by a radioreceptor assay technique and the therapeutic as well as noxious effects of these drugs.

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## SITE OF ACTION OF ANTICHOLINERGIC DRUGS

In recent years, the mechanisms whereby anticholinergic drugs reduce the acute EPS caused by neuroleptics has been reasonably well characterized by neuroanatomic and neurophysiologic studies (Calne *et al.*, 1975). Current evidence suggests that the therapeutic effects of neuroleptics derive from their blockade of dopamine receptors, particularly the D-2 subtype (Creese *et al.*, 1976; Kebabian and Calne, 1979) within the striatum; these receptors mediate the inhibitory effects of dopamine on cholinergic interneurons (figure 1). Blockade of the striatal D-2 receptors results in disinhibition of the cholinergic neurons; and the increased turnover and release of acetylcholine causes an excessive stimulation of their postsynaptic striatal muscarinic cholinergic receptors (Racagni *et al.*, 1976). Fortunately, the dopaminergic-cholinergic synaptic sequence appears to be unique to the extrapyramidal system and therefore pharmacologic restitution of cholinergic tone by co-administration of muscarinic receptor antagonists does not disturb the therapeutic action of neuroleptics that presumably occurs at dopamine synapses in the cortico-limbic projections. While this description of striatal cholinergic-dopaminergic neuronal synaptic relationships appears operationally valid, it does not adequately reflect the emerging evidence of the topographic organization of the striatal connections or the second-order interactions between striatal neurons and dopaminergic afferents.

Recent studies have uncovered major cholinergic projections from the magnocellular nuclei in basal forebrain including the nucleus basalis of Meynert, diagonal band of Broca and medial septal nucleus that innervate the cerebral cortex and hippocampal formation (Johnston *et al.*, 1981). Evidence is emerging that this cholinergic system appears to play a critical role in higher cognitive functions. Destruction of the hippocampal cholinergic pathway in experimental animals profoundly impairs recent memory (Olton and Feustle, 1981). And the basal forebrain cholinergic pathways selectively degenerate in Alzheimer's dementia, a disorder characterized by a profound deterioration of higher cognitive functions in man (Whitehouse *et al.*, 1982). Since anticholinergic drugs impair recent memory (Drachman, 1971) and, at high doses, cause more global disruptions of cognitive functions (Longo, 1966), interference with neurotransmission at the cortico-hippocampal cholinergic synapses is the likely site of this effect of anticholinergics.

The muscarinic receptors, the predominant population of receptors mediating the central effects of acetylcholine, have been well characterized by means of ligand-binding techniques (Birdsall and Hulme, 1976). These receptors exhibit relatively uniform characteristics with regard to antagonist interactions throughout major brain regions (Birdsall *et al.*, 1980). Displace-

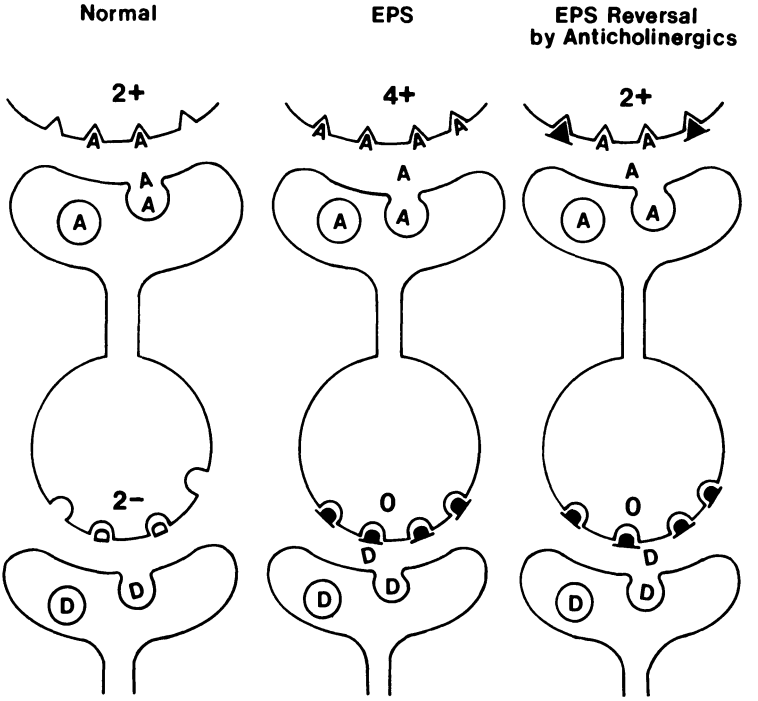


**Figure 1.** Schematic representation of the interaction between neuroleptics and anticholinergics at striatal dopaminergic synapse. Dopaminergic terminals in the striatum release dopamine (D) that inhibits the activity of cholinergic interneurons. Neuroleptics (●), by blocking the D-2 receptor, disinhibit the cholinergic neurons, resulting in increased stimulation of muscarinic receptors by acetylcholine (A). Muscarinic antagonists (▲) can normalize muscarinic receptor activation and thereby reduce EPS.

ment studies with agonists reveal a subpopulation of sites uniformly labeled by antagonists that can be resolved into low, high and 'super' high affinity compounds (Birdsall *et al.*, 1978). The most commonly used antagonist ligand to label the muscarinic receptors is [$^3$H]-quinuclidinyl benzilate (QNB); this ligand exhibits a subnanomolar affinity for the receptors with an extremely slow rate of dissociation that results in a very favorable ratio of specific to non-specific binding. The compelling correlation between the affinity of muscarinic antagonists for the [$^3$H]-QNB labeled sites and their ability to antagonize the physiologic effects of cholinergic agonists has established that this

ligand is an excellent probe for the antagonist conformation of central muscarinic receptors (Yamamura and Snyder, 1974). Furthermore, cross-species studies indicate a high degree of conservancy of muscarinic receptor characteristics as revealed by the specific binding of [$^3$H]-QNB.

## CHARACTERIZATION OF THE RADIORECEPTOR ASSAY FOR ANTICHOLINERGICS

Since several radioreceptor assays for psychotropic medications have been developed to detect these drugs on the basis of their interactions with specific receptor sites (Ferkany and Enna, 1982), we felt that the muscarinic receptor labeled with [$^3$H]-QNB might serve as an effective probe for detecting drugs on the basis of their anticholinergic properties. Preliminary experiments indicated that the addition of 200 µl of drug-free human serum to the 2 ml buffer mix used for measuring the specific binding of [$^3$H]-QNB to suspensions of membranes prepared from the rat forebrain caused a 29 ± 2% inhibition of total binding and a 30 ± 5% inhibition of non-specific binding of [$^3$H]-QNB (0.3 nM) to the receptor sites (Tune and Coyle, 1980). Thus, the ratio of total to non-specific binding remained constant at 8.5:1. Displacement curves generated by the addition of increasing concentrations of drugs known to have varying potency as anticholinergics revealed comparable $K_I$'s in the absence or in the presence of 200 µl of drug-free human serum. Notably, the assay detected the classical muscarinic antagonists such as benztropine as well as the anticholinergic properties of drugs with other primary effects such as diphenhydramine, amitriptyline and thioridazine. In order to have a norm against which individual displacement values for serum could be compared, inhibition curves with atropine in the presence of drug-free human serum were run for each assay. Thus, values for serum samples could be expressed in terms of the equivalent amount of atropine that produced comparable inhibition of the specific binding of [$^3$H]-QNB.

To validate the assay, serum levels of nortriptyline in patients receiving this drug as an antidepressant were measured both by the muscarinic receptor assay and by gas-liquid chromatography (Tune and Coyle, 1981). Since the gas chromatographic method measures total serum levels of nortriptyline, the nortripytline was extracted from serum in heptane-isoamyl alcohol (99:1) and then back-extracted in 0.1 N HCl for its detection on the basis of its muscarinic blocking properties (figure 2). Although the serum levels of nortriptyline ranged over 10-fold in seven patients (25 - 305 µg $l^{-1}$), there was an excellent correlation between the values obtained with the radioreceptor assay and those determined by gas-liquid chromatography ($r$ = 0.99; $p < 0.001$).

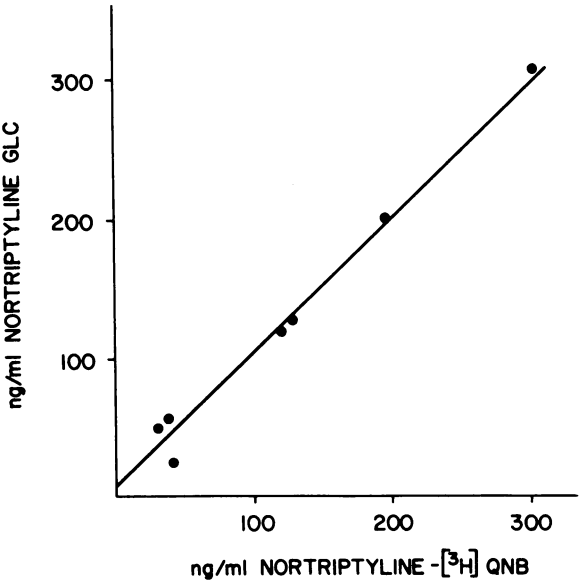


**Figure 2.** Correlation between serum nortriptyline levels measured by gas chromatography (GLC) and by the radioreceptor assay. Nortriptyline was extracted from serum obtained from seven patients being treated for unipolar depression ($r = 0.99$; $N = 7$; $p < 0.001$).

Since whole serum has been used in the assay, only anticholinergic drugs dissociated from serum proteins are free to compete with [$^3$H]-QNB at the muscarinic receptors. Because the serum is diluted 10-fold with the assay buffer, the equilibrium between free and protein-bound drug is shifted, resulting in a constant increase in the unbound drug. To assess the degree of protein binding of benztropine, we examined the relationship between the values measured with whole serum added and the levels obtained in split samples, in which the serum proteins were first precipitated by treatment with perchloric acid. The values for whole serum, as a percent of the total amount of anticholinergic in the perchloric extract of the serum, varied from 1.4 to 10% in samples from 34 patients receiving benztropine. Nevertheless, the two values correlated significantly ($p < 0.05$) and an average of 95% of the benztropine was bound to serum proteins under the conditions of the assay. Since free drug represents that available to interact with receptors *in vivo*, we felt that the measurements of whole serum gave a better insight to the physiologically active component of the anticholinergic drugs.

## ACUTE EXTRAPYRAMIDAL SYMPTOMS AND SERUM NEUROLEPTIC AND ANTICHOLINERGIC LEVELS

In a cross-sectional study, the relationship between serum levels of anticholinergics and the total daily dose of benztropine was examined in 49 patients who were receiving this drug in addition to neuroleptics. No correlation was observed between the serum levels of the anticholinergics and total daily doses of benz-

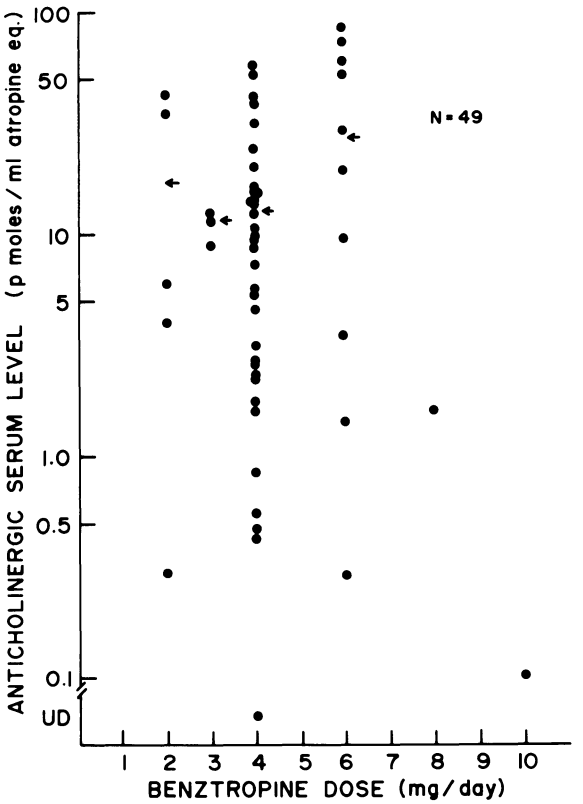


**Figure 3.** Relationship between total daily dose of benztropine and serum levels of free anticholinergics. Serum samples were obtained from 49 inpatients who were receiving a fixed dose of benztropine for at least 4 days prior to phlebotomy. Results for the radioreceptor assay are expressed in terms of the amount of atropine (pmol) that produced the same amount of inhibition. Arrows indicate the mean level for each daily dose.

tropine. To the contrary, serum anticholinergic activity varied on the average of 10-fold from the lowest to the highest level in patients receiving 2, 4 and 6 mg per day of benztropine (figure 3). In six patients, who were followed serially with increasing oral doses of benztropine, a markedly non-linear relationship between daily dose and serum anticholinergic levels was observed. In most cases, 2 mg increments in oral dose were associated with several-fold increases in the serum level of anticholinergic activity.

A cohort of 109 patients, who were receiving at least 400 mg per day of chlorpromazine equivalents (Davis, 1976) were evaluated for the presence and severity of EPS with the DiMascio scale; and serum levels of neuroleptics were determined by the radioreceptor assay of Creese and Snyder (1977). In this cross-sectional study, the patients' drug doses were determined by their treating physician; and the majority of these patients were receiving haloperidol as the neuroleptic. Notably, a poor and non-significant correlation between serum levels of neuroleptics and the severity of EPS was observed ($r$ = 0.029; $N$ = 109; $p > 0.1$). This finding is consistent with clinical observations that the development of EPS is a poor predictor of therapeutic doses of neuroleptics (Alpert *et al.*, 1978) and that individuals vary in vulnerability to the EPS-inducing effects of neuroleptics (Chase *et al.*, 1970).

For 76 of these patients, whose serum levels of anticholinergics had been measured in the same samples, there was a highly significant inverse relationship between the severity of EPS and the serum levels of anticholinergics (figure 4) detected by the radioreceptor assay ($r$ = 0.441; $p < 0.005$). Of patients with serum levels less than 10 pmol $ml^{-1}$ of atropine equivalents, 44 experienced EPS with a DiMascio score greater than or equal to 2 while only 9% of patients with serum levels in excess of 10 nmol $l^{-1}$ of atropine equivalents experienced significant EPS ($\chi^2$ = 8.9; $N$ = 76; $p < 0.005$). The value, 10 nmol $l^{-1}$ atropine equivalents, is approximately five times the $K_I$ of atropine for the muscarinic receptor in the assay and thus reflects a serum level associated with substantial blockade of muscarinic receptors. To rule out an artifactual skew in the serum neuroleptic levels that might account for the low incidence of EPS in patients with higher serum anticholinergic levels, the relationship between serum neuroleptic and serum anticholinergic levels was plotted. However, no significant correlation was observed in the whole population ($r$ = 0.16) or in those patients experiencing EPS ($r$ = - 0.21) or free of EPS ($r$ = 0.25).

Since the mechanism of action of anticholinergics in reversing EPS involves correcting the neuroleptic-induced cholinergic dysfunction in the striatum (Calne *et al.*, 1975), we were curious whether the ratio of anticholinergic activity to dopamine receptor

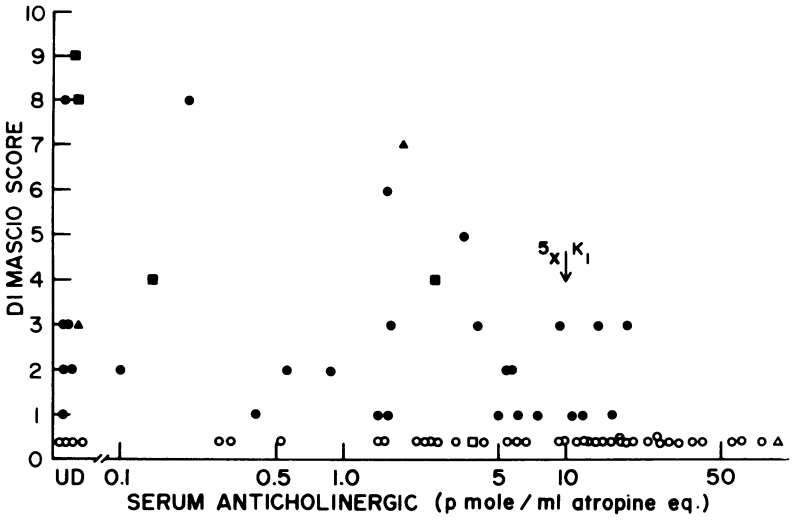


**Figure 4.** Correlation between acute extrapyramidal side-effects and serum anticholinergic levels. Seventy-six patients, who were receiving both neuroleptics (at least 400 mg per day of chlorpromazine or its equivalent) and anticholinergic medication for at least 4 days of constant dosage, were rated for EPS with the DiMascio scale and a blood sample was obtained. Results for the radioreceptor assay are presented in terms of the amounts of atropine (pmol) that produce the same degree of inhibition of specific binding of [$^3$H]-QNB. Open symbols are EPS-negative and closed symbols are EPS-positive patients. UD, undetectable serum levels. Circles, benztropine; squares, trihexyphenidyl; triangles, diphrenhydramine. 5 x $K_I$ indicates the concentration of atropine equivalent to five times the $K_I$ for atropine ($r$ = 0.441; $N$ = 76; $p < 0.005$).

blocking activity might play a critical role in control of EPS. In other words, we wondered whether EPS resulting from low serum neuroleptic levels might be corrected by low anticholinergic levels whereas high serum neuroleptic levels would require high anticholinergic levels (figure 5). However, plotting the relationship between EPS and the ratio of serum anticholinergics to serum neuroleptics, both measured by the radioreceptor assay techniques, did not reveal a significant inverse correlation ($r$ = 0.257; $0.1 > p > 0.05$; $N$ = 76). Nevertheless, patients with serum anticholinergic/neuroleptic ratios of 0.5 or greater had a significantly

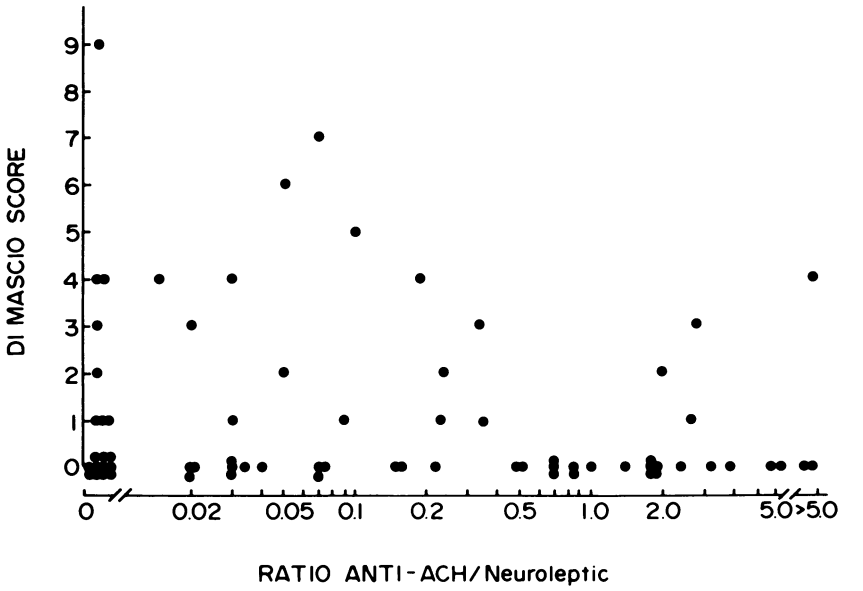


**Figure 5.** Correlation between acute extrapyramidal side-effects and the ratio of anticholinergic to neuroleptic drug levels. The results from 76 patients were used to examine the relationship between EPS and the ratio of serum anticholinergic to neuroleptic levels. Anticholinergic and neuroleptic levels were converted to similar units ($\mu g\ l^{-1}$ atropine equivalents and $\mu g\ l^{-1}$ chlorpromazine equivalents) to calculate the ratio ($r$ = 0.257; 0.2> $p$ > 0.05; $N$ = 76).

lower incidence of EPS as compared to those with a lower ratio ($\chi^2$ = 7.5; $p < 0.02$). Thus, an 'ideal ratio' between anticholinergics and neuroleptics does not appear to be involved in EPS control although an excess of anticholinergic is associated with a more favorable response.

## COGNITIVE EFFECTS OF ANTICHOLINERGIC DRUGS

As discussed above, the anticholinergic drugs not only interfere with cholinergic neurotransmission in the striatum but also with the function of the cholinergic projections to the cerebral cortex and hippocampal formation, which are thought to play an important role in cognitive functions. Accordingly, we wondered whether the serum levels of anticholinergics associated with reduction in EPS

Table 1. Effects of anticholinergics on recent memory function in schizophrenic patients

| Parameter | Mean ± S.E.M. | Correlation coefficients | | |
|---|---|---|---|---|
| | | Atropine equivalents | Chlorpromazine equivalents | Recent memory |
| Mini PSE | 4.7 ± 0.8 | 0.05 | 0.07 | - 0.29 |
| Atropine equivalents (nmol $l^{-1}$) | 12.0 ± 2.5 | - | 0.12 | - 0.51* |
| Chlor-promazine equivalents (μg $l^{-1}$) | 32.0 ± 10 | - | - | 0.20 |
| Recent memory | 7.2 ± 0.2 | - | - | - |

The correlation coefficients for the various parameters measured in 24 patients are presented. Recent memory refers to the average number of correct responses for 15 trials (Tune *et al.*, 1982).
* $p < 0.01$.

might also result in impairments in recent memory. A cohort of 24 patients, all of whom were receiving neuroleptics and satisfied Research Diagnostic Criteria (Spitzer *et al.*, 1975) for schizophrenia, were examined with regard to the severity of schizophrenic symptoms as measured by the Mini-Present State Exam of Wing (1974), verbal intelligence quotient (verbal IQ) as measured by the Wexler Adult Inventory Scale (WAIS) and performance on a recent memory task (Tune *et al.*, 1982). Serum levels of neuroleptics and anticholinergics were measured by the radioreceptor assay techniques. All of the patients were in remission; and none of the patients were disoriented to person, place or time. No significant correlation was observed between the serum levels of anticholinergics or neuroleptics with regard to the severity of schizophrenic symptoms (table 1). However, a significant inverse correlation between serum levels of anticholinergics and performance on the recent memory test was demonstrated ($r = 0.51$; $p < 0.01$; $N = 24$). Notably, the serum levels of anticholinergics measured in these patients were within the range associated with control of EPS; thus, it appears that serum levels of anticholinergics effective in reducing EPS may also cause subtle but significant impairments in higher cognitive functions in the absence of frank delirium.

These findings have received additional support from a prospective study of post-operative delirium in patients undergoing cardiac surgery (Tune *et al.*, 1981). In this study, patients were examined for cognitive function by the Mini-Mental Status Exam (Folstein *et al.*, 1975) and were rated for delirium by clinical assessment. After surgery, blood was obtained at the time of clinical evaluation for measurement of serum anticholinergic levels. When all the samples taken throughout the course of the study were analyzed, 14 out of 16 samples obtained from patients who were rated as clinically delirious had serum anticholinergic levels greater than 7.5 nmol $l^{-1}$ of atropine equivalents while only five of 33 samples obtained from cognitively intact patients had serum levels greater than this ($\chi^2 = 23.8$; $p < 0.001$). Comparison of the change in Mini-Mental State Examination from before surgery with serum anticholinergic levels revealed a highly significant inverse correlation ($r = 0.83$; $N = 24$; $p < 0.01$). Thus, as serum levels of anticholinergic drugs increased, there was a progressive impairment in higher cognitive functions as assessed by the difference in scores before and after surgery. While multiple factors contribute to post-operative delirium in patients undergoing cardiac surgery, these results suggest that drugs with anticholinergic effects may play a significant contributory role.

## CONCLUSION

Radioreceptor assays have attracted increasing interest because of their purported ability to detect drugs on the basis of their receptor site specific mechanisms of action (Ferkany and Enna, 1982). In addition, these assays are relatively inexpensive and technically easy to perform. The radioreceptor assay based upon muscarinic antagonist inhibition of the specific binding of [$^3$H]-QNB clearly has important advantages. Extensive studies carried out to characterize the binding site for [$^3$H]-QNB have demonstrated that this ligand labels a homogeneous population of sites, with regard to antagonist interactions, that correlates well with regard to physiologic affinity of antagonist to block muscarinic receptor activation (Birdsall and Hulme, 1976; Birdsall *et al.*, 1978, 1980). And the muscarinic antagonist activities of a host of drugs including neuroleptics, antihistamines and antidepressants, whose primary mechanism of action likely does not involve the cholinergic system, have been well described (Richelson and Divinitz-Romero, 1977). Thus, a serum assay utilizing the [$^3$H]-QNB labeled muscarinic receptor will detect drugs regardless of chemical structure on the basis of their ability to block muscarinic receptors. Finally, the high affinity of [$^3$H]-QNB for the muscarinic receptor and the favorable ratio of specific to non-specific binding ensures that the assay is not only specific but also extremely sensitive.

Results obtained thus far with the muscarinic radioreceptor assay for anticholinergic drugs in serum indicate a marked variation among patients in their disposition of anticholinergic drugs used to treat neuroleptic-induced acute EPS; thus, serum levels vary over a range of 10-fold or more in patients given the same dose of benztropine, a finding consistent with other psychotropic drugs including anticonvulsants and antidepressants (Eadie and Tyrer, 1974; Rish *et al.*, 1979). The variation in serum levels attained with standard doses of anticholinergics may explain, in part, the variability in clinical response of EPS to anticholinergic treatment. Furthermore, dose-response studies in individual patients indicate a non-linear relationship between oral dose and serum levels of anticholinergics with small increments in dose often associated with large increases in serum levels. These dose-level curves are reminiscent of those reported for phenytoin (Eadie and Tyrer, 1974).

Clinical experience indicates that individuals vary considerably in their susceptibility to developing EPS when treated with neuroleptics. In our cross-sectional study, serum level of neuroleptic measured by radioreceptor assay correlated poorly with the presence and severity of EPS. It is important to note that fluphenazine decanoate is associated with quite low constant serum levels of neuroleptic but a moderately high level of EPS (Tune *et al.*, 1979). One source of variation in susceptibility to EPS may involve differences among individuals in the ability of their nigro-striatal dopaminergic pathway to increase in activity to compensate for striatal D-2 receptor blockade (Chase *et al.*, 1970). Nevertheless, a highly significant inverse correlation was demonstrated between serum levels of anticholinergics in patients receiving neuroleptics and EPS severity. A serum level of anticholinergic which would be associated with substantial muscarinic receptor blockade (10 nmol $l^{-1}$ of atropine equivalents) was associated with a low incidence of EPS. When these data were expressed in terms of the ratio of muscarinic receptor blocking activity to dopamine receptor blocking activity, it was apparent that a high ratio favored EPS control. The results of these studies suggest that for those patients who develop EPS, oral doses of anticholinergics that result in serum levels approaching 10 nmol $l^{-1}$ of atropine equivalents should eliminate or markedly attenuate EPS.

Current evidence suggests that the muscarinic receptors in the striatum are identical to those in the cortex with regard to antagonist characteristics. Thus, it would be expected that muscarinic receptor blockade, which may be optimal for attenuating EPS, may nevertheless significantly impair cortico-hippocampal cholinergic neurotransmission. The results of a cross-sectional study support this concern since serum anticholinergic levels correlated significantly with impairments in performance on a recent memory test but were unrelated to psychiatric symptom

severity in schizophrenic outpatients (Tune *et al.*, 1982). Impairments in cognitive functions were also observed in surgical patients in serum ranges of anticholinergics that were near those associated with reduction in EPS (Tune *et al.*, 1981). While the results of our studies have pointed to a critical level of serum anticholinergics which is effective in eliminating or attenuating EPS, this level encroaches upon that associated with impairments in higher cognitive functions. Thus, one must consider both the benefits as well as the liabilities associated with anticholinergic drug use to control neuroleptic-induced EPS.

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# Clinical effects related to the serum concentrations of thioridazine and its metabolites

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## INTRODUCTION

Neuroleptic drugs have vastly improved the treatment of psychotic disorders and increased our capacity to ameliorate severe symptoms and to rehabilitate patients. The first substance used, chlorpromazine, has been followed by a variety of others, mostly without major differences in antipsychotic activity. These drugs do, however, differ in respect to type and frequency of induced side-effects. Since some side-effects are concentration-dependent, it is important to keep both acute and maintenance doses minimal without loss of therapeutic effect. With the aid of routine serum determinations, we have therefore sought to optimize the treatment with a commonly used neuroleptic drug, thioridazine. Earlier studies of the metabolism (Mårtensson *et al.*, 1975), protein binding (Nyberg *et al.*, 1978) and metabolite characteristics (Axelsson and Mårtensson, 1977) of this drug have provided the basis for the present study, in which our aim has been to explore any correlations between the serum concentrations of thioridazine and its metabolites on the one hand, and clinical effects, including side-effects, on the other.

## METHODS

### Patients

Patients with acute paranoid psychosis were screened for inclusion in the study on admission to Psychiatric Department III, Lillhagen Hospital. Those with affective disorders or dementia were excluded. Extensive examinations, including laboratory tests and ECG recordings, showed good physical health without abuse of alcohol and narcotics in the 65 patients, 29 men and 36 women, who

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were accepted. With no age restriction used, their ages ranged from 16 to 78 (mean 50) years.

During the observation period of 20-50 days from admission to discharge from hospital, thioridazine was the only drug given. With variable dose schedules determined by the attending psychiatrist, the mean daily dose ranged between 123 and 1007 mg and the maximum between 200 and 1200 mg. The medication was given in equal amounts at 7 a.m., 12 noon, 4 p.m. and 8 p.m. After a week constant medication (steady-state level), blood samples were obtained before the first dose of the day. The sampling procedure was repeated once a week, before any change in dosage and in connection with discharge from hospital. The total serum concentrations of thioridazine and its main metabolites, side-chain sulfoxide, side-chain sulfone and ring sulfoxide, were determined by use of gas chromatography (Mårtensson *et al.*, 1975).

## Rating of Therapeutic Effect

Before the start of treatment and at the end of the observation period, the psychiatric symptoms were rated according to the Comprehensive Psychopathological Rating Scale (CPRS) (Asberg *et al.*, 1978). An independent psychiatrist performed the ratings without knowledge of the doses and serum concentrations involved. Separate registrations were made of the total CPRS score and the score for paranoid symptoms alone. The percentage of the admission score still remaining at the end of the observation period served as a measure of the therapeutic effect.

Comparative analyses were made of patients at or below the age of 40 years ($n$=21) and those above ($n$=44). Patients with a mean total serum concentration of thioridazine between 1 and 2 $\mu$mol $l^{-1}$ were separately studied concerning the influence of dose and concentration of metabolites on the clinical outcome.

## Evaluation of Side-Effects

Side-effects were especially studied in 38 patients, 14 men and 24 women between the ages of 16 and 78 (mean 59) years. Both objective signs and subjective reports of the side-effects most commonly seen during treatment with neuroleptics (table 1) were rated according to frequency and intensity (Axelsson and Mårtensson, 1980). A slight degree of a reported or observed side-effect was given the score 1, a moderate degree the score 2 and a severe degree the score 3. The tremor rating, based on objective findings only, was given the score 1 for finger tremor with extended hands, 2 for finger and hand tremor with extended hands and 3 for continuously observable hand tremor.

Body weight and cardiovascular parameters were registered on admission, during treatment, in connection with blood sampling and

Table 1. Rated side-effects

| | |
|---|---|
| Drowsiness | Sweating |
| Headache | Blurred vision |
| Sensibility disturbance | Palpitation |
| Paresthesia | Vertigo |
| Tremor | Postural hypotension |
| Rigidity | Nausea/vomiting |
| Hyperkinesia | Constipation |
| Hypokinesia | Diarrhea |
| Dyskinesia | Itching |
| Acute dystonia | Dermatological reactions |
| Akathisia | Menstrual disturbances |
| Inhibition of micturition | Gynecomastia |
| Dry mouth | Galactorrhea |
| Salivation | Altered libido |
| Nasal congestion | Inhibition of ejaculation |

at discharge. Owing to poor cooperation, some patients could not be included on every occasion. Systolic and diastolic blood pressures and heart rates (erect and recumbent) were recorded, and the orthostatic blood pressure change was calculated from these values. ECG recordings (XII leads) were performed at the Medical Centre of Lillhagen Hospital throughout the observation period. An independent physician made the ECG analyses, which included measures of Q-S and P-Q time and heart rate. With the aid of a new classification system (Axelsson and Aspenström, 1982), two types of T-wave changes were distinguished: type I with rounded (grade 1), plateau-shaped (grade 2) or bifid (grade 3) T-waves; and type II with diphasic or inverted T-waves.

Only the most prevalent side-effects, seen in a significant number of patients, are presented in this study.

## Statistics

Non-parametric correlation analyses were performed by use of Pitman's test (Bradley, 1968). The technique suggested by Mantel was used to test the partial correlation between two variables and eliminate the influence of a third (Mantel, 1963). Quadratic regression analysis was applied to the calculations of non-linear relationships. In comparisons between groups of patients, Wilcoxon's test for two samples was used.

The mean dose was defined as accumulated dose divided by number of days of the observation period. The mean serum concentration was calculated from the area under the time-concentration curve.

## RESULTS

### Serum Concentrations

The total serum concentrations of thioridazine, side-chain sulfoxide, side-chain sulfone and ring sulfoxide were in the ranges 0.35-6.44, 0.32-4.53, 0.11-1.63 and 0.81-12.19 μmol $l^{-1}$, respectively.

### Therapeutic Effect

The total CPRS score remaining at the end of the observation period was, in mean, 37% (range 0-121%). In 50 of the 65 patients, there was a reduction of at least 50%.

On average 22% (range 0-100%) of the paranoid score on admission was still present at the time of discharge. Absence of paranoid symptoms was noted in 37 out of 65 patients, and in 57 the paranoid score was reduced by 50% or more.

#### *Correlations between therapeutic effect, dose and age*

Positive correlations were seen in all patients between mean daily dose and therapeutic effect as reflected by both the total CPRS score ($p<0.01$) and the paranoid score ($p<0.05$). A negative correlation was found between age and dose. With age taken into consideration, linear and quadratic regression analyses showed no correlation of significance between dose and therapeutic effect.

#### *Correlations between therapeutic effect and total serum concentration of thioridazine*

Positive correlations were found between therapeutic effect and serum concentration of thioridazine in patients aged 40 or less, but not in those above 40 years (table 2). The correlation between serum concentration and total remaining CPRS score satisfied best a non-linear relation as verified by the difference between the correlation coefficients of the quadratic and linear regression analyses, which were 0.58 ($p<0.02$) and 0.46 ($p<0.05$), respectively. The corresponding figures for the paranoid score were 0.63 ($p<0.01$, quadratic) and 0.45 ($p<0.05$, linear).

Comparisons were made between groups of patients at a given total serum concentration of thioridazine in order to explore the possibility of an optimal serum concentration interval, indicated by the quadratic regression analysis. The patients aged 40 or less were classified according to serum concentration of thioridazine, which was below 1.0 μmol $l^{-1}$ in six patients, between 1.0 and 2.0 μmol $l^{-1}$ in 10 and above 2 μmol $l^{-1}$ in five. The classifi-

Table 2. Therapeutic effect at different serum concentration intervals of total thioridazine in patients aged 40 years or less, and in those above the age of 40

| Age range | Concentration interval (μmol $l^{-1}$) | Remaining total CPRS score (%) | |
|---|---|---|---|
| | | Mean | Range |
| 40 years or less | <1.0 | 45 | 23-84 |
| | 1.0-2.0 | 22 | 4-61 |
| | >2.0 | 14 | 8-20 |
| Above 40 years | <0.7 | 63 | 52-76 |
| | 0.7-2.0 | 37 | 4-104 |
| | >2.0 | 45 | 0-121 |

cation of the patients above 40 years of age gave four patients with serum concentrations of thioridazine below 0.7 μmol $l^{-1}$, 27 in the interval between 0.7 and 2.0 μmol $l^{-1}$ and 13 above 2.0 μmol $l^{-1}$.

Among the patients at or below the age of 40, the therapeutic response was clearly better in those with serum concentrations above 2.0 μmol $l^{-1}$ than in those below 1.0 μmol $l^{-1}$ ($p<0.01$). The patients in the interval between 1.0 and 2.0 μmol $l^{-1}$ tended to respond better to treatment than those below 1.0 μmol $l^{-1}$ ($p<0.10$), but differed little from those above 2.0 μmol $l^{-1}$. In the patients above 40 years, the therapeutic effects were distinctly more pronounced in patients with serum concentrations between 0.7 and 2.0 μmol $l^{-1}$ than in those below 0.7 μmol $l^{-1}$ ($p<0.02$). No other differences in therapeutic response were found between the groups of older patients. Table 2 shows the mean and range of the remaining total CPRS scores at the various concentration intervals of thioridazine.

*Influence of dose and metabolite concentrations on therapeutic effect at a given concentration of total thioridazine*

Since positive correlations were found between the thioridazine concentration and dose, between the thioridazine concentration and therapeutic effect and between dose and therapeutic effect, the serum concentration of thioridazine was used as background variable in the correlation analysis of dose and therapeutic effect. The serum concentrations of thioridazine and metabolites and the therapeutic effect were also intercorrelated. The 31 patients with serum concentrations between 1.0 and 2.0 μmol $l^{-1}$ were therefore especially studied. In this group of patients between the

ages of 16 and 78 (mean 50) years, the serum concentration of thioridazine was, in mean, 1.50 (range 1.02-1.99) μmol $l^{-1}$, equally distributed over the dose intervals. The mean dose ranged between 154 and 1007 (mean 502) mg.

Patients with a dose above 600 mg of thioridazine showed a better therapeutic effect than those with a dose below 400 mg ($p<0.01$). For further exploration of this finding, the serum concentration/dose ratio was calculated in each case. Patients with low concentration/dose ratios, below 3 x $10^{-3}$ μM $mg^{-1}$ ($n$=15), showed better therapeutic effect, reflected by the percent remaining total CPRS score of, in mean, 25 (range 4-61), than patients with concentration/dose ratios above 3 x $10^{-3}$ μM $mg^{-1}$ ($n$=16), where the score was, in mean, 43 (range 5-104) ($p<0.03$).

The ratio between the metabolite/thioridazine concentrations was also calculated in each of the 31 patients involved in this part of the study. The following values were obtained: side-chain sulfoxide, mean 1.04, range 0.37-2.0; side-chain sulfone, mean 0.33, range 0.08-0.62; ring sulfoxide, mean 2.91, range 1.14-7.73. When patients with high and low ratios, determined according to the median values (side-chain sulfoxide 0.95, side-chain sulfone 0.33 and ring sulfoxide 2.70), were compared regarding therapeutic effect, no significant difference was revealed. There was, however, a tendency toward difference in the case of side-chain sulfoxide, reflected by the total remaining CPRS score, which in patients with low side-chain sulfoxide/thioridazine ratios was, in mean, 39 (range 4-104), while it was 29 (range 4-87) in patients with high ratios. Comparison of patients with side-chain sulfoxide/thioridazine ratios above and below 1.0 at serum concentrations of thioridazine between 1.0 and 2.0 μmol $l^{-1}$ showed marked differences. The patients with a ratio above 1.0 had a total remaining CPRS score of, in mean, 20 (range 4-46) ($n$=12); those with a ratio below 1.0 had a corresponding score of 43 ($n$=19) ($p<0.01$).

## Side-Effects

### *Type*

The side-effects found to be most frequent were dry mouth, tremor, drowsiness, nasal congestion, increase in body weight, decrease in blood pressures, increase in diastolic orthostatic blood pressure, decrease in heart rate and ECG T-wave changes of type I. These symptoms were seen in about three-fourths of the patients and were most often of a slight degree (score 1). The increase in body weight during the observation period was, in mean, 3.7 (range 1-8) kg, or 6% (range 1-18%) more than the weight recorded on admission. The systolic and diastolic blood pressures and the heart rate decreased by, in mean, 11% (range 6-14%). All patients but one showed ECG T-wave changes of type I.

*Variation with time*

The side-effects differed regarding variation in intensity during the course of treatment. Dry mouth, drowsiness and nasal congestion reached their maximum intensity after 10-19 days of medication, after which time they decreased. The orthostatic blood pressure change followed a similar pattern with a peak value after about 7 days of treatment and returned to pre-treatment level toward the end of the observation period. The onset of tremor, decrease in blood pressures and ECG T-wave changes of type I did, however, occur immediately after the start of treatment and remained constant throughout the observation period. The changes in body weight and heart rate followed a pattern of successive accentuation during treatment.

*Intercorrelations and correlations to other variables*

Tremor was more often observed in women than in men ($p<0.05$), and the same variation with sex was indicated by the dry mouth score. Women below the age of 50 showed a greater increase in body weight than older women ($p<0.02$), and tended to increase more in weight than men below the age of 50 ($p<0.10$). Significant intercorrelations were found between the mean scores for dry mouth, drowsiness and nasal congestion, none of which were correlated to increase in body weight. The weight increase was, however, correlated to therapeutic effect reflected by the total CPRS score, which more often was reduced by 50% in patients with increase in body weight (18 out of 22) than in those without weight gain (2 out of 9) ($p<0.01$, fourfold table test). The diastolic blood pressure in the erect position was more often decreased in women (18 out of 22) than in men (3 out of 6) ($p<0.04$).

*Concentration dependence*

Inter-individual correlation analysis showed that patients with dry mouth had higher serum concentrations of side-chain sulfoxide ($p<0.02$) and side-chain sulfone ($p<0.01$) than patients without this side-effect. The serum concentration of side-chain sulfone was also higher in patients with tremor than in those without ($p<0.05$).

Positive correlations were found between changes in systolic and diastolic blood pressures (values on admission minus values at the end of the observation period) on the one hand, and the serum concentration of ring sulfoxide on the other ($p<0.05$ and $p<0.003$, respectively).

No significant intercorrelations were found between the other variables investigated, or between patients with and without other types of side-effects.

Table 3. Values of $p$ for the correlation analysis between drug concentrations and side-effects

| Variable | Serum concentrations | | | |
|---|---|---|---|---|
| | Thioridazine | Thioridazine side-chain sulfoxide | Thioridazine side-chain sulfone | Thioridazine ring sulfoxide |
| Dry mouth | n.s. | n.s. | n.s. | n.s. |
| Tremor | n.s. | $p<0.03$ | n.s. | $p<0.01$ |
| Drowsiness | n.s. | n.s. | n.s. | n.s. |
| Nasal congestion | n.s. | n.s. | n.s. | n.s. |
| Body weight | n.s. | n.s. | n.s. | n.s. |
| Heart rates (erect, recumbent) | $p<0.002$ | $p<0.02$ | $p<0.02$ | $p<0.002$ |
| Blood pressures (systolic, diastolic, erect, recumbent) | $p<0.002$ | $p<0.002$ | $p<0.002$ | $p<0.002$ |
| Orthostatic blood pressure (diastolic) | $p<0.01$ | $p<0.002$ | $p<0.006$ | $p<0.005$ |
| ECG T-wave changes of type I | $p<0.001$ | $p<0.01$ | $p<0.001$ | $p<0.0001$ |

n.s. = non-significant

The results of the intra-individual correlation analysis are given in table 3. All cardiovascular parameters showed strong, positive correlations at all drug concentrations. The tremor score was positively correlated to the sulfoxide metabolites. Dry mouth, drowsiness, nasal congestion and body weight showed no significant correlations.

## CONCLUDING REMARKS

The following conclusions summarize our findings of direct interest to the clinician during acute treatment of paranoid psychosis with thioridazine alone.

### Therapeutic Effect

For optimal antipsychotic effect, patients aged 40 years or less should have a serum concentration of total thioridazine above 2.0 $\mu mol\ l^{-1}$. This level is usually reached with a daily dose of 800 mg.

Patients aged 40 years or less may respond favorably within the concentration interval 1.0-2.0 $\mu mol\ l^{-1}$, provided that this level is reached with a high dosage (concentration/dose ratio below 0.3) or with a side-chain sulfoxide/thioridazine ratio exceeding 1.0.

Patients above the age of 40 should have a serum concentration of total thioridazine between 0.7 and 2.0 $\mu mol\ l^{-1}$ for optimal therapeutic effect. At concentrations above or below this level, the response to the drug is generally unsatisfactory.

### Side-Effects

The most pronounced side-effects during acute treatment with thioridazine are dry mouth, tremor, drowsiness, nasal congestion, increase in body weight, decrease in blood pressures, decrease in heart rates and ECG T-wave changes of type I.

Drowsiness, dry mouth, nasal congestion and decrease in diastolic/orthostatic blood pressure are temporary at constant serum concentrations of thioridazine and its metabolites.

Women are more prone to develop side-effects than are men.

Increase in body weight is a favorable prognostic sign.

Tremor is significantly correlated to the concentrations of side-chain sulfoxide, side-chain sulfone and ring sulfoxide, but not to thioridazine.

The cardiovascular side-effects are strongly correlated to the serum concentrations of thioridazine and its main metabolites.

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# Plasma levels of perphenazine related to clinical effect and extrapyramidal side-effects

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## INTRODUCTION

Many investigators have attempted to evaluate a correlation between the therapeutic effect and the plasma concentration of neuroleptic drugs (Buyze *et al.*, 1973; Davis *et al.*, 1974; Mjørndal and Oreland, 1971; Wiles and Franklin, 1978). Unfortunately, simultaneous registration of the degree of side-effects is often lacking. However, clinical experience shows that development of side-effects of neuroleptic drugs, especially extrapyramidal reactions, may have such substantial negative influence on the effort of resocialization that the total situation remains unsatisfactory in spite of amelioration of the psychotic symptoms (Bolvig Hansen *et al.*, 1981). Therefore, it is of the utmost importance to handle neuroleptics in a way which gives maximal therapeutic effect with a minimum of side-effects.

## MATERIAL AND METHODS

In light of the above-mentioned problems, we have done three clinical pharmacological studies involving acute psychotic inpatients, who have not received neuroleptic treatment within the last four months prior to the studies. Patients with signs of affective disorders, disturbed consciousness and alcohol or drug abuse were excluded. Informed consent was obtained from all the patients in accordance with Helsinki Declaration II.

As a first step we investigated 26 patients (16 males and 10 females) during a four-week period of treatment to evaluate the correlation between plasma levels of perphenazine and the degree

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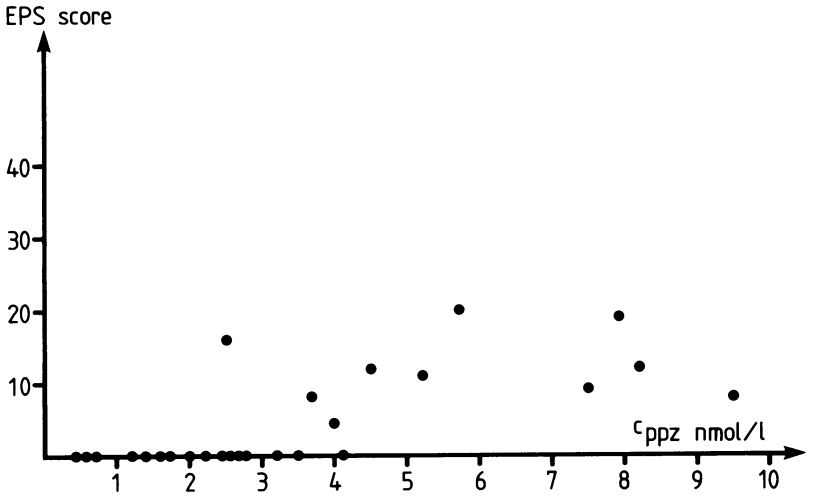


**Figure 1.** A single plasma concentration value of perphenazine from each of 26 patients plotted versus the degree of total extrapyramidal side-effects (EPS), which are expressed as the sum of scale points from Simpson and Angus Rating Scale plus the degree of akathisia and dystonia quantitated by means of a four-point scale. Plasma concentrations eliciting side-effects differ significantly from those not provoking side-effects ($p<0.02$), using the Mann-Whitney test.

of total extrapyramidal side-effects. All patients were given oral perphenazine treatment continuously with the dosage individually determined on the basis of body weight. The dosage was kept at a constant level during the entire investigation period. The medication was given t.i.d. in equal doses with constant intervals (8 h). Blood samples to determine the perphenazine concentration were drawn at the end of a dose interval (minimum values). No other medication was allowed except occasionally for nitrazepam. The psychiatrist and the chemist were kept blind to each other's results.

Secondly, we investigated another 26 patients (17 males and 9 females) during a five-week period with the aim of evaluating the correlation between plasma levels and the degree of antipsychotic effect. All patients were given oral perphenazine treatment continuously and the medication was likewise given in equal doses with constant intervals (8 h), with blood samples drawn at the end of a dose interval. Based on the perphenazine concentration determined after three days of treatment, the doses were adjusted to give concentrations of either below 3 nmol $l^{-1}$ or between 5 and

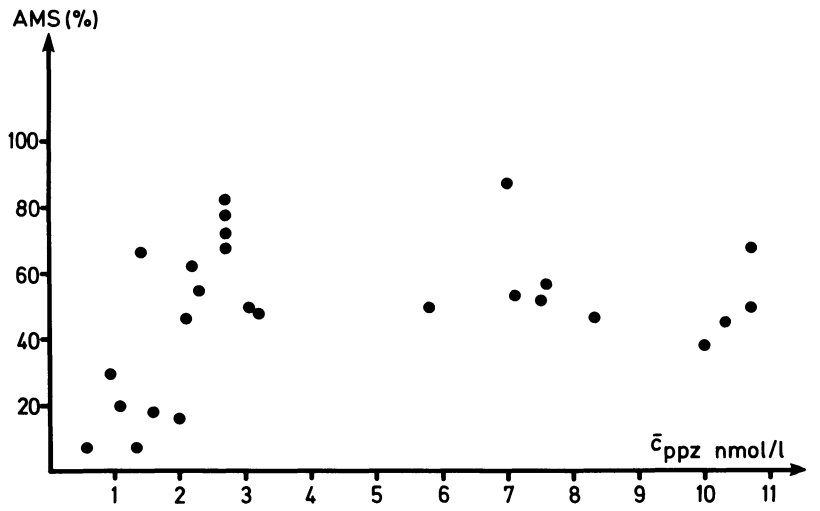


**Figure 2.** Average plasma concentrations of perphenazine for each of 26 patients plotted versus the degree of antipsychotic effect (AMS), which is the amelioration expressed in percent of the initial value, as judged from the Brief Psychiatric Rating Scale. Plasma concentrations above 2 nmol $l^{-1}$ induce a significantly better therapeutic outcome than do concentrations below this value ($p<0.005$), using the Mann-Whitney test.

10 nmol $l^{-1}$. To camouflage the allocation from the psychiatrist, all patients received from the beginning biperidine, 2 mg t.i.d. The only additional medication allowed was occasional use of nitrazepam. The psychiatrist and the chemist were kept blind to each other's results.

Finally, we investigated 23 patients (15 males and 8 females) selected using the above-mentioned criteria in order to evaluate the differences in clinical effects between depot preparations of perphenazine-enanthate and the new perphenazine-decanoate. Injections were given either fortnightly or weekly. The only additional medication allowed was, occasionally, nitrazepam. The psychiatrist and the chemist were kept blind to each other's results.

## RESULTS

In figure 1, the plasma level of perphenazine for each of the 26 patients is plotted versus the degree of total extrapyramidal side-effects. Dividing the patients into two clinical groups

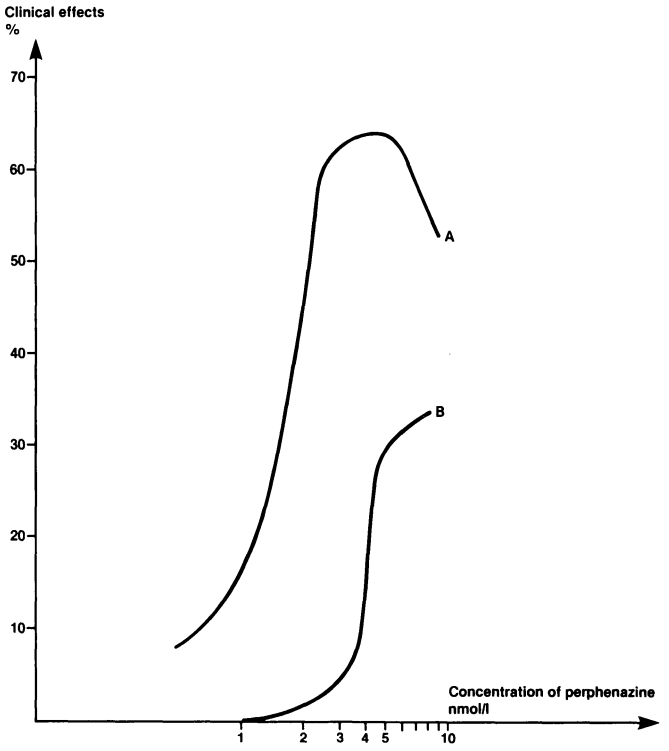


**Figure 3.** Clinical effects versus logarithmic plasma concentration scale. Curve A gives the amelioration expressed in percent of the initial values as judged from the Brief Psychiatric Rating Scale (data from figure 2). Curve B gives the total extrapyramidal side-effect scores expressed in percent of the maximal obtainable value (48 points) (data from figure 1).

(showing or not showing side-effects, respectively), a significant difference in plasma levels between the groups was found ($p<0.02$), using the Mann-Whitney test. However, a rather poor correlation ($R=0.64$) between the perphenazine plasma concentration and the intensity of the total extrapyramidal side-effects was found.

Figure 2 illustrates the relation between therapeutic outcome and the plasma levels of perphenazine for each of the other 26 patients. Patients with low levels (<2 nmol $l^{-1}$) generally achieved a substantially poorer clinical response compared to those with higher concentrations ($p<0.005$), using the Mann-Whitney test. There was also a tendency to a weaker antipsychotic effect among the patients in the high plasma concentration group (>5

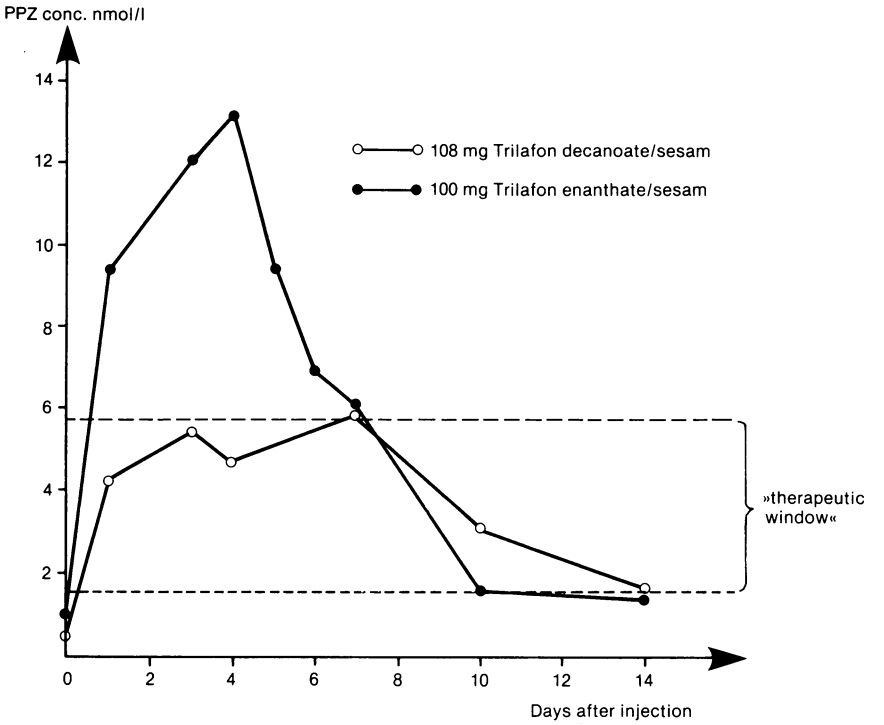


**Figure 4.** Plasma concentrations of perphenazine in a single patient after injection of 100 mg perphenazine-enanthate and 108 mg perphenazine-decanoate, respectively. The broken lines give the limits for the optimal plasma concentration range. During the enanthate period extrapyramidal side-effects appeared. No side-effects were seen during the decanoate period.

nmol $l^{-1}$) than among those with plasma concentrations in the range 2-3 nmol $l^{-1}$. This difference was not statistically significant ($p>0.05$), using the Mann-Whitney test.

The relationship between the antipsychotic effect and the risk of eliciting extrapyramidal side-effects at different plasma levels is shown in a 'dose-response' curve (figure 3). It can be seen that a maximal therapeutic response is achieved before the incidence of extrapyramidal side-effects increases drastically.

Based on the results from the oral studies one might, therefore, conclude that it is possible to obtain a maximal therapeutic effect without provoking extrapyramidal side-effects. The optimal plasma concentration range seems to be between 2 and 3 nmol $l^{-1}$ (figure 3).

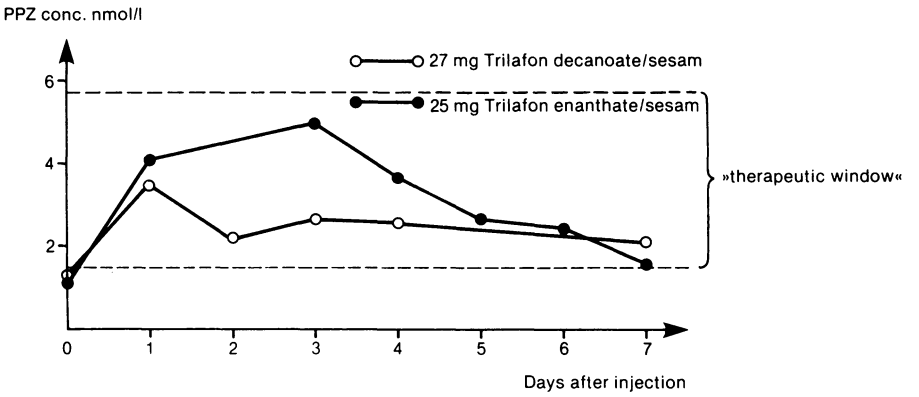


**Figure 5.** Plasma concentrations of perphenazine in a single patient after injection of 25 mg perphenazine-enanthate and 27 mg perphenazine-decanoate, respectively. The broken lines give the optimal plasma concentration range. No side-effects were seen in either of the two depot periods.

Patients treated continuously with oral perphenazine had a constant ratio of 1.8 between maximum and minimum plasma concentration within an 8 h dose interval (Bolvig Hansen and Larsen, 1977). This means that a minimum concentration of e.g. 3 nmol $l^{-1}$ corresponds to a maximum concentration of 5.4 nmol $l^{-1}$. Consequently, the operational plasma concentration range for depot administration should be about 2-6 nmol $l^{-1}$ for attainment of maximal therapeutic effect without extrapyramidal side-effects.

Figure 4 shows typical plasma profiles of perphenazine after injection of a standard dose of perphenazine-enanthate and perphenazine-decanoate, respectively. As seen, the enanthate curve far exceeds the upper limit of the optimal concentration range resulting in appearance of extrapyramidal side-effects. In order to avoid the rather often seen extrapyramidal side-effects during perphenazine-enanthate treatment, we lowered the dose and shortened the injection intervals to one week. In figure 5 typical plasma concentration profiles of perphenazine after injection of a low dose of perphenazine-enanthate and perphenazine-decanoate, in a single patient, are shown. As seen, the enanthate curve now lies within the optimal therapeutic range. Consequently, no extrapyramidal phenomena occurred.

## CONCLUSION

When treating acute psychotic states with perphenazine, it is in most cases possible to achieve an excellent therapeutic effect

without provoking extrapyramidal side-effects. During oral treatment given in three equal daily doses with constant intervals, one should guide the plasma level into the range of 2-3 nmol $l^{-1}$ as measured at the end of the dose interval. Using depot preparations of perphenazine, the plasma concentrations should lie within the limits of 2 and 6 nmol $l^{-1}$.

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# Saliva haloperidol concentrations in schizophrenic patients: relation to serum haloperidol and prolactin concentrations

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## INTRODUCTION

Measurement of drugs in saliva has been proposed as a non-invasive means by which serum drug concentrations can be assessed (Horning *et al.*, 1977). The concentrations of many drugs in saliva are equal to or less than the concentrations of free (non-protein-bound) drug in plasma or serum (Horning *et al.*, 1977; Stephen *et al.*, 1980). However, recent studies have shown that neuroleptics such as chlorpromazine (May *et al.*, 1981) and haloperidol (Yamazumi and Miura, 1981) are present in saliva in concentrations exceeding the total plasma concentration of drug.

Some of the variables which can affect saliva drug concentrations include: (1) the amount of drug bound to blood proteins or lipids; (2) the physio-chemical properties of the drug including its $pK_a$ and its lipophilicity; (3) the extent to which the drug is actively transported from serum into saliva; (4) the flow dependence of the drug from serum into saliva; and (5) the pH difference between blood and saliva (Horning *et al.*, 1977; Stephen *et al.*, 1980). Which, if any, of the above variables account for high saliva versus serum neuroleptic concentrations is unknown.

One of the benefits of measuring drugs and other compounds in saliva is the ease with which samples can be collected (Poland and Rubin, 1982). More importantly, however, is the possibility that saliva drug concentrations may be pharmacologically more relevant than serum or plasma concentrations. In fact, May *et al.* (1981) reported that saliva chlorpromazine concentrations 24 h after the first dose related more strongly to clinical outcome than did plasma concentrations. Previous studies have shown a strong positive association between serum haloperidol and serum prolactin

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concentrations (Rubin and Hays, 1980; Rubin *et al.*, 1980; Poland and Rubin, 1981). Strong correlations between saliva and serum haloperidol and between saliva haloperidol and serum prolactin would suggest that, under appropriate conditions, saliva haloperidol measurements might prove to be clinically useful. Accordingly, we undertook the present study to determine the relationships among serum and saliva haloperidol concentrations and serum prolactin levels in chronically medicated patients.

## MATERIALS AND METHODS

Paired serum and saliva samples were obtained from 27 chronically medicated schizophrenic patients in the morning, approximately 10 h after their last dose of medication. After rinsing his/her mouth with water, each subject was asked to allow saliva to accumulate. One to two milliliters of mixed saliva was collected by expectoration into small polypropylene funnels inserted into 12x75 mm polypropylene tubes. The saliva samples were centrifuged for 30 min at 500*g* to remove particulate matter, and the supernatant was frozen. Immediately after the saliva was collected, a blood sample was obtained by venipuncture. The blood was allowed to clot overnight at 4°C and centrifuged the next morning at 500*g* for 30 min. The serum was removed and frozen for subsequent haloperidol and prolactin measurements.

Serum and saliva haloperidol were measured by radioimmunoassay (RIA) as described previously (Poland and Rubin, 1981). This RIA does not cross-react appreciably with any known haloperidol metabolite and only cross-reacts to a limited extent (0.32%) with reduced haloperidol, a metabolite which is present in serum at approximately equal concentrations to that of the parent compound but with only one-tenth the neuroleptic activity of haloperidol (Forsman and Ohman, 1979; Hays *et al.*, 1980). Prolactin was measured by RIA using the hormone kit provided by Dr A.F. Parlow on behalf of the National Pituitary Agency.

To achieve a Gaussian distribution for the haloperidol and prolactin values, the data were log-transformed prior to the calculation of Pearson correlation coefficients (Zivin and Bartko, 1976). The nominal significance levels were corrected for the multiple correlations (Jacobs, 1976).

## RESULTS

Table 1 shows the serum and saliva haloperidol concentrations, the saliva-to-serum ratios of haloperidol, and the serum prolactin concentrations of 24 chronically medicated patients during pharmacokinetic steady-state conditions. There was a significant correlation between the serum and saliva haloperidol concentrations

Table 1. Serum and saliva haloperidol (HAL) concentrations, saliva-to-serum HAL ratios, and serum prolactin (PRL) concentrations in 24 chronically medicated patients during pharmacokinetic steady-state conditions

| Subject | Serum HAL ($\mu g\ l^{-1}$) | Saliva HAL ($\mu g\ l^{-1}$) | Saliva-to-serum ratio | PRL ($\mu g\ l^{-1}$) |
|---|---|---|---|---|
| 1 | 6 | 3 | 0.50 | 25 |
| 2 | 7 | 2 | 0.29 | 24 |
| 3 | 10 | 4 | 0.40 | 103 |
| 4 | 11 | 102 | 9.27 | 63 |
| 5 | 12 | 26 | 2.17 | 296 |
| 6 | 15 | 58 | 3.87 | 62 |
| 7 | 15 | 22 | 1.47 | 7 |
| 8 | 16 | 110 | 6.88 | 59 |
| 9 | 22 | 131 | 5.95 | 57 |
| 10 | 22 | 4 | 0.18 | 166 |
| 11 | 24 | 53 | 2.21 | 55 |
| 12 | 25 | 67 | 2.68 | 36 |
| 13 | 26 | 61 | 2.35 | 51 |
| 14 | 26 | 55 | 2.12 | 18 |
| 15 | 27 | 56 | 2.07 | 37 |
| 16 | 28 | 36 | 1.29 | 70 |
| 17 | 28 | 60 | 2.14 | 23 |
| 18 | 31 | 23 | 0.74 | 39 |
| 19 | 34 | 109 | 3.20 | 137 |
| 20 | 34 | 111 | 3.26 | 59 |
| 21 | 35 | 86 | 2.43 | 28 |
| 22 | 51 | 448 | 8.78 | 32 |
| 23 | 60 | 472 | 7.87 | 240 |
| 24 | 66 | 40 | 0.61 | 97 |
| Range | 6-66 | 2-472 | 0.2-9.36 | 7-296 |

($r$ = +0.70, $p$<0.005), but there was no significant correlation either between serum haloperidol and serum prolactin ($r$ = +0.23) or between saliva haloperidol and serum prolactin ($r$ = +0.21).

Table 2 shows weekly serum and saliva haloperidol concentrations, saliva-to-serum haloperidol ratios, and serum prolactin levels in three chronically medicated patients studied repeatedly during pharmacokinetic steady-state conditions. For the three subjects, serum haloperidol concentrations were relatively stable, but saliva haloperidol concentrations were more variable, particularly for subjects 25 and 27. The saliva-to-serum haloperidol ratios also were relatively constant within each subject, except for sample 6 of subject 25 and sample 2 of subject 27.

Table 2. Weekly serum and saliva haloperidol (HAL) concentrations, saliva-to-serum HAL ratios, and serum prolactin (PRL) concentrations in three chronically medicated patients during pharmacokinetic steady-state conditions

Subject 25 - 10 mg haloperidol per day

| Sample | Serum HAL ($\mu g\ l^{-1}$) | Saliva HAL ($\mu g\ l^{-1}$) | Saliva-to-serum ratio | Serum PRL ($\mu g\ l^{-1}$) |
|---|---|---|---|---|
| 1 | 41 | 10 | 0.24 | 146 |
| 2 | 22 | 5 | 0.23 | 166 |
| 3 | 42 | 28 | 0.67 | 136 |
| 4 | 21 | 4 | 0.19 | 131 |
| 5 | 25 | 8 | 0.32 | 157 |
| 6 | 64 | 73 | 1.14 | 179 |
| 7 | 30 | 24 | 0.80 | 151 |
| 8 | 25 | 13 | 0.52 | 142 |
| Range | 21-64 | 4-73 | 0.23-1.14 | 131-179 |

Subject 26 - 5 mg haloperidol per day

| Sample | Serum HAL ($\mu g\ l^{-1}$) | Saliva HAL ($\mu g\ l^{-1}$) | Saliva-to-serum ratio | Serum PRL ($\mu g\ l^{-1}$) |
|---|---|---|---|---|
| 1 | 7 | 3 | 0.43 | 24 |
| 2 | 4 | 3 | 0.75 | 14 |
| 3 | 12 | 6 | 0.50 | 41 |
| 4 | 5 | 3 | 0.60 | 22 |
| Range | 4-12 | 3-6 | 0.43-0.75 | 14-41 |

Subject 27 - 5 mg haloperidol per day

| Sample | Serum HAL ($\mu g\ l^{-1}$) | Saliva HAL ($\mu g\ l^{-1}$) | Saliva-to-serum ratio | Serum PRL ($\mu g\ l^{-1}$) |
|---|---|---|---|---|
| 1 | 12 | 26 | 2.17 | 17 |
| 2 | 21 | 500 | 23.8 | 24 |
| 3 | 28 | 142 | 5.07 | 37 |
| 4 | 20 | 50 | 2.50 | 15 |
| Range | 12-28 | 26-500 | 2.17-23.8 | 15-37 |

Table 3 shows the within-subject correlations between serum and saliva haloperidol, serum haloperidol and serum prolactin, and saliva haloperidol and serum prolactin for the three subjects of table 2. The within-subjects serum-to-saliva correlations were comparable to the across-subjects correlation of these two measures ($r$ = +0.70). The saliva-to-serum haloperidol ratios for each of the three subjects also were relatively stable. Excluding sample 6 (subject 25) and sample 2 (subject 27), the within-subjects haloperidol ratios were less variable than the across-subjects haloperidol ratios (table 1). In contrast to the saliva-to-serum drug correlations, both the serum and saliva haloperidol-to-prolactin correlations were much higher within subjects than they were across subjects (see above).

Table 3. Within-subject correlations among serum and saliva haloperidol (HAL) and serum prolactin (PRL)

| Correlations | Subject 25 | Subject 26 | Subject 27 |
|---|---|---|---|
| Serum HAL vs Saliva HAL | $r$ = +0.87 | $r$ = +0.88 | $r$ = +0.65 |
| Serum HAL vs Serum PRL | $r$ = +0.61 | $r$ = +0.98 | $r$ = +0.73 |
| Saliva HAL vs Serum PRL | $r$ = +0.41 | $r$ = +0.84 | $r$ = +0.60 |

## DISCUSSION

The present study confirms and extends the work of Yamazumi and Miura (1981) on saliva haloperidol concentrations. Although the haloperidol RIA used by those investigators probably measured reduced haloperidol as well as haloperidol itself (Rubin and Poland, 1981), the results of their study are similar to ours. They found a positive across-subjects correlation between saliva and serum haloperidol concentrations ($r$ = +0.75) which was similar to that found in our subjects ($r$ = +0.70). In addition, many of their subjects showed saliva-to-serum haloperidol ratios of 5 or more, which also is comparable to the ratios found in some of our subjects.

The presently accepted view is that, for many drugs, the salivary concentration reflects the unbound drug concentration in plasma, which presumably is the fraction of drug in blood that is pharmacologically active. For many drugs the correspondence between saliva and free plasma concentrations has been shown to be close (Horning *et al.*, 1977) and, depending upon the drug, the saliva concentration often is equal to or less than the free drug concentration in plasma, but not greater than the total drug concentration. Thus, drug saliva-to-plasma ratios are usually 1 or less.

However, as indicated by the present study, for most patients the saliva-to-serum haloperidol ratios were greater than 1, and thus the saliva haloperidol concentrations were obviously greater than the amount of free haloperidol in serum, which is approximately 8% of the total (Forsman and Ohman, 1977). Part of the reason for the elevated saliva haloperidol concentrations is due to trapping of haloperidol in its ionic form. Saliva generally is more acidic than plasma, so that a greater percentage of the haloperidol in saliva is positively charged and not able to diffuse back into blood. However, haloperidol has a $pK_a$ of 8.25, so that even at a saliva pH of 6, the trapping effect would not account entirely for the high saliva concentrations in some of the patients. Interestingly, chlorpromazine and desmethylimipramine also are concentrated in saliva. Chlorpromazine sequesters in saliva up to concentrations 100-fold higher than that found in plasma (May *et al.*, 1981), and desmethylimipramine concentrations in saliva can be up to 16 times higher than serum concentrations (Cooper *et al.*, 1981). Again, the free concentrations of both chlorpromazine and desmethylimipramine are 10% or less. The reasons for the high saliva concentrations of these three psychoactive drugs are unknown.

We previously have found a significant correlation between serum haloperidol and serum prolactin levels in chronically medicated patients (Rubin *et al.*, 1980; Rubin and Poland, 1981; Poland and Rubin, 1981). However, in the present study we found no significant across-subjects correlation between serum haloperidol and serum prolactin. The large inter-subject variability in the prolactin response to neuroleptic drug administration may account in part for this finding (Rubin and Hays, 1980). Although only three subjects were studied, the within-subjects correlations between serum haloperidol and serum prolactin were much higher.

Prolactin concentrations were measured to determine if saliva haloperidol correlated better with serum prolactin than did serum haloperidol. If this were so, then saliva levels might in some way have reflected free serum haloperidol concentrations. However, saliva haloperidol correlated less well with serum prolactin than did serum haloperidol, suggesting that saliva haloperidol is not a good measure of pharmacologically relevant haloperidol concentrations. More specifically, subject 27 had a saliva haloperidol concentration which fell from 500 $\mu g\ l^{-1}$ in sample 2 to 142 $\mu g\ l^{-1}$ in sample 3, while serum haloperidol concentrations increased 33% between sample 2 and sample 3 (table 2). The corresponding serum prolactin concentrations also increased between samples 2 and 3, showing a positive relationship with serum haloperidol but a negative relationship with saliva haloperidol. These data also suggest that saliva haloperidol is not a good reflection of serum free haloperidol, but additional studies need to be performed on larger samples of patients before this issue can be settled.

**Acknowledgements**

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# Section Four

# Clinical Pharmacokinetics of Lithium and Antidepressants

# Significance of the serum lithium concentration and the treatment regimen for wanted and unwanted effects of lithium treatment

Mogens Schou[1]

## INTRODUCTION

Lithium (Li) treatment was the first psychiatric therapy to be monitored through determination of serum concentrations of the drug. In the beginning this served to guard against overdosage and poisoning, but later it was used to ensure sufficient levels for achievement of effect. When Li started being used for prophylactic purposes, i.e. for prevention or alleviation of manic-depressive recurrences, the monitoring procedure became particularly important, because under these circumstances no symptoms were present, and dosage adjustment could therefore not be based on clinical assessment.

## SERUM LITHIUM UNDER STANDARDIZED CONDITIONS

The lithium ion is absorbed readily from the gastrointestinal tract; it is not bound to proteins; it penetrates into tissues with varying rapidities; and its apparent distribution volume corresponds to about 70% of the body. Elimination takes place almost exclusively through the kidneys with an elimination half-life of about 15-30 h.

The Li concentration in blood serum is used for monitoring purposes. The serum concentration is clearly not identical with the Li concentration in those - unknown - brain cells in which the ion exerts its therapeutic and prophylactic actions, but there is reason to suppose that the serum concentration can be used as at least an indicator of that concentration.

Owing to differences in the rates of Li absorption, distribution and elimination, the serum Li concentration fluctuates throughout the day with peak values 2-4 h after each intake, followed by gradual and largely exponential fall until the next

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intake. Administration with one or two daily intakes is customary. Studies carried out by Amdisen in our Institute (Amdisen, 1980) indicate that the serum concentration which is best suited for monitoring purposes is that obtained in blood samples drawn in the morning 12 h after the last intake of Li. At this time the serum concentration is least influenced by individual differences in gastrointestinal absorption and renal elimination. The samples should be drawn under steady-state conditions, i.e. about one week after start of treatment and after dosage changes. When, in the following, I use the terms 'serum Li' or 'serum Li concentration' without further qualification, I am referring to the serum concentration determined under these standardized circumstances.

It has been debated whether the Li concentration in erythrocytes might reflect the Li concentration in brain cells better than does the serum Li concentration, and erythrocyte Li levels or erythrocyte/plasma Li ratios have been suggested as predictors of response to Li or of Li toxicity. However, the evidence presented in support of these notions has, in my opinion, not been sufficiently strong to justify the rather elaborate procedure of erythrocyte Li determinations as a routine procedure.

Determination of the Li concentration in saliva has also been proposed as an aid in treatment monitoring, especially for remote parts of developing countries where the drawing of blood samples might present difficulties. The proposal has in fact been made many times, but I do not know of any place where the procedure is in fact used.

## SERUM LITHIUM RESPONSE CURVE FOR PROPHYLACTIC EFFECT

Whereas in pharmacology in general it is customary to deal with dose-response curves, we can, when Li treatment is concerned, advantageously replace these with serum Li - response curves, because the renal Li clearance shows such pronounced individual variation that different patients may require markedly different Li dosages in order to achieve the same serum Li level. Response to Li may be either wanted or unwanted. I shall deal first with wanted response, which in this connection means response to prophylactic Li treatment, i.e. prevention or alleviation of manic and depressive relapses through Li administration over months and years.

Figure 1 shows how a serum Li - response curve for prophylactic effect *might* look, but it should be emphasized that what is shown is a schematic approximation based on uncertain and mostly retrospective information. It corresponds largely to the graph published by Baastrup (1980), which shows serum Li concentrations in 75 patients given successful Li treatment. The curve shown here takes into account that some patients do not respond to Li.

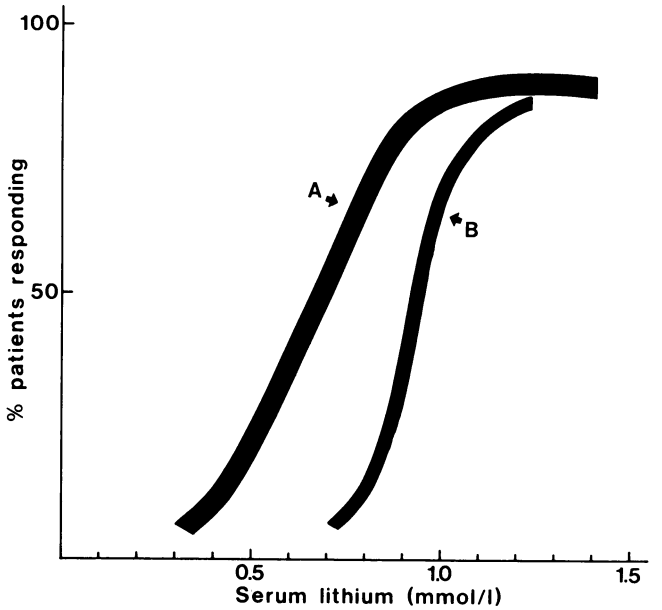


**Figure 1.** Serum lithium response curve for the prophylactic effect of lithium (curve A) and for lithium-induced polyuria (curve B). The graphs are schematic approximations.

The exact shape and location of a serum Li - response curve can be determined only through prospective studies in which patients are allocated randomly to different serum Li levels over the expected effective concentration range and maintained at these levels for sufficiently long periods to permit calculation of degrees of prophylactic effectiveness. Such studies involve considerable practical difficulties, and their ethical justification seems debatable.

A lower limit for effective serum Li levels of about 0.4 mmol $l^{-1}$ corresponds to Baastrup's experiences and is also in accordance with data presented by Hullin (1981). His study was prospective and showed that patients maintained at serum Li levels below 0.4 mmol $l^{-1}$ had a markedly higher frequency of relapse than patients maintained at higher serum Li levels.

An upper limit is more difficult to establish because of the increasing frequency of side-effects with increasing dosage and serum levels, but there is at least no evidence to support the notion of further prophylactic benefit at levels higher than about 1.1-1.3 mmol $l^{-1}$ nor of a 'therapeutic window' with falling ef-

ficiency at higher levels. None of Baastrup's successfully treated patients had serum levels higher than 1.1 mmol $l^{-1}$ and curve A has accordingly been drawn between the points 0.4 and 1.1 mmol $l^{-1}$.

The relationship between serum Li and prophylactic outcome may, in fact, not be quite so simple. Sarantidis and Waters (1981) found that patients with an excellent prophylactic response had a higher average serum Li level (0.74 mmol $l^{-1}$) than patients with only a fair prophylactic response (average serum Li 0.63 mmol $l^{-1}$), but then they found that patients with a poor response also had a high level (0.79 mmol $l^{-1}$), possibly because in these patients the dosage had been 'pushed' in order to achieve effect but without this actually being obtained. Sashidharan *et al.* (1982) studied serum Li levels from 53 patients with varying outcomes of prophylactic Li treatment and found that the levels for both those with poor outcome and those with good outcome spread over the full range between 0.3 and 1.3 mmol $l^{-1}$. In fact, those with a favorable outcome spent significantly less time at serum Li levels above 0.9 mmol $l^{-1}$ than did those with a poor outcome; an interpretation of this finding is made difficult by the retrospective nature of the study.

Curve A in figure 1 is drawn in such a way that it becomes horizontal at a level lower than 100% response. This merely reflects that some patients do not respond to the treatment. The frequency of non-response, partial or total, is difficult to estimate, because it depends so much on the wideness of indications on which Li treatment is started.

## SERUM LITHIUM RESPONSE CURVES FOR SIDE-EFFECTS

Serum Li - response curves for side-effects of Li are generally located at a somewhat higher concentration range than that of the serum Li response curve for wanted effects. This is fortunate for the patients.

In figure 1, curve B has been superimposed on curve A. Curve B might, in principle, represent any of the Li-induced side-effects; as shown here it reflects the situation for one of the more frequent side-effects of long-term Li treatment, namely impairment of renal water reabsorption. As before, we are not dealing with a precisely 'titrated' curve but rather with a schematic diagram which reflects the outcome of different studies with different approaches.

Various measures are available of the extent to which Li treatment inhibits the response of the distal tubules and collecting ducts to the action of the antidiuretic hormone. Vestergaard and Amdisen (1981) determined maximum urine osmolality after administration of a vasopressin analog, DDAVP (desmopressin acetate), and introduced this value as the dependent variable in a multiple regression analysis with the following predictor vari-

ables: age, sex, serum Li, Li dosage, duration of Li treatment, concomitant treatment with neuroleptic drugs, and concomitant treatment with antidepressant drugs. Data from 147 patients were employed. The renal concentrating ability, as expressed by the maximum urine osmolality, was found to be significantly and positively correlated with the serum Li concentration and with the duration of the Li treatment, but not with any of the other independent variables. The serum Li concentration ranged from 0.4 to 1.1 mmol $l^{-1}$, average value 0.87 mmol $l^{-1}$. In another study Vestergaard and Thomsen (1981) employed data from the same patient group for multiple regression analysis but now used as the dependent variable the expression $V/C$Li, where $V$ is the urine flow and $C$Li is the renal Li clearance. This expression is a reliable indicator of water handling in the distal parts of the nephron (Thomsen, 1978; Thomsen *et al.*, 1981), and the multiple regression analysis once again showed significant association with the serum Li concentration and the duration of the Li treatment.

A third study indicating that the effect of Li on the distal parts of the nephron is closely associated with the serum Li concentration is that carried out by Penney *et al.* (1981). They demonstrated increased urinary excretion of arginine vasopressin, the antidiuretic hormone in man, and found the logarithm of the urine arginine vasopressin excretion significantly correlated with the serum Li concentration. In this study the serum Li concentration ranged from 0.4 to 1.2 mmol $l^{-1}$ and averaged 0.63 mmol $l^{-1}$.

Confirmation of the location and of the steepness of the slope of curve B in figure 1 can further be obtained by comparison of polyuria frequencies in different clinics. It has been a striking observation in the Li-kidney studies of recent years that impairment of renal concentrating ability occurs with high frequency in some clinics and with low frequency in others. Vestergaard and Amdisen (1981) found the renal concentrating ability markedly decreased in their patients, treated at the Psychiatric Hospital in Aarhus. Hullin *et al.* (1979) from Leeds found only a moderate lowering of maximum urine osmolality in their Li-treated patients. Treatment duration was approximately the same in the two clinics, but the patients had been maintained at different serum Li levels: in Aarhus, at an average value of 0.85 mmol $l^{-1}$; in Leeds, at an average value of only 0.59 mmol $l^{-1}$. It seems likely that this 40% difference in serum level may have played at least a contributory role for the different affection of renal concentrating ability in the two patient groups.

In the Aarhus hospital, we have now taken notice of these findings and within recent years adjusted treatment guidelines so that patients are now maintained at serum levels which are about 25% lower than those employed previously. Rather than aiming at serum levels between 0.8 and 1.0 mmol $l^{-1}$ we now adjust dosages to concentrations within the range 0.6-0.8 mmol $l^{-1}$, and it is our

expectation for the coming years that prophylactic efficacy will remain largely unchanged but that Li-induced polyuria and perhaps other side-effects may become less frequent.

## INDIVIDUAL ADJUSTMENT OF LITHIUM DOSAGES AND SERUM LITHIUM LEVELS

The therapeutic range of Li is, as indicated, rather narrow. Doses and serum Li concentrations required for therapeutic and prophylactic effect are not very much lower than concentrations which produce side-effects and there is often overlapping so that some patients can be kept free of relapses only at the cost of suffering side-effects such as tremor, weight gain, thirst and polyuria. It is therefore important that each patient is maintained at the serum Li concentration which is optimum for him or her, i.e. the concentration which produces a maximum of prophylactic protection with a minimum of side-effects. The curves in figure 1 indicate that 0.6-0.8 mmol $l^{-1}$ is a useful range to aim for initially in the treatment or in groups of patients, but it should be understood that for the individual patient the optimum range may be still narrower, and fine adjustment of dosages and serum Li levels to the individual optimum is a very important task for the physician. Since we are dealing with prophylactic treatment, such adjustment may take a long time, since dosage changes have to be followed by long periods of observation in order to see whether the desired result was obtained.

By 'fine' adjustment I really mean *fine*. Time and again I have been amazed at how much difference it may make for a patient's life quality, if his or her Li dosage is raised by as little as one-half or one Li tablet and the serum concentration correspondingly increased by one-tenth of a millimole per liter. This may take away slight but troublesome manic and depressive mood changes. In other cases a reduction of serum Li by one-tenth of a millimole per liter may effectively remove or reduce troublesome side-effects. It is perhaps difficult for patients and physicians to understand that such seemingly small changes can be so important, and I have sometimes wondered whether it would be didactically preferable if serum Li levels were presented in micromoles per liter rather than in millimoles per liter. A change of serum Li from 700 to 800 μmol $l^{-1}$ somehow looks more impressive than a change from 0.7 to 0.8 mmol $l^{-1}$, and such a change may, as I have indicated, make all the difference for the patient's well-being.

## SIGNIFICANCE OF THE TREATMENT REGIMEN

Li is usually administered in one or two, sometimes three, daily doses, and both conventional and slow-release or retard tablets

are in use. The choice of treatment regimen determines the course of the serum Li concentration over the day. This is illustrated in figure 2, which shows the mean serum Li concentration around the clock in a group of patients from the Psychiatric Hospital in Aarhus and in a group from the Psychiatric Clinic at Rigshospitalet in Copenhagen. In Aarhus the patients had been given slow-release tablets twice daily, morning and evening. In Copenhagen they had been treated with conventional tablets given once daily, in the evening. It will be noted that fluctuations were much wider in the Copenhagen group, with the highest concentrations more than three times the lowest, whereas in Aarhus the highest value was only 1.4 times the lowest. One may also note that in Aarhus the maximum value was reached 4-5 h after the intake of Li, whereas in Copenhagen the peak value was reached about 2 h after the intake.

Now, which of these two curves is clinically preferable? This has been the subject of friendly debate between the two clinics. We in Aarhus felt that the avoidance of high concentration peaks and hence of possible peak-associated side-effects would offer most advantage. Our colleagues in Copenhagen felt that the achievement of low concentrations before the next intake of Li would offer the organism a chance of restitution that might

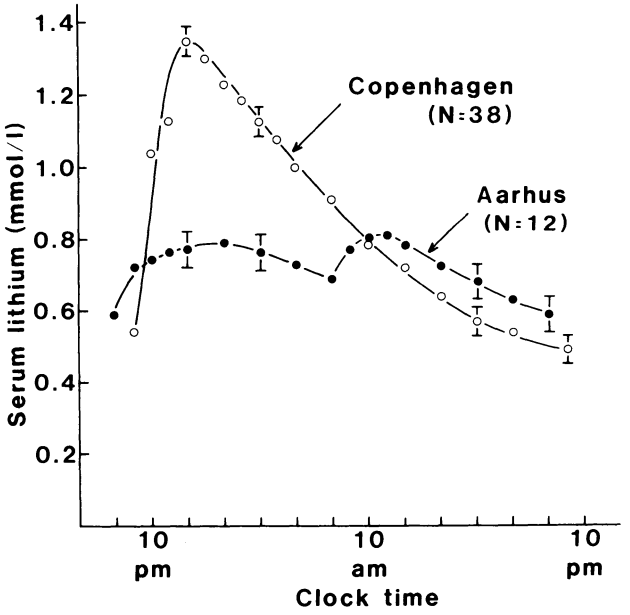


Figure 2. Mean serum lithium concentrations around the clock in two groups of patients given different treatment regimens (see text). Data from Lauritsen *et al.* (1981)

outweigh any disadvantages of the high peaks. Last year we carried out a quantitative comparison with respect to the degree of polyuria in the two groups (Schou *et al.*, 1982). The comparison was based on the assumption, unproven but not unreasonable, that prophylactic efficacy was roughly the same in the two clinics. Since the two groups differed as regards various patient and treatment features, we subjected the data to multiple regression analysis with urine flow as the dependent variable and with age, sex, treatment duration, and serum Li as predictor variables; to this we added the treatment regimen as a further predictor variable, allotting the dummy values of 0 and 1 to each of the two regimens.

I do not mind admitting that the outcome of the study came as a surprise to both clinics (table 1). Each of our groups had expected its own treatment regimen to produce less polyuria than the other regimen. The data showed fairly pronounced polyuria in both groups, median urine flows being between 2 and 3 1 per 24 h. Our patients in Aarhus in fact had more polyuria than the patients in Copenhagen, but the difference was not large and only marginally significant. A significant difference appeared when we compared values for the more specific indicator of distal water handling, *V/C*Li (Thomsen, 1978), and only with this expression as the dependent variable did the treatment regimen add significantly to the explained variation in the multiple regression analysis.

These results have shaken my belief in the superiority of the Aarhus regimen, at least as far as polyuria is concerned, but it has perhaps not quite convinced me that the Copenhagen regimen is

Table 1. Patient and treatment features, urine flow and distal water handling (mean values) in two groups of patients given different lithium treatment regimens (data from Schou *et al.*, 1982)

| | Aarhus | Copenhagen |
|---|---|---|
| Treatment regimen | Slow-release, twice daily | Conventional, once daily |
| Number of patients | 95 | 28 |
| F/M | 45/50 | 21/7 |
| Age (years) | 43 | 51 |
| Body weight (kg) | 74 | 70 |
| Duration of treatment (years) | 6.5 | 8.0 |
| 12h-SLi (mmol $l^{-1}$) | 0.82 | 0.87 |
| Creatinine clearance (ml $min^{-1}$) | 99 | 90 |
| Urine flow (l/24 h) | 2.83 | 2.38* |
| *V/C*Li | 0.099 | 0.076** |

*$p = 0.05$; **$p < 0.05$.

better. Definitive conclusions in this respect must await within-clinic trials with random allocation of treatment regimens and with assessment of not only side-effects but also therapeutic and prophylactic effects of the treatment. Perhaps in the future we shall see much less polyuria and polydipsia in our Li-treated patients in Aarhus as we reduce the Li doses and serum levels by about 25%.

## EPILOGUE

Determination of serum Li concentrations is a useful aid for monitoring and guidance of Li treatment, but the values reported by the laboratory should not be accorded magical significance. The patient's mental and physical well-being is more important for the choice of dosage level than is the location of the serum Li concentration at one or the other end of the recommended range. Individual adjustment of the dosage to the optimum level is the essential feature; maintenance of this level can then be supported through serum Li determinations.

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# Hydroxy metabolite concentrations: role of renal clearance

William Z. Potter[1], Elizabeth A. Lane[1] and Matthew V. Rudorfer[1]

## INTRODUCTION

It is generally emphasized that basic lipophilic psychoactive drugs are cleared primarily through metabolism, not renal clearance (Potter *et al.*, 1981). Kidney disease or other sources of alterations of renal clearance are not reported to affect plasma concentrations of drugs such as the tricyclic antidepressants, although the clearance of unmetabolized drugs such as lithium will clearly be affected (Amdisen, 1977). From any but an academic point of view this would be the limit of interest in renal clearance if extensively metabolized parent drugs were the only biologically active forms.

We have known for years however (reviewed in Garattini *et al.*, 1975) that many psychoactive drugs are metabolized to biologically active compounds. More recently it has become established that hydroxylated but unconjugated biologically active metabolites exist in significant steady-state concentrations both in plasma and brain (for review, see Potter and Calil, 1981). The majority of evidence supporting a pharmacodynamic role of hydroxy metabolites, both in terms of therapeutic and side- or toxic effects, stems from investigations with the tricyclic antidepressants (TCAs), although a study of direct clinical efficacy has only been obtained for a hydroxyphenothiazine (Kleinman *et al.*, 1978). This paper focuses primarily on the TCAs but will consider relevant data from studies with another class of basic lipophilic drugs, the beta-adrenergic antagonists.

It was shown over a decade ago that in man the majority of a dose of TCA was metabolized to hydroxy metabolites which were excreted in the urine (Crammer *et al.*, 1969). A few years later,

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Alexanderson and Borgå (1973), as part of a remarkably comprehensive series of studies on nortriptyline pharmacokinetics at the Karolinska Institute, showed that there was a high renal clearance of unconjugated as well as conjugated 10-hydroxynortriptyline. Subsequent studies carried out through the same laboratory have replicated and extended this finding (Kragh-Sørensen *et al.*, 1977; Mellström *et al.*, 1981).

More recently, numerous laboratories almost concurrently demonstrated that unconjugated hydroxy metabolites of TCAs might have clinical relevance in light of their preclinical biological activity and the documentation of significant steady-state concentrations in man (reviewed in Potter and Calil, 1981). Table 1 shows relative steady-state concentrations of biologically active hydroxy metabolites following a variety of TCAs. In order to carry out mechanistic pharmacodynamic investigations with TCAs, it is necessary to understand better the determinants of the steady-state concentrations of their hydroxy metabolites. Our special emphasis here is on the renal clearance of these compounds. This, however, can only be understood in terms of an overall pharmacokinetic model which is presented below.

Table 1. Average steady-state concentrations of tricyclic antidepressants and active metabolites. Concentrations are corrected to a 100 mg daily dose

| Administered drug | Average concentration for 100 mg/day dose | | | Reference |
|---|---|---|---|---|
| | AT | NT | OH-NT | |
| Amitriptyline (AT) | 58 | 62 | - | Potter *et al.* (1981) |
| Nortriptyline (NT) | - | 110 | - | Potter *et al.* (1981) |
| | | 131 | 172 | Kragh-Sørensen *et al.* (1977) |
| | IMI | DMI | OH-DMI | |
| Imipramine (IMI) | 45 | 92 | 35* | Potter *et al.* (1982) |
| Desipramine (DMI) | 39 | 55 | | Potter *et al.* (1981) |
| | CI | DCI | OH-DCI | |
| Chlorimipramine (CI) | 61 | 128 | 63 | Linnoila *et al.* (1982) |
| | 42 | 99 | - | Träskmann *et al.* (1979) |

OH-NT = 10-hydroxynortriptyline
OH-DMI = 2-hydroxydesipramine
OH-DCI = 8-hydroxychlordesipramine
DCI = desmethylchlorimipramine
* Concentrations in children

## THE DETERMINANTS OF METABOLITE CONCENTRATION

In a number of recent studies, the variability in clearance of a drug to a particular metabolite (Mellström *et al.*, 1981) or the elimination clearance of a metabolite (Kitanaka *et al.*, 1982) have been studied with a view to making generalized statements about variability in metabolite concentration. None of these clearances, alone, determines the variability in metabolite concentration. Rather, all of them must be integrated into an overall relationship with all of the clearances that affect metabolite concentration:

$$C_m = \frac{fm_{p \to m} \times (\text{dose rate})_p}{Cl_m} \quad (1)$$

$$= \frac{Cl_{p \to m} \times (\text{dose rate})_p}{(Cl_{p \to m} + Cl_{p \to \text{other}})\, Cl_m} \quad (2)$$

where $C_m$ = steady-state concentration of metabolite, $fm_{p \to m}$ = fraction of parent drug (p) metabolized to the metabolite of interest (m), $(\text{dose rate})_p$ = constant dose rate of parent drug, $Cl_m$= elimination clearance of metabolite, $Cl_{p \to m}$ = clearance of parent drug to metabolite (m), and $Cl_{p \to \text{other}}$ = clearance of parent drug to other metabolites.

First, variability in the clearance of parent drug to metabolite causes variability in metabolite concentration in the same way that it causes variability in fraction metabolized to that metabolite. Assuming that the clearances of parent drug to this metabolite and to other metabolites vary independently, the fraction metabolized varies directly with clearance of parent drug to metabolite, but in a non-linear manner. That is, higher values of this clearance result in a higher value of the numerator and the denominator as seen by inspection of equation (2). The impact of high values of clearance of parent drug to metabolite upon fraction metabolized, and therefore upon metabolite concentration, depends upon the *absolute value* of fraction metabolized. This relationship is also crucial to understanding the effect of wide variations in rate of clearance to concentrations of metabolite. Although the clearance of parent drug to metabolite can have any value, the fraction metabolized is limited to values between 0 and 1. Furthermore, if one begins with high absolute values of fraction metabolized, large variability in clearance of parent drug to the metabolite causes much less variability in fraction metabolized (figure 1). For example, if the ultimate fraction metabolized is approximately 0.9, a two-times range in rate of clear-

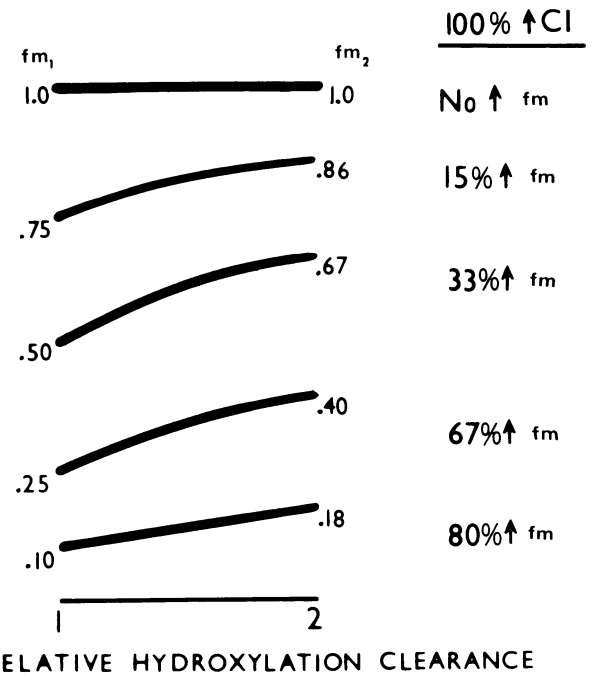


**Figure 1.** The range of fraction of parent drug metabolized to hydroxy metabolite for a twofold range in clearance of parent drug to hydroxy metabolite. Note that at larger values of *fm* a 100% higher hydroxylation clearance corresponds to a smaller percentage increase in *fm*.

ance of parent drug to the metabolite will produce very little variability in fraction metabolized (i.e. 0.90 to 0.95). On the other hand, if the usual fraction metabolized to a specific metabolite is in the range of 0.10, a two-times range in the rate of clearance of parent drug to the metabolite will correspond to an almost twofold range in fraction metabolized (0.10 to 0.18).

Secondly, variability in the elimination clearance of the metabolite affects metabolite concentration in an inverse manner (equations (1) and (2)). If the metabolite is eliminated by a renal and a metabolic route and it is appropriate to sum the respective clearances, the metabolite concentration becomes:

$$C_m = \frac{fm_{p\rightarrow m} \times (\text{dose rate})_p}{Cl_{m,\,\text{renal}} + Cl_{m,\,\text{metabolic}}} \qquad (3)$$

For instance, age-dependent decreases in renal function usually result in similar decreases in the renal clearances of metabolites. The impact of a decrease in renal clearance of a metabolite upon the metabolite concentration depends upon the relative values of metabolite renal clearance ($Cl_{m,renal}$) and metabolite clearance by metabolism ($Cl_{m,metabolic}$); that is, upon the fraction of the metabolite excreted unchanged. The denominator of equation (3) may thus be considered as being composed of fractional pathways of metabolite clearance which must add up to 1.0. Hence, if 50% of the metabolite is usually excreted unchanged (i.e. 50% via $Cl_{m,renal}$) then 50% will be metabolized. A comparison population in which renal function is reduced to 10% of normal, such as the aged, could proportionately reduce the fraction excreted unchanged in urine in this case from 50% to 5%. The proportional value of the denominator would now be 0.05 + 0.50 or 0.55. The reciprocal of this is 1.82; thus, a 10-fold reduction in fraction excreted in the urine would increase the metabolite concentration by 82% (equation (3)). If only 10% of the metabolite were excreted unchanged and renal clearance were decreased to 10% of normal, the metabolite concentration would, however, only be about 10% higher (reciprocal of [(0.10 x 0.10)+ 0.90]).

To summarize, the metabolite concentration

(a) varies directly, but non-linearly, with clearance of parent drug to the metabolite;
(b) is more sensitive to variability in clearance of parent drug to the metabolite when the fraction of parent drug metabolized via that pathway is small;
(c) varies inversely with renal clearance of metabolite;
(d) is most sensitive to variability in renal clearance when the fraction of metabolite excreted unchanged is large.

## APPLICATION TO ACTIVE METABOLITES OF TRICYCLIC ANTIDEPRESSANTS

### Nortriptyline

The hydroxylation of nortriptyline has been shown to range over 10-fold when poor and efficient hydroxylators were compared (Mellström *et al.*, 1981). The fraction of nortriptyline metabolized to hydroxynortriptyline was also reported to be higher in the efficient hydroxylators (60-75%) than in the poor hydroxylators (24-49%). The 10-fold range in hydroxylation clearance, therefore, corresponded to a threefold range in fraction metabolized. These figures are consistent with the clearance of parent drug by other pathways being constant between subjects (figure 1). On the basis of equation (1) one might expect this three-times variability in fraction metabolized to be reflected in a three-

times variability in steady-state metabolite concentration (or in area under the curve (AUC) of metabolite after a single dose). However, no such relationship between metabolite concentration and hydroxylation clearance was observed (Mellström *et al.*, 1981). One must conclude that the metabolite AUC was not correlated with hydroxylation clearance ($Cl_m$) because of the independent way in which the elimination clearance of hydroxynortriptyline varied.

In the same set of data, we see that 30-60% of hydroxynortriptyline formed from nortriptyline was excreted unchanged. Therefore, age-dependent decreases in renal function in such a group would be expected to result in a maximum 40-200% increase in metabolite concentration as can be calculated from equation (3) according to the examples provided above. Such predicted increases are consistent with the relative elevation of the ratio of hydroxynortriptyline to nortriptyline reported by Bertilsson *et al.* (1979) in older subjects.

**Desipramine**

In an initial series of studies of concentrations of active forms of tricyclic antidepressant following drug treatment with desipramine (Kitanaka *et al.*, 1982), we found elevated plasma concentrations of the 2-hydroxy metabolite of desipramine in a group of elderly depressed women as compared to younger patients and healthy volunteers. We also observed a marked increase in the ratio of hydroxydesipramine to desipramine in the elderly as reported for the hydroxynortriptyline-to-nortriptyline ratio. This finding was associated with a markedly diminished renal clearance of hydroxydesipramine in the older patients (figure 2). In this study, the fraction of hydroxydesipramine excreted unchanged was not calculated so that it is not possible to use equation (3) to calculate the predicted increase in the plasma concentration of hydroxydesipramine given the observed decrease in renal clearance.

Recently, we have explored the issue of the renal clearance of 2-hydroxydesipramine more fully in a prospective study. The preliminary results from this study are summarized here.

There were 27 subjects of whom 14 were female ranging in age from 20 to 58 years (mean of 36.4 ± 13.3 years). All but two were pre-menopausal. The remaining 13 male subjects ranged in age from 29 to 55 years, with a mean of 42.3 ± 8.4 years. Subjects revealed no medical or psychiatric abnormalities by history, physical examination or blood tests (including liver chemistry), and took no routine medication. None used alcohol on a daily basis. Two males were smokers, one smoking a pipe and the other smoking cigars.

A five-day protocol was conducted on an outpatient basis. On the first day, each subject took an oral dose of 100 mg desipramine after an overnight fast. Venous blood samples for desipramine

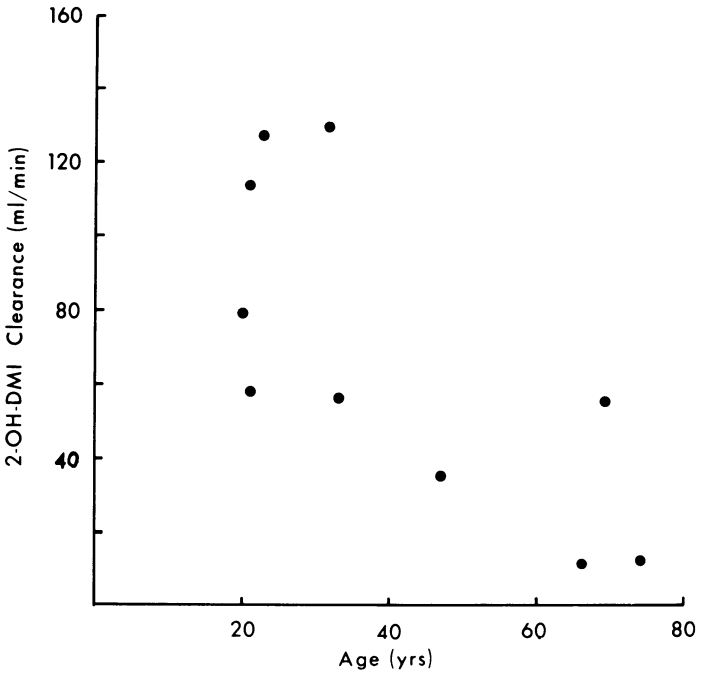


**Figure 2.** Decreased renal clearance of 2-hydroxydesipramine with age ($r$ = -0.73, $p < 0.05$) for young normal volunteers and young and old depressed patients (from Kitanaka *et al.*, 1982).

and 2-hydroxydesipramine concentration were drawn just before and at intervals up to 96 h after the dose. Disposable plastic syringes and heparinized polypropylene tubes were used instead of Vacutainers®. Serial 24 h urine collections were performed by all subjects for five days beginning with the time of dosage.

Plasma and urinary concentrations of desipramine and 2-hydroxydesipramine were determined by the high-performance liquid chromatography assay of Sutfin and Jusko (1979) with previously described modifications (Kitanaka *et al.*, 1982). Urine samples were assayed both before and after overnight incubation with glucuronidase (pH 4.5) at 37°C.

Renal clearance of hydroxydesipramine was calculated as the total amount of this metabolite excreted unchanged in urine divided by the total AUC hydroxydesipramine in plasma. For the first 27 subjects studied, mean clearance was 3.92 ± 1.14 ml $min^{-1}$ $kg^{-1}$ with a range of 1.68 to 6.49 ml $min^{-1}$ $kg^{-1}$. There was a significant negative correlation ($r$ = -0.56, $p < 0.005$) between age and hydroxydesipramine renal clearance for the group as a whole, with

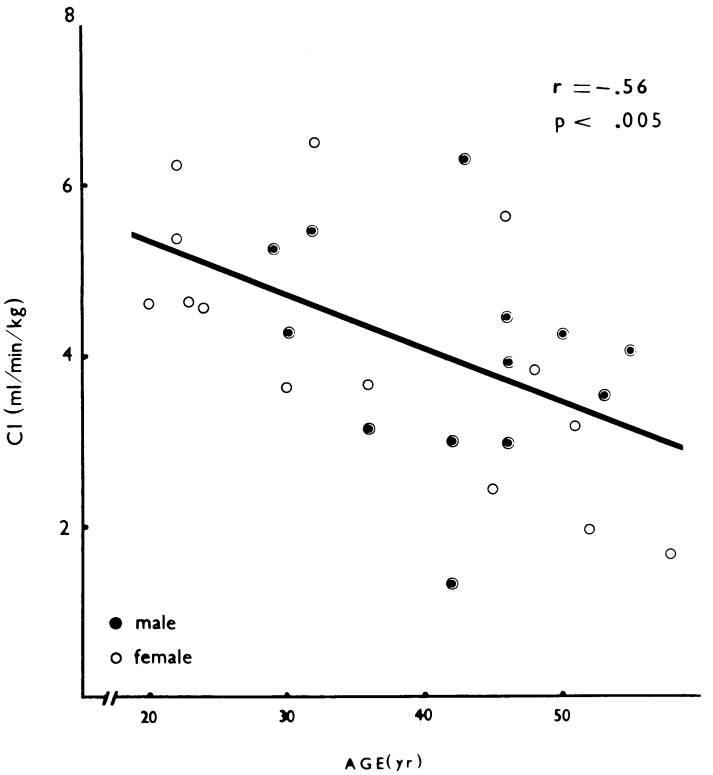


**Figure 3.** Decreased renal clearance with age for a group of 27 normal volunteers. Open symbols = women; solid symbols = men ($r$ = -0.56, $p$ < 0.005).

a pronounced sex difference (figure 3). The 14 female subjects, ranging in age from 20 to 58 years, showed a more marked correlation ($r$ = -0.69, $p$ < 0.01) than did the 13 males (29 to 55 years of age) whose age-clearance association failed to reach significance ($r$ = -0.30).

Assuming that hydroxydesipramine is eliminated in urine only in the unchanged form or conjugated to glucuronide, the fraction of hydroxydesipramine eliminated unchanged in urine may be taken as the amount of hydroxydesipramine excreted unchanged divided by the sum of unchanged plus glucuronidated hydroxydesipramine assayed in urine. By this method, the fraction of hydroxy metabolite of desipramine excreted unchanged is 0.32 ± 0.09 in 21 subjects, slightly lower than has been reported for the 10-hydroxy metabolite of nortriptyline (Alexanderson and Borgå, 1973; Mellström *et al.*, 1981) (table 2). Given this range of the frac-

Table 2. Fraction of hydroxy metabolite excreted unconjugated (single dose)

| | | | Reference |
|---|---|---|---|
| 10-OH-NT | 0.47 ± 0.07 | ($N$ = 6) | Alexanderson and Borgå (1973) |
| | 0.42 ± 0.12 | ($N$ = 8) | Mellström *et al.*(1981) |
| 2-OH-DMI | 0.32 ± 0.09 | ($N$ = 21) | This work |

tion of hydroxydesipramine which is excreted unchanged (up to 50%), a one-half decrease in the average renal clearance of hydroxydesipramine over the 20-58 years age range should correspond to a 12-35% increase in the single dose or steady-state AUC of hydroxydesipramine.

In these subjects, the AUC of hydroxydesipramine did tend to increase with age. Significantly more of the subjects aged 42-58 years had a metabolite AUC greater than 600 ng $ml^{-1}$ h compared with the subjects aged 20-32 years ($\chi^2$, $p < 0.05$). The median hydroxydesipramine AUC for the older group was 768 ng $ml^{-1}$ h (range 438 to 1634) and for the younger group was 565 ng $ml^{-1}$ h (range 408 to 909), representing an average increase of 36% which is at the upper end of our prediction based on an average 50% reduction in hydroxydesipramine renal clearance.

The fraction of desipramine metabolized to hydroxydesipramine (*fm*) was obtained by summing the total amounts of unbound plus conjugated hydroxydesipramine excreted in the urine and dividing by the dose of desipramine. In 25 individuals, the mean *fm* was 0.38 ± 0.09, similar to that reported for nortriptyline (Alexanderson and Borgå, 1973; Mellström *et al.*, 1981) (table 3).

Therefore, the fraction of desipramine metabolized to hydroxydesipramine and, thereby, the hydroxydesipramine AUC is also sensitive to variability in the hydroxylation clearance of desipramine. Nevertheless, for this group of subjects, the fraction metabolized was apparently independent of hydroxylation

Table 3. Fraction of drug metabolized to hydroxy metabolite (single dose)

| | | | | Reference |
|---|---|---|---|---|
| NT | 10-OH-NT | 0.49 ± 0.03 | ($N$ = 6) | Alexanderson and Borgå (1973) |
| | | 0.43 ± 0.18 | ($N$ = 8) | Mellström *et al.*(1981) |
| DMI | 2-OH-DMI | 0.38 ± 0.09 | ($N$ = 25) | This work |

clearance, despite an eightfold range of hydroxylation clearance. This apparent independence is similar to that seen by Mellström *et al.* (1981) for the AUC of hydroxynortriptyline after nortriptyline.

Our findings that a large amount of unconjugated hydroxydesipramine is excreted unchanged and that there is substantial renal clearance of hydroxydesipramine add to the small, but similar, literature for other antidepressant or cardiovascular compounds. It was originally shown by Alexanderson and Borgå (1973) that the renal clearance of hydroxynortriptyline was over 100 ml $min^{-1}$. A mean renal clearance of 80.5 ml $min^{-1}$ was reported for the same metabolite by Kragh-Sørensen *et al.* (1977). It was therefore expected (Potter *et al.*, 1981) that the renal clearance of these hydroxy metabolites would be affected by processes which alter kidney function such as aging (Vestal, 1978). Thus, as mentioned above, there is a decreased renal clearance of hydroxydesipramine in elderly depressed patients accompanied by an increase in the plasma hydroxydesipramine-to-desipramine ratio at steady state (Kitanaka *et al.*, 1982).

This was interpreted to mean that the rate of desipramine hydroxylation was not altered since desipramine concentrations were the same in the elderly as in younger populations. Also, as noted above, preliminary results from our subsequent, more detailed, investigation of single-dose desipramine pharmacokinetics support this interpretation although the age range was more limited. Higher plasma concentrations of the hydroxy metabolite of metoprolol, a beta-receptor antagonist used in cardiovascular disease, have also been reported in elderly versus young volunteers (Lundborg *et al.*, 1982), a finding that has likewise been ascribed to the lowering of glomerular filtration rate (GFR) with age.

Little systematic data in this regard have been collected on subjects with other sources of decreased excretory function such as renal disease. Those which are available, however, are consistent with the results seen in healthy aging individuals. Renal clearance of hydroxymetoprolol correlated directly with GFR in four patients with impaired renal function (Hoffmann *et al.*, 1980). In an initial study of 20 patients with chronic renal failure who were administered a single dose of nortriptyline, Dawling *et al.* (1981) found no changes in parent drug clearance or half-life in patients as compared to that in controls given the same dose. They did speculate that hydroxy metabolites of nortriptyline might accumulate during renal insufficiency and proceeded to demonstrate subsequently this phenomenon in some of these same patients (Braithwaite and Dawling, 1981). For a given dose of nortriptyline, mean plasma concentrations of unconjugated and, more markedly yet, of conjugated 10-hydroxynortriptyline, were higher in the renal patients than in healthy volunteers.

This finding is consistent with the pharmacokinetic model (equation (3)) since the clearance of conjugated 10-hydroxynortriptyline may be assumed to be dependent on excretion alone and not on further metabolism. Of course, a certain fraction of the conjugate could be excreted via bile as well as urine.

Returning to the plasma pharmacokinetics of biologically active metabolites, there is another detail to be considered and that is related to plasma binding. In the calculation of urinary clearances of hydroxy metabolites as described above, or as reported in the literature by dividing the excretion rate by the mean steady-state concentration of total (bound plus free) drug in plasma (Alexanderson and Borgå, 1973; Kragh-Sørensen *et al.*, 1977; Kitanaka *et al.*, 1982), allowance is not made for the fact that bound drug does not diffuse through the glomerulus. Use of free drug concentrations (at most 20% of total plasma hydroxy metabolite concentration (Potter and Calil, 1981)) in the denominator when computing clearance will yield at least a fivefold increase in calculated renal clearance. The resulting value of greater than 500 ml $min^{-1}$ greatly exceeds GFR, indicating active secretion of unconjugated hydroxy tricyclic metabolite. Thus, factors interfering with active secretion, independent of age or renal failure, might also be expected to increase concentration of OH tricyclics selectively. Such possible specific effects on active secretion have not yet been investigated.

These observations also remind one that changes in the plasma binding of hydroxy metabolites can also affect their renal clearance. As described in the general case by Levy (1980), assuming that renal tubular secretion is a function of the free plasma concentration of compound and that secretion is not limited by the flow rate of plasma perfusing the renal tubules, then the renal clearance of total drug will be linearly and directly related to the free fraction of a compound. Although the free fraction of at least tertiary and secondary amine TCAs does not generally cover more than a twofold range (Potter *et al.*, 1981), there are sometimes extremes. Again, there is no direct evidence that this mechanism plays a significant role in the variability of the renal clearance of hydroxy metabolites of TCAs. One situation that might be expected to produce altered binding of some drugs, uremia, appears to have no effect on that of desipramine (Reidenberg *et al.*, 1971) or the structurally similar tetracyclic antidepressant, maprotiline (Lynn *et al.*, 1981) and may likewise have no effect on the binding of hydroxydesipramine. It will be interesting to see if patients with elevated $\alpha_1$-acid glycoprotein who show increased binding of parent TCAs (Piafsky *et al.*, 1978) will also have a reduced free fraction of the active hydroxy metabolites. If so, one would predict decreased renal clearance of the hydroxy metabolite and a selective increase in its plasma concentration.

## CONCLUSIONS

In summary, there are active hydroxy metabolites of TCAs which are at least partially dependent on renal excretion for their elimination. Multiple factors can influence the renal clearance of these active metabolites both at the level of glomerular filtration and active secretion. In real-life situations, however, the less than 50% fraction of hydroxy TCA metabolites excreted unchanged in most individuals limits the possible consequences of major alterations in kidney function. At most, one is likely to find a doubling of the unconjugated hydroxy TCA metabolite steady-state concentration secondary to reduced renal clearance. Such an increase would be likely to have significant pharmacodynamic effects only for individuals in whom the hydroxy represents a large proportion of total active compound. One would, therefore, predict that alterations in urinary clearance might have the greatest functional significance in patients treated with nortriptyline or amitriptyline since imipramine, desipramine and chlorimipramine tend to produce relatively low steady-state concentrations of conjugated hydroxy metabolites.

More generally, this paper has demonstrated how the application of pharmacokinetic theory and relatively simple pharmacokinetic models can produce pharmacodynamically relevant information concerning the expected limits of effects. The clinical psychopharmacological literature contains many instances of undue emphasis being placed on factors that might influence pharmacokinetic behavior of parent drug or metabolite. Here, we have not only identified a number of variables but attempted to assess their likely relevance to real-life situations by pharmacokinetically appropriate prospective study design and reanalysis of earlier findings.

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# Stereospecific 10-hydroxylation of nortriptyline - genetic aspects and importance for biochemical and clinical effects

Leif Bertilsson[1], Britt Mellström[1], Conny Nordin[2], Bo Siwers[2] and Folke Sjöqvist[1]

## INTRODUCTION

The plasma level of the tricyclic antidepressant nortriptyline is an important determinant for its clinical effects (cf. Sjöqvist *et al.*, 1980). The therapeutic effect is related to the plasma level in a curvilinear manner, with poor outcome both at low and very high plasma concentrations (Asberg *et al.*, 1971). There are two main reasons why nortriptyline has become one of the most thoroughly investigated antidepressants: (a) analytical methods were developed very early for measurement of low plasma levels of this secondary amine, and (b) it was initially thought that nortriptyline had no active metabolites (in contrast to the tertiary amines amitriptyline and imipramine). It was later shown that the major metabolite of nortriptyline, i.e. 10-hydroxy-nortriptyline, is almost as potent as the parent drug in inhibiting the uptake of norepinephrine (NE) in rat brain slices (Bertilsson *et al.*, 1979). The *E*- and *Z*-10-OH-nortriptyline isomers (figure 1) were equipotent in this respect. It was also shown that the plasma levels of 10-OH-nortriptyline often exceeded those of the parent drug during nortriptyline treatment. Levels of 10-OH-nortriptyline and nortriptyline in CSF were comparable, showing that the hydroxy metabolites pass into the central nervous system (*loc. cit*).

We are now reporting on the regulation of the nortriptyline hydroxylation in healthy subjects and in patients treated for depression as well as preliminary observations on the interrelationships between the pharmacokinetics of nortriptyline and 10-OH-nortriptyline, and biochemical and clinical effects.

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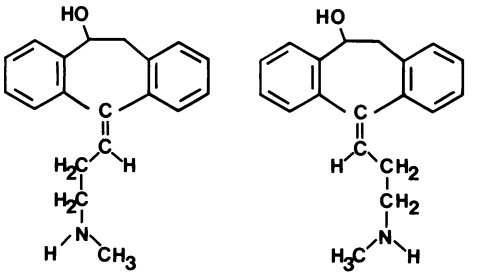


**Figure 1.** Chemical formulas of the isomeric *E*- and *Z*-10-OH-nortriptyline.

## GENETIC REGULATION OF THE 10-HYDROXYLATION OF NORTRIPTYLINE

Alexanderson *et al.* (1969) showed that the steady-state plasma levels of nortriptyline were very similar within pairs of monozygotic twins. In dizygotic twins and in monozygotic twins treated with some other drugs, e.g. barbiturates, the levels of nortriptyline could be quite different within pairs. As the plasma level of nortriptyline is dependent to a major extent on the rate of 10-hydroxylation (Alexanderson and Borgå, 1973), it can be concluded that this metabolic pathway is genetically controlled, but may be affected by concomitant drug treatment.

During recent years polymorphic 4-hydroxylation of debrisoquine (Mahgoub *et al.*, 1977) and *N*-oxidation of sparteine (Eichelbaum *et al.*, 1979) have been demonstrated. We later showed that these two polymorphic oxidations are regulated by similar genetic factors and possibly the same subunit of cytochrome P-450 is involved in both metabolic reactions (Eichelbaum *et al.*, 1982). We have also compared the polymorphic oxidation of debrisoquine and sparteine with the metabolism of nortriptyline using crossover studies in healthy volunteers. There was a strong correlation between the debrisoquine metabolic ratio (debrisoquine/4-hydroxy-debrisoquine in urine during 6 h following a single 10 mg dose of debrisoquine) and the total plasma clearance of nortriptyline after a single dose (Spearman rank coefficient of correlation $r_s$ = - 0.83; $n$ = 8; $p$ = 0.01) (Bertilsson *et al.*, 1980a). By measuring the excretion of nortriptyline metabolites in urine, it was shown that the metabolic clearance of nortriptyline by hydroxylation in the *E*-position, but not in the *Z*-position, correlated even better to the debrisoquine metabolic ratio ($r_S$ = - 0.88; $p < 0.01$) (Mellström *et al.*, 1981). This implies that common enzymatic mechanisms are involved in the hydroxylation of debrisoquine and in the *E*-10-hydroxylation of nortriptyline. The strong correlation ($r_S$ = 0.96) between the total plasma clearance of nortriptyline

and the metabolic clearance by *E*-10-hydroxylation shows that this metabolic reaction is important for the disposition of the drug (Mellström *et al.*, 1981). The *Z*-isomer is a minor metabolite compared to *E*-10-OH-nortriptyline and constitutes only 5-22% of total 10-OH-nortriptyline.

In the clinical study that will be discussed below, patients who had been treated with nortriptyline for depression for at least three weeks were phenotyped with debrisoquine *after* the treatment period. As seen in figure 2 there was a significant correlation ($r_S$ = 0.71; $n$ = 8; $p < 0.05$) between the steady-state plasma level of nortriptyline (per dose unit) and the debrisoquine metabolic ratio. The plasma levels of unconjugated or total (unconjugated plus conjugated) 10-OH-nortriptyline were not significantly related to the debrisoquine metabolic ratio ($r_S$ = -0.36, $n$ = 8; and $r_S$ = - 0.14, $n$ = 7). While plasma levels of nortriptyline are dependent on the rate of hydroxylation, the levels of 10-OH-nortriptyline are determined by its rate of formation, its rate of conjugation and its renal excretion. In a similar study (Bertilsson and Aberg-Wistedt, 1983), it was shown that the steady-state plasma level of desipramine (which is hydroxylated in the aromatic 2-position) and the debrisoquine metabolic ratio correlated significantly ($r_S$ = 0.92; $n$ = 10; $p < 0.01$). These results imply that (a) the hydroxylation of debrisoquine, desipramine and nortriptyline may be mediated by the same isoenzyme of the cytochrome P-450 system and (b) the debrisoquine phenotyping test predicts plasma levels of both desipramine and nortriptyline fairly well. Alexanderson (1972) has previously shown in a crossover study that the metabolism of these two tricyclic antidepressants correlate strongly within individuals.

## TREATMENT OF DEPRESSION WITH NORTRIPTYLINE

In an ongoing investigation 16 depressed patients have been treated with 50 mg nortriptyline t.i.d. (25 mg b.i.d. in one old patient) for at least 3 weeks after 4-7 days of placebo treatment. The severity of depression was rated with a subscale of the Comprehensive Psychopathological Rating Scale (CPRS) (Asberg *et al.*, 1978) once a week. Lumbar punctures were performed before and after 3 weeks of nortriptyline treatment. The punctures were performed using a standardized technique (Nordin *et al.*, 1982) early in the morning after at least 8 h of bed rest. Twelve milliliters were drawn with the needle between the fourth and fifth lumbar vertebrae. The levels of 5-HIAA, HVA and MHPG in CSF were determined by mass fragmentography (Bertilsson, 1981). Plasma was drawn once a week and several times during a dosage interval after 3 weeks of treatment. Nortriptyline and 10-OH-nortriptyline in plasma and CSF were analyzed by mass fragmentography (Borgå *et al.*, 1972).

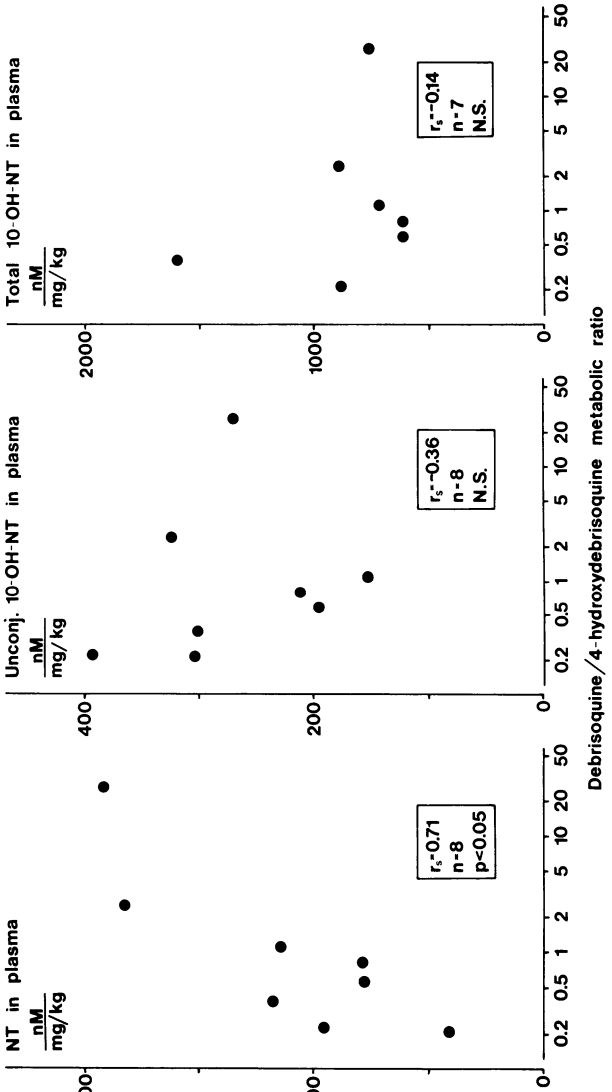


**Figure 2.** Relationships between the steady-state plasma levels of the parent drug nortriptyline (NT) unconjugated 10-OH-nortriptyline (10-OH-NT) and total (unconjugated plus conjugated) 10-OH-nortriptyline and the debrisoquine metabolic ratio (logarithmic scale). Nortriptyline and its metabolites were measured by mass fragmentography and debrisoquine and its 4-hydroxy metabolite were measured by gas chromatography.

## Plasma and CSF Levels of Nortriptyline and 10-OH-Nortriptyline

As previously shown (Bertilsson *et al.*, 1979), the plasma levels of 10-OH-nortriptyline were high in some patients and low in others during treatment of depression with nortriptyline. There was, however, little variation in the plasma levels of both nortriptyline and 10-OH-nortriptyline during a dosage interval as illustrated in two patients in figure 3. This was true in patients with both a high (patient 9) and a low (patient 10) ratio of nortriptyline and 10-OH-nortriptyline (figure 3).

In 15 patients the ratio between the concentrations in CSF and plasma was similar for nortriptyline (10.4 ± 2.0%) and unconjugated 10-OH-nortriptyline (11.5 ± 2.4%). There was a strong correlation between the CSF and plasma levels for both nortriptyline ($r$ = 0.93; $p < 0.001$) and 10-OH-nortriptyline ($r$ = 0.71; $p < 0.01$) (figure 4). Evidently both plasma and CSF concentrations are of a similar order of magnitude for 10-OH-nortriptyline and nortriptyline. The inter-individual variation in plasma protein binding for both nortriptyline and 10-OH-nortriptyline is small and thus the total plasma concentrations should reflect the concentrations at receptor sites.

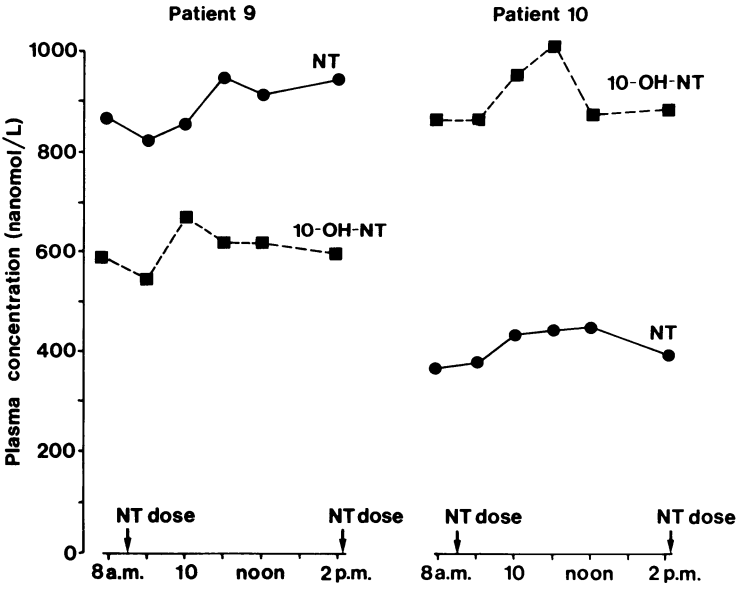


Figure 3. Plasma levels of nortriptyline (NT) and 10-OH-nortriptyline (10-OH-NT) (unconjugated) during a dosage interval in two patients treated with 50 mg nortriptyline t.i.d.

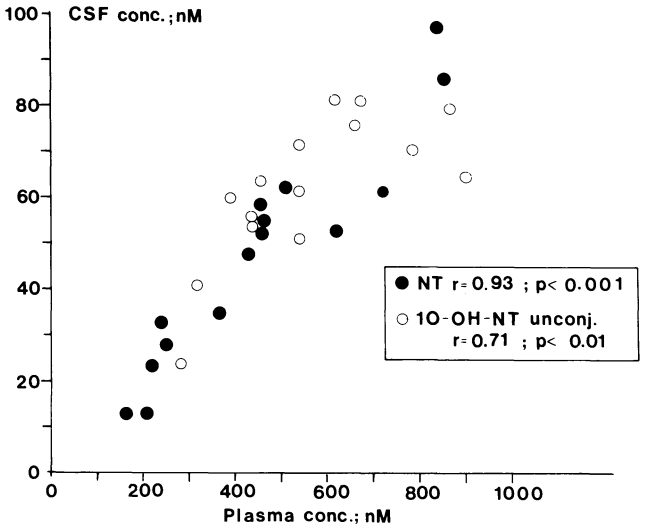


**Figure 4.** CSF and plasma levels of nortriptyline (NT) and 10-OH-nortriptyline (10-OH-NT) (unconjugated) in 15 patients treated with nortriptyline for 3 weeks.

## Biochemical and Clinical Effects of Nortriptyline Treatment

Both nortriptyline and 10-OH-nortriptyline are potent inhibitors of NE uptake with little effect on serotonin (5-HT) uptake (Bertilsson *et al.*, 1979). After 3 weeks of nortriptyline treatment of 15 depressed patients the CSF level of the NE metabolite decreased by 36.3% ($p < 0.001$) (table 1). The effect on the 5-HT metabolite 5-HIAA was less pronounced (-19.7%; $p < 0.01$) and there was no effect on the dopamine metabolite HVA (table 1). This confirms the marked effect of nortriptyline treatment on noradrenergic neurons (Bertilsson *et al.*, 1974). Treatment with the potent inhibitors of 5-HT uptake, chlorimipramine and zimelidine, have a more pronounced effect on 5-HIAA than on MHPG (Bertilsson *et al.*, 1974, 1980b).

In two consecutive studies (Asberg *et al.*, 1973, 1976), it has been shown that the levels of 5-HIAA in CSF are bimodally distributed in a group of untreated depressed patients. This indicates that endogenous depression is biochemically heterogeneous. The relatively selective NE uptake inhibitor nortriptyline seemed to have a more favorable effect in patients with a pre-treatment 5-HIAA level in CSF above 15 $\mu g\ l^{-1}$ than in patients with lower CSF 5-HIAA (Asberg *et al.*, 1973). A working hypothesis was formulated that patients with a low 5-HIAA in CSF have a disturbance in serotonergic neurons while patients with higher levels have disturbed noradrenergic functions. Studies with

Table 1. Amine metabolite levels in CSF before and during nortriptyline treatment in 15 patients (mean ± S.D.; nmol $l^{-1}$)

| Amine metabolite | Before treatment | During treatment | Change (%) | Significance |
|---|---|---|---|---|
| 5-HIAA | 92.3 ± 42.0 | 74.1 ± 35.0 | -19.7 | $p < 0.01$ |
| HVA | 201 ± 123 | 183 ± 125 | - 9.0 | n.s. |
| MHPG | 49.4 ± 10.6 | 31.4 ± 5.4 | -36.3 | $p < 0.001$ |

chlorimipramine (Träskman *et al.*, 1979), desipramine and zimelidine (Aberg-Wistedt *et al.*, 1982) have given partial support for this hypothesis.

In the present study where 16 depressed patients were treated with nortriptyline, the severity of depression after 3 weeks of treatment was significantly ($p < 0.01$) higher in the patients with a pre-treatment CSF 5-HIAA below 15 μg $l^{-1}$ (mean CPRS score 11.7 ± S.D. 3.6; $n = 6$) than in patients with a higher pre-treatment 5-HIAA (CPRS score 5.4 ± 3.9; $n = 10$). No definite conclusion should, however, be drawn from this as the former group was more

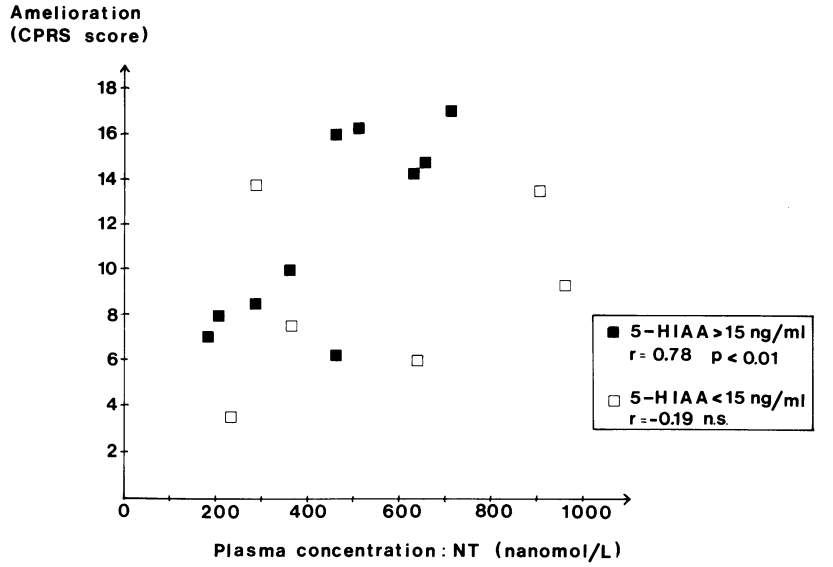


**Figure 5.** Relationship between the amelioration of depression and plasma concentration of nortriptyline (NT) after 3 weeks of treatment of 16 patients. The patients have been subgrouped according to the pre-treatment CSF level of 5-HIAA.

depressed (CPRS score 20.2 ± 2.1) than the latter group (CPRS score 16.6 ± 2.1) before treatment ($p < 0.01$).

In this relatively small sample of 16 patients there was no linear (or curvilinear) relationship between the amelioration and plasma level of nortriptyline (figure 5). However, among the 10 patients with a pre-treatment 5-HIAA above 15 ng $ml^{-1}$ there was a positive correlation ($r = 0.78$; $p < 0.01$) (figure 5). An even stronger relationship emerged when the amelioration was correlated with the plasma concentration of nortriptyline plus 0.57 x (10-OH-nortriptyline) ($r = 0.84$; $p < 0.01$). The factor 0.57 was the relative potency of 10-OH-nortriptyline and nortriptyline found previously in studies *in vitro* (Bertilsson *et al.*, 1979). The absence of a curvilinear relationship may be due to the fact that none of the patients with a high 5-HIAA had high levels of nortriptyline and/or 10-OH-nortriptyline in plasma. These preliminary results indicate that nortriptyline treatment has a positive effect in patients with high pre-treatment levels of 5-HIAA in CSF and that both nortriptyline and 10-OH-nortriptyline may contribute to this effect.

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# The fate of amitriptyline and its metabolites, taking into account their binding in plasma

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## INTRODUCTION

At the last meeting in this series, it became clear that the pharmacological activity of the hydroxylated metabolites ought to be considered in future clinical studies on antidepressants (Potter, 1981). Some of the genetic aspects of the metabolism of these antidepressants have been reviewed by Bertilsson *et al.* (1981).

The penetration of drugs into tissue for metabolism and for pharmacological action partly depends on their binding to plasma proteins and blood cells. Indeed, these blood constituents may act as vehicles and, thus, facilitate elimination by metabolism or through the kidney.

Saliva may be considered to be an ultrafiltrate of blood in those cases where drugs are not actively transported. The analysis of drugs in saliva therefore represents a useful tool for studying their binding to blood constituents.

The present study deals with some of these aspects of the clinical pharmacokinetics of antidepressants.

## SALIVA - AND FREE PLASMA CONCENTRATION OF TRICYCLIC ANTIDEPRESSANTS

The analysis of psychotropic drugs in saliva seems not to be a tool of clinical interest. Actually, as demonstrated with lithium,

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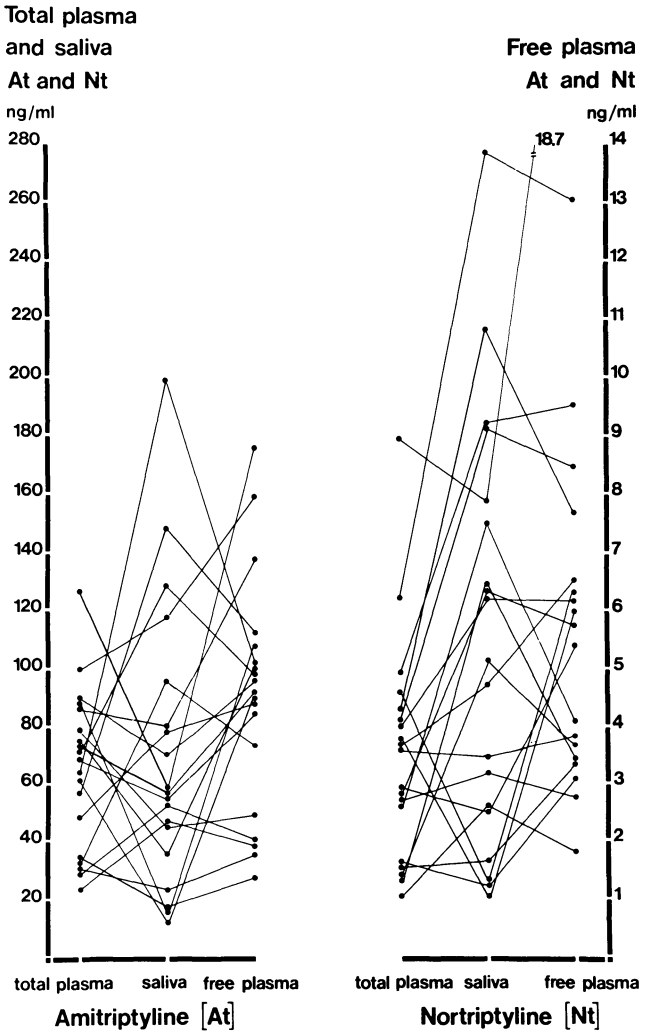


**Figure 1.** Distribution of amitriptyline (At) and nortriptyline (Nt) in total plasma, plasma dialyzate and saliva of 19 patients treated with At (reproduced from Baumann *et al.* 1982a, with permission of Karger AG, Basel).

patients generally prefer blood samplings to collection of saliva. Besides, the strong anticholinergic action of some classic tricyclics seriously hinders the collection of saliva for analytical purposes. Nevertheless, psychotropic drugs such as amitriptyline and nortriptyline probably reach saliva by diffusion, and measure-

Table 1. Calculation of the coefficients of correlation (Pearson) between values of amitriptyline (AT) and nortriptyline (NT), respectively, in plasma and saliva of 19 patients

| Parameters | Coefficient of correlation | Significance |
|---|---|---|
| Free AT vs total AT | 0.8499 | $p \leqslant 0.001$ |
| Free NT vs total NT | 0.9451 | $p \leqslant 0.001$ |
| Saliva AT vs total AT | 0.1124 | n.s |
| Saliva NT vs total NT | 0.5121 | $p \leqslant 0.05$ |
| Saliva AT vs free AT | 0.3813 | n.s. |
| Saliva NT vs free NT | 0.6164 | $p \leqslant 0.01$ |
| Saliva AT/NT vs total AT/NT | 0.8114 | $p \leqslant 0.001$ |
| Saliva AT/NT vs free AT/NT | 0.8119 | $p \leqslant 0.001$ |
| Free AT/NT vs total AT/NT | 0.9232 | $p \leqslant 0.001$ |
| log(free AT) vs log(saliva AT) | 0.4479 | n.s. |
| log(free NT) vs log(saliva NT) | 0.5395 | $p \leqslant 0.05$ |

ment of these drugs in this body fluid may give useful information about the relative importance of plasma protein binding for the diffusion process of the drugs. Two reports deal with concentration of amitriptyline (Jeffrey and Turner, 1978) and nortriptyline (Kragh-Sørensen and Larsen, 1980) in plasma and saliva, but neither of these studies takes the free plasma levels into account. Jeffrey and Turner (1978) found a significant correlation between the logarithmic drug concentrations of total plasma and saliva, whereas Kragh-Sørensen and Larsen (1980) found that the saliva/plasma ratio varied from 2 to 4, intra- and inter-individually.

Baumann *et al.* (1982a) observed in 19 amitriptyline-treated patients that in saliva amitriptyline and nortriptyline varied between 20 and 300% of their respective total plasma levels (figure 1). Free plasma levels were 2-30% of the concentrations measured in saliva, and salivary levels of amitriptyline and nortriptyline thus poorly reflect the respective free or total plasma levels. The calculated coefficients of correlation do not encourage drug monitoring in saliva for clinical use (table 1). On the other hand, a highly significant correlation existed between free and total plasma drug levels. The relationships observed for the ratios amitriptyline/nortriptyline between the different compartments allow us to presume a common mechanism for the transport of amitriptyline and nortriptyline into saliva. Indeed, both compounds are weak bases with $pK_a$ values of 9.85 and 10.8 respectively (Maître *et al.*, 1980). As such, their diffusion into saliva depends on its pH, varying between 5.45 and 7.8 (Mucklow *et al.*, 1978). According to these authors and also

Danhof and Breimer (1978), saliva levels of weak bases depend not only on the pH of saliva, but also on the binding of the drug to plasma proteins:

$$\frac{C_s}{C_p} = \frac{1 + 10^{(pK_a - pH_s)}}{1 + 10^{(pK_a - pH_p)}} \times \frac{f_p}{f_s} \qquad (1)$$

where $C_s$, $C_p$ = concentration of drug in saliva and in plasma, respectively, $pH_s$, $pH_p$ = saliva and plasma pH, respectively, $f_s$, $f_p$ = fraction of drug unbound in saliva and in plasma, respectively, and (free $C_p$) = unbound concentration of drug in plasma.

As $pH_p = 7.4$, $f_s = 1$, $f_p = (\text{free } C_p)/C_p$, $pH_s < 7.8$, $pK_a > 9$, then

$$1 + 10^{(pK_a - pH_s)} \simeq 10^{(pK_a - pH_s)}$$
$$1 + 10^{(pK_a - pH_p)} \simeq 10^{(pK_a - pH_p)}$$

and so

$$C_s = \frac{10^{(pK_a - pH_s)}}{10^{(pK_a - pH_p)}} \times (\text{free } C_p)$$

This equation yields

$$\log C_s = - pH_s + 7.4 + \log(\text{free } C_p)$$

This equation clearly demonstrates that salivary levels of amitriptyline and nortriptyline depend on free plasma levels, and salivary pH. Salivation must be stimulated to get a pH 7.4, at which free plasma level would be equal to that found in saliva. Indeed, most often salivary pH is slightly acidic (5.4-6.8) and thus would lead to an 'extraction' of amitriptyline and nortriptyline from plasma. This mechanism explains why saliva levels are quite similar to total plasma levels (Jeffrey and Turner, 1978). From a theoretical point of view, the increase of saliva pH by one unit reduces the concentration of the drugs in saliva by a factor of 10. When the pH of saliva is calculated from experimental data obtained from amitriptyline and nortriptyline separately, good concordance was found (Baumann *et al.*, 1982a). However, it was not clear whether the calculated pH values reflected the actual pH values. Probably, some other factors which interfere with this model have to be considered: deposit of the drugs on the buccal mucosa or in the salivary ducts and unspecific binding to macromolecules in saliva could thus lead to erroneous results. The salivary flow rate may be a determinant for the accumulation of drug in saliva through an increase in its pH. On the other hand, the question remains open whether differences exist in the secretion of the drugs in saliva of parotic or submandibular origin. It is also important to carry out the collection of samples in the post-absorption phase, when there are no differences in the concentrations of the drugs between venous and arterial blood (Posti, 1982).

These points underline the difficulties encountered in introducing clinical drug monitoring in saliva for weak bases like tricyclics or neuroleptics.

The hypothesis that the concentration of drug available for the active site depends on free rather than total plasma levels has led to numerous *in vitro* studies concerning the binding of amitriptyline and nortriptyline to plasma proteins (Borgå *et al.*, 1969; Alexanderson and Borgå, 1972; Brinkschulte and Breyer-Pfaff, 1980; Burch *et al.*, 1981). Using another approach, Baumann *et al.* (1982a) studied the binding of 'endogenous' amitriptyline and nortriptyline in patients treated with clinical doses of amitriptyline. Results from these investigations showed an inter-individual variation in binding with a factor of about 2 in untreated subjects without organic disease. The binding may be subject to even greater variability under the influence of competing drugs or in physical illness.

The preliminary results of this study, in which 17 patients already have been studied, show that the mean amitriptyline concentrations in total plasma are slightly higher than the corre-

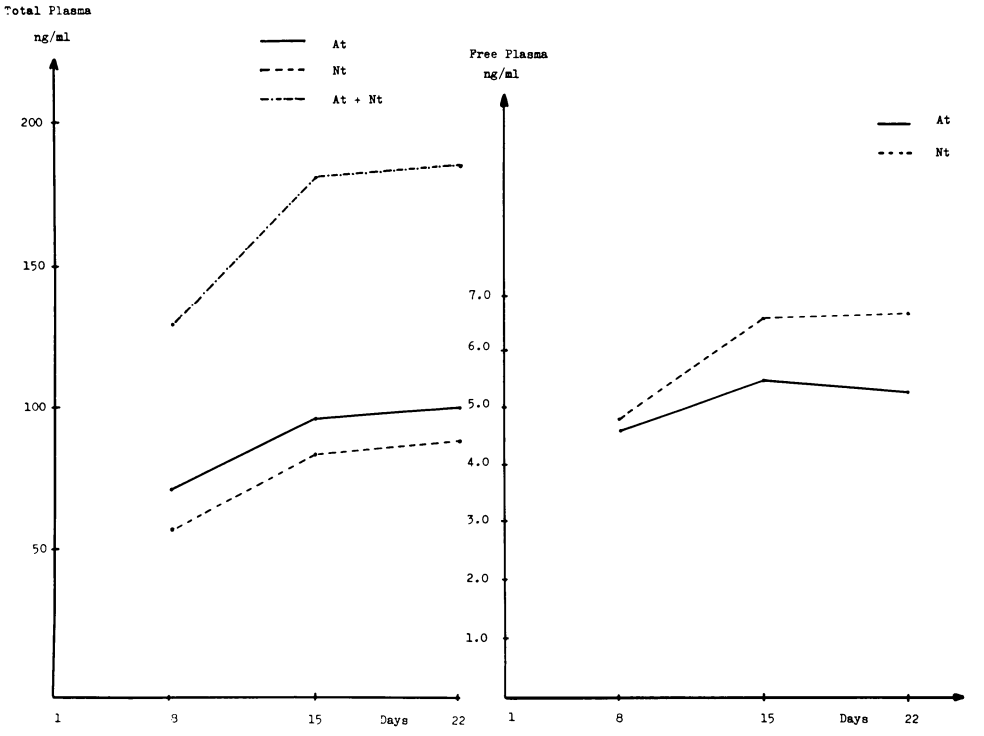


**Figure 2.** Total and free plasma amitriptyline (At) and nortriptyline (Nt) in 17 patients.

sponding nortriptyline levels, while the inverse is true for free plasma levels (figure 2). This confirms earlier *in vitro* studies showing that nortriptyline is bound to plasma proteins to a lesser extent than amitriptyline.

## STEADY-STATE LEVELS OF HYDROXYLATED METABOLITES

The hydroxylated metabolites of tricyclic antidepressants have gained interest, since it was shown that they exert a central effect in inhibiting the uptake of norepinephrine (Bertilsson *et al.*, 1979), and that the concentration of unconjugated hydroxylated metabolites in plasma may exceed that of the parent compounds (Alvan *et al.*, 1977; Garland *et al.*, 1979; for review, see Potter, 1981). These polar metabolites are probably bound to plasma proteins to a lesser extent than are the parent compounds, and consequently their pharmacological availability in plasma is higher.

Figure 3 presents various concentrations of amitriptyline and its metabolites in a patient treated for three weeks with 150 mg amitriptyline (Laroxyl). The amitriptyline level in plasma exceeded that of hydroxy-amitriptyline by a factor of 3, while the inverse relationship was noticed for nortriptyline and hydroxy-nortriptyline. However, the free plasma concentration of OH-amitriptyline in this case was slightly higher than the free concentration of the parent compound. Very striking is the fact that, on day 22, the free nortriptyline concentration was only 4.5 $\mu g\ l^{-1}$, but that of OH-nortriptyline reached a level of 42 $\mu g\ l^{-1}$. In this patient, free OH-amitriptyline and OH-nortriptyline were 25-40% of their corresponding total plasma concentration. In saliva, the hydroxy metabolite levels were higher than amitriptyline and nortriptyline in plasma, respectively. This finding demonstrates the relevance of free plasma drug levels for diffusional processes.

The phenotype of hydroxylation is one of the principal factors responsible for the inter-individual variation of the metabolism of the drugs in the liver. In the field of antidepressant drugs, its role has been clearly demonstrated for nortriptyline (Bertilsson *et al.*, 1980; Mellström *et al.*, 1981) and recently also for amitriptyline (Balant-Gorgia *et al.*, 1982). The patient presented in figure 3 did not show abnormal hydroxylation.

These preliminary results confirm the necessity of investigations including hydroxylated metabolites and protein binding studies. As already pointed out by Potter (1981), such studies should help to clarify discrepancies in the literature with regard to concentration-response relationships.

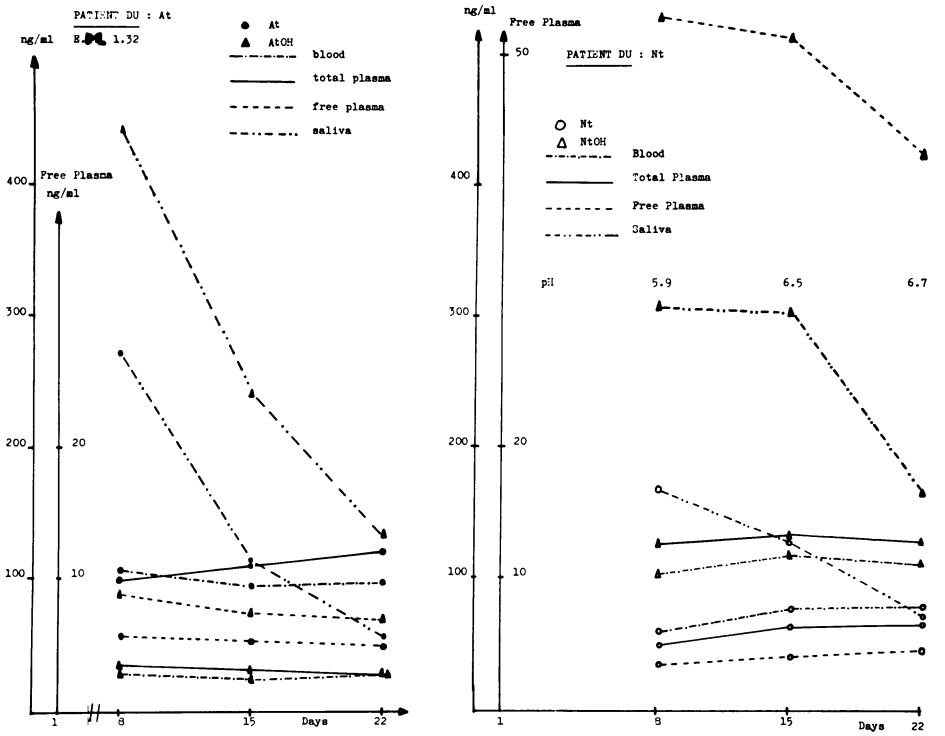


**Figure 3.** Whole blood, total and free plasma and saliva levels of amitriptyline (At) and its metabolites in a patient treated with 150 mg Laroxyl® daily for three weeks.

## THE EFFECT OF AMITRIPTYLINE ON ALPHA$_1$-ACID GLYCOPROTEIN (AAG)

Several blood constituents have been found to be responsible for the plasma protein binding of tricyclic antidepressants. In particular, these basic drugs exhibit high affinity for AAG (Piafsky and Borgå, 1977; Brinkschulte and Breyer-Pfaff, 1980). The clinical significance of the drug binding to this protein has recently been discussed (de Leve and Piafsky, 1981).

Carroll *et al.* (1981) reported an increase of AAG in five depressed women after imipramine treatment. Pre-treatment AAG values were slightly elevated in comparison to controls.

Independently, in a study where AAG was measured by immunodiffusion at the beginning and after three weeks of treatment with 150 mg amitriptyline daily in 16 primary depressive patients without somatic disease, we observed a statistically significant increase ($p < 0.01$) of the AAG levels (Baumann *et al.*, 1982b). The initial and the final mean values remained within the normal

range (50-140 mg per 100 ml) (figure 4). No change in albumin levels was noticed. For control, the same measurements were performed in 16 freshly admitted patients with varying diagnoses. Their pharmacological treatment included neuroleptics, antidepressants (except amitriptyline) and anti-Parkinson agents. Neither albumin nor AAG concentrations in plasma increased or decreased significantly during the three weeks in this group (figure 4). This drug effect seems not to be very specific, as anti-epileptic drugs also cause an increase in AAG concentration (Routledge *et al.*, 1981). At the present time, no satisfactory explanation of this effect can be offered.

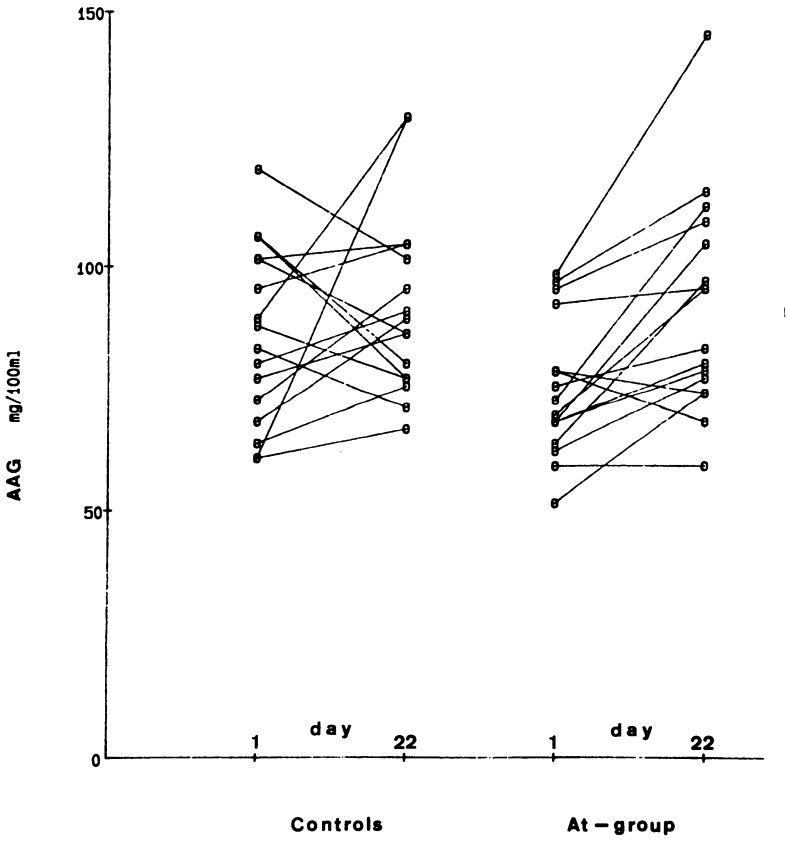


**Figure 4.** Alpha$_1$-acid glycoprotein levels in 16 depressive patients treated for three weeks with 150 mg Laroxyl® daily and in 16 controls. AAG was measured before (day 1) and during treatment (day 22) in the depressive patients (from Baumann *et al.*, 1928b).

AAG occurs in three variants and in up to eight polymorphic forms. In 12 depressive patients treated for three weeks with amitriptyline, the number of bands and their relative intensities remained stable within each patient (Tinguely *et al.*, 1982), even if the AAG levels rose. This finding is not quite unexpected, as polymorphism is under genetic control and not markedly influenced by environmental factors.

## SUMMARY AND CONCLUSION

Recent investigations have demonstrated the inter-individual differences in plasma protein binding and in the metabolism of tricyclic antidepressants. This means that, in clinical research, the relevance of these variables for blood level monitoring should be examined. The determination of free plasma drug levels still represents a technical problem. The present study shows that saliva level monitoring proves the importance of the unbound fraction of the drug in plasma for diffusional processes. In addition, a detailed case study confirms the need for measuring also the hydroxylated metabolites in the different compartments of the blood. Finally, a treatment with amitriptyline produces an increase in AAG concentration in plasma, which probably is of little relevance for the steady-state levels of the drug, but which encourages further research about the relationship between amitriptyline and glycoproteins.

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# Protein binding of imipramine and related compounds

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## INTRODUCTION

Protein binding of drugs is often mentioned as a subject of importance. In relation to psychotropic drugs it is, however, difficult to find works substantiating the importance in relation to either clinical or pharmacokinetic aspects. One of the reasons for this is probably that most works deal with quantitative rather than qualitative binding measurements. Another reason could be that the many different techniques that have been used have led to different results, and difficulties in establishing the possible clinical significance of such measurements.

## METHODOLOGICAL PROBLEMS

The most commonly used techniques are equilibrium dialysis (ED), ultrafiltration (UF), and measurement of CSF/plasma ratio. In studies of protein binding of a particular drug, ED generally yields relatively high free fraction measurements whereas UF generally yields relatively low free fractions. CSF/plasma ratio tends to yield results lying between those obtained by ED and UF.

Results from protein binding studies of imipramine and carbamazepine illustrate these tendencies (table 1).

Another problem, probably also primarily related to the techniques, is the difference in inter-individual variation found in different studies. As seen from table 1, the range of inter-individual variation in protein binding of imipramine found in different studies varies from less than twofold to more than fourfold. With carbamazepine the differences seem less dramatic.

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Table 1. Results from studies on plasma protein binding of imipramine and carbamazepine

Imipramine

| Method | *N* | % free | | Reference |
|---|---|---|---|---|
| | | Mean | Range | |
| ED 37°C | 26 | 15 | 5-23 | Glassman *et al.* (1973) |
| ED 37°C | 8 | 13.7 | - | Pruitt and Dayton (1971) |
| ED 37°C | 23 | 7.9 | 6.1-11.1 | Piafsky and Borgå (1977) |
| CSF/plasma | 12 | 9.5 | 4.4-12.9 | Muscettola *et al.* (1978) |
| UF 20°C | 5 | 4.2 | - | Borgå *et al.* (1969) |

Carbamazepine

| Method | *N* | % free | | Reference |
|---|---|---|---|---|
| | | Mean | Range | |
| ED 37°C | 8 | 27.9 | 26.0-29.8 | Rawlins *et al.* (1975) |
| ED 24°C | 12 | 25.9 | - | McAuliffe *et al.* (1977) |
| CSF/plasma | 10 | 24 | 19-34 | Johannesen *et al.* (1976) |
| CSF/plasma | 11 | 18.2 | 13.9-33.3 | Meinardi (1972) |
| CSF/plasma | 19 | 21.0 | - | Johannesen and Strandjord (1972) |
| UF 37°C | 24 | 18.2 | 10.3-29.7 | Hooper *et al.* (1975) |
| UF 22°C | 6 | 23.9 | - | Di Salle *et al.* (1974) |
| UF 22°C | 14 | 14 | 19-34 | Johannesen *et al.* (1976) |

As it must be assumed that the inter-individual variation in protein binding is more important in clinical practice than the absolute degree of binding, it seems reasonable to focus on this problem.

The various problems related to the use of ED have been discussed in detail elsewhere (Kristensen and Gram, 1982).

It seems worth while to mention the important factors to be controlled when using this technique. Probably the most important factors are pH and dilution. To obtain reproducible results, the variation in pH must be less than 0.1 pH unit during dialysis (Henry *et al.*, 1981; Brinkschulte and Breyer-Pfaff, 1979). Calculation of free fraction implies dividing concentration of drug on the buffer side by the concentration on the serum side of the membrane, and it is therefore necessary to correct for the increased volume at the serum side caused by the colloidal osmotic pressure. This, of course, is more important the longer the

dialysis lasts. When dilution of serum becomes more than about 5%, corrections should be made, otherwise a too low degree of protein binding will be found. In specific studies precise information on other factors such as temperature, membrane qualities, radiochemicals, buffer, concentration of drugs, storage of samples and the use of serum or plasma should be listed in order to evaluate the results properly.

## MEASUREMENT IN DILUTED SERUM

For large-scale studies, it may be possible to use diluted serum for ED. By diluting serum and drug to the same degree, the ratio between drug and proteins remains constant. Then it can be derived from the law of mass action that free fraction of the drug ($\alpha$) in different dilutions of serum and drug can be calculated from measurement of free fraction at only one concentration (dilution) of drug and protein. The equation to be used is

$$\alpha_1 = \frac{\alpha}{\alpha + y - \alpha y} \qquad (1)$$

where $\alpha$ is the free fraction in undiluted serum and $\alpha_1$ is the free fraction at a particular dilution of drug and protein, and $y$ is the relative concentration after dilution. For example, with imipramine: $\alpha = 0.109$ in undiluted serum ($y = 1$), and $\alpha_1$ in a 10-times diluted medium can be calculated:

$$\alpha_1 = \frac{0.109}{0.109 + 0.10 - 1.09 \times 0.1} = 0.550$$

The theory has been tested with imipramine and mianserin and the results seem to confirm the predictions (figure 1). The advantages of this method seem to be that less volume of blood samples is used, dilution becomes insignificant, pH is easy to control, and equilibrium is reached within less time. The disadvantages of this method are a somewhat lower precision, and the method has limited application for drugs bound less than 50% in undiluted serum.

## PROTEIN BINDING OF IMIPRAMINE IN HEALTHY SUBJECTS

The plasma protein binding studies of most psychotropic drugs have been done with relatively small numbers of subjects and it is not possible to see if age, sex, or other factors influence the degree of drug binding. Therefore, we have studied 145 healthy subjects evenly distributed with respect to age, sex, smoking habits, and use of oral contraceptives.

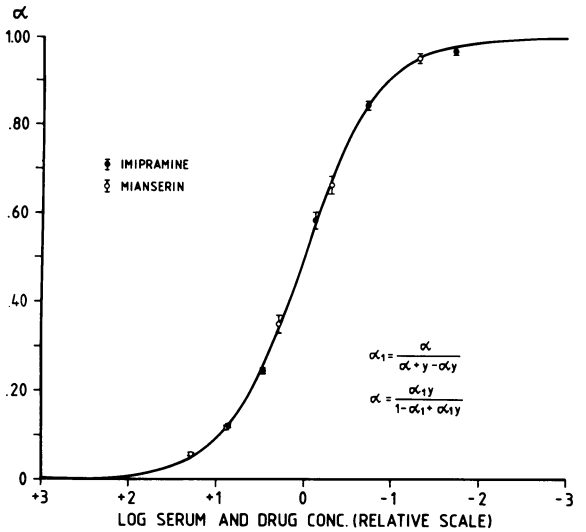


**Figure 1.** Drug protein binding in diluted serum. The $x$-axis represents a *relative* log concentration. The curve has been constructed according to equation (1) (see text).

In figure 2, the distribution of free fraction values in men and women is shown. The range of inter-individual variation is less than twofold and the mean value of free fraction is 0.11. In women there was a trend toward higher binding in the elderly (>50 years). No difference between smokers and non-smokers was observed and no definite effects of oral contraceptives could be observed.

To evaluate the impact of inter-individual differences in concentration of 12 plasma proteins these were measured in subjects with relatively high ($n$ = 17) and low ($n$ = 18) binding respectively. Other groups (Bickel, 1975; Piafsky and Borgå, 1977; Brinkschulte and Breyer-Pfaff, 1980) have demonstrated binding of basic drugs to albumin and orosomucoid, but the inter-individual differences in degree of binding have not been explained fully by differences in concentration of these proteins. Other factors thus appear to be involved. These may be binding to other proteins or interference from endogenous compounds such as steroids and electrolytes.

We therefore examined the possible binding to other proteins by measuring the concentration of the following proteins: $\alpha_1$-antitrypsin, $\alpha_2$-HS-glycoprotein, ceruloplasmin, prealbumin, $\beta_2$-glycoprotein I, complement $C3_c$, haptoglobulin, hemopexin, albumin, $\alpha_1$-acid-glycoprotein (orosomucoid) and apolipoproteins A and B. The radial immunodiffusion technique (Behring Werke) was used. Significantly higher concentrations of orosomucoid, complement $C3_c$

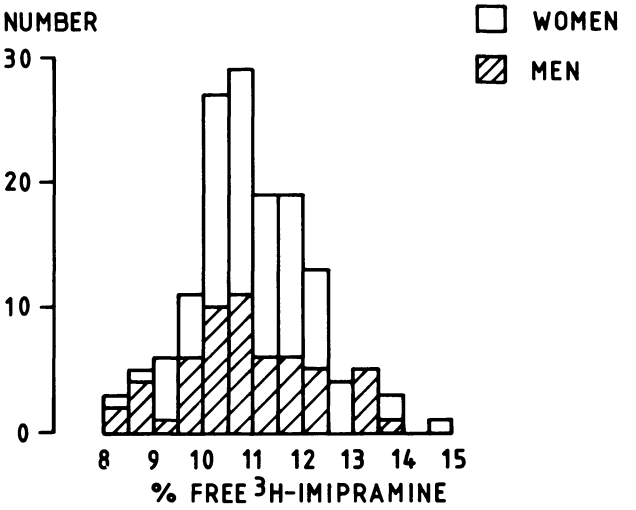


**Figure 2.** Free fraction of $^{3}$H-imipramine in serum from 145 healthy subjects.

and apolipoprotein B were found in the high binding group. No statistically significant difference in concentration of the other proteins was found; in particular, the albumin concentration was the same in the two groups.

In the low binding group, a positive correlation between orosomucoid and apolipoprotein B was found whereas there was a negative correlation in the high binding group. This indicates that in some subjects the high binding may be caused by high concentration of apolipoprotein B and in others by high concentration of $\alpha_1$-acid-glycoprotein. There was a positive correlation between these two proteins and complement $C3_c$. When the concentration of the three proteins was added (figure 3), the discrimination between the two groups seemed to become better. The results thus indicate significant binding to $\alpha_1$-acid-glycoprotein and apolipoprotein B. Whether there is a binding to complement $C3_c$ has to be evaluated further because the present results may just as well reflect co-variation with the other two proteins.

## MIANSERIN PROTEIN BINDING

Whereas acidic drugs seem to bind almost exclusively to albumin, basic drugs also bind to several other proteins, making the qualitative aspects much more complicated. In order to compare the protein binding of imipramine with the basic, tetracyclic, antidepressive drug mianserin, 43 sera from the imipramine study were tested for mianserin binding (figure 4).

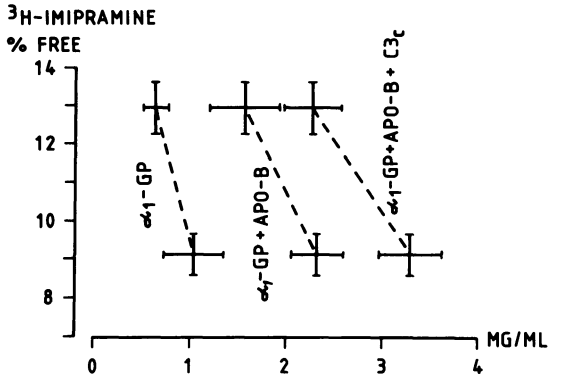


**Figure 3.** Discrimination between groups of healthy subjects with low and high serum protein binding of $^3$H-imipramine by addition of concentrations of $\alpha_1$-acid-glycoprotein ($\alpha_1$-GP), apolipoprotein B (APO-B) and complement $C3_c$. Mean value ± standard deviation.

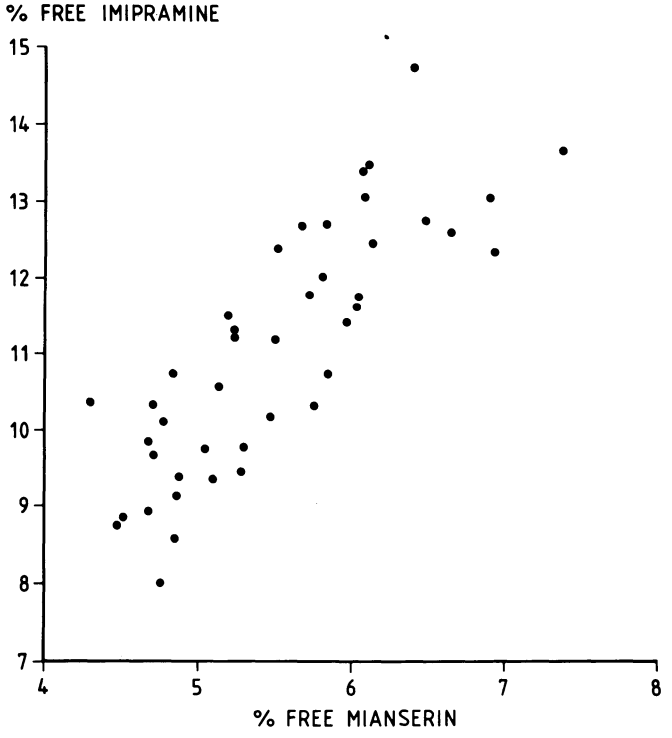


**Figure 4.** Serum protein binding of imipramine and mianserin in 43 healthy subjects.

A rather high degree of correlation was found between the binding of mianserin and imipramine. There must, however, be some difference in binding of the two compounds because the imipramine-free fraction was, on average, 0.11 and mianserin-free fraction was, on average, 0.055. It is thus indicated that high binding of one basic drug is associated with high binding of another basic drug. This would be expected if the drugs bind to the same proteins, but with different affinity, or to different binding sites on the same protein molecules.

## CARBAMAZEPINE AND CARBAMAZEPINE-EPOXIDE

Carbamazepine is used mainly as an anti-epileptic drug, but it is also used for some other purposes, such as treatment of mania (Ballenger and Post, 1978; Okuma *et al.*, 1979). Structurally, it is a tricyclic compound, somewhat related to imipramine. Carbamazepine is weakly acidic and the main metabolite, carbamazepine-epoxide, has an almost neutral reaction. Carbamazepine-epoxide has been shown (Frigerio and Morselli, 1975) to have anticonvulsive activity almost equal to carbamazepine in rats. Attempts (Dam *et al.*, 1977) to evaluate the anticonvulsive effects of the epoxide metabolite by measuring total concentrations of carbamazepine and carbamazepine-epoxide in humans have not been successful. The reason for this might be the variance in plasma level/effect relationship caused by inter-individual variations in protein binding. To study this problem, blood samples were drawn before the morning dose in 17 epileptic patients on long-term carbamazepine monotherapy. The results are shown in figure 5.

A relatively weak linear correlation between total carbamazepine and total epoxide concentrations was found, whereas the free concentration strictly followed a non-linear correlation curve. This curve may be constructed assuming saturable kinetics for the epoxide elimination. This is a rather surprising finding and calls for further investigations, especially larger number of patients. However, the very limited variation in carbamazepine/carbamazepine-epoxide ratio when free fraction was measured indicates little or no genetic variation of this metabolic step. If the hypothesis of saturable kinetics for the epoxide metabolite holds true, it might explain some unexpected effects and side-effects of carbamazepine seen in patients reaching high serum concentrations of carbamazepine (Höppener *et al.*, 1980). A small increase in free carbamazepine concentration may be followed by a large increase in free carbamazepine-epoxide concentration in such patients.

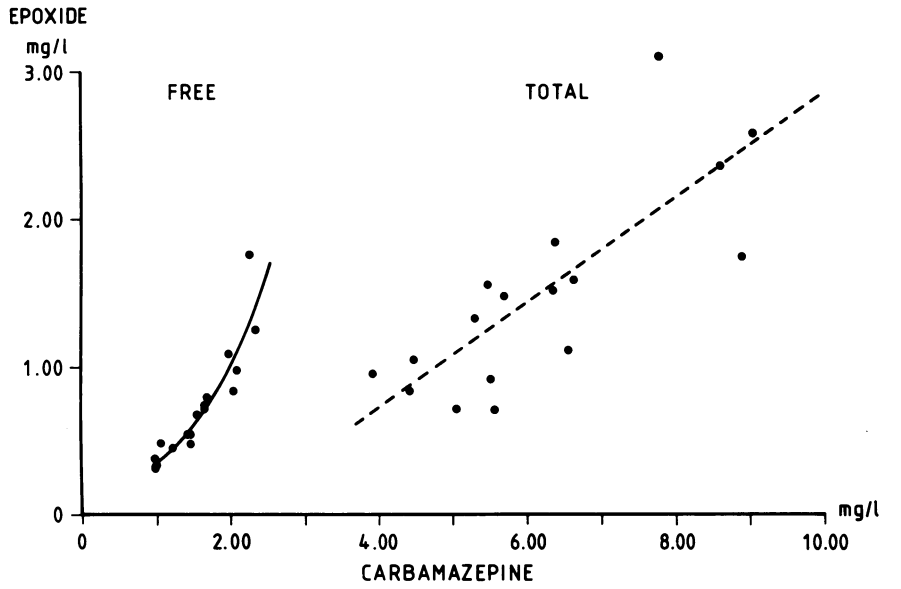


**Figure 5.** Total and free concentrations of carbamazepine and carbamazepine-epoxide in 17 epileptic patients in chronic treatment with carbamazepine.

## CONCLUSION

The inter-individual variation in protein binding of imipramine and related compounds is potentially important from both a pharmacokinetic and a clinical point of view. Both the range of variation in degree of binding and the quality of binding might influence results from studies on plasma level/effect relationships.

One of the difficulties in evaluation of results of protein binding studies is the many different techniques used. Even when the same type of technique is used, different results can be obtained because of the many variables that have to be controlled. This implies that detailed information on the technique used is necessary to evaluate the result.

As described for imipramine and mianserin, the inter-individual variation in binding is less than twofold in healthy subjects. It therefore seems improbable that the degree of protein binding is of importance in clinical practice. The differences in protein concentrations revealed in the imipramine high and low binders indicate, however, that the quality of binding could be of importance. If the binding to a particular protein is strong enough (high affinity) to prevent extraction of the drug by the liver, variation in the concentration of this protein will influence the

elimination of the drug. These aspects probably are much more relevant in patients suffering from somatic illnesses leading to abnormal serum protein patter (Piafsky *et al.*, 1977; Kates *et al.*, 1977).

In pharmacokinetic and pharmacogenetic studies it may be an advantage to use concentration of free drug instead of total. As described in the carbamazepine study, the correlation of carbamazepine and the carbamazepine-epoxide free concentrations is much better than the correlation of total concentrations. The 'noise' of inter-individual variance in degree of binding can apparently conceal some inter-relations between parent substance and metabolites.

**Acknowledgements**

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# Section Five

# Receptors and Transmitters Related to the Effects of Antidepressants

# Relationships between receptor affinities of different antidepressants and their clinical profiles

Hakan Hall[1]

## INTRODUCTION

The mechanism of action of antidepressant drugs is not well understood. The classical tricyclic antidepressant drugs as well as some newer antidepressants have been developed as inhibitors of the presynaptical re-uptake of the neurotransmitters norepinephrine (NE) (Glowinski and Axelrod, 1964; Iversen, 1965; Carlsson *et al.*, 1966) and 5-hydroxytryptamine (5-HT) (Carlsson *et al.*, 1969; Ross and Renyi, 1969). It has been hypothesized that the therapeutic action of the antidepressant drugs is due to an increased availability of NE and 5-HT at postsynaptic receptors in the brain as the result of the re-uptake blockade (Schildkraut, 1965; Coppen, 1967; van Praag, 1974). However, in recent years some antidepressants (e.g. mianserin and iprindole) have been developed which lack effect or have only very slight effect on the uptake mechanisms.

Extensive studies of the pharmacological effects of antidepressant drugs, tricyclics as well as non-tricyclics, have shown that several of them exert multiple effects on the pre- and postsynaptic systems in the brain. Thus it has been shown that antidepressants of various structural types inhibit the activation of histamine-sensitive adenylate cyclase, an effect that was suggested to be part of the therapeutic effect (Kanof and Greengard, 1978). Furthermore, a number of antidepressants block the muscarinic receptors (Snyder and Yamamura, 1977), which has been suggested to be associated with the well known anticholinergic side-effects rather than with the therapeutic action. Several of these drugs are, in addition, potent inhibitors of 5-HT receptors, histaminergic receptors and adrenergic receptors (Hall and Ogren, 1981). These findings indicate that the increased

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availability of neurotransmitters induced by the inhibition of their re-uptake of neurotransmitters could be counteracted by a simultaneous postsynaptic receptor blockade. The net effect of a drug with these multiple effects is not necessarily an increased neuronal firing in the postsynaptic neuron.

Generally, the depressive syndrome is not alleviated immediately after the administration of antidepressant drugs. There is normally a lag period of one to three weeks before a significant amelioration superior to that of placebo can be seen. This has focused attention on the effect of prolonged treatment on the mechanisms regulating the receptor responses in animals.

It has been observed that several of the antidepressant drugs on repeated administration produce a decreased density of β-adrenoceptors in rat cerebral cortex as studied by NE- or isoprenaline-stimulated adenylate cyclase (Vetulani *et al.*, 1976) or by radiolabeling of the β-adrenoceptors (Banerjee *et al.*, 1977; Wolfe *et al.*, 1978). Furthermore, 5-$HT_1$ and 5-$HT_2$ receptors and $\alpha_2$-adrenoceptors are also affected by long-term treatment with antidepressants (Reisine, 1981). It has been proposed recently that changes in pre- and postsynaptic receptors on long-term treatment are involved in the mechanism of action of the therapeutic effect of the antidepressant drugs (Fuxe *et al.*, 1982). In the present study, I will discuss the effects of antidepressants on receptors, both acutely and subchronically. Emphasis will be put especially on the possibility of predicting the clinical profile, with regard to both wanted effects and unwanted side-effects, based upon the pharmacological profile obtained in animal experiments.

## ACUTE EFFECTS ON RECEPTORS

### General

It has been shown by several authors that tricyclic antidepressant drugs inhibit the binding of radiolabeled ligands to various receptors in the brain (Hall and Ogren, 1981; Snyder and Yamamura, 1977; U'Prichard *et al.*, 1978). For example, imipramine, desipramine and amitriptyline are potent inhibitors of $\alpha_1$-adrenoceptors, histamine-$H_1$ receptors and muscarinic receptors. In addition to these receptors, clomipramine interacts with dopamine-$D_2$ receptors. Many antidepressants of the non-tricyclic type do not inhibit the neuronal receptors to the same extent as do the tricyclics. Thus, zimelidine, nomifensine and alaproclate do not interact acutely with any receptor studied so far, while other antidepressants of the new generation, such as iprindole and mianserin, do. No antidepressant drug interacts acutely with the β-adrenergic receptor.

We have in an earlier work (Hall and Ogren, 1981) constructed a correlation matrix for comparison of the receptors, based on the

above-mentioned results. Correlations were found between several receptor types, indicating similarities in their recognition sites (table 1).

Of special interest for the present study is the close similarity between the muscarinic receptors and the histamine-$H_1$ receptors, which have both been suggested to be involved in the development of side-effects in the clinical use of antidepressants (Snyder and Yamamura, 1977; Ogren *et al.*, 1981).

Table 1. Correlation coefficients in log-log regression analysis of $IC_{50}$ values of 13 different antidepressants in various receptor binding systems (data from Hall and Ogren, 1981)

| Receptor comparison | Correlation coefft. |
|---|---|
| d-LSD vs 5-$HT_1$ | 0.94 |
| Muscarinic vs $histamine_1$ | 0.93 |
| $Alpha_1$ vs $dopamine_2$ | 0.86 |
| d-LSD vs $dopamine_2$ | 0.81 |
| d-LSD vs $histamine_1$ | 0.81 |
| $Alpha_1$ vs muscarinic | 0.80 |

Other comparisons: correlation coefficient < 0.80.

## Muscarinic Receptors

One of the main drawbacks in the use of tricyclic antidepressant drugs are the anticholinergic side-effects caused by these drugs. The side-effects related to peripheral anticholinergic action are, for example, dry mouth, constipation, mydriasis and urinary retention. Side-effects related to the central anticholinergic action are, for example, confusion, disturbed concentration, disorientation, delusions and hallucinations. It can be suggested that these side-effects are due to the antagonizing effect of tricyclic antidepressants on the cholinergic receptors in the brain and in the periphery.

The antimuscarinic potencies of drugs can easily be estimated using the $^3$H-QNB binding assay (Yamamura and Snyder, 1974; Snyder and Yamamura, 1977). The relative potencies of some antidepressants in this assay are shown in table 2 (Hall and Ogren, 1981; see also Snyder and Yamamura, 1977). It is evident that the tricyclic antidepressants and mianserin are potent antimuscarinic drugs while others are more or less devoid of activity on the muscarinic receptor in the CNS.

In the periphery, the anticholinergic effects can be evaluated using the acetylcholine-induced contractions of the guinea-pig ileum. As can be seen from table 2, there is a high corre-

Table 2. Muscarinic receptor binding affinity and anticholinergic effects of some antidepressant drugs

| | Inhibition of $^3$H-QNB binding[a] ($IC_{50}$, μM) | Blockade of AcCh-induced contractions[b] ($ED_{50}$, μmol $kg^{-1}$) | Saliva secretion after 150 mg drug[c] (% of placebo) |
|---|---|---|---|
| Alaproclate | 79.5 | 53.8 | n.d. |
| Amitriptyline | 0.069 | 0.48 | 27.8 |
| Clomipramine | 0.184 | 3.63 | 35.1 |
| Desipramine | 0.848 | 4.23 | n.d. |
| Imipramine | 0.181 | 3.53 | 33.2 |
| Iprindole | 2.37 | 18.0 | n.d. |
| Maprotiline | 0.650 | 7.59 | 44.6 |
| Mianserin | 0.566 | 13.9 | 53.0* |
| Nomifensine | 48.8 | 157.0 | 80.7 |
| Nortriptyline | 0.180 | 2.78 | 42.2 |
| Norzimelidine | 19.8 | 32.5 | n.d. |
| Zimelidine | 33.7 | 40.9 | 79.3 |
| | $r$=0.91, $p$ < 0.001 | $r$=0.91, $p$ < 0.001 | |
| | $r$=0.99, $p$ < 0.001 | | |

[a] From Hall and Ögren (1981).
[b] From Ögren and Hall (1983).
[c] From Rafaelsen *et al.* (1981).
* dose 60 mg.

Correlation coefficients and p values from regression analysis (logarithms of columns 1 and 2). As mianserin was given in a lower dose in the saliva secretion study, the other values for mianserin were corrected (x 2.5) before the analysis was carried out.

lation between the central muscarinic receptor binding affinity and the peripheral effects ($r$ = 0.91, $p$ < 0.001, log-log regression analysis).

Very few studies have been performed to quantify anticholinergic side-effects in the clinic. However, Rafaelsen *et al.* (1981) made a quantitative measurement of saliva secretion after the administration of antidepressants to healthy volunteers. I have compared the percent inhibition relative to placebo of whole mouth saliva secretion 10 h after the administration of antidepressants (all 150 mg single dose except mianserin 60 mg single dose). As can be seen from table 2 and figure 1, there was a highly significant correlation between the inhibition of $^3$H-QNB

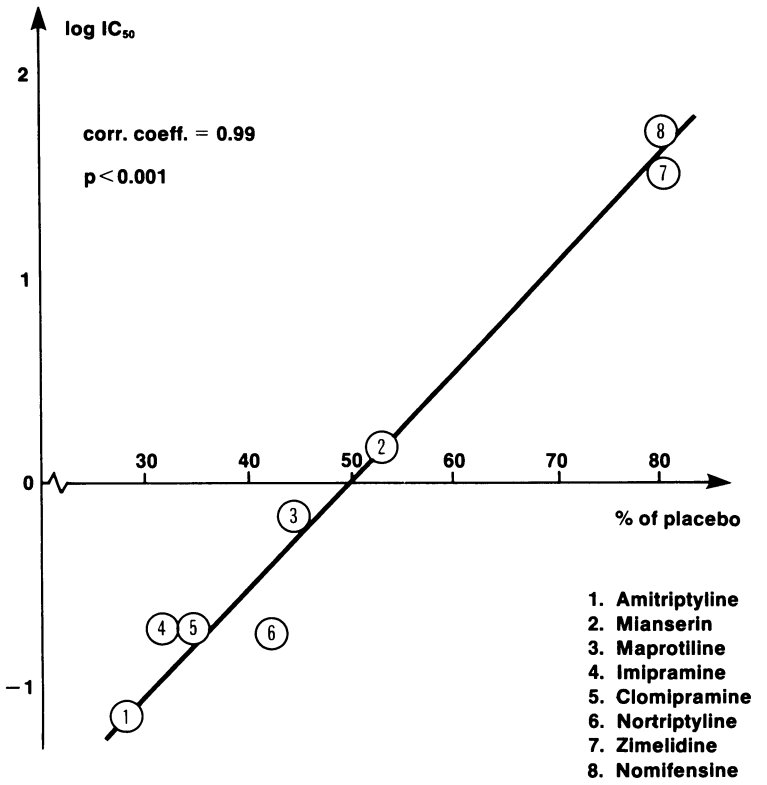


Figure 1. Inhibition of $^{3}$H-QNB binding versus percent inhibition of saliva secretion. (Data from Hall and Ogren (1981) and from Rafaelsen *et al.* (1981).)

binding in rat brain and the inhibition of whole mouth saliva secretion in man ($p < 0.001$) and between the inhibition of acetylcholine-induced contractions of the guinea-pig ileum and the inhibition of the whole mouth saliva secretion in man ($p < 0.001$). These results imply that $^{3}$H-QNB binding experiments can be of great value for the prediction of antimuscarinic side-effects, regardless of these being central or peripheral.

## Histaminergic Receptors

The effects of drugs on histamine-$H_1$ receptors in the brain were assessed by their potency to displace specific $^{3}$H-mepyramine binding from rat cortical homogenates (Hall and Ogren, 1981) (table 3). The results show that the tricyclic antidepressants

Table 3. Antihistaminergic effects of some drugs

| | Inhibition of $^3$H-mepyramine binding[a] ($IC_{50}$,nM) | Block of histamine-induced contraction of guinea-pig ileum[b] ($IC_{50}$,nM) | Inhibition of histamine-sensitive adenylate cyclase[c] ($IC_{50}$,nM) |
|---|---|---|---|
| Alaproclate | 23200 | 42000 | >10000 |
| Amitriptyline | 6 | 130 | 49 |
| Brompheniramine | 20 | 130 | 1100 |
| Clomipramine | 64 | | 53 |
| Desipramine | 457 | 1100 | 280 |
| Imipramine | 29 | 160 | 140 |
| Iprindole | 250 | | 200 |
| Maprotiline | 25 | 530 | |
| Mianserin | 6 | 330 | 65 |
| Nomifensine | 8870 | 75000 | |
| Nortriptyline | 48 | | |
| Norzimelidine | 8290 | 42000 | |
| Zimelidine | 2900 | 24000 | >10000 |

[a] From Hall and Ogren (1981).
[b] From Ogren and Hall (1983).
[c] From Kanof and Greengard (1978) and Dolphin and Greengard (personal communication).

had a high affinity for $^3$H-mepyramine binding sites, some being approximately as active as brompheniramine.

It is well known that antihistaminergic drugs may be used as sedatives in man. It may reasonably be suggested that antidepressants cause sedation through inhibition of histamine-$H_1$ receptors. We have tried to estimate the sedative action of the antidepressant drugs by using a number of different animal tests, such as decrease in locomotor activity, grip strength, passivity, rotarod performance, loss of righting reflex, etc. (Ogren *et al.*, 1981). The drugs were then ranked and the mean rank was used as an estimate of the relative sedative effect of the drug. A correlation ($r$ = 0.92, $p < 0.01$) was found between these mean rank orders and the $IC_{50}$ for inhibition of $^3$H-mepyramine binding (figure 2), which indicates a clear association between the histamine-$H_1$ receptor interaction and sedative effects in animals.

For antidepressant drugs, Ogren *et al.* (1981) have made a rough estimate of the incidence of sedative effects in the clinic

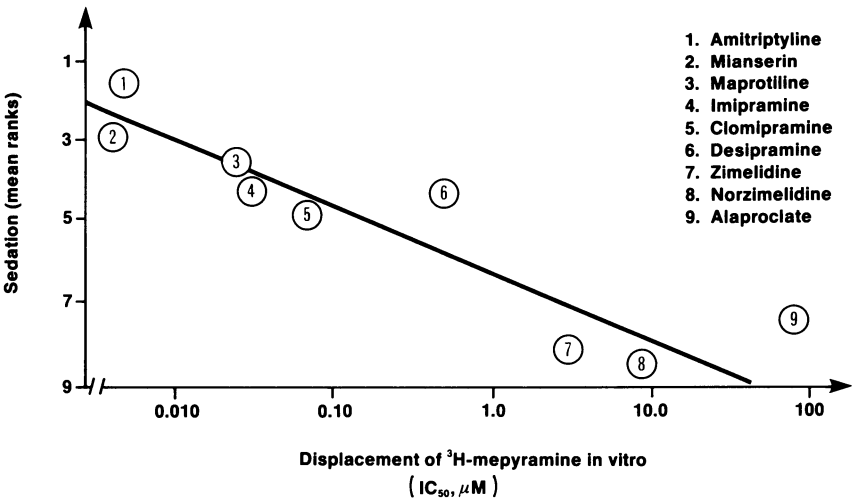


**Figure 2.** Relationship between sedative effects of antidepressants and central $H_1$-receptor blocking properties

by interpolation of data from the literature. These data correlate very well ($r$ = - 0.97) with the inhibition of $^3$H-mepyramine binding caused by these drugs. It can thus be suggested that study of the effects of antidepressants on $^3$H-mepyramine binding can predict the incidence of sedative effects in the clinic.

A number of antidepressant drugs have been shown to be potent inhibitors of histamine-sensitive adenylate cyclase in hippocampal homogenates of the guinea-pig, an effect that has been suggested to be mediated by histamine-$H_2$ receptors (Kanof and Greengard, 1978). Some of the new, non-tricyclic antidepressants, such as zimelidine and alaproclate, are devoid of activity on histamine-mediated adenylate cyclase (table 3). This indicates that blockade of histamine-$H_2$ receptors is not important for antidepressant activity as was originally suggested (Kanof and Greengard, 1978).

## Alpha-Adrenoceptors

The tricyclic antidepressant drugs are generally believed to enhance NE activity in the brain by increasing the synaptic concentration of NE due to the inhibition of its neuronal uptake. However, since several antidepressants block α-adrenoceptors at low concentrations (table 4), it is suggested that several antidepressants in fact may reduce noradrenergic activity in the brain. However, NE activity could be enhanced by an interaction

Table 4. Effects of antidepressants on alpha-adrenoceptors and on NE re-uptake

| | Inhibition of binding of | | Inhibition of |
|---|---|---|---|
| | [$^3$H]WB4101[a] ($IC_{50}$, μM) | [$^3$H]Clonidine[a] ($IC_{50}$, μM) | NE re-uptake[b] ($IC_{50}$, μM) |
| Alaproclate | 19.1 | 105 | > 34 |
| Amitriptyline | 0.022 | 0.550 | 0.054 |
| Clomipramine | 0.035 | 4.12 | 0.060 |
| Desipramine | 0.250 | 10.6 | 0.003 |
| Imipramine | 0.097 | 5.04 | 0.028 |
| Iprindole | 6.81 | 6.70 | 2.6 |
| Maprotiline | 0.350 | 15.5 | 0.075 |
| Mianserin | 0.067 | 0.126 | 0.10 |
| Nomifensine | 1.27 | 4.56 | 0.007 |
| Nortriptyline | 0.088 | 3.87 | 0.013 |
| Norzimelidine | 0.953 | 9.30 | 0.76 |
| Zimelidine | 1.18 | 3.35 | 2.7 |

[a] From Hall and Ögren (1981).
[b] From Ross and Renyi (1975) and from Ross (personal communication).

of the antidepressants with the presynaptic $\alpha_2$-adrenoceptor. There is evidence that mianserin, which is a very weak NE uptake inhibitor *in vivo* (Leonard, 1974), might cause an increase of NE release due to this interaction with presynaptic $\alpha_2$-adrenoceptors (Baumann and Maître, 1977; Harper and Hughes, 1979).

The complexity of the cardiovascular system makes it practically impossible to predict the effect of drugs on, for example, heart rate, ECG and resting blood pressure (table 5) (Burgess, 1981). Nevertheless, it is quite clear that the antidepressants of the new generation, such as nomifensine and zimelidine, elicit fewer side-effects, which could be due to an overall more selective action on uptake with much less effect on the α-receptors.

Blockade of α-adrenoceptors causes a number of effects on the cardiovascular system, such as postural hypotension, tachycardia, increased cardiac output and decreased peripheral resistance (Nickerson and Hollenberg, 1967, cited in Burgess, 1981). However, as pointed out above, the antidepressant drugs may influence the cardiovascular system through other mechanisms, such as anticholinergic effects, blockade of NE or 5-HT re-uptake or local anesthetic effect (table 5). The only cardiovascular effect that might be directly related to $\alpha_1$-adrenoceptor blockade is postural hypotension (Nickerson and Hollenberg, 1967). No clearcut corre-

Table 5. Potential effects of antidepressants on the CVS. From Burgess (1981)

| | |
|---|---|
| (1) Anticholinergic[a] | (1) Sinus bradycardia/tachycardia<br>(2) Junctional rhythm |
| (2) Norepinephrine re-uptake blockade[b] | (1) Sinus bradycardia<br>(2) Increase in PR interval<br>(3) AV junctional rhythm<br>(4) AV dissociation<br>(5) Ventricular arrhythmias<br>(6) Hypertension |
| (3) 5-HT re-uptake blockade[c] | (1) Hypertension<br>(2) Sinus bradycardia/tachycardia<br>(3) Increase in PR interval<br>(4) AV junctional tachycardia |
| (4) Local anesthetic/ quinidine-like activity[d] | (1) Increase in QRS duration<br>(2) Increase in Q-Tc duration<br>(3) Decreased contractility |
| (5) α-Adrenoceptor blockade[e] | (1) Postural hypotension<br>(2) Tachycardia<br>(3) Increased cardiac output<br>(4) Decreased peripheral resistance |

[a] Gravenstein *et al.*, (1969).
[b] Innes and Nickerson (1975).
[c] James *et al.*, (1975).
[d] Heisenbuttel and Bigger (1970).
[e] Nickerson and Hollenberg (1967).

lation between the effects of antidepressants on, for example, blood pressure and $\alpha_1$-adrenoceptor blockade is seen, although most antidepressants with α-adrenoceptor blocking potency cause decreased blood pressure at therapeutic doses. Neither zimelidine nor nomifensine, which are both devoid of activity on the α-adrenoceptors, causes changes in mean arterial blood pressure at doses as low as the tricyclics (Lindbom and Forsberg, 1981). However, mianserin, which is a potent inhibitor of both $\alpha_1$- and $\alpha_2$-adrenoceptors (Hall and Ogren, 1981), does not cause any decreased blood pressure, which is probably due to the fact that the inhibition of the presynaptic $\alpha_2$-adrenoceptors counteracts the effect on the $\alpha_1$-adrenoceptors.

Peroutka *et al.* (1977) have suggested that there is a significant correlation between the sedative effects of neuroleptic drugs and their ability to block α-adrenoceptors. We can find a significant correlation between the $IC_{50}$ values for $\alpha_1$-adrenoceptor binding sites ($^3$H-WB4101) and the $ED_{50}$ for suppression of locomotion (Spearman rank correlation coefficient, $r_s$ = 0.71,

$p < 0.025$; data on locomotion from Toneby, personal communication). The antidepressants which do not effect locomotion in mice (alaproclate, nomifensine, norzimelidine and zimelidine) were very weak inhibitors of $^3$H-WB4101 binding in the rat cortex homogenates. However, as is pointed out above, histaminergic blockade is highly correlated to sedation, and the Spearman rank correlation coefficient, $r_s$, is even higher between $^3$H-mepyramine binding and suppression of locomotion.

### Dopaminergic Receptors

Most antidepressants are weak inhibitors of the dopaminergic receptors. The only antidepressants with some potency are clomipramine ($IC_{50}$ = 268 nM) as seen in $^3$H-spiroperidol binding tests (Hall and Ogren, 1981) and imipramine ($K_i$ = 180 nM) and mianserin ($K_i$ = 620 nM) in $^3$H-haloperidol binding tests (Burt *et al.*, 1976). The antidopaminergic effect of these antidepressants cannot be directly related to any clinical effects or side-effects. The inhibition of dopamine uptake caused by nomifensine which increases the activity of dopamine at the receptors may be the cause of the stimulatory effects of nomifensine at higher doses.

### Serotonergic Receptors

In acute studies some antidepressant drugs interfere with both central and peripheral 5-$HT_1$ and 5-$HT_2$ receptors. However, for the 5-HT uptake inhibitors, the potencies of postsynaptic blockade are much lower than those of the uptake inhibition (table 6). The newer non-tricyclic 5-HT selective uptake inhibitors are practically devoid of activity on the postsynaptic 5-HT receptors. The acute blockade of serotonergic receptors by some antidepressants has not been related to any effects or side-effects of the antidepressants in the clinic.

### Other Receptors

With very few exceptions, the acute administration of antidepressants has been found not to interact with other receptors in the central nervous system (Hall and Ogren, 1981).

## UPTAKE BINDING SITES

During recent years, binding of radiolabeled antidepressants has been described. The binding sites involved are probably closely related to the uptake sites of 5-HT (Raisman *et al.*, 1979a, b,

Table 6. Effects of some drugs on 5-HT mechanisms as studied *in vivo* and *in vitro*

| | *In vitro* receptor binding[a] ($IC_{50}$, μM) | | *In vivo* block of head twitches[b] ($ED_{50}$, μmol $kg^{-1}$) | Block of serotonin-induced contractions[b] of rat uterus ($IC_{50}$, μM) | Block of 5-HT re-uptake[c] ($IC_{50}$, μM) |
|---|---|---|---|---|---|
| | ($^3$H)d-LSD | ($^3$H)5-HT | | | |
| Alaproclate | >100 | >100 | >110 | 2.96 | 0.37 |
| Amitriptyline | 0.150 | 1.52 | 15 | 0.39 | 0.18 |
| Clomipramine | 0.917 | 21.2 | 53 | 0.15 | 0.018 |
| Desipramine | 3.45 | 16.1 | 110 | 0.30 | 1.10 |
| Imipramine | 1.35 | 24.6 | 63 | 0.29 | 0.14 |
| Iprindole | 5.80 | 15.2 | >110 | 0.43 | 10.0 |
| Maprotiline | 3.05 | 15.8 | >80 | 0.23 | 7.2 |
| Mianserin | 0.097 | 1.21 | 18 | 0.0006 | 11.0 |
| Nomifensine | 3.47 | 9.88 | 30 | 0.037 | 6.6 |
| Nortriptyline | 0.302 | 1.00 | 30 | 0.044 | 0.87 |
| Norzimelidine | 14.7 | 63.1 | >110 | 0.93 | 0.030 |
| Zimelidine | 10.9 | 33.2 | >110 | 1.12 | 0.24 |

[a] From Hall and Ogren (1981).
[b] From Ogren *et al.*, (1979).
[c] From Ross and Renyi (1975) and Ross (personal communication).

1980; Langer and Briley, 1981; Hall *et al.*, 1982b) and of NE (Langer *et al.*, 1981; Lee and Snyder, 1981). The tricyclic antidepressants and also some new, non-tricyclic, antidepressants have been developed as inhibitors of presynaptic neurotransmitter uptake. A close correlation can therefore also be seen between inhibition of $^{3}$H-imipramine binding and the clinical efficacy of the 5-HT uptake inhibitors (Langer and Briley, 1981), as well as between inhibition of $^{3}$H-desipramine binding and the clinical efficacy of the NE uptake inhibitors (Lee and Snyder, 1981).

## EFFECTS ON RECEPTOR NUMBER

Long-term treatment of rats with antidepressants causes a down-regulation of the central cortical β-adrenoceptor response measured as NE- or isoprenaline-stimulated adenylate cyclase activity (Vetulani *et al.*, 1976; Banerjee *et al.*, 1977; Sulser, 1982). Extensive studies have shown that antidepressants of various types (NE and 5-HT uptake inhibitors, MAO inhibitors, atypical antidepressants) and also other antidepressant treatment (REM sleep deprivation, electroconvulsive treatment) cause subsensitivity of the β-adrenergic system in the brain (Sulser, 1982). However, the extent of down-regulation varies greatly between antidepressant drugs and apparently no correlation can be seen between recommended clinical dose and down-regulatory effect of the drugs (table 7).

Recently, other changes in receptor number and affinity have been reported. Thus both 5-$HT_1$ and 5-$HT_2$ receptors are affected (Peroutka and Snyder, 1980; Fuxe *et al.*, 1979, 1982; Hall *et al.*, 1982a). The 5-$HT_2$ receptors are down-regulated similar to the β adrenoceptor, while long-term treatment with both NE and 5-HT

**Table 7.** Comparison of recommended full daily dose in the clinic and down-regulatory effect on central beta-adrenoceptors upon long-term treatment in rats

| | Recommended full daily dose (mg per day) | Effect on beta-adrenoceptors (% of control)† |
|---|---|---|
| Amitriptyline | 250-300 | 83.7 (10) |
| Desipramine | 150-200 | 59.5 (5) |
| Mianserin | 120 | 85.6 (10) |
| Zimelidine | 200-300 | 79.0 (10) |
| Alaproclate* | 200-600 | 96.5 (10) |
| Amiflamine* | < 5 | 89.0 (10) |

* Drug not yet launched. Possible full daily dose.

† from Hall *et al.*, (1983). Dose (mg/kg per day) given for three weeks to rats within brackets

uptake inhibitors induces one new low-affinity 5-$HT_1$ binding site (Fuxe *et al* , 1979, 1982).

Other receptors that have been reported to be changed upon long-term treatment with antidepressants are $\alpha_2$-adrenoceptors (up-regulation) (Reisine *et al.*, 1982), $\alpha_2$-adrenoceptors (down-regulation) (Smith *et al.*, 1981; Vetulani, 1982), dopamine autoreceptors (down-regulation) (Reisine, 1981), and $^3$H-imipramine binding sites (down-regulation) (Raisman *et al.*, 1980).

The change in various receptor numbers and/or affinities after long-term treatment with antidepressants does not directly correlate to the clinical effect or to any side-effect. However, since most antidepressive treatments so far cause changes in at least one type of receptor (β-adrenoceptors), it has been suggested that this effect may be part of the therapeutic mechanism (Vetulani, 1982).

## CONCLUSION

Several antidepressant drugs elicit multiple direct effects on neurotransmitter receptors. Since side-effects often occur directly upon administration in contrast to the therapeutic effect which generally occurs after 2-3 weeks of treatment, it has been suggested that the side-effects are the result of direct receptor blockade. In the present paper, I have tried to correlate the incidence of side-effects with the affinity of the various drugs to neurotransmitter receptors. In a few, obvious cases this has been possible (anticholinergic side-effects vs blockade of muscarinic receptors; sedation vs histamine-$H_1$ blockade; postural hypotension vs $\alpha_1$-adrenoceptor blockade). Other effects or side-effects seem to be impossible to predict on data from receptor binding studies. This is mainly due to the fact that many drugs are multiple receptor blockers and exert multiple side-effects, while others are very weak overall receptor blockers and elicit very few side-effects. It may, however, be wise to use receptor binding studies in the design of new drugs. The antidepressants of the new generation are a good example of where this technique has led to the development of drugs with more specific action.

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# Antidepressants and ∝-adrenoceptors

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## INTRODUCTION

Although research into the biochemical and pharmacological effects of psychotropic agents has been largely responsible for the development of hypotheses concerning the etiology of depressive illness, the precise mechanism of action of antidepressant drugs is still a matter for conjecture. The widely quoted hypothesis of monoamine deficiency in depression, whereby antidepressants act rapidly to enhance the availability of norepinephrine (NE) and/or serotonin (5-HT) in the brain via decreased catabolism or inhibition of monoamine uptake (Schildkraut, 1965), is now seen to be an oversimplification (Charney *et al.*, 1981b; Sugrue, 1981a; Waldmeier, 1981). Thus, some newer antidepressants neither inhibit monoamine oxidase (MAO) nor significantly affect monoamine uptake, while several highly effective uptake inhibitors appear not to be useful in the treatment of depression. Furthermore, the time course of drug effects on monoamine availability, which are immediate, are inconsistent with the clinical improvement, which may take two to three weeks. In order to accommodate some of these difficulties, the hypothesis has been extended to include dopamine (Randrup and Braestrup, 1977), and research has focused on the effects of chronic administration of antidepressants, particularly the adaptive changes induced in the sensitivity or density of monoamine receptors (Charney *et al.*, 1981b; Waldmeier, 1981).

In addition to their well documented acute effects on monoamine uptake and MAO (Maxwell and White, 1978; Randrup and Braestrup, 1977), antidepressants interact directly with a number of central receptors including muscarinic, histaminergic, serotonergic and adrenergic receptors (Hall and Ogren, 1981; Sugrue, 1981a). Interactions with muscarinic (Snyder and Yamamura, 1977) or histamine $H_1$-receptors (Richelson, 1979; Diffley *et al.*, 1980)

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are probably more associated with the anticholinergic or sedative side-effects respectively of antidepressants than with their therapeutic action. Suggestions that inhibition by antidepressants of $H_2$-sensitive adenylate cyclase in brain is involved in their therapeutic action (Kanof and Greengard, 1978) are discouraged by the similar potency of neuroleptic phenothiazines in this respect and by the high selectivity of antidepressants for $H_1$- rather than $H_2$- receptors (Snyder, 1980). Furthermore, adaptive changes in sensitivity or density have not been observed for muscarinic or histaminergic receptors following chronic treatment with antidepressants (Maggi *et al.*, 1980a; Peroutka and Snyder, 1980). As far as central 5-HT receptors are concerned, many antidepressants behave as classicial 5-HT antagonists in both pharmacological (Maj, 1981) and receptor binding studies (Tang and Seeman, 1980; Hall and Ogren, 1981), while upon chronic administration they appear to reduce the density of 5-HT receptors of the $5\text{-HT}_2$ type (Peroutka and Snyder, 1980). It is possible that the therapeutic action of some antidepressants may in part be due to a reduced functional activity of some central serotonergic systems.

Antidepressant drugs exert manifold effects upon central noradrenergic systems (Sugrue, 1981a). Following acute administration or *in vitro*, many antidepressants strongly inhibit neuronal re-uptake of NE, are potent antagonists at postsynaptic $\alpha_1$-adrenoceptors but in general weak antagonists at presynaptic $\alpha_2$-adrenoceptors, and do not interact at all with β-adrenoceptors. Chronic administration appears to reduce the density of cortical $\alpha_2$- and β-receptors leading to a condition of subsensitivity to agonists. The effects of antidepressants on NE uptake (Maxwell and White, 1978) and β-adrenoceptor function (Sulser *et al.*, 1978) have been reviewed elsewhere. It is the purpose of the present paper to review the roles played by $\alpha_1$- and $\alpha_2$-adrenoceptors in the mechanisms of action of antidepressants.

## CLASSIFICATION AND FUNCTION OF α-ADRENOCEPTORS

There are two basic types of α-adrenoceptor, $\alpha_1$ and $\alpha_2$, which differ in their selectivity for the α-antagonists prazosin and yohimbine and for the α-agonists clonidine, α-methylnorepinephrine and phenylephrine (van Zwieten and Timmermans, 1980; Exton, 1982). $\alpha_1$-Receptors are located postsynaptically, are blocked more potently by prazosin than by yohimbine, and generally have low affinity for clonidine and α-methylnorepinephrine but high affinity for phenylephrine. On the other hand, $\alpha_2$-adrenoceptors are located both pre- and postsynaptically, are blocked more potently by yohimbine, and have high affinity for clonidine and α-methylnorepinephrine but low affinity for phenylephrine (table 1). The terms pre- and postsynaptic describe the location of α-adrenoceptors, while the $\alpha_1/\alpha_2$ subdivision is based upon selectivity for

Table 1. Characteristics of α-adrenoceptors

| Receptor type | Location | Selective agonists | Selective antagonists |
|---|---|---|---|
| $\alpha_1$ | postsynaptic | phenylephrine | prazosin |
| $\alpha_2$ | presynaptic | clonidine<br>α-methylnorepinephrine | yohimbine |
| $\alpha_2$ | postsynaptic | clonidine<br>α-methylnorepinephrine | yohimbine |

(1) Receptor binding studies using $^3$H-clonidine or $^3$H-yohimbine do not differentiate between pre- and postsynaptic $\alpha_2$-adrenoceptors.
(2) Clonidine acts presynaptically to lower NE turnover, but postsynaptically to lower blood pressure and stimulate growth hormone secretion.

agonists and antagonists. The endogenous catecholamines NE and epinephrine are non-selective agonists, that is they are about equally active at both $\alpha_1$- and $\alpha_2$-adrenoceptors.

Postsynaptic α-adrenoceptors are located at the target cell and represent classical receptors in the traditional sense. Their stimulation by an agonist causes a physiological or pharmacological response, involving changes in cell calcium fluxes for $\alpha_1$-receptors and a decrease in intracellular cyclic AMP for $\alpha_2$-receptors. Presynaptic $\alpha_2$-adrenoceptors appear to mediate a negative feedback mechanism which leads to inhibition of transmitter release probably by restricting the calcium available for the excitation-secretion coupling (Langer, 1977; Starke *et al.*, 1977; Westfall, 1977). Thus, stimulation of presynaptic $\alpha_2$-receptors by agonists, including NE itself, reduces the amount of NE released per nerve impulse from its storage sites in the neuron. Accordingly, NE in the synaptic cleft inhibits its own release, while α-sympatholytic agents increase the amount of NE released per nerve impulse as a result of blockade of presynaptic $\alpha_2$-receptors. Both $\alpha_1$- and $\alpha_2$-adrenoceptors are widely distributed in the brain, particularly in the cortex (Maggi *et al.*, 1980b). However, central postsynaptic $\alpha_2$-adrenoceptors appear to be mainly involved in mediating hypotensive actions in the brainstem.

## ACUTE EFFECTS OF ANTIDEPRESSANTS ON CENTRAL α-ADRENOCEPTORS

### $\alpha_1$-Adrenoceptors

A number of antidepressants have been studied for blockade of central $\alpha_1$-adrenoceptors, using the antagonistic ligand WB-4101 (tables 2 and 3). The use of different brain areas and species,

**Table 2.** Inhibition of $^3$H-WB-4101 binding ($\alpha_1$-adrenoceptors) by tricyclic antidepressants. All values are given in nM

| Drug | Rat brain | Rat cortex | | Calf cortex |
|---|---|---|---|---|
| | $K_i$ [a] | $K_i$ [b] | $IC_{50}$ [c] | $IC_{50}$ [d] |
| Tertiary amines | | | | |
| Amitriptyline | 23 | 24 | 22 | 46 |
| Clomipramine | 55 | - | 35 | 130 |
| Doxepin | 23 | 23 | - | 24 |
| Imipramine | 58 | 58 | 97 | 160 |
| Trimipramine | - | - | - | 67 |
| Secondary amines | | | | |
| Desipramine | 148 | 150 | 250 | 440 |
| Maprotiline | - | - | 350 | 250 |
| Nortriptyline | 71 | 71 | 88 | 130 |
| Protriptyline | 277 | 280 | - | 420 |
| Reference α-antagonists | | | | |
| Phentolamine ($\alpha_1/\alpha_2$) | - | 3.6 | - | - |
| Prazosin ($\alpha_1$) | - | 0.49 | - | - |
| Yohimbine ($\alpha_2$) | - | 480 | - | - |

[a] U'Prichard *et al.* (1978).
[b] Maggi *et al.* (1980a).
[c] Hall and Ogren (1981).
[d] Tang and Seeman (1980).

$IC_{50}$ values (nM) for inhibition of NE uptake in rat brain synaptosomes (Randrup and Braestrup, 1977): amitriptyline (20), clomipramine (44), imipramine (20), trimipramine (1000), desipramine (1.5), maprotiline (20), nortriptyline (11), protriptyline (3).

the quoting of results as inhibitory concentrations ($IC_{50}$) or as inhibition constants ($K_i$) and the non-standardization of receptor binding techniques suggest that comparisons between antidepressant drugs should be made with confidence only within individual studies. Nevertheless, the different sets of results appear to be consistent.

Most of the drugs studied, particularly tricyclic antidepressants (table 2) had a high affinity for the antagonistic $\alpha_1$-adrenoceptor. The most potent antidepressants included doxepin, amitriptyline, clomipramine, imipramine and trimipramine, which all have a tertiary amino group in the side chain. These drugs inhibited binding at about the same concentrations as they inhibited NE uptake (table 2), with the exception of trimipramine which was very much more potent as an $\alpha_1$-antagonist than as an uptake inhibitor. Secondary amino tricyclics, on the other hand, were relatively weak $\alpha_1$-antagonists and much more effective as inhibitors of NE uptake (table 2).

Table 3. Inhibition of $^3$H-WB-4101 binding ($\alpha_1$-adrenoceptors) by atypical antidepressants. All values are given in nM

| Drug | Rat brain $K_i$ [a] | Rat cortex $K_i$ [b] | Rat cortex $IC_{50}$ [c] | Calf cortex $IC_{50}$ [d] |
|---|---|---|---|---|
| Selective 5-HT uptake inhibitors | | | | |
| Alaproclate | - | - | 19100 | - |
| Fluoxetine | 8000 | > 1000 | - | - |
| Zimelidine | - | - | 1180 | 1210 |
| Others | | | | |
| Iprindole | 9600 | > 1000 | 6810 | 6150 |
| Mianserin | - | 86 | 67 | 56 |
| Nomifensin | - | - | 1270 | 980 |
| Trazodone | - | 68 | - | - |
| Reference α-antagonists | | | | |
| Phentolamine ($\alpha_1/\alpha_2$) | - | 3.6 | - | - |
| Prazosin ($\alpha_1$) | - | 0.49 | - | - |
| Yohimbine ($\alpha_2$) | - | 480 | - | - |

[a] U'Prichard *et al.* (1978).
[b] Maggi *et al.* (1980a).
[c] Hall and Ogren (1981).
[d] Tang and Seeman (1980).

Of the atypical antidepressants, only mianserin and trazodone showed inhibition of WB-4101 binding at concentrations below 1 μM (table 3). Most of the compounds, especially the selective 5-HT uptake inhibitors alaproclate, fluoxetine and zimelidine, as well as other atypicals like iprindole and nomifensin, were virtually inactive. Mianserin and trazodone were intermediate in potency between the two groups of tricyclic antidepressants.

In assessing the significance of the apparent central $\alpha_1$-antagonism displayed by some antidepressants, it is important to bear in mind that even the most potent compounds like doxepin and amitriptyline are at least 50-fold less potent than the specific $\alpha_1$-antagonist prazosin (table 2). Furthermore, they are about 10-fold less potent in receptor binding studies with WB-4101 than is the non-selective α-antagonist phentolamine (Maggi *et al.*, 1980a). Nevertheless, it is likely that the $\alpha_1$-adrenolytic activity shown by many antidepressants is in part responsible for their ability to produce hypotensive side-effects. Thus, the tertiary amines imipramine, amitriptyline and clomipramine, which are among the most potent antidepressants for central $\alpha_1$-adrenoceptor antagonism, are also more prone to cause orthostatic hypotension than is the secondary amine nortriptyline (Glassman and Bigger, 1981).

Significant correlations have been noted between the sedative effects of antidepressants and their ability to block $\alpha_1$-adrenoceptors, suggesting an involvement of $\alpha_1$-receptors in the production of sedation (U'Prichard *et al.*, 1978; Snyder, 1980; Hall and Ogren, 1981). However, a similar rank order of potency exists for the blockade by tricyclic antidepressants of both central (Richelson, 1979; Diffley *et al.*, 1980) and peripheral (Figge *et al.*, 1979) histamine $H_1$-receptors as holds for blockade of $\alpha_1$-adrenoceptors, and central $H_1$-receptors are believed to mediate the production of sedation by antihistamines (Uzan *et al.*, 1979). Suggestions have also been made that an inverse relationship exists between blockade of $\alpha_1$-adrenoceptors and the ability of antidepressants to produce psychomotor activation (U'Prichard *et al.*, 1978; Snyder, 1980). Some evidence does exist to support this, since those atypical antidepressants which block $\alpha_1$-adrenoceptors are also sedative, for example mianserin and trazodone, whereas those which do not block $\alpha_1$-adrenoceptors tend to be activating, such as nomifensin and zimelidine. Again, however, the so-called activating drugs also happen to have less antihistamine properties (Hall and Ogren, 1981), which could explain their apparent lack of sedation and tendency toward psychomotor activation.

Table 4. Inhibition of $^3$H-clonidine binding ($\alpha_2$-adrenoceptors) by tricyclic antidepressants. All values are given in nM

| Drug | Rat cortex | | Calf cortex |
|---|---|---|---|
| | $K_i$ [a] | $IC_{50}$ [b] | $IC_{50}$ [c] |
| Tertiary amines | | | |
| Amitriptyline | 630 | 550 | 850 |
| Clomipramine | - | 5040 | 7430 |
| Doxepin | 890 | - | 2000 |
| Imipramine | 3400 | 4120 | 4930 |
| Trimipramine | - | - | 1700 |
| Secondary amines | | | |
| Desipramine | 7600 | 10600 | 9400 |
| Maprotiline | - | 15500 | 16770 |
| Nortriptyline | 1700 | 3870 | 2980 |
| Protriptyline | 14000 | - | 10360 |
| Reference α-antagonists | | | |
| Phentolamine ($\alpha_1/\alpha_2$) | 2.5 | - | - |
| Prazosin ($\alpha_1$) | 6400 | - | - |
| Yohimbine ($\alpha_2$) | 73 | - | - |

[a] Maggi *et al.* (1980a).
[b] Hall and Ogren (1981).
[c] Tang and Seeman (1980).

### $\alpha_2$-Adrenoceptors

Antidepressants are in general weak antagonists at central $\alpha_2$-adrenoceptors as determined by displacement of binding of the agonistic ligand clonidine (tables 4 and 5). $IC_{50}$ and $K_D$ values tend to be in the 1-10 μM range, comparable to those of prazosin which is pharmacologically inactive at the $\alpha_2$-receptor (van Zwieten and Timmermans, 1980). All antidepressants are clearly less potent than the non-selective α-antagonist phentolamine and, with the exception of mianserin, they are less potent than the selective $\alpha_2$-antagonist yohimbine. There appears to be no correlation between the affinities of antidepressants for the $\alpha_1$-antagonistic and the $\alpha_2$-agonistic binding sites, except that tertiary amine tricyclics generally tend to be more potent than secondary amine tricyclics at both receptors (table 4). All antidepressants, with the possible exception of mianserin, appear to be selective for the $\alpha_1$-antagonistic binding site.

In addition to their effects on NE uptake, antidepressants may raise synaptic NE levels by increasing NE release from the presynaptic neuron, that is via presynaptic $\alpha_2$-adrenoceptor blockade (Collis and Shepherd, 1980). This activity has been confirmed pharmacologically for amitriptyline and mianserin, which are the most potent presynaptic $\alpha_2$-antagonists in receptor binding experiments (table 5). Thus, mianserin has been shown to raise the

Table 5. Inhibition of $^3$H-clonidine binding ($\alpha_2$-adrenoceptors) by atypical antidepressants. All values are given in nM

| Drug | Rat cortex | | Calf cortex |
|---|---|---|---|
| | $K_i$ [a] | $IC_{50}$ [b] | $IC_{50}$ [c] |
| Selective 5-HT uptake inhibitors | | | |
| Alaproclate | - | $> 10^5$ | - |
| Fluoxetine | 9200 | - | - |
| Zimelidine | - | 3350 | 610 |
| Others | | | |
| Iprindole | 11400 | 6700 | 16000 |
| Mianserin | 35 | 126 | 12 |
| Nomifensin | - | 4560 | 2480 |
| Trazodone | 2100 | - | - |
| Reference α-antagonists | | | |
| Phentolamine ($\alpha_1/\alpha_2$) | 2.5 | - | - |
| Prazosin ($\alpha_1$) | 6400 | - | - |
| Yohimbine ($\alpha_2$) | 73 | - | - |

[a] Maggi *et al.* (1980a).
[b] Hall and Ogren (1981).
[c] Tang and Seeman (1980).

amount of NE released in response to nerve stimulation of tissues previously incubated with $^3$H-NE, both in central (Baumann and Maître, 1977) and peripheral (Harper and Hughes, 1979; Doggrell, 1980) tissues, and similar effects have been observed for amitriptyline in peripheral tissue (Hughes, 1978; Collis and Shepherd, 1980). Mianserin has also been shown after acute administration to reverse the electrophysiological (Svensson *et al.*, 1981), behavioral (Robson *et al.*, 1978; Clineschmidt *et al.*, 1979; Hunt *et al.*, 1981), cardiovascular (Doxey *et al.*, 1978; Robson *et al.*, 1978; Cavero *et al.*, 1980) and biochemical (Baumann and Maître, 1977; Fludder and Leonard, 1979a; Sugrue, 1980) effects of the $\alpha_2$-agonist clonidine. However, although mianserin has consistently been shown to be the antidepressant with the most potent effects at $\alpha_2$-adrenoceptors, its selectivity for $\alpha_2$- over $\alpha_1$-receptors is doubtful. Indeed all of the studies mentioned above have tended to show about equipotency at $\alpha_1$- and $\alpha_2$-receptors. Furthermore, while being the most potent of the antidepressants at peripheral $\alpha_2$-adrenoceptors, mianserin is also somewhat more effective at peripheral $\alpha_1$-adrenoceptors (table 6). Nevertheless, since mianserin is a very weak inhibitor of NE uptake *in vivo* despite moderately potent effects *in vitro* (Goodlett *et al.*, 1977), presynaptic $\alpha_2$-blockade could be a way by which the drug raises synaptic NE levels.

## CHRONIC EFFECTS OF ANTIDEPRESSANTS ON CENTRAL α-ADRENOCEPTORS

The acute effects of antidepressants on α-adrenoceptors may not be relevant to their therapeutic action, and attention has turned in

**Table 6.** Antagonistic potencies of antidepressants at peripheral pre- and postsynaptic α-adrenoceptors (Brown *et al.*, 1980)

| Compound | Presynaptic activity[a] | Postsynaptic activity[b] | Ratio pre/post |
|---|---|---|---|
| Amitriptyline | $8.6 \times 10^{-6}$ | $5.8 \times 10^{-8}$ | 0.007 |
| Nortriptyline | $1.1 \times 10^{-5}$ | $8.5 \times 10^{-7}$ | 0.08 |
| Mianserin | $6.5 \times 10^{-7}$ | $1.8 \times 10^{-7}$ | 0.28 |
| Trazodone | $1.1 \times 10^{-6}$ | $1.8 \times 10^{-7}$ | 0.16 |
| Viloxazine | $1.3 \times 10^{-5}$ | $> 3.5 \times 10^{-5}$ | - |
| Yohimbine | $7.9 \times 10^{-9}$ | $8.9 \times 10^{-7}$ | 113 |
| Prazosin | $2.9 \times 10^{-6}$ | $1.4 \times 10^{-9}$ | 0.0005 |

[a] Concentration (M) producing 20% reversal of inhibition by cocaine of electrically induced contractions of rat vas deferens.
[b] Concentration (M) producing 50% inhibition of the effects of NE on rat anoccygeus muscle.

recent years to their influence on receptor systems during chronic administration. The ability of many antidepressants to decrease the binding of postsynaptic β-adrenoceptors and 5-HT receptors in rat brain does not extend to a large extent to α-adrenoceptor binding (Charney *et al.*, 1981b; Sugrue, 1981a).

### $\alpha_1$-Adrenoceptors

Although there are reports of reduced binding of WB-4101 in rat vas deferens following chronic desipramine treatment (Wetzel *et al.*, 1981) and even of increased binding in mouse brain after long-term amitriptyline treatment (Rehavi *et al.*, 1980), the consensus of opinion is that $\alpha_1$-adrenoceptor binding is largely unaffected by chronic treatment with antidepressants. Thus, chronically administered antidepressants of various classes including desipramine, imipramine, amitriptyline, iprindole, pargyline and fluoxetine fail to alter WB-4101 binding in rat brain (Bergstrom and Kellar, 1979; Rosenblatt *et al.*, 1979; Peroutka and Snyder, 1980; Tang *et al.*, 1981). This conclusion is at variance with both the electrophysiological and behavioral evidence for enhanced responsiveness toward NE at postsynaptic $\alpha_1$-adrenoceptors. Long-term treatment with imipramine, clomipramine, amitriptyline, desipramine and iprindole has enhanced responses to iontophoretic NE in a number of projection areas of the brain which are mediated through postsynaptic α-adrenoceptors, for example in the dorsal lateral geniculate (Menkes and Aghajanian, 1981) and the facial motor nucleus (Menkes *et al.*, 1980). An enhanced response to iontophoretic NE has also been observed in rat amygdaloid neurons after long-term desipramine, imipramine or iprindole, but the amygdala contains NE neurons which may have both α- and β- characteristics (Wang and Aghajanian, 1980). In behavioral studies (Maj *et al.*, 1979a,b; Charney *et al.*, 1981a,b), chronic antidepressant treatment enhances responsiveness to catecholamine agonists. Despite the lack of changes in binding, it appears that central postsynaptic $\alpha_1$-adrenoceptors become supersensitive to the effects of NE following long-term treatment with many antidepressants.

### $\alpha_2$-Adrenoceptors

The effects of chronic antidepressant treatment on $\alpha_2$-adrenoceptors are important *per se*, and because of suggestions that $\alpha_2$-adrenoceptor sensitivity is mediated by β-receptors in a type of homeostatic control mechanism for central NE function (Maggi *et al.*, 1980b) and that β-receptor desensitization results from a gradually developing subsensitivity of presynaptic $\alpha_2$-receptors (Crews and Smith, 1978). Central $\alpha_2$-adrenoceptor subsensitivity,

however, is produced by only some and not all antidepressants and is by no means a general mechanism of action. Moreover, supersensitivity has also been reported.

Electrophysiological responses of NE neurons in the locus coeruleus to the $\alpha_2$-agonist clonidine were reduced by chronic treatment with desipramine, imipramine and zimelidine but not by mianserin, iprindole or clomipramine (Svensson *et al.*, 1981; Svensson and Scuvee-Moreau, 1981). After chronic desipramine treatment, locus coeruleus neurons of rats failed to respond to a single dose of desipramine which in untreated animals markedly decreased neuronal firing (McMillen *et al.*, 1980). However, although chronic desipramine treatment is reported to reduce $\alpha_2$-adrenoceptor sensitivity in rat heart (Crews and Smith, 1978), the response of brain $\alpha_2$-receptors is at best equivocal (see Charney *et al.*, 1981a). Certainly, $^3$H-clonidine binding in rat cortex was unaffected by long-term treatment with desipramine, mianserin or iprindole (Sugrue, 1981b; Tang *et al.*, 1981), although it was reduced by the MAOIs clorgyline, pargyline and tranylcypromine (Cohen *et al.*, 1980; Sugrue, 1981b). There was no evidence that inverse reciprocal modulation of central adrenoceptors occurred, since some of the treatments which failed to alter $^3$H-clonidine binding were highly effective in reducing β-adrenoceptor density. The effects of amitriptyline on $^3$H-clonidine binding are equivocal, with reports of unchanged (Tang *et al.*, 1981) or decreased binding (Smith *et al.*, 1981).

Interactions of antidepressants with the behavioral and biochemical effects of clonidine are equivocal because the doses used have sometimes been in excess of those (25-100 μg $kg^{-1}$, i.p.) required to activate presynaptic $\alpha_2$-receptors selectively (see Sugrue, 1981a). Long-term treatment with desipramine attenuated suppression of rat locomotor activity induced by a dose of clonidine known to be selective for $\alpha_2$-adrenoceptors (Spyraki and Fibiger, 1980), and also attenuated the clonidine-induced reduction of rat brain levels of the NE metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) (McMillen *et al.*, 1980; Sugrue, 1981c). The ability of chronic desipramine to attenuate biochemical responses to clonidine appears to depend upon the frequency (twice daily) and duration (5-9 days) of desipramine administered as well as the dose of clonidine (Sugrue, 1981a,c). The use of low-dose clonidine has also shown that long-term treatment with amitriptyline, trazodone or iprindole does not attenuate the clonidine-induced reduction in MHPG (Sugrue, 1981a).

Mianserin, despite its potent antagonism at presynaptic $\alpha_2$-adrenoceptors, failed to alter biochemical responses to low-dose clonidine in rats given daily doses of 10 mg $kg^{-1}$ for 9 to 15 days, although it was effective both acutely and after 5 days of treatment (Sugrue, 1980). Reports that chronic mianserin treatment antagonizes both the behavioral and biochemical effects of clonidine involved the use of doses of the $\alpha_2$-agonist well in excess of 100 μg $kg^{-1}$, which probably also stimulated $\alpha_1$-

adrenoceptors (Fludder and Leonard, 1979a,b; Tang *et al.*, 1979). Furthermore, mianserin given chronically failed to reverse the acute hypotensive and biochemical responses to a single dose of 300 μg clonidine in healthy volunteers (Elliott *et al.*, 1981).

There is also evidence that some antidepressants can cause supersensitivity of $\alpha_2$-adrenoceptors. The density of $\alpha_2$-receptors has been reported to be increased by long-term desipramine (Johnson *et al.*, 1980). Both desipramine (Schoffelmeer and Mulder, 1982) and mianserin (Cerrito and Raiteri, 1981) appear to increase the release of NE from brain tissue after chronic administration to rats, an action mediated through presynaptic $\alpha_2$-adrenoceptors.

## ANTIDEPRESSANTS AND HUMAN α-ADRENOCEPTORS

Studies of α-adrenoceptors in man have necessarily assessed peripheral rather than brain receptor responses, and have generally had to rely upon indirect and possibly non-specific measures of receptor responses such as blood pressure, growth hormone and cortisol secretion, and NE metabolite levels. The data have been extensively reviewed and lead to opposite conclusions - blood pressure responses appear to support the presence of supersensitive peripheral postsynaptic α-adrenoceptors in depression while the hormone data support a subsensitivity of these same receptors (Cohen *et al.*, 1980; Charney *et al.*, 1981b; Sugrue, 1981a; Waldmeier, 1981). More recent research has concentrated on the role of presynaptic $\alpha_2$-adrenoceptors in depression, being based upon the hypothesis that such receptors are supersensitive in depression and may be down-regulated or desensitized by antidepressant treatment (Cohen *et al.*, 1980; Smith *et al.*, 1981).

Binding sites for $^3$H-clonidine or $^3$H-yohimbine on human platelet membranes seem to satisfy the biochemical and pharmacological requirements of a physiological $\alpha_2$-adrenoceptor and appear to be similar in nature to central $\alpha_2$-adrenoceptors in several species including humans (Garcia-Sevilla *et al.*, 1981a,b). Using $^3$H-clonidine as an agonistic ligand, platelet $\alpha_2$-adrenoceptor density ($B_{max}$) in drug-free patients with major depressive disorder was shown to be significantly higher than that in normal subjects, although the affinity of clonidine for the receptor ($K_D$) was not different between the groups (table 6). There was no relation between values of $B_{max}$ and the severity of depression. Treatment of six patients with imipramine or amitriptyline led to significant decreases in both the density of $\alpha_2$-receptors and the affinity of clonidine for the receptor (table 6). Since tricyclics competitively inhibit $^3$H-clonidine binding *in vitro* with an increased $K_D$ and unchanged $B_{max}$ these effects are probably specific for long-term treatment. However, the relevance of these findings to the mechanism of antidepressant action is marred by the observation that similar decreases in $B_{max}$ and $K_D$ were observed in one

Table 7. $\alpha_2$-Adrenoceptor binding to human platelet membranes[a]

| Authors | $^3$H-ligand | Normal subjects | | Patients with major depressive disorder[b] | | | |
|---|---|---|---|---|---|---|---|
| | | | | Drug-free | | After treatment[c] | |
| | | $K_D$ | $B_{max}$ | $K_D$ | $B_{max}$ | $K_D$ | $B_{max}$ |
| Garcia-Sevilla *et al.* (1981b) | clonidine | 5.0 ($n$=21) | 34.2* ($n$=21) | 5.5 ($n$=17) | 45* ($n$=17) | | |
| | | | | 6.3$^{\dagger}$ ($n$=6) | 50$^{\S}$ ($n$=6) | 3.3$^{\dagger}$ ($n$=6) | 34$^{\S}$ ($n$=6) |
| Daiguji *et al.* (1981) | yohimbine | 0.92 ($n$=9) | 240 ($n$=9) | 0.97 ($n$=11) | 204 ($n$=11) | - | - |

[a] $K_D$ = dissociation constant (nM), $B_{max}$ = maximal binding capacity (fmol per mg protein), $n$ = number of subjects.
[b] Research Diagnostic Criteria.
[c] Four patients received imipramine 175-250 mg per day for 4 weeks, two patients received amitriptyline, either 125 mg per day for 4 weeks or 150 mg per day for 3 weeks.
* $p < 0.005$; $^{\dagger}$ $p < 0.05$; $^{\S}$ $p < 0.005$.

patient who failed to respond to imipramine treatment (Garcia-Sevilla *et al.*, 1981b) and in two other imipramine-treated patients with agoraphobia and panic attacks but no significant depression (Garcia-Sevilla *et al.*, 1981a). Furthermore, using $^{3}$H-yohimbine as an antagonistic ligand, Daiguji *et al.* (1981) were unable to demonstrate any differences in $K_D$ or $B_{max}$ values between normal subjects and drug-free depressed patients (table 7). It is also likely that the $\alpha_2$-adrenoceptors labeled by clonidine and yohimbine are not presynaptic in nature, since the major physiological NE functions of platelets (for example, aggregation) involve postsynaptic $\alpha_2$-adrenoceptors (Exton, 1982). In a study using the non-selective $\alpha$-adrenoceptor ligand $^{3}$H-dihydroergocryptine, Wood and Coppen (1982) showed that drug-free depressed patients had a reduced number of binding sites ($B_{max}$) when compared with normal controls.

Some support for the hypothesis of supersensitive $\alpha_2$-adrenoceptors in depression is provided by a placebo-controlled study in which the effects of clonidine on plasma MHPG levels (a presynaptic effect) and blood pressure (a postsynaptic effect) were evaluated in depressed patients before and after long-term desipramine treatment (Charney *et al.*, 1981a). The effects of a single dose of clonidine (1 to 5 $\mu g\ kg^{-1}$) on both MHPG and blood pressure were attenuated by long-term desipramine treatment, which also significantly reduced plasma MHPG levels. There was no association between therapeutic response, pretreatment MHPG levels and clonidine effects on MHPG levels and blood pressure, during either placebo or drug treatment. However, in a follow-up study by the same group (Charney *et al.*, 1982) the ability of acute clonidine to lower plasma MHPG and reduce blood pressure did not differ between healthy subjects and drug-free depressives but the depressed patients showed a blunted growth hormone response to clonidine, implying normal sensitivity of presynaptic $\alpha_2$ receptors in depression but decreased sensitivity of postsynaptic $\alpha_2$-adrenoceptors. Since the same group (Sternberg *et al.*, 1982) has also demonstrated that clonidine fails to lower plasma MHPG levels in schizophrenic patients but does so in healthy subjects, while lowering blood pressure equally in both groups, it is difficult to accept the exclusivity of presynaptic $\alpha_2$-adrenoceptor subsensitivity for depression. Furthermore, in another placebo-controlled study, but in healthy subjects, biochemical and blood pressure responses to a single 300 $\mu g$ dose of clonidine were unaffected by chronic treatment with mianserin (Elliott *et al.*, 1981).

## COMMENT

Acutely administered antidepressants exert manifold actions on monoaminergic systems. These acute effects may not be relevant to the mechanism of antidepressant activity, because many other drugs

with similar pharmacological actions are not known to be antidepressant and there is no correlation between the doses required for acute pharmacological effects and those for clinical efficacy. Thus, as far as α-adrenoceptors are concerned, the moderately potent $\alpha_1$-antagonism exhibited by tricyclic antidepressants, mianserin and trazodone is far exceeded by the classical antagonists prazosin and phentolamine, which are not known to be antidepressant. Furthermore, clinically effective antidepressants such as nomifensin, iprindole, fluoxetine and zimelidine hardly interact with $\alpha_1$-adrenoceptors. It is more likely that such interactions are concerned with the side-effects of some antidepressants. Acute interactions with $\alpha_2$-adrenoceptors are absent for most clinically effective antidepressants, which are highly selective for $\alpha_1$-adrenoceptors, except for mianserin which, though non-selective, is more potent at $\alpha_2$-adrenoceptors than the standard antagonist yohimbine. It is not yet clear whether specific $\alpha_2$-antagonists have antidepressant properties.

The delayed onset of therapeutic effects exhibited by all antidepressants suggests that the pharmacological effects of long-term treatment may be more important than those following acute administration. The ability of long-term desipramine treatment to produce $\alpha_2$-adrenoceptor subsensitivity in rat brain is not shared by other proven or putative antidepressants, including the potent $\alpha_2$-antagonist mianserin, and it is too early to say whether or not the ability of desipramine to reduce platelet $\alpha_2$-adrenoceptor sensitivity in depressed patients extends to other drugs. The lack of uniform effects on presynaptic NE function suggests that such effects cannot explain the clinical activity of all antidepressants.

In contrast to the variable presynaptic actions of chronic antidepressant treatment, alterations in postsynaptic $\alpha_1$-adrenoceptor sensitivity have been consistently observed with a variety of drugs, though in the absence of changes in receptor density. It is possible that the effects of antidepressants on postsynaptic NE receptors, including $\alpha_1$- and β-adrenoceptors, represent a final common pathway for antidepressant activity. These postsynaptic changes may well be produced by the operation of different mechanisms depending upon the particular antidepressant.

It is tempting to speculate that, leaving aside effects on serotonergic and possibly other systems, depression is related to a functional hyperactivity of NE neuronal systems in the brain. The hypersensitive receptors may serve to amplify incoming stimuli and thereby result in central excitability. Antidepressant treatment will result in a desensitization of hyper-responsive NE receptor functioning, leading to a reduction in the amplification mechanism that translates sensory input into behavioral and physiological events. Such speculation may necessitate an overhaul of the original catecholamine hypothesis of depression which attributed the illness to a deficiency of NE at central synapses.

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# Antidepressants: effects on histaminic and muscarinic receptors

Elliott Richelson[1]

## INTRODUCTION

Antidepressant drugs of many chemical classes are antagonists of several different types of neurotransmitter receptors. Among the first known of these receptor interactions were the antagonisms of histamine $H_1$ and muscarinic acetylcholine receptors. In fact, the first antidepressant, imipramine hydrochloride, was originally synthesized for use as an antihistamine (Kuhn, 1970). More recently, antidepressants have been shown, in addition, to be antagonists *in vitro* of histamine $H_2$, $\alpha_1$-adrenergic, $\alpha_2$-adrenergic and serotonergic receptors. This paper will present the evidence that antidepressants antagonize histamine $H_1$, histamine $H_2$ and muscarinic acetylcholine receptors in brain and elsewhere. In addition the relevance of these data for the treatment of depression and other diseases will be discussed.

It is well established that acetylcholine is a neurotransmitter at nicotinic and muscarinic receptors in the nervous system. An antimuscarinic drug when given to patients may cause a number of effects, most commonly, dry mouth, blurred vision, constipation and sinus tachycardia. Histamine, on the other hand, has a number of different functions in the body and in addition may also serve as a neurotransmitter (Taylor and Richelson, 1981). Histamine causes its effects by activating two different types of receptors (histamine $H_1$ and histamine $H_2$). Classically, histamine $H_1$ receptors are involved with anaphylactic and allergic reactions, while histamine $H_2$ receptors are involved with the secretion of gastric acid.

When an antihistaminic ($H_1$) drug is given to patients, sedation and drowsiness often occur. It is presumed that these are effects of histamine $H_1$ antagonism in the brain and that brain histamine $H_1$ receptors are involved with mechanisms of arousal.

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## METHODS FOR ASSAY OF HISTAMINIC AND MUSCARINIC RECEPTORS

Histamine $H_1$ and muscarinic receptors have been successfully assayed by biological techniques as well as by radioligand binding procedures. For histamine $H_2$ receptors there are a number of different biological assays; however, radioligand binding techniques have not been successful in identifying these receptor sites, despite several attempts to do so.

Ideally, to study a receptor or drug-receptor interaction, both biological and radioligand binding procedures should be used in combination. By using biological assays to corroborate the results from radioligand binding assays, it may be possible to avoid such problems as underestimation of the affinity of a high-affinity ligand for its receptor by having too high a receptor concentration in a binding assay (as discussed below); or the measurement of meaningless binding as, for example, to glass fiber filters or to talcum powder (Hollenberg and Cuatrecasas, 1979).

The classical biological assay for histamine $H_1$ and muscarinic receptors is the agonist-induced contraction of the guinea-pig ileum *in vitro*. Another, less classical, approach that we have used extensively makes use of cultured murine neuroblastoma cells (clone N1E-115) (Richelson *et al.*, 1978; Richelson, 1978a, b). These cells contain, among other things, histamine $H_1$ and muscarinic receptors which, when activated, cause a marked and transient increase in intracellular cyclic GMP levels. Because receptor-mediated cyclic GMP synthesis occurs only with intact cells, the availability of a clone that has these properties has greatly aided the study of these receptors and their interactions with psychotropic drugs.

For the histamine $H_2$ receptor, agonist-induced stimulation of cyclic AMP in tissue slices (Tuong *et al.*, 1980), homogenates (Green and Maayani, 1977; Kanof and Greengard, 1978) or vesicles (Psychoyos, 1981) from brain has been used as a biological assay for this receptor. As discussed later in this paper, the affinities of psychotherapeutic drugs for histamine $H_2$ receptors in homogenates are different from those for receptors in intact cells (Tuong *et al.*, 1980; Schwartz *et al.*, 1981).

Radiolabeled pyrilamine (also called mepyramine) binds to histamine $H_1$ receptors in brains of many different species and in smooth muscle with an equilibrium dissociation constant ($K_D$) of around 1 nM (Hill *et al.*, 1977, 1978; Tran *et al.*, 1978). Recently, [$^3$H]doxepin, a tricyclic antidepressant, and perhaps the most potent histamine $H_1$ antagonist known ($K_D$ = 20-50 pM), has been shown to identify histamine $H_1$ receptors in rat brain (Tran *et al.*, 1981; Taylor and Richelson, 1982) and in human brain (Kanba and Richelson, unpublished data). Unlike [$^3$H]pyrilamine, this radioligand binds to two classes of sites in rat brain (figure 1) and it is the high-affinity site that has the characteristics of a histamine $H_1$ receptor.

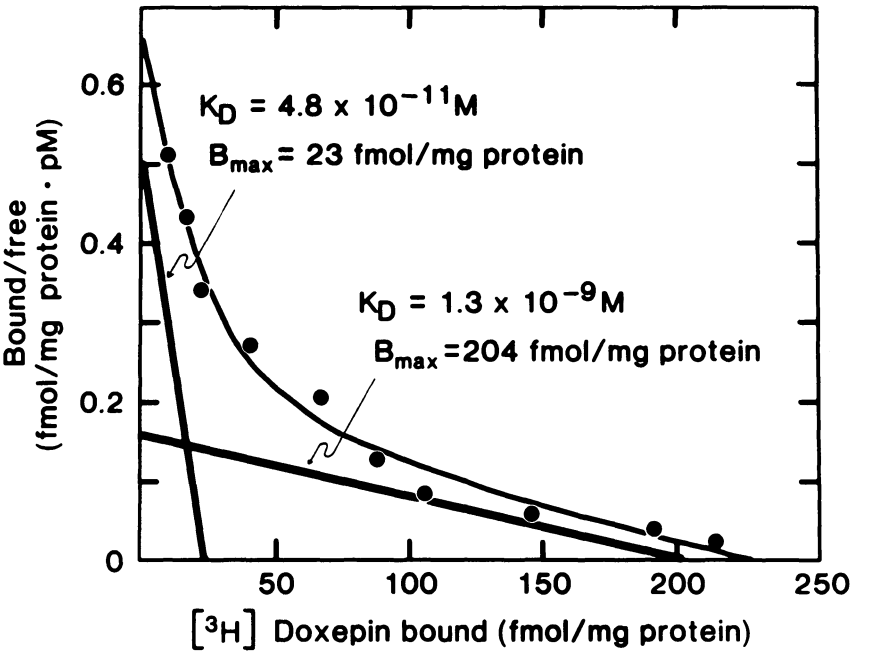


**Figure 1.** Rosenthal (Scatchard) analysis of [$^3$H]doxepin binding to rat brain. Reproduced from Taylor and Richelson (1982) with the permission of Elsevier Biomedical Press.

Radioactively labeled antagonists of histamine $H_2$ receptors (mostly [$^3$H]cimetidine) have been used in receptor binding studies in an attempt to identify these receptors (Rosenfeld *et al.*, 1976; Burkard, 1978; Devoto *et al.*, 1980; Kendall *et al.*, 1980; Rising *et al.*, 1980). However, in the reported work, the binding of [$^3$H]cimetidine lacks the pharmacological characteristics that one would expect if it were binding to a histamine $H_2$ receptor; and this binding is uniformly distributed throughout the brain. Thus, it is likely that this binding of [$^3$H]cimetidine is not to histamine $H_2$ receptors.

## COMPETITIVE ANTAGONISM BY ANTIDEPRESSANTS OF HISTAMINIC ($H_1$ AND $H_2$) AND MUSCARINIC RECEPTORS

### Histamine $H_1$ Receptors

As noted above, it was known from the outset that antidepressants of the tricyclic type were antihistamines (Kuhn, 1970). Just how

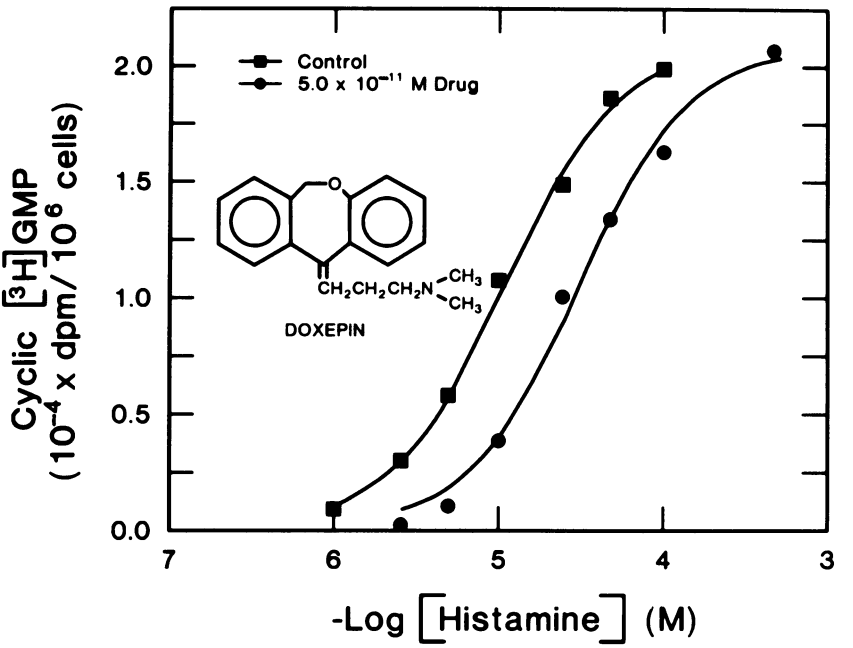


**Figure 2.** Effect of doxepin on histamine $H_1$ receptor-mediated cyclic GMP synthesis by murine neuroblastoma cells.

potent these compounds were as histamine $H_1$ antagonists was learned about 20 years after the first use of these drugs as antidepressants. With our assay using intact cultured nerve cells (Richelson *et al.*, 1978; Richelson, 1978a), we obtained equilibrium dissociation constants for tricyclic antidepressants and histamine $H_1$ receptors by dose-ratio analyses of dose-response curves (e.g. figure 2) (Richelson, 1978b, 1979). Our results (table 1) showed that all of these antidepressants were competitive antagonists of histamine $H_1$ receptors and that doxepin (figure 2) was perhaps the most potent histamine $H_1$ antagonist known (table 1), being, for example, about 800 times more potent than diphenhydramine at histamine $H_1$ receptors. Since our first reports of these results, we have added to the list of antidepressants studied (table 1) and still find doxepin to be the most potent compound tested.

Because we found that doxepin and amitriptyline were orders of magnitude more potent as antagonists of histamine $H_1$ receptors than a number of classical antihistaminics, we used another system, the guinea-pig ileum bioassay, to confirm these results (Figge *et al.*, 1979). $K_D$ values for tricyclic antidepressants

Table 1. Antidepressants and related compounds: equilibrium dissociation constants for histamine $H_1$ and muscarinic receptors

| Compound | Receptor | |
|---|---|---|
| | Histamine $H_1$[a] (pM) | Muscarinic[b] (nM) |
| Doxepin | 32 | 80 |
| Trimipramine | 100 | 58 |
| Amitriptyline | 130 | 18 |
| Mianserin | 310 | 820 |
| Maprotiline | 1000 | 570 |
| Butriptyline | 1800 | 35 |
| Amoxapine | 6800 | 1000 |
| Nortriptyline | 7000 | 150 |
| Imipramine | 10000 | 90 |
| 2-Hydroximipramine | 14500 | 1210 |
| Clomipramine | 29600 | 37 |
| Protriptyline | 35000 | 25 |
| Trazodone | 73300 | 324000 |
| Desipramine | 260000 | 198 |
| Iprindole | 320000 | 2100 |
| Didesmethylimipramine | 1090000 | 590 |
| Diphenhydramine | 25000 | 180 |
| Atropine | > 1000000 | 2 |

[a] Data from Richelson (1978b, 1979, and unpublished).
[b] Data from El-Fakahany and Richelson (1983).

determined from their competitive antagonism of histamine-induced contractions of the guinea-pig ileum were essentially the same as those found with the use of murine neuroblastoma cells.

After we reported our data for tricyclic antidepressants, another laboratory (Tran *et al.*, 1978) reported that tricyclic antidepressants were potent inhibitors of the binding of [$^3$H]-pyrilamine to histamine $H_1$ receptors of rat brain. The results reported by Tran *et al.* (1978) largely confirmed our work. However, there were some interesting discrepancies. For the high-affinity compounds (doxepin, amitriptyline and nortriptyline), their calculated equilibrium dissociation constants were at least one order of magnitude higher (indicating a lower affinity for these compounds in the binding assays). For the low-affinity compounds (imipramine, protriptyline and desipramine), our data from both murine neuroblastoma cells and guinea-pig ileum agreed very well with their data. In our radioligand binding studies

using [$^3$H]pyrilamine (Taylor and Richelson, 1980), we showed that these discrepant results for the high-affinity compounds resulted from the use in the assay of too high a receptor concentration which results in the overestimation of equilibrium dissociation constants.

In radioligand binding assays this equilibrium dissociation constant is often called an inhibitor constant, $K_i$, when it is calculated by the Cheng-Prusoff formula (Cheng and Prusoff, 1973) from the concentration of non-radioactively labeled drug giving 50% inhibition of binding ($IC_{50}$) of the radioligand. However, in general, whenever the affinity of the radiolabeled ligand is less than the affinity of the unlabeled ligand (as is the case, for example, of [$^3$H]pyrilamine being displaced by doxepin), incorrect estimates of the relative affinities may be obtained (Munson and Rodbard, 1980). However, even larger discrepancies from the true values occur when the $K_D$ of a non-radioactively labeled compound is in the range of the receptor concentration in the assay, because the $IC_{50}$ then becomes a function of the receptor concentration as well (Taylor and Richelson, 1980). For example, the $IC_{50}$ for the competition between [$^3$H]pyrilamine and doxepin can be increased about 40-fold by increasing the receptor concentration by only threefold!

Using [$^3$H]pyrilamine and relatively low concentrations of histamine $H_1$ receptors from rat brain, we obtained $IC_{50}$ data for six antidepressants (Taylor and Richelson, 1980). The calculated inhibitor constants ($K_i$) for these compounds showed a significant correlation with their equilibrium dissociation constants derived from the inhibition of either histamine $H_1$ receptor-mediated cyclic GMP formation by murine neuroblastoma cells or histamine $H_1$ receptor-mediated contractions of the guinea-pig ileum. In addition, in [$^3$H]pyrilamine binding studies with homogenates of feline brain (Taylor *et al.*, 1982), $K_D$ values for doxepin, amitriptyline and imipramine were very close to those values obtained with receptors from mouse, rat and guinea-pig. All these data obtained from different species, with the use of vastly different assays, strongly suggest that the true $K_D$ values for the tricyclic antidepressants are as listed in table 1 and that there are no species differences in this characteristic (i.e. binding of tricyclic antidepressants) of histamine $H_1$ receptors.

Since doxepin has such a uniquely high affinity for histamine $H_1$ receptors, we and others have studied the characteristics of its binding to tissue from rat (Tran *et al.*, 1981; Taylor and Richelson, 1982) and human brain (Kanba and Richelson, unpublished data). Rosenthal (Scatchard) analyses of binding data indicated the presence of two distinct binding sites for [$^3$H]doxepin (figure 1) in rat brain. In our studies (Taylor and Richelson, 1982), the $K_D$ of the high-affinity site was 20-50 pM, values similar to those obtained with the several different assays described above. The $K_D$

for the low-affinity site was about 40 nM and the maximum number of low-affinity binding sites was about 50 times the number of high-affinity sites. The regional distribution of the high-affinity sites correlated with the known distribution of histamine $H_1$ receptors and, from the results of competition studies between various drugs and [$^3$H]doxepin for the high-affinity site, we demonstrated that [$^3$H]doxepin was binding to histamine $H_1$ receptors. However, the low affinity site has not been identified.

There were some curious results with our binding studies. The maximum number of high-affinity [$^3$H]doxepin binding sites was about 10% of that found for [$^3$H]pyrilamine binding to histamine $H_1$ receptors in the same preparations. In addition, various tricyclic antidepressants were very potent competitors at the high-affinity [$^3$H]doxepin site with affinities for this site higher than those for the [$^3$H]pyrilamine site. These data suggested to us that [$^3$H]doxepin is binding to a subclass of histamine $H_1$ receptors. However, without a biological correlate of these binding studies, we do not know whether this conclusion is valid. It is too soon in our studies of [$^3$H]doxepin binding to human brain to know whether similar results will be obtained with this species.

### Histamine $H_2$ Receptors

Within a short period of time, two different laboratories using essentially similar assays independently reported that tricyclic and other types of antidepressants were more potent than cimetidine as competitive antagonists of histamine $H_2$ receptors (Green and Maayani, 1977; Kanof and Greengard, 1978). One group (Kanof and Greengard, 1978) even suggested that antagonism of histamine $H_2$ receptors by antidepressants was responsible for their therapeutic effects, an idea that is unlikely since most neuroleptics are more potent than tricyclic antidepressants in this assay system. In addition, cimetidine causes depression in some patients (Jefferson, 1979).

The assay used for these studies was histamine-stimulated adenylate cyclase activity in homogenates of guinea-pig brain (hippocampus and cortex). Since antidepressants exhibit high affinity for histamine $H_2$ receptors only in this assay system and not in assays using intact cell preparations from the guinea-pig hippocampus (Tuong *et al.*, 1980) or from porcine skin (Iizuka *et al.*, 1976), there appears to be a major problem with these data. In addition, antidepressants have little or no antagonistic effect in other biological assays of histamine $H_2$ receptors such as histamine-mediated gastric acid secretion or increase in heart rate (Bohman *et al.*, 1980; Schwartz *et al.*, 1981). The reasons for this discrepancy are unknown. However, it is clear that more research is required to know whether antidepressants are in fact potent histamine $H_2$ antagonists.

## Muscarinic Receptors

Like the antihistaminic ($H_1$) property of tricyclic antidepressants, the antimuscarinic property of these compounds has been known since the 1950s. However, the first papers to report equilibrium dissociation constants for some of these compounds were published only in the last 15 years (Brimblecombe and Green, 1967; Atkinson and Ladinsky, 1972; Shein and Smith, 1978). These data were derived in experiments which made use of muscarinic receptor-mediated contractions of the guinea-pig ileum (Brimblecombe and Green, 1967; Shein and Smith, 1978) or rat fundal strips (Atkinson and Ladinsky, 1972).

With the advent of radioligand binding technology, many laboratories have assessed the antagonistic potency of antidepressants at muscarinic receptors of brain from non-human species. Using a biological assay, we have, in addition, determined the equilibrium dissociation constants for antidepressants and muscarinic receptors of murine neuroblastoma cells (Richelson and Divinetz-Romero, 1977; Petersen and Richelson, 1982).

The question frequently arises, however, whether data for these drugs derived from studies with non-human tissues are applicable to human brain, the true site of action of these psychotherapeutic compounds. Therefore, using human brain tissue, we determined the equilibrium dissociation constants for a series of

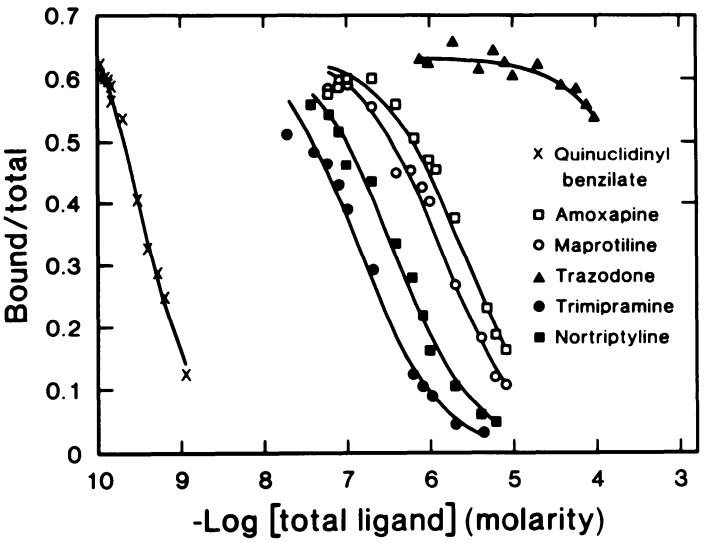


**Figure 3.** Competition by antidepressants for [$^3$H]quinuclidinyl benzilate binding to muscarinic receptors of human caudate nucleus.

antidepressants and related compounds (El-Fakahany and Richelson, 1983). A total of 22 compounds were tested for their ability to antagonize the binding of 1-[$^3$H]quinuclidinyl benzilate to muscarinic receptors in homogenates of human caudate nucleus (e.g. figure 3).

Equilibrium dissociation constants for these drugs and muscarinic receptors (table 1 lists 16 of the compounds studied) spanned over four orders of magnitude, with the tertiary amine tricyclic antidepressant amitriptyline ($K_D$ = 324 μM) the least potent.

We compared subsets of the data for antidepressants and related compounds obtained in the human brain studies with data derived by us using the murine neuroblastoma bioassay, with data derived by Shein and Smith (1978) using the guinea-pig ileum bioassay, and with data derived by Golds *et al.*, (1980) using radioligand binding and rat brain. In all three comparisons there was a significant correlation and the slope of the regression lines were all close to 1. We concluded from these analyses that there were no species differences between human, mouse, rat and guinea-pig in the affinities of antidepressants for muscarinic receptors.

## CLINICAL IMPLICATIONS OF THE ANTIHISTAMINIC AND ANTIMUSCARINIC PROPERTIES OF ANTIDEPRESSANTS

### Treatment of Depression

The histamine $H_1$ antagonism by antidepressants very likely is responsible for the sedation and drowsiness caused by some of these compounds. This can be a useful property for some patients who are both agitated and depressed and in this sense can be therapeutic. However, for other patients this sedation and drowsiness may become intolerable, necessitating a change to an antidepressant that is low on the list for histamine $H_1$ antagonism. This property of antidepressants will also potentiate the actions of central depressant drugs such as alcohol and minor tranquilizers.

It has been suggested that the antimuscarinic property of antidepressants is responsible for their mood-elevating effect (Janowsky *et al.*, 1972; Davis *et al.*, 1978). Abuse of antimuscarinic drugs for their euphoriant and other effects also supports this idea (Bluhm and Koller, 1981). Evidence presented here does not support this hypothesis since there is such a broad range of antimuscarinic potencies among the antidepressants with some compounds being practically devoid of activity (table 1).

The antimuscarinic property of antidepressants more likely reflects their propensity to cause adverse effects in patients, such as dry mouth, blurred vision, urinary retention and consti-

pation. When an antimuscarinic side-effect becomes a problem in a patient or when such side-effects are to be avoided, the clinician should choose an antidepressant that has low affinity for muscarinic receptors (table 1).

### Treatment of Other Diseases

The remarkable potency of antidepressants at blocking histamine $H_1$ receptors has led to their use in the treatment of allergic diseases (Sullivan, 1982). In addition, antidepressants are being used to treat peptic ulcer disease (Mangla and Pereira, 1982; Richelson, 1983) and their efficacy for treating ulcers may be related to their antihistaminic ($H_2$) and antimuscarinic properties.

## SUMMARY

In summary, knowledge of the potency of antidepressants at blocking histaminic and muscarinic receptors gives a rational basis for understanding interactions of these drugs with other drugs and for understanding some of their side-effects. In addition, these data from basic research studies are suggesting new uses for these compounds.

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# Antidepressants and components of the beta adrenoceptor system: studies on zimelidine

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## INTRODUCTION

Treatment of rats with a number of antidepressants for one to several weeks leads to a decrease in the density of beta adrenoceptors in the cerebral cortex and other brain regions (Bergstrom and Kellar, 1979; Maggi *et al.*, 1980). This reduction is accompanied by a concomitant decrease in the beta adrenoceptor-mediated cyclic AMP accumulation (Vetulani *et al.*, 1976; Wolfe *et al.*, 1978). Similar beta adrenoceptor alterations are also evoked by other antidepressant treatments. Thus, treatment with electroconvulsive therapy and monoamine oxidase inhibitors also induce beta adrenoceptor down-regulation (Gillespie *et al.*, 1979; Sellinger-Barnette *et al.*, 1980). The antidepressant treatments, however, have to be administered for a clinically relevant period of time before the changes are observable, whereas a single treatment does not change the number of beta adrenoceptors nor the associated cyclic AMP accumulation (Sarai *et al.*, 1978; Vetulani *et al.*, 1976; Wolfe *et al.*, 1978). We have found that deprivation of REM sleep, which is as effective as imipramine in the alleviation of depression (Vogel *et al.*, 1975), causes a reduction of beta adrenoceptor-mediated cyclic AMP accumulation in rat 'limbic' forebrain, without changing the density of beta adrenoceptors in this brain region (Klysner and Geisler, 1983). In these animals that were submitted to deprivation of REM sleep for one week, no change in either beta adrenoceptor number or cyclic AMP synthesis was observed in the cerebral cortex or in the cerebellum. In contrast, Mogilnicka *et al.* (1980) found that 72 h of REM sleep deprivation leads to a reduced density of beta adrenoceptors in the rat cerebral cortex.

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Treatment with reserpine, which in some persons may provoke a serious depression, causes an opposite change, i.e. the number of cerebral beta adrenoceptors is increased after reserpine administration. Concomitant treatment of rats with reserpine and lithium, giving a plasma lithium concentration of approximately 1 mmol $l^{-1}$, prevents the increase in beta adrenoceptor density due to reserpine treatment (Treiser and Kellar, 1979), an effect which has been proposed to be related to the ability of lithium to prevent the occurrence of depressive episodes. Concomitant treatment with lithium and imipramine does not prevent the beta adrenoceptor down-regulation brought about by imipramine (Rosenblatt *et al.*, 1979). We have observed that chronic treatment of rats with lithium, giving a plasma lithium concentration of 0.5-0.6 mmol $l^{-1}$, has no effect on the alteration in the number of $^{3}$H-dihydroalprenolol ($^{3}$H-DHA) binding sites in the cerebral cortex after reserpine and imipramine treatment, although this lithium treatment does counteract the rise in isoprenaline-stimulated cyclic AMP accumulation after reserpine treatment (A. Geisler and R. Klysner, unpublished).

Such studies suggest a relationship between down-regulation of beta adrenoceptors and alleviation of depression, and suggest that the function of these receptors may be increased in the brain of patients suffering from depressive illness. A few studies have focused on determination of beta adrenoceptor number and function in blood cells from depressed patients. Thus, Pandey *et al.* (1979) found that isoprenaline-stimulated cyclic AMP formation in a mixed leukocyte preparation was significantly reduced in patients with major depressive disorders in comparison to the isoprenaline-induced response in leukocytes from normal subjects. In a study performed by Extein *et al.* (1979) the number of beta adrenoceptors in isolated lymphocytes was lower in both depressed and manic patients compared to euthymic patients and control persons, and the same pattern of changes was observed when isoprenaline-stimulated cyclic AMP production was studied. The treatments given, i.e. desipramine and lithium, did not significantly alter $^{3}$H-DHA binding to the beta adrenoceptors, nor did they alter isoprenaline-stimulated cyclic AMP formation in the blood cells. However, in a study by Lerer *et al.* (1981), it was found that salbutamol given as an antidepressant did reduce beta adrenoceptor-mediated rise in the blood level of cyclic AMP. This cyclic AMP is thought to originate from beta adrenoceptors in blood vessels. It should be remembered, however, that alterations in the number and function of beta adrenoceptors in blood cells and blood vessels may depend on such local factors as the level of beta adrenoceptor agonists in the blood (epinephrine and salbutamol), and may therefore not give a true reflection of beta adrenoceptor function in the brain of these patients. An indication that beta adrenoceptor alterations after antidepressant treatments may in fact be confined to the brain comes from the

study of Frazer *et al.* (1978) in which no changes in isoprenaline-stimulated cyclic AMP formation was evident in the heart and diaphragm of animals treated with desipramine, although the same response was clearly reduced in the cerebral cortex.

The molecular events leading to a down-regulation of beta adrenoceptors in rat brain after antidepressant treatments are unknown. It is possible that increased availability of norepinephrine (NE) at the beta adrenoceptor due to inhibition by antidepressants of presynaptic NE uptake mechanisms, or due to a decreased number of $alpha_2$ adrenoceptors during antidepressant treatment, leading to increased NE release, may eventually result in a down-regulation of the beta adrenoceptors. In line with the latter assumption is the observation that a combined treatment with an antidepressant and an alpha adrenoceptor blocking drug results in a faster down-regulation of cerebral beta adrenoceptors than treatment with the antidepressant drug alone (Crews *et al.*, 1981; Wiech and Ursillo, 1980). However, Sugrue (1981) found that, although beta adrenoceptors in the cerebral cortex were down-regulated by various antidepressant treatments, only some of these treatments led to a reduction in $alpha_2$ adrenoceptor binding in this tissue. With regard to the notion that inhibition of presynaptic NE uptake by antidepressants results in beta adrenoceptor down-regulation, it is difficult to explain why treatment with cocaine does not decrease the number of beta adrenoceptors (Sellinger-Barnette *et al.*, 1980), even when given as chronic infusion (Sethy and Harris, 1981).

In addition, it seems complicating that some antidepressant treatments down-regulate both the number of beta adrenoceptors and associated cyclic AMP formation, while other treatments reduce the beta adrenoceptor-mediated cyclic AMP accumulation without altering the number of receptors. As mentioned above, we have found that deprivation of REM sleep decreased isoprenaline-mediated cyclic AMP accumulation in rat 'limbic' forebrain without affecting the number of beta adrenoceptors and, also, that low plasma lithium levels antagonized the effect of reserpine on beta adrenoceptor-mediated cyclic AMP formation without counteracting the reserpine-induced increase in beta adrenoceptor density. Furthermore, Mishra *et al.* (1979) observed a decreased NE-stimulated cyclic AMP accumulation in rat cerebral cortex after nisoxetine treatment without alteration of beta adrenoceptor binding, and Mishra *et al.* (1980) found that treatment with the new antidepressant zimelidine (Coppen *et al.*, 1979; Aberg-Wistedt *et al.*, 1981) had no effect on the density of beta adrenoceptors in rat cerebral cortex, yet inhibiting NE-stimulated cyclic AMP accumulation. In contrast, Ross *et al.* (1981) found that zimelidine dose-dependently reduced the number of beta adrenoceptors in rat cerebral cortex, and also Sethy and Harris (1981) found a down-regulation of beta adrenoceptor density after zimelidine treatment.

Since such discrepancies may arise from the use of beta adrenoceptor antagonists to determine the number of beta adrenoceptors and the use of beta adrenoceptor agonists to assess cyclic AMP accumulation, we have in the present study investigated the effect of zimelidine treatment on components of the beta adrenoceptor system, i.e. the binding of agonist and antagonist to the receptor and the associated cyclic AMP accumulation. Further, we have evaluated the fluoride-stimulated adenylate cyclase activity in order to assess possible changes in the GTP-binding protein (Citri and Schramm, 1980), which participates in the receptor-mediated cyclic AMP formation.

## MATERIALS AND METHODS

Male Wistar rats weighing 180-200 g were treated twice a day with 10 mg $kg^{-1}$ zimelidine dihydrochloride monohydrate, intraperitoneally. Controls received a corresponding volume of saline. After 14 days the treatment was discontinued and 24 h after the last zimelidine administration the rats were decapitated and dissected in a cold room (4°C). Most of the cerebral cortex on the dorsal and lateral aspect of the brain was used. Tissue for binding studies and fluoride-stimulated adenylate cyclase activity was frozen and stored at -80°C, while tissue for isoprenaline-stimulated cyclic AMP formation was processed immediately.

### $^3$H-DHA Binding

Binding of $^3$H-DHA was essentially as described by Bylund and Snyder (1976). The tissue was diluted to approximately 5 mg $ml^{-1}$. However, the incubation at 23°C was extended to 25 min, and non-specific binding was defined as the binding of $^3$H-DHA that could be displaced by 0.3 µmol $l^{-1}$ L-propranolol. When studying agonist binding, GTP was added to a final concentration of 0.3 mmol $l^{-1}$. The number of beta adrenoceptor sites ($B_{max}$) and their affinity for $^3$H-DHA was assessed by Scatchard analysis (Scatchard, 1949). The Scatchard analysis was made by linear regression.

### Fluoride-Stimulated Adenylate Cyclase

Tissue for fluoride-stimulated cyclic AMP formation was thawed at 4°C and processed as previously described (Laursen *et al.*, 1977) with the following exceptions: the tissue was diluted in 50 volumes of buffer and incubated for 40 min at 0°C followed by addition of NaF or water. The incubation continued for 20 min at 30°C and then cyclic AMP formation was started by addition of ATP (final concentration, 0.5 mmol $l^{-1}$). After 2.5 min, incubation

was stopped by boiling for 3 min. The boiled tissue was centrifuged at 4°C and the supernatant was stored at -20°C until determination of cyclic AMP.

### Isoprenaline-Stimulated Cyclic AMP Accumulation

Cerebral tissue for determination of cyclic AMP accumulation was chopped with a microtome to a thickness of 300 μm. After a 45° rotation of the table the tissue was chopped again. Thereafter the tissue was suspended in 20 ml of a Krebs-Ringer buffer with the following composition: NaCl, 122 mM; KCl, 3 mM; $CaCl_2$, 1.3 mM; $MgSO_4$, 1.2 mM; $KH_2PO_4$, 0.4 mM; D-glucose, 10 mM; $NaHCO_3$, 25 mM; $Na_2EDTA$, 10 mM. This buffer had previously been gassed with 95% $O_2$ - 5% $CO_2$ to a pH value of 7.3-7.4. The tissue preparation was then pre-incubated for 60 min at 37°C in an atmosphere of 95% $O_2$ - 5% $CO_2$ with changes of buffer every 20 min. After 60 min the tissue suspension was diluted and 500 μl aliquots were transferred to beakers continuously gassed with 95% $O_2$ - 5% $CO_2$. Buffer or isoprenaline and buffer or Rolipram were added and the incubation was continued for 15 min. The incubation was stopped by boiling the tissue for 10 min. The samples were then spun in the cold and the clear supernatant was stored at -20°C until determination of cyclic AMP. Cyclic AMP was determined by a competitive protein binding method (Geisler *et al.*, 1977). The protein content in the preparations was determined by the method of Lowry *et al.* (1951). Statistical analysis was performed by the Mann-Whitney rank sum test for unpaired samples. $^3$H-Dihydroalprenolol was obtained from New England Nuclear, Boston, Mass., DL-isoproterenol sulfate from Sigma Chemical Corp., St Louis, Mo., and guanosine 5'-triphosphate (GTP) was obtained from Boehringer, Mannheim, FRG. L-Propranolol was a generous gift from Imperial Chemical Industries, UK, and zimelidine dihydrochloride monohydrate was generously supplied by Astra Läkemedel AB, Södertälje, Sweden. All other agents used were of reagent grade. Rolipram (ZK 62.711) was generously supplied by Schering AG, Berlin/Bergkamen, FRG.

**Table 1.** Effect of 14 days of zimelidine treatment on $^3$H-DHA binding to rat cerebral cortex

| | $B_{max}$ (fmol/mg protein) | $K_d$ (nmol/mg protein) |
|---|---|---|
| Control | 256 ± 19 | 0.76 ± 0.07 |
| Zimelidine | 220 ± 13 (N.S.) | 0.84 ± 0.10 (N.S.) |

Means ± S.E.M. There were six animals in each group. Treated animals received zimelidine (10 mg $kg^{-1}$ b.i.d.) and controls received 0.9% NaCl.
N.S. = not significant compared to corresponding controls.

**RESULTS**

Treatment with zimelidine for 14 days was not found to change the affinity of beta adrenoceptors in the cerebral cortex for $^{3}$H-DHA, and although there was a trend toward a decrease in receptor density after the treatment, this did not, however, reach statistical significance (table 1). For both animals treated with zimelidine and control animals, displacement of specifically bound

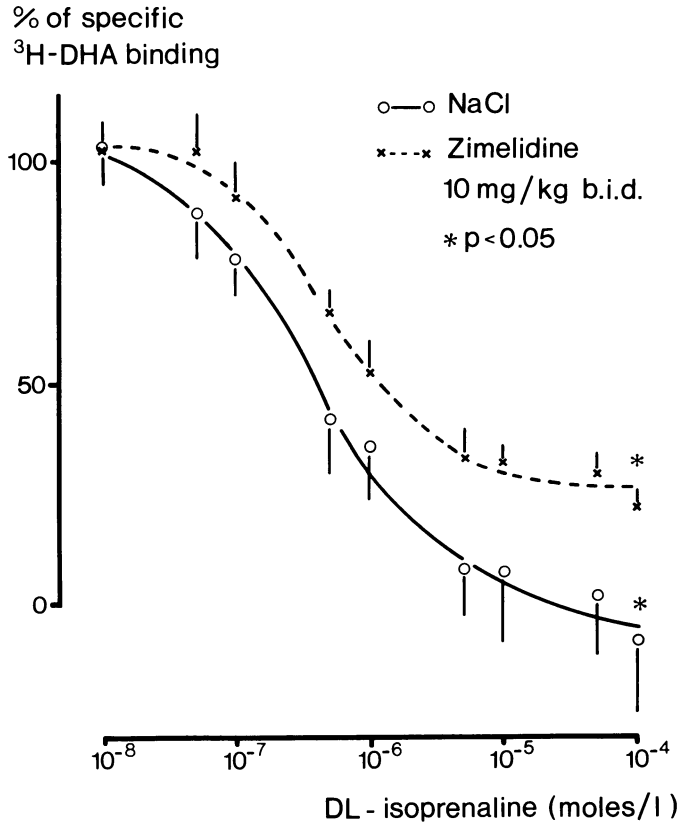


**Figure 1.** Displacement of specifically bound $^{3}$H-DHA (4 nmol $l^{-1}$) by increasing concentrations of DL-isoprenaline. Means ± S.E.M. There were six animals in each group. Rats were treated with zimelidine 10 mg $kg^{-1}$ b.i.d. or saline. The specific binding of $^{3}$H-DHA was defined for each animal as the binding that could be displaced by 0.3 µmol $l^{-1}$ of L-propranolol.

$^3$H-DHA (4 nmol $l^{-1}$) was performed with increasing concentrations of DL-isoprenaline. The specifically bound $^3$H-DHA was defined for each animal as the binding that could be displaced by 0.3 μmol $l^{-1}$ of L-propranolol. By this approach it was found that high concentrations of isoprenaline (0.1 mmol $l^{-1}$) displaced less specifically bound $^3$H-DHA in the zimelidine-treated group than in the control group, indicating a reduction of agonist binding sites after zimelidine treatment (figure 1). The affinity for isoprenaline, however, did not seem to be altered by the treatment, since $IC_{50}$ values were the same in the zimelidine-treated and in the control group. Zimelidine treatment was found to reduce isoprenaline-stimulated cyclic AMP accumulation in slices of cerebral cortex when measured as percent stimulation over basal values (table 2). This effect was only evident when the potent phosphodiesterase inhibitor Rolipram (Schwabe *et al.*, 1976) was present in the assay medium. The effect of zimelidine treatment on cyclic AMP formation was not due to a direct effect of zimelidine, since zimelidine added *in vitro* (0.1 mmol $l^{-1}$) had no effect on isoprenaline-stimulated cyclic AMP accumulation with Rolipram added to the assay medium. The same changes in cyclic AMP accumulation after zimelidine treatment as described above were seen when the absolute values were considered, although the changes did not reach statistical significance (table 3). Stimulation of cyclic AMP formation in homogenized cerebral cortex by NaF (10 mmol $l^{-1}$) was not changed by zimelidine treatment. Thus, in control animals the basal adenylate cyclase activity was 101 ± 7 pmol cyclic AMP/mg protein (mean ± S.E.M.), while the corresponding value for zimelidine-treated animals was 102 ± 7. The fluoride-stimulated activity was 317 ± 26 and 299 ± 27 pmol cyclic AMP/mg protein for control and zimelidine-treated animals, respectively. Each group comprised 16 animals.

**Table 2.** Effect of 14 days of zimelidine treatment on cyclic AMP accumulation in slices of rat cerebral cortex

| | **Isoprenaline** $1x10^{-4}$ M | **Isoprenaline** $1x10^{-4}$ M + **Rolipram** $1x10^{-5}$ M |
|---|---|---|
| Control | 236 ± 40 | 203 ± 35 |
| Zimelidine | 176 ± 27 (N.S.) | 102 ± 10* |

* $p<0.02$ compared to NaCl.
Results are expressed as percent stimulation above the basal level.
Means ± S.E.M. There were 13-14 animals in each group. Rats received zimelidine 10 mg $kg^{-1}$ b.i.d. or 0.9% NaCl.

**Table 3.** Effect of 14 days of zimelidine treatment on cyclic AMP accumulation in slices of rat cerebral cortex

| | Basal | Isoprenaline $1x10^{-4}$ M | Rolipram $1x10^{-5}$ M | Isoprenaline $1x10^{-4}$ M + Rolipram $1x10^{-5}$ M |
|---|---|---|---|---|
| Control | 7.7 ± 1.2 | 15.0 ± 2.2 | 19.6 ± 2.5 | 32.9 ± 3.6 |
| Zimelidine | 10.0 ± 1.0 | 15.9 ± 2.1 | 24.3 ± 1.4 | 25.3 ± 1.9 |
| | (N.S.) | (N.S.) | (N.S.) | (N.S.) |

Results are expressed as pmol cAMP/mg protein.
Means ± S.E.M. Rats received zimelidine 10 mg $kg^{-1}$ b.i.d. or 0.9% NaCl. There were 13-14 animals in each group.
The figures for isoprenaline and isoprenaline + Rolipram give increase above basal values.
N.S. = not significant compared to corresponding controls.

## DISCUSSION

In this study it was found that chronic treatment with zimelidine leads to a decreased beta adrenoceptor-mediated cyclic AMP accumulation in rat cerebral cortex. This supports the notion that all antidepressant treatments decrease beta adrenoceptor-mediated cyclic AMP formation in the rat cerebrum. The diminished cyclic AMP synthesis after zimelidine treatment is accompanied by a reduced binding of agonist to the beta adrenoceptor, and although the agonist binding was assessed indirectly by displacement of an antagonist, the data indicate a reduction of the number of agonist binding sites, but unaltered affinity for the agonist. In contrast, the antagonist binding was not found to be significantly changed by the zimelidine treatment. Determination of agonist binding sites may be more physiologically relevant, but it should be emphasized that in other studies antidepressants have been able to bring about a decreased beta adrenoceptor antagonist binding, although in many cases not of the same magnitude as the decrease in cyclic AMP formation. Consequently, the lack of correlation between the reduced antagonist binding and the reduction of cyclic AMP accumulation may be due to different changes in agonist and antagonist binding.

This study dose not give any indication of alterations in GTP binding proteins associated with cyclic AMP formation after zimelidine treatment, since the fluoride stimulation of cyclic AMP production did not differ between the control group and the zimelidine-treated group. It cannot, however, be excluded that a change did take place in the GTP binding proteins associated with beta adrenoceptor function, since these probably only constitute a minor part of the GTP binding proteins present in the cerebral cortex.

In most studies on the influence of antidepressant treatments on beta adrenoceptor-mediated cyclic AMP accumulation, inhibitors of cyclic AMP degradation have not been included in the assay medium. It is therefore not clear if changes in phosphodiesterase occur after antidepressant treatments, either as an adaptation to the altered beta adrenoceptor function or as a direct effect of antidepressants on phosphodiesterases. This possibility should be borne in mind since it has been shown that antidepressants can bind to calmodulin, a phosphodiesterase regulator (Reynolds and Claxton, 1982).

In the present study it has been necessary to include the potent phosphodiesterase inhibitor Rolipram in order to demonstrate the changes in isoprenaline-stimulated cyclic AMP accumulation after zimelidine treatment. Therefore, further studies on the effects of zimelidine and other antidepressants on the various components of the beta adrenoceptor system may serve to elucidate the changes occurring in beta adrenoceptor function after antidepressant treatment.

**SUMMARY**

The antidepressant drug zimelidine given for 2 weeks decreased agonist binding to beta adrenoceptors without significantly affecting antagonist binding in rat cerebral cortex. Further, zimelidine lowered the isoprenaline-stimulated cyclic AMP accumulation in slices of cerebral cortex, but only when a phosphodiesterase inhibitor was included in the assay medium. This study supports the assumption that antidepressant drugs reduce beta adrenoceptor function in the brain, but differences in the effect of various antidepressants on the functional components of the beta adrenoceptor system may occur.

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# ß-Adrenoceptor agonists enhance the functional activity of brain 5-hydroxytryptamine: relationship to antidepressant activity

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## INTRODUCTION

Salbutamol, a $\beta_2$-adrenoceptor agonist, has been reported to be a clinically effective antidepressant (Jouvent *et al.*, 1977; Lecrubier *et al.*, 1980; Lerer *et al.*, 1981). It has been suggested that salbutamol may be producing its therapeutic effect by an action on brain β-adrenoceptors (Lerer *et al.*, 1981; Kostowski, 1981). The question arises as to how such an action might be translated into an antidepressant effect and whether other β-adrenoceptor agonists might have a neuropharmacological spectrum of activity similar to that of salbutamol.

Repeated electroconvulsive shock given as electroconvulsive therapy is an effective antidepressant treatment in man (see Kendell, 1981). Repeated electroconvulsive shock in animals produces enhanced 5-hydroxytryptamine-mediated (5-HT) behavioral responses in experimental animals (see Costain *et al.*, 1979). There is also some evidence (Stolz and Marsden, 1982) that chronic tricyclic antidepressant administration, given the right experimental conditions, can produce enhancement of some 5-HT-mediated behaviors.

Salbutamol has been shown to increase 5-HT-mediated behavior in rats (Ortmann *et al.*, 1981; Cowen *et al.*, 1982). Here we wish to describe some of the effects of salbutamol and other β-adrenoceptor agonists on 5-HT-mediated behaviors and to describe in greater detail the effects of clenbuterol [4-amino-L-(*tert*-butylamine)methyl-3,5-dichlorobenzylalcohol-hydrochloride] which is also a $\beta_2$-adrenoceptor agonist (Engelhardt, 1976). It is very liposoluble and therefore enters the brain with ease (von Kopitar and Zimmer, 1976).

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## METHODS

### Animals

Adult male Sprague-Dawley derived rats and male $C_{57}$ Bl 01a mice were used. Animals were housed in groups of six in a 0800-2000 h light-dark cycle at constant temperature (20 ± 1°C) and fed on an *ad libitum* diet of modified 41B pellets and tap water. Behavioral studies were performed between 0930 and 1730 h.

### Behavioral Studies

5-HT-mediated behaviors in rats were examined in the following ways. Groups of three rats were pre-treated with the β-adrenoceptor agonist simultaneously with tranylcypromine (10 mg $kg^{-1}$) and 30 min later given L-tryptophan (50 mg $kg^{-1}$). In other experiments, pairs of rats were pre-treated with the appropriate β-adrenoceptor agonist and 15 min later given either quipazine (25 mg $kg^{-1}$) or 5-MeODMT (2 mg $kg^{-1}$). Hyperactivity responses produced by these treatments were measured on Automex meters. Results were analyzed using Student's unpaired *t* test.

Individual behavioral changes which occur following quipazine administration were rated separately. Forepaw treading, headweaving, hindlimb abduction and Straub tail were scored by the method described by Deakin and Green (1978) and Green *et al.* (1981); i.e 0 = absent, 1 = equivocal, 2 = present, 3 = severe. Results were analyzed using Wilcoxon non-parametric statistics.

Dopamine-mediated behavioral responses were tested using the unilateral nigrostriatal-lesioned rat model of Ungerstedt (1971a, b). 6-Hydroxydopamine (8 μg) was infused unilaterally into the substantia nigra as previously described by Heal *et al.* (1980). Circling activity to both apomorphine and metamphetamine was measured in glass rotameter bowls. Circling during 1 min was recorded at 10 min intervals following injection. Only rats which circled more than 5 turns per min to both apomorphine (2 mg $kg^{-1}$) and metamphetamine (2 mg $kg^{-1}$) were used. Results were analyzed using Student's paired *t* test and analysis of variance.

The 5-hydroxytryptophan-induced (5-HTP) head twitch responses were determined as follows: Mice received carbidopa (25 mg $kg^{-1}$ i.p.) followed 15 min later by clenbuterol (0.5 mg $kg^{-1}$) and 5-HTP (10 mg $kg^{-1}$ i.p.) after a further 15 min. Control animals received saline instead of clenbuterol. Head twitches were counted for 2 min every 15 min during the 60 min period following 5-HTP.

Brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) were measured by the method of Curzon and Green (1970). The concentration of brain 5-HT was measured 60 min after administration of

the monoamine oxidase inhibitor tranylcypromine (5 mg $kg^{-1}$ i.p.) in rats given either saline or clenbuterol (0.5 mg $kg^{-1}$ or 5 mg $kg^{-1}$ i.p.) 60 min earlier and the rate of 5-HT synthesis calculated by the method of Neff and Tozer (1968). Plasma total and ultrafilterable tryptophan was determined as previously described (Curzon *et al.*, 1972).

## DRUGS

All drugs were administered i.p. dissolved in 0.9% saline or suspended in 1% carboxymethylcellulose in saline. Carbidopa (5 mg $ml^{-1}$) and 5-HTP (20 mg $ml^{-1}$) were dissolved in a small volume of 0.1 N HCl before being diluted with normal saline to give a final concentration of 30 mM HCl.

## RESULTS

### Effect of β-Adrenoceptor Agonists on 5-HT-Mediated Behaviors

None of the β-adrenoceptor agonists administered alone, at doses employed in these experiments, stimulated locomotor activity or produced stereotyped behaviors. Therefore, the β-agonists themselves are not capable of producing the various behavioral syndromes produced by increased functional activity of 5-HT.

Clenbuterol at a dose of 5 mg $kg^{-1}$ administered 15 min before quipazine (25 mg $kg^{-1}$) produced marked enhancement of the behav-

**Table 1.** The effect of β-adrenoceptor agonists on the 5-HT-mediated hyperactivity produced by quipazine

| Pre-treatment | Activity counts (mean ± S.D.) | | | (*n*) |
|---|---|---|---|---|
| Saline | 2344 | ± | 265 | (7) |
| Clenbuterol (0.25 mg $kg^{-1}$) | 3093 | ± | 191 | (4)† |
| Clenbuterol (5 mg $kg^{-1}$) | 3516 | ± | 238 | (3)† |
| Saline (pH 3) | 2183 | ± | 372 | (7) |
| Salbutamol (5 mg $kg^{-1}$) | 2396 | ± | 623 | |
| Terbutaline (5 mg $kg^{-1}$) | 2203 | ± | 359 | (3) |
| Salbutamol (20 mg $kg^{-1}$) | 3030 | ± | 178 | (3)* |
| Terbutaline (20 mg $kg^{-1}$) | 3541 | ± | 785 | (3)* |

The β-adrenoceptor agonist was injected intraperitoneally (i.p.) 15 min before quipazine, 25 mg $kg^{-1}$ i.p. Activity counts are the number of counts for the 50 min following quipazine administration recorded by pairs of rats placed on Automex meters. Bracketed figure is number of pairs of animals tested.

Different from appropriate saline control: $*p < 0.01$, $\dagger p < 0.001$.

ioral response. This enhancement was detected by an increase in automated counts recorded on the Automex meters and also by scoring the behavioral responses of headweaving, forepaw treading and hindlimb abduction.

Clenbuterol was effective in increasing 5-HT-mediated behavioral responses at a dose of 0.25 mg $kg^{-1}$. In contrast, neither salbutamol nor terbutaline enhanced the 5-HT hyperactivity syndrome produced by quipazine at a dose of 5 mg $kg^{-1}$. At a dose of 20 mg $kg^{-1}$ they did, however, produce an enhanced response (see table 1).

Clenbuterol (5 mg $kg^{-1}$) given 15 min *after* quipazine produced significant enhancement of the hyperactivity syndrome. At a lower dose (0.5 mg $kg^{-1}$), it failed to do so. The timing of the clenbuterol administration in relation to its potency in enhancing 5-HT-mediated behaviors may, therefore, be crucial.

Clenbuterol (5 mg $kg^{-1}$) also enhanced the hyperactivity produced by the 5-HT agonist, 5-MeODMT (0.2 mg $kg^{-1}$) and also that produced by tranylcypromine (10 mg $kg^{-1}$) with L-tryptophan (50 mg $kg^{-1}$), a combination known to increase brain 5-HT and its spillover into functional activity (see table 2).

Table 2. The effect of clenbuterol on the hyperactivity syndromes produced by 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), and tranylcypromine (TCP) and L-tryptophan

| Treatment | | | Activity counts (mean ± S.D.) | | | (*n*) |
|---|---|---|---|---|---|---|
| Saline | + | 5-MeODMT | 807 | ± | 65 | (4) |
| Clenbuterol | + | 5-MeODMT | 1152 | ± | 92 | (4)† |
| Saline | + | TCP/L-tryptophan | 2306 | ± | 420 | (4) |
| Clenbuterol | + | TCP/L-tryptophan | 3492 | ± | 393 | (4)* |

Clenbuterol (5 mg $kg^{-1}$) was injected 15 min before 5-MeODMT (2 mg $kg^{-1}$) or together with TCP (10 mg $kg^{-1}$) 30 min before L-tryptophan (40 mg $kg^{-1}$). Results show the mean ± S.D. of activity counts in the 20 min following 5-MeODMT, or the 90 min following tryptophan. Animals were tested either in pairs (5-MeODMT) or in groups of three (TCP/L-tryptophan). The bracketed figure refers to the number of groups of animals tested.

Different from appropriate saline control: $*p < 0.01$, $\dagger p < 0.001$.

## Effect of Clenbuterol on 5-HTP-Induced Head Twitches in Mice

Clenbuterol (0.5 mg $kg^{-1}$) injected 15 min before 5-HTP (100 mg $kg^{-1}$) produced a significant enhancement of the mouse head twitch.

### Effect of β-Adrenoceptor Antagonists on Clenbuterol-Induced Enhancement of Quipazine Hyperactivity

The β-adrenoceptor antagonists were administered 15 min before the s.c. injection of clenbuterol (2 mg $kg^{-1}$). Quipazine (25 mg $kg^{-1}$) was administered 15 min later.

Metoprolol (5 mg $kg^{-1}$) blocked the clenbuterol-induced enhancement of quipazine hyperactivity. Atenolol (5 mg $kg^{-1}$) and butoxamine (5 mg $kg^{-1}$) were without effect (table 3). None of the β-adrenoceptor antagonists significantly reduced the baseline responses to quipazine (table 3).

Table 3. Effect of β-adrenoceptor antagonists on clenbuterol-induced enhancement of quipazine hyperactivity

| Pre-treatment | | | Activity counts (mean ± S.D.) | (*n*) |
|---|---|---|---|---|
| Saline | + | saline | 2108 ± 448 | (3) |
| Saline | + | clenbuterol | 2908 ± 189 | (4)* |
| Metoprolol | + | saline | 2005 ± 333 | (5) |
| Metroprolol | + | clenbuterol | 2298 ± 310 | (5) |
| Butoxamine | + | saline | 1723 ± 372 | (3) |
| Butoxamine | + | clenbuterol | 3355 ± 570 | (3)† |
| Atenolol | + | saline | 2090 ± 74 | (3) |
| Atenolol | + | clenbuterol | 2671 ± 230 | (3)† |

The β-adrenoceptor antagonists were injected i.p. in a dose of 5 mg $kg^{-1}$ 15 min before either saline or clenbuterol 2 mg $kg^{-1}$ subcutaneously. Quipazine 25 mg $kg^{-1}$ i.p. was administered 15 min later. Activity counts were recorded as described in table 1. The bracketed figure is the number of pairs of animals tested.

Different from appropriate saline control: $*p < 0.05$, $\dagger p < 0.02$.

### Effect of Clenbuterol Pre-Treatment on Dopamine-Mediated Behavioral Responses

Rats with unilateral lesions of the substantia nigra were first pre-treated with clenbuterol (5 mg $kg^{-1}$). After 15 min the circling responses were tested with either metamphetamine (0.5 mg $kg^{-1}$) or apomorphine (0.5 mg $kg^{-1}$). Clenbuterol administration did not alter the ipsilateral circling response to metamphetamine. The contralateral circling response to the dopamine agonist apomorphine did not differ from controls at any timepoint or in total rotations during the 60 min period of the experiment. However, the rate of decline of response to apomorphine appeared to be slower in the clenbuterol-treated rats.

Table 4. Effect of clenbuterol on brain tryptophan and indoleamine concentration and metabolism

| Brain concentration (g/g brain) | Saline | Clenbuterol ($0.5$ mg $kg^{-1}$) | Clenbuterol ($5.0$ mg $kg^{-1}$) |
|---|---|---|---|
| Tryptophan | 2.03 ± 0.20 (10) | 3.86 ± 0.23 (10)† | 9.14 ± 0.97 (4)† |
| 5-Hydroxyindoleacetic acid | 0.50 ± 0.02 (9) | 0.59 ± 0.03 (5)* | 0.61 ± 0.01 (8)§ |
| 5-Hydroxytryptamine | 0.32 ± 0.01 (7) | 0.32 ± 0.02 (5) | 0.36 ± 0.01 (5)* |
| 5-HT after tranylcypromine | 0.48 ± 0.01 (10) | 0.56 ± 0.02 (6)† | 0.58 ± 0.01 (6)§ |
| Synthesis rate ($\mu g\ g^{-1}h^{-1}$) | 0.16 | 0.24 | 0.22 |

Results expressed as mean ± S.E.M. with the number of observations in brackets. Tryptophan, 5-HT and 5-HIAA concentrations were measured 60 min after clenbuterol. Brain 5-HT concentration was also measured 60 min after tranylcypromine.

Different from saline-treated controls: *$p < 0.025$, †$p < 0.01$, §$p < 0.001$.

### Effect of *p*-Chlorophenylalanine (PCPA) Pre-Treatment on Clenbuterol-Induced Enhancement of Quipazine-Mediated Hyperactivity

Rats were injected with PCPA (200 mg $kg^{-1}$ i.p.) at 1530 h on day 1 and day 2. On day 3 the animals were injected with clenbuterol (0.5 mg $kg^{-1}$ i.p.) or saline followed 15 min later by quipazine (25 mg $kg^{-1}$ i.p.).

Pre-treatment with PCPA did not affect the enhancement by clenbuterol of quipazine-induced hyperactivity. Biochemical investigations showed that pre-treatment with PCPA produced a 65% reduction in whole brain 5-HT concentrations.

### Effect of Prazosin Pre-Treatment on the Clenbuterol-Induced Enhancement of Quipazine-Mediated Hyperactivity

Prazosin (3 mg $kg^{-1}$ i.p.) was administered 15 min before clenbuterol (0.5 mg $kg^{-1}$ i.p.) or saline and quipazine (25 mg $kg^{-1}$) after a further 15 min. Although prazosin pre-treatment reduced the quipazine-induced hyperactivity response in its own right, an enhancement of this quipazine response was still seen in rats pre-treated with clenbuterol. Thus, $\alpha_1$-adrenoceptor inhibition had no effect on the enhancement of 5-HT function by clenbuterol.

### Brain and Plasma Indole Concentrations Following Clenbuterol

Clenbuterol (0.5 mg $kg^{-1}$) did not alter whole brain 5-HT concentrations 50 min later but a dose of 5 mg $kg^{-1}$ produced a modest increase (table 4). Both doses increased brain 5-HIAA concentration. There was an increase in whole brain 5-HT content following tranylcypromine which was greater in rats pre-treated with clenbuterol, suggesting that there was an increase in brain 5-HT turnover.

Both doses of clenbuterol increased the concentration of tryptophan in the brain.

Clenbuterol produced about a doubling of plasma free fatty acid concentration, a marked decrease in plasma total tryptophan concentration, a small but not statistically significant increase in plasma free tryptophan concentration and a 170% increase in the plasma free tryptophan as a percentage of plasma total tryptophan (table 5).

Since clenbuterol increased brain tryptophan and brain 5-HIAA concentrations, the effect of the peripheral $\beta_1$-adrenoceptor antagonist atenolol (5 mg $kg^{-1}$) given 15 min before clenbuterol (5 mg $kg^{-1}$) on the increase in brain 5-HIAA concentration was examined. Atenolol alone did not alter brain 5-HIAA concentration but it completely blocked the rise produced by clenbuterol (table 6).

Table 5. Plasma free fatty acid and tryptophan concentration 30 min after clenbuterol (5 mg $kg^{-1}$)

| Plasma concentration | Saline ($n$ = 6) | Clenbuterol (5 mg $kg^{-1}$) ($n$ = 6) |
|---|---|---|
| Free fatty acid (mEq $l^{-1}$) | 0.57 ± 0.07 | 1.17 ± 0.08* |
| Total tryptophan (μg $ml^{-1}$) | 17.41 ± 1.1 | 8.75 ± 0.7* |
| Free tryptophan (μg $ml^{-1}$) | 3.26 ± 0.30 | 4.32 ± 0.60 |
| Free tryptophan (%) | 18.72 ± 1.5 | 48.47 ± 4.4* |

Different from saline-treated controls: * $p < 0.001$.

Table 6. Effect of atenolol on the clenbuterol-induced rise of brain 5-HIAA

| | Brain 5-HIAA concentration (μg/g brain) | |
|---|---|---|
| Saline | 0.50 ± 0.02 | (9) |
| Clenbuterol (5 mg $kg^{-1}$) | 0.61 ± 0.01 | (8)* |
| Atenolol (5 mg $kg^{-1}$) | 0.53 ± 0.02 | (4) |
| Atenolol (5 mg $kg^{-1}$) + clenbuterol | 0.49 ± 0.03 | (4) |

Brain 5-HIAA concentration was measured 60 min after clenbuterol. Different from saline, atenolol or atenolol plus clenbuterol: * $p < 0.001$.

## DISCUSSION

Although salbutamol and terbutaline both enhanced 5-HT-mediated behaviors, they were less potent in doing so than clenbuterol. This is probably because they are less lipid-soluble and therefore do not enter the brain as readily as clenbuterol. Delini-Stula *et al.* (1979) showed that salbutamol potentiated 5-HTP-induced head twitch. Fenoterol and terbutaline were also shown to enhance this behavior (Ortmann *et al.*, 1981). We have now shown that clenbuterol is active in enhancing head twitch behavior.

Metoprolol, which enters the brain, blocked the clenbuterol-induced enhancement of quipazine hyperactivity while atenolol, which has limited brain penetration (Day *et al.*, 1977), did not. The precise nature of the receptor upon which clenbuterol may be acting to enhance 5-HT-mediated behaviors is difficult to interpret. While clenbuterol, salbutamol and related agonists are classified as $\beta_2$-adrenoceptor agonists, the effect of clenbuterol

was blocked by metoprolol, which is a relatively selective $\beta_1$-adrenoceptor antagonist, but not by the $\beta_2$-adrenoceptor antagonist butoxamine. Although the selectivity of β-adrenoceptor agonists is not absolute and may be dose-related, it is odd that butoxamine was inactive. From these limited experiments, it seems that clenbuterol may produce enhancement of 5-HT-mediated responses by activation of central $\beta_1$-adrenoceptors, but more experiments need to be done on this point.

It is important to note that none of the β-adrenoceptor agonists administered alone induced behaviors like those produced by increasing brain 5-HT function. It seems unlikely, therefore, that the β-adrenoceptor agonists produce their enhancement of 5-HT-mediated responses by acting directly upon 5-HT receptors. This possibility has to be considered because non-selective β-adrenoceptor antagonists reduce 5-HT-mediated responses (Costain and Green, 1978), perhaps by direct blockade of 5-HT receptors (Middlemiss *et al.*, 1977).

The effect upon dopamine-mediated behaviors of clenbuterol was insignificant compared with its effect upon 5-HT-mediated behaviors. There was a slight slowing of the rate of decline of apomorphine-induced circling; in other experiments, clenbuterol has been known to increase slightly apomorphine-induced hyperactivity, but the effects are minor as compared with the effects on 5-HT-mediated behaviors.

Careful observation showed that clenbuterol enhanced those quipazine-induced behaviors thought to be specifically mediated by 5-HT, i.e. headweaving, forepaw treading and hindlimb abduction, as well as more generalized hyperactivity. In addition, clenbuterol has now been shown to enhance the 5-HTP-induced head twitch, as does salbutamol (Ortmann *et al.*, 1981), so that clenbuterol is active in various behavioral models of central 5-HT function.

Waldmeier (1981) found increased levels of 5-HIAA in rat brain, striatum and cortex after clenbuterol (0.3 mg $kg^{-1}$ i.v.) and also found increased 5-HT levels in the striatum. Erdö *et al.* (1982) found no change in rat whole brain 5-HT and 5-HIAA levels after salbutamol administration. In the present study, clenbuterol has been shown to elevate whole brain 5-HT concentrations (at the high dose of 5 mg $kg^{-1}$) and to increase brain 5-HT synthesis rate. Our investigations imply that increased plasma level of non-esterified (or free) fatty acids probably displace tryptophan from plasma albumin binding sites, increase plasma free tryptophan concentrations, and result in an increase in brain tryptophan concentrations, producing a resulting increase in brain 5-HT synthesis and metabolism (Curzon and Fernando, 1976). An alternative explanation might be that, if clenbuterol causes increased secretion of insulin by stimulation of pancreatic cell β-adrenoceptors, the increased plasma levels of insulin may produce a fall in the plasma concentration of branched-chain amino

acids competing with tryptophan for transport in the brain, which may then facilitate tryptophan transport into the brain (Munro *et al.*, 1975), with subsequent increase in 5-HT synthesis and turnover.

We believe, though, that there is little causative relationship between clenbuterol-induced changes in brain 5-HT metabolism and the clenbuterol-induced enhancement of 5-HT-mediated behaviors. Behavioral enhancement still occurs in rats treated with PCPA to lower brain 5-HT concentrations. Additionally, the action of direct 5-HT agonists, such as quipazine and 5-methoxy-*N*,*N*-dimethyltryptamine, which are also enhanced by clenbuterol, speak against the involvement of a presynaptic increase in 5-HT synthesis and release from nerve terminals in the phenomenon of enhancement of 5-HT-mediated behavioral responses by clenbuterol. Atenolol blocked the clenbuterol-induced rise in brain 5-HIAA concentration but not the enhancement of quipazine-mediated behaviors and it seems likely that atenolol blocks the metabolic effects of clenbuterol on plasma free fatty acid concentrations and the changes in plasma and brain tryptophan.

It is not yet clear how β-adrenoceptor agonists such as salbutamol and clenbuterol enhance 5-HT-mediated behavioral responses. The experiments reported here seem to suggest that clenbuterol is not acting on presynaptic mechanisms involved in 5-HT synthesis and release. This is not to say that β-agonists by acting on norepinephrine (NE) receptors in 5-HT cell bodies might not influence the activity of 5-HT neurons and increase the turnover of 5-HT (Baraban and Aghajanian, 1981), but this does not seem to be the mechanism by which clenbuterol enhances 5-HT behavioral responses.

One must, therefore, ask whether clenbuterol could increase the sensitivity of 5-HT receptors. As far as the brain is concerned, we have no information on that point.

It seems more likely, however, that clenbuterol acts upon that sequence of neural mechanisms by which activation of 5-HT receptors is translated into a behavioral response. We have seen no impressive enhancement of dopamine-mediated behaviors by clenbuterol. Therefore, the enhancement of 5-HT-mediated behaviors does not seem to involve a dopaminergic mechanism despite the known interactions of dopaminergic and serotonergic mechanisms in the production of the 5-HT hyperactivity syndrome (Green and Grahame-Smith, 1974).

It will be necessary, however, to define those receptors upon which clenbuterol is acting before we can understand the mechanisms by which it enhances 5-HT-mediated behaviors. It is distinctly odd that a β-agonist with predominantly $\beta_2$ activity should be blocked by an antagonist with mainly $\beta_1$-antagonist activity. Simplistically this might mean that either the brain receptors responsible for interacting with the so-called $\beta_2$-agonists are unlike those receptors classified as $\beta_1$ or $\beta_2$ in the periphery,

or that clenbuterol, a so-called $\beta_2$-agonist, is not actually acting on β-adrenoceptors, i.e. NE or epinephrine receptors, but on some other related type of amine receptor which, fortuitously, is blocked by metoprolol.

Apart from these neuropharmacological speculations, how do the findings described in this paper have relevance to the antidepressant action of $\beta_2$-agonists? The most information on clinical efficacy is on salbutamol and no adequate trials of the antidepressant activity of clenbuterol have yet been reported. If, however, the antidepressant activity of salbutamol is related to enhancement of brain 5-HT function, then clenbuterol should be a more potent antidepressant than salbutamol. Many lines of investigation suggest that there may be abnormalities of 5-HT function in the brains of endogenously depressed patients and that in certain patients treatments which enhance 5-HT function, such as L-tryptophan, tryptophan plus a monoamine oxidase inhibitor, 5-HTP, 5-HTP plus a specific 5-HT uptake inhibitor (such as chlorimipramine), are effective antidepressant therapies. Because of these lines of evidence, it is of great interest to observe that the so-called $\beta_2$-agonists have a degree of selectivity in enhancing 5-HT function. Assuming that the $\beta_2$-agonists are acting on some type of NE receptor in an area of the brain, it would be of great interest in terms of the various monoamine theories of depression to sort out neuropharmacologically the way in which noradrenergic mechanisms are modulating 5-HT functional activity.

There are further interesting analogies and paradoxes. The functional activity of rat brain 5-HT is increased by repeated electroconvulsive shock, yet in these circumstances cortical β-adrenoceptor number and their function (i.e. NE-sensitive adenylate cyclase activity) are decreased. This would not be expected if the 'degree' of 5-HT-mediated behavior was directly dependent on β-adrenoceptor sensitivity and activity. In addition, the functional activity of brain 5-HT is inhibited by non-selective lipophilic β-adrenoceptor antagonists, such as propranolol (Costain and Green, 1978). This does fit the hypothesis that in some way β-adrenoceptor function is positively correlated to 5-HT function. Against this, however, is the observation that, although lesions of the locus coeruleus and ventral and dorsal noradrenergic bundles prevent the enhancement of 5-HT-mediated behaviors by repeated ECS, such lesions do not in themselves inhibit or alter the 5-HT-mediated behaviors described (Green and Deakin, 1980). Thus these 5-HT-mediated behaviors are not dependent upon the functioning of the noradrenergic projections to the cortex.

At the moment we cannot construct an overall hypothesis which fits together the following points:

(1) $\beta_2$-Agonists enhance behavioral responses to 5-HT agonists.
(2) This enhancement is blocked by a $\beta_1$-antagonist but not by a $\beta_2$-antagonist.

(3) Repeated electroconvulsive shock (given like ECT) down-regulates cortical β-adrenoceptor function but enhances 5-HT function. Antidepressant drug administration down-regulates cortical β-adrenoceptor function but enhances certain aspects of 5-HT function.
(4) Non-selective lipophilic β-adrenoceptor antagonists (e.g. propranolol) inhibit 5-HT-mediated behavioral responses.

Within these analogies and paradoxes, a great deal remains to be revealed about the inter-relationships of brain 5-HT and noradrenergic function.

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# Effects of antidepressant treatments on 'whole body' norepinephrine turnover

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## INTRODUCTION

Monoamine theories of depression postulate that a relative lack of either norepinephrine (Schildkraut, 1965; Bunney and Davis, 1965) or serotonin (Ashcroft *et al.*, 1966; Coppen, 1967) at certain critical synapses in the central nervous system (CNS) are associated with symptoms of 'endogenous' depression. Based on these theories, Prange *et al.* (1974) have developed a 'permissive monoamine' hypothesis of bipolar affective disorders. They suggest that a continuously low serotonergic tone permits wide fluctuations in CNS norepinephrinergic activity, low activity being associated with depressive and high with manic symptoms. Janowsky *et al.* (1972) have emphasized that a cholinergic-norepinephrinergic imbalance may be important in generating affective symptoms. In their view, an increased cholinergic tone and a reduced norepinephrinergic activity is characteristic of depression, whereas a relatively low cholinergic tone and a high norepinephrinergic activity is associated with mania.

The possible role of monoamines in affective disorders was originally suggested by side-effects of antihypertensive treatments such as reserpine, which deplete CNS monoamines (Brodie and Costa, 1962) and produce depressions in susceptible individuals (Achor *et al.*, 1955). Moreover, the classic antidepressant drugs, tricyclics and monoamine oxidase inhibitors (MAOIs), block, respectively, the re-uptake (Carlsson *et al.*, 1969a, b) or enzymatic degradation of norepinephrine, serotonin and several other monoamines.

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Maas (1975) summarized the literature concerning the biochemistry and pharmacology of affective disorders and concluded that there could be two biochemical subtypes of depressions: one associated with a relative lack of norepinephrine and another with a relative lack of serotonin. Two clinical studies, which involved treatment with desipramine or zimelidine, relatively specific uptake inhibitors of norepinephrine or serotonin respectively, in an intended crossover design failed to support this postulate (Stewart *et al.*, 1980; Aberg, 1981). In both studies, a large majority of patients responded well to the first active treatment regardless of its transmitter specificity.

Animal experiments investigating receptor changes in the CNS produced by different chronic antidepressant treatments have demonstrated desensitization of norepinephrinergic postsynaptic β and presumed presynaptic $\alpha_2$ adrenoceptors, serotonin receptors and, in some instances, presynaptic dopamine autoreceptors (for review, see Charney *et al.*, 1981). The most recent studies have found that direct manipulations of the norepinephrine or serotonin neuronal systems at the specific receptor level can cause secondary functional changes in the other system (Przegalinsky *et al.*, 1981; Hallberg *et al.*, 1982; Grahame-Smith *et al.*, 1982).

Thus, contrary to earlier suggestions, the transmitter specificity of certain antidepressants may not be helpful in parceling out the significance of a given neurotransmitter to the pathology of depression in a given patient. Consequently, attempts to define 'norepinephrine and serotonin depressions' may be futile. Indeed, our recent data in schizophrenics and murderers which follow upon previous findings in other groups of subjects support that low 5-hydroxyindoleacetic acid, already reported to be associated with suicidal depressions and impulsive psychopathy (Asberg *et al.*, 1976; Brown *et al.*, 1979), is not characteristic of depression, but rather is primarily associated with violent behavior toward oneself or others (Ninan *et al.*, unpublished; Virkkunen *et al.*, unpublished).

In light of the above uncertainties concerning the role of serotonin and the preponderance of norepinephrine theories in the field, we focused our efforts to quantitate effects of antidepressant treatments on norepinephrine metabolism in depressed patients. We are measuring indices of dopamine and serotonin metabolism as well, because of the above-mentioned animal and clinical data suggestive of important interactions between the monoaminergic neuronal systems. This communication, however, deals mainly with the results involving norepinephrine. Our strategy has been to use the most specific monoamine re-uptake inhibiting antidepressants clinically available (Carlsson *et al.*, 1969a,b; Ogren *et al.*, 1981), and to compare them with clorgyline, a specific MAO type A inhibitor (Johnston, 1968) and the 'non-specific' treatments, lithium and electroconvulsive therapy. All patients were followed longitudinally during a lengthy pre-

drug placebo period and during steady-state kinetics on each drug. We measured the monoamines and their main metabolites in the urine, because this is currently the best way to investigate the turnover of these transmitters in man.

## PATIENTS AND METHODS

Twenty-one severely depressed patients, 17 women and four men, participated in our studies (Linnoila *et al.*, 1982a, b, 1983, and unpublished). All of them underwent a minimum 5-week initial placebo period and were subsequently treated with either clorgyline, desipramine, ECT, lithium or zimelidine. Clorgyline was administered in doses of 5 or 10 mg per 24 h to four women with a primary, major bipolar affective disorder (Spitzer *et al.*, 1978). Three men and nine women, six unipolar and six bipolar, participated in the desipramine-zimelidine trial. Based on single dose kinetics, the dose of desipramine was titrated to produce plasma levels between 300 and 450 $\mu mol\ l^{-1}$ (Potter *et al.*, 1981). Zimelidine was administered as a 200-300 mg/24 h dose. Seven women and one man, four bipolar and four unipolar, took part in the ECT-lithium study. In all studies, where a crossover design was partially used (four patients in the desipramine-zimelidine trial and three patients in the ECT-lithium study), an adequate washout period was allowed between the treatments. Three patients participated in more than one study on separate admissions. Throughout the studies, all patients were maintained on a strictly controlled low monoamine diet (Muscettola *et al.*, 1977).

The severities of depression, mania and psychosis were rated every morning and night by trained psychiatric nurses with the Bunney and Hamburg (1963) scale.

Urinary norepinephrine, normetanephrine, 3-methoxy-4-hydroxyphenylglycol (MHPG) and vanilmandelic acid (VMA) were quantified with mass fragmentography (Karoum and Neff, 1982). Twenty-four hour urine samples (7 a.m. - 7 a.m.), with volumes in excess of 900 ml, obtained during the last two weeks of placebo treatment and during steady-state kinetics on the antidepressants as well as between days 7 to 14 after the ECTs, were stored at -20°C and preserved with 3% sodium metabisulfite until analyzed. The means of three to four urine collections were used in the statistical analyses. Urinary norepinephrine, normetanephrine, MHPG and VMA outputs were added to produce an indicator of 'whole body' norepinephrine turnover (Linnoila *et al.*, 1982a).

The urinary norepinephrine and metabolite outputs and 'whole body' norepinephrine turnovers during placebo and active treatments were compared by parametric analysis of variance for repeated measurements, when appropriate, and with Student's *t*-test for related samples. The mood ratings were compared using the Mann-Whitney *U*-test. Two-tailed probabilities were used in all comparisons.

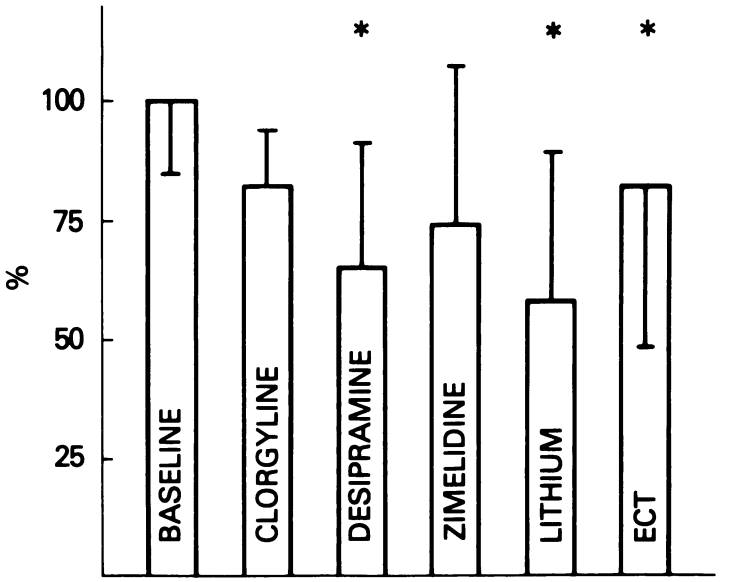


**Figure 1.** Effect of antidepressants on 24-hour mean ± S.D. urinary norepinephrine (NE) output. $*p < 0.05$, Student's $t$-test for related samples, two-tailed probability.

## RESULTS

Clorgyline significantly reduced urinary MHPG and VMA output and 'whole body' norepinephrine turnover, but it increased normetanephrine output (Figures 1-5). Desipramine significantly reduced urinary norepinephrine, MHPG and VMA output and whole body norepinephrine turnover, but zimelidine reduced urinary MHPG output only (Figures 1-5). Lithium reduced all indices of norepinephrine metabolism to a significant degree, and ECT reduced norepinephrine and normetanephrine output and tended to reduce 'whole body' norepinephrine turnover as well.

Clorgyline, ECT and lithium reduced mean depression ratings significantly ($p < 0.01$) but only two depressed patients responded to treatment with desipramine or zimelidine, respectively. Furthermore, clorgyline tended to stabilize mood in rapidly cycling bipolar patients (Potter *et al.*, 1982). No differences could be found in the antidepressant-induced changes of urinary norepinephrine and metabolite outputs between the responders and non-responders. However, the patients showing a favorable therapeutic outcome during desipramine and zimelidine treatments had relatively low pre-treatment MHPG outputs.

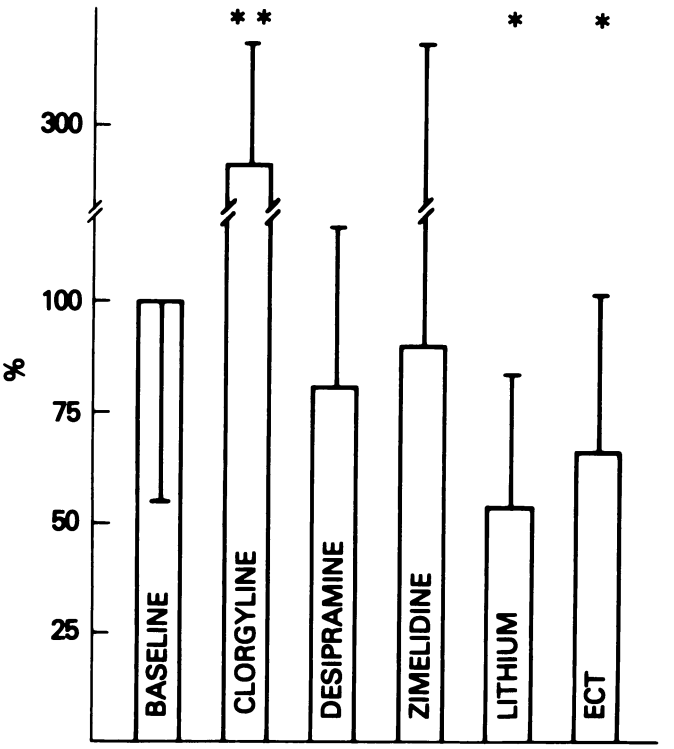


**Figure 2.** Effect of antidepressants on 24-hour mean ± S.D. urinary normetanephrine (NM) output. $*p < 0.05$; $**p < 0.01$; Student's $t$-test for related samples, two-tailed probability.

## DISCUSSION

The main finding of this series of studies was that five different antidepressant treatments all affected norepinephrine metabolism in man. Moreover, the different effects of some of these treatments on the relative amounts of norepinephrine and its main metabolites excreted in the urine allowed us to 'fingerprint' their mechanism of action in depressed patients.

Seemingly our results are hard to reconcile with the norepinephrine theory of depression and the animal studies demonstrating desensitization of β and $\alpha_2$ adrenoceptors during administration of antidepressants (Charney *et al.*, 1981). After all, neither the theory nor the animal experiments necessarily predict that antidepressants would reduce total norepinephrine metabolism as a result of reduced presynaptic release of the transmitter (Linnoila

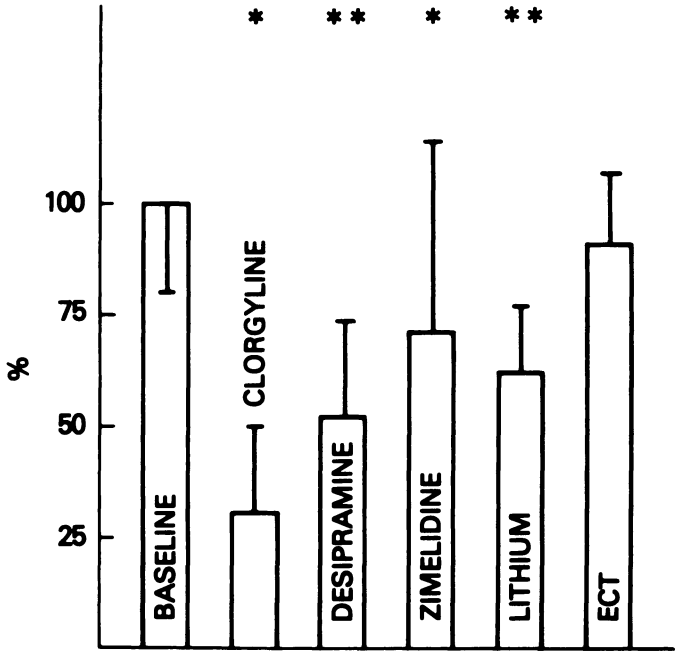


**Figure 3.** Effect of antidepressants on 24-hour mean ± S.D. urinary 3-methoxy-4-hydroxyphenylglycol (MHPG) output. *$p < 0.05$; **$p < 0.01$; Student's $t$-test for related samples, two-tailed probability.

*et al.*, 1982b). However, in spite of reducing 'whole body' norepinephrine turnover, desipramine may actually increase the amount of intrasynaptic norepinephrine and potentiate its effects in man, as indicated by an increased plasma norepinephrine concentration, heart rate and blood pressure in healthy volunteers receiving the drug (Ross *et al.*, unpublished). Thus, the reduced 'whole body' norepinephrine turnover, in depressed patients on desipramine, is consistent with an adaptation of the noradrenergic neuron system to increased intrasynaptic transmitter. The mechanism of this adaptation could involve both a reduced firing rate of presynaptic norepinephrine neurons and subsensitization of presynaptic $\alpha_2$ adrenoceptors as observed in experimental animals treated with desipramine (Svensson and Usdin, 1978), depending on the balance of intrasynaptic norepinephrine increase and $\alpha_2$ adrenoceptor subsensitivity.

Over and beyond the absolute reduction in 'whole body' norepinephrine turnover produced by desipramine, the drug modestly increased the relative amount of norepinephrine excreted as normetanephrine. This finding is compatible with a blockade of nor-

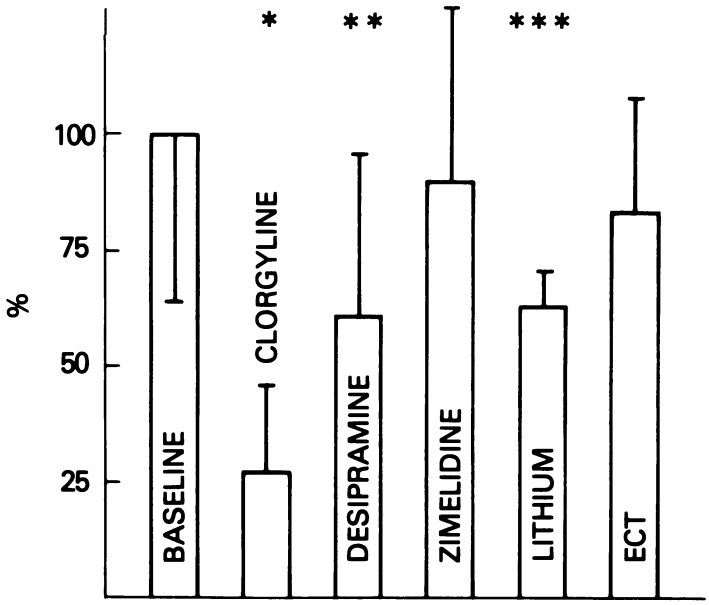


**Figure 4.** Effect of antidepressants on 24-hour mean ± S.D. urinary vanilmandelic acid (VMA) output. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, Student's $t$-test for related samples, two-tailed probability.

epinephrine re-uptake, which is maintained even during chronic administration (Linnoila *et al.*, 1983; Ross *et al.*, unpublished).

Clorgyline reduced 'whole body' norepinephrine turnover more than any other antidepressant that we have studied (Linnoila *et al.*, 1982a). This drug has been demonstrated to increase the cerebrospinal fluid ratio of norepinephrine to dopamine-β-hydroxylase in man. The relative decrease of dopamine-β-hydroxylase has been suggested to indicate a reduced firing of norepinephrinergic neurons (Lerner *et al.*, 1979). Our findings are in agreement with this interpretation. Furthermore, like desipramine, clorgyline presumably increases intrasynaptic norepinephrine concentration as indicated by the greatly enhanced normetanephrine output during clorgyline treatment. Thus, the mechanism by which clorgyline could reduce presynaptic norepinephrine neuronal firing would be similar to that of desipramine.

Clorgyline most markedly reduced the absolute and relative outputs of the deaminated metabolites of norepinephrine. These effects on norepinephrine and metabolite output pattern are consistent with MAO inhibition. Moreover, the drug did not change the urinary output of phenylethylamine, which is a preferred substrate of MAO type B. Therefore, our data with multiple para-

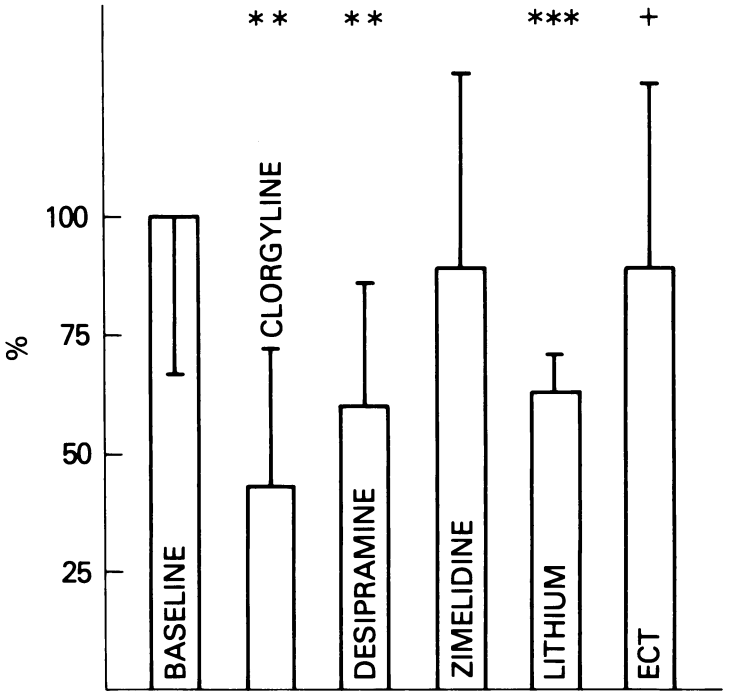


**Figure 5.** Effect of antidepressants on mean ± S.D. 'whole body' norepinephrine (N) turnover. $^{+}p < 0.07$; $^{**}p < 0.01$; $^{***}p < 0.001$, Student's $t$-test for related samples, two-tailed probability.

meters (Linnoila *et al.*, 1982a) provide direct support for the previous conclusion that clorgyline is a specific MAO type A inhibitor *in vivo* which was based on measures of MHPG and platelet MAO type B (Murphy *et al.*, 1979).

Lithium reduced the urinary outputs of norepinephrine and all of the measured metabolites. The reduction of 'whole body' norepinephrine turnover produced by lithium was only slightly less than that produced by clorgyline. Interestingly, the two drugs that have mood stabilizing effects in bipolar patients (lithium and clorgyline (Potter *et al.*, 1982)), produced no more reduction in 'whole body' norepinephrine turnover than desipramine, which may even precipitate mood cycles (Extein *et al.*, 1979). Because lithium reduced proportionately both the extracellular and intracellular metabolites of norepinephrine, our data do not provide evidence for lithium-induced increases of norepinephrine re-uptake in depressed patients as has been suggested in animal experiments (Schildkraut, 1974). Rather, lithium may inhibit tyrosine hyd-

roxylase, since it reduces 'whole body' dopamine turnover as well as that of norepinephrine (Linnoila, Karoum and Potter, unpublished). If this finding holds, then our attempts to elucidate the relative importance of dopamine and norepinephrine in bipolar affective disorders by measuring urinary catecholamines during lithium treatment will be futile.

ECT reduced urinary norepinephrine and normetanephrine outputs and tended to reduce 'whole body' norepinephrine turnover. The mechanism by which ECT produces these changes remains obscure. Interestingly, depressed patients have been reported to 'spillover' more norepinephrine from sympathetic nervous system synapses than volunteers (Esler *et al.*, 1982). Our data suggest that such 'spillover' may be specifically reduced by ECT.

It is also unclear how zimelidine might reduce urinary MHPG output without altering ratios between norepinephrine and its metabolites. We currently speculate that this could be secondary to a zimelidine-mediated increase of inhibitory serotonergic input to the locus coeruleus - a speculation for which we are attempting to provide experimental evidence.

Although our results can be interpreted to indicate that the five antidepressant treatments reduce presynaptic norepinephrine release, we cannot conclude that this effect is a necessary component of their antidepressant mechanism of action. This is because the clinical antidepressant effect of these treatments usually does not appear until at least two weeks of administration whereas our animal and healthy volunteer data (Garrick *et al.*, unpublished; Ross *et al.*, unpublished) suggest that the effects of clorgyline and desipramine on norepinephrine metabolism are immediate. Thus, an adaptation of the CNS to a reduced presynaptic norepinephrine release could be involved in producing an antidepressant effect. In phobic patients, who often respond to antidepressants within days, the reduction of presynaptic norepinephrine release can be postulated to be therapeutic *per se*. In hyperactive children who respond rapidly to treatment with tricyclic antidepressants but lose the favorable therapeutic effect within weeks, an adaptation to the reduced release of presynaptic norepinephrine can be postulated to be counter-therapeutic (Linnoila *et al.*, 1980). Further parallel biochemical studies in depressed and phobic patients as well as hyperactive children should help us to understand better the ultimate mechanisms of action of current antidepressant treatments.

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# Association of $^3$H-imipramine binding with serotonin uptake and of $^3$H-desipramine binding with norepinephrine uptake: potential research tools in depression

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## INTRODUCTION

The study of the classical pharmacological receptors with receptor binding techniques is by now a well established methodology. Receptor binding techniques have also been successfully employed to study and characterize sites of drug action which possess the properties of pharmacological receptors. One such example concerns the discovery and characterization of the high-affinity binding sites for $^3$H-diazepam which are related to the site of action of benzodiazepines in the central nervous system (Squires and Braestrup, 1977; Möhler and Okada, 1977). Using the same approach, we have recently studied the high-affinity binding of two radioactively labeled tricyclic antidepressants, $^3$H-imipramine and $^3$H-desipramine, with the aim of investigating the mechanism of action of tricyclic antidepressant drugs and to attempt to identify their sites of action and their possible relationship to the biochemistry of affective disorders.

## CHARACTERIZATION OF THE $^3$H-IMIPRAMINE BINDING SITE

The high-affinity binding site for $^3$H-imipramine is present in membranes prepared from various regions of the brain of several species, including man (Raisman *et al.*, 1979a,b, 1980; Langer *et al.*, 1981a, 1982). $^3$H-Imipramine also binds saturably and with high affinity to human platelets (Briley *et al.*, 1979; Langer *et al.*, 1980a).

As shown in table 1, the high-affinity binding of $^3$H-imipramine to membranes of the rat cerebral cortex is inhibited by tricyclic antidepressants and by non-tricyclic inhibitors of neuronal uptake of 5-HT in the nanomolar range. Serotonin is the

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Table 1. Inhibition of $^3$H-imipramine binding in membranes of the rat cerebral cortex

| | $IC_{50}$ (nM) | Hill coefficient |
|---|---|---|
| Imipramine | 7 | 0.91 |
| Desipramine | 177 | 1.11 |
| Chlorimipramine | 25 | 1.19 |
| Nortriptyline | 200 | 0.92 |
| Amitriptyline | 25 | 0.96 |
| Citalopram | 30 | 0.64 |
| Fluoxetine | 50 | 0.58 |
| Norzimelidine (Z) | 200 | 0.55 |
| Serotonin | 2000 | 0.49 |

$^3$H-Imipramine binding was measured in rat cortical membranes at 2.5 nM $^3$H-imipramine in the presence of various concentrations of the drugs. $IC_{50}$'s were calculated from semilog plots, and Hill coefficients according to Braestrup and Nielsen (1980). Each value was calculated from 8 to 10 points, which were repeated at least three times.

Table 2. Stereospecific inhibition of $^3$H-imipramine binding by the optical isomers of femoxetine and paroxetine

| | $IC_{50}$ (nM) | $n$ |
|---|---|---|
| Paroxetine α (-) | 6.2 ± 0.25 | 7 |
| Paroxetine α (+) | 63.5 ± 6.8 | 4 |
| Paroxetine β (-) | 288.0 ± 17.0 | 4 |
| Paroxetine β (+) | 66.4 ± 18.9 | 4 |
| Femoxetine α (-) | 135.0 ± 35.0 | 4 |
| Femoxetine α (+) | 68.7 ± 7.6 | 4 |

$^3$H-Imipramine binding was measured in rat cortical membranes at 2.5 nM $^3$H-imipramine in the presence of various concentrations of the drugs. $IC_{50}$'s were calculated from semilog plots and are given as means ± S.E.M. $n$ = number of determinations.

only neurotransmitter that inhibits the high-affinity binding of $^3$H-imipramine (table 1). While the tricyclic antidepressants inhibit the binding of $^3$H-imipramine competitively, 5-HT and the non-tricyclic inhibitors of 5-HT uptake inhibit the binding of $^3$H-imipramine in a complex manner (Sette *et al.*, 1983), with Hill coefficients significantly below unity (table 1). Studies using the optical isomers of paroxetine and femoxetine (table 2) have confirmed that $^3$H-imipramine binding is stereoselective for the

isomers which are active at inhibiting the uptake of serotonin. Similar results were previously reported for the (*Z*) and (*E*) isomers of zimelidine and norzimelidine (Langer *et al.*, 1980c).

A significant correlation was reported between the potency of 17 antidepressant and non-antidepressant drugs to inhibit neuronal uptake of 5-HT and $^3$H-imipramine binding in the rat hypothalamus (Langer *et al.*, 1980b). No such correlation was obtained between the inhibition of norepinephrine (NE) uptake and $^3$H-imipramine binding (Langer *et al.*, 1980b). Compatible with these results is the fact that the distribution of $^3$H-imipramine binding sites in the brain is closely correlated with the density of serotonergic innervation, in the rat brain (Palkovits *et al.*, 1981) and in the human brain (Langer *et al.*, 1981a).

Following electrolytic lesions of the dorsal raphe in the rat and subsequent to degeneration of serotonergic nerve terminals in the hypothalamus, striatum and cerebral cortex the endogenous levels of 5-HT are reduced by approximately 50% (Sette *et al.*, 1981) while the levels of dopamine (DA) and NE remain essentially unchanged. In these three areas of the brain and following the electrolytic lesions of the dorsal raphe the $B_{max}$ of $^3$H-imipramine binding was reduced in parallel with the decrease in 5-HT levels, indicating that these binding sites are located on serotonergic nerve terminals (Sette *et al.*, 1981). Similar results were obtained when the neurotoxin 5,7-dihydroxytryptamine was used to produce selective degeneration of serotonergic nerve terminals (Gross *et al.*, 1981; Brunello *et al.*, 1982).

Our results suggest that $^3$H-imipramine binds to sites associated with the 5-HT uptake system but different from the substrate recognition site for 5-HT as shown schematically in figure 1. It is possible that a site modulating the neuronal uptake of 5-HT is present on serotonergic nerve endings, and that this site (labeled by $^3$H-imipramine) is different from the substrate recognition site for the transmitter, 5-HT (figure 1). One can envisage the existence of a presynaptic site that modulates neuronal uptake in analogy with the presynaptic autoreceptors which modulate the release of their neurotransmitter (Langer, 1980). While the release-modulating presynaptic autoreceptors are acted upon by the neurotransmitter itself, the sites modulating 5-HT uptake may be acted upon by a co-transmitter or by an, as yet, unknown endogenous substance probably acting like imipramine (figure 1) and present at the synapse or in the circulation. The possible significance of this substance which inhibits the uptake of $^3$H-5-HT and the binding of $^3$H-imipramine remains to be established. It would certainly be of interest to isolate and determine the chemical properties of this endogenous factor (IDF = imipramine displacing factor) which inhibits $^3$H-imipramine binding and $^3$H-5-HT uptake. At present, it remains to be clarified whether the IDF is a known or a novel substance.

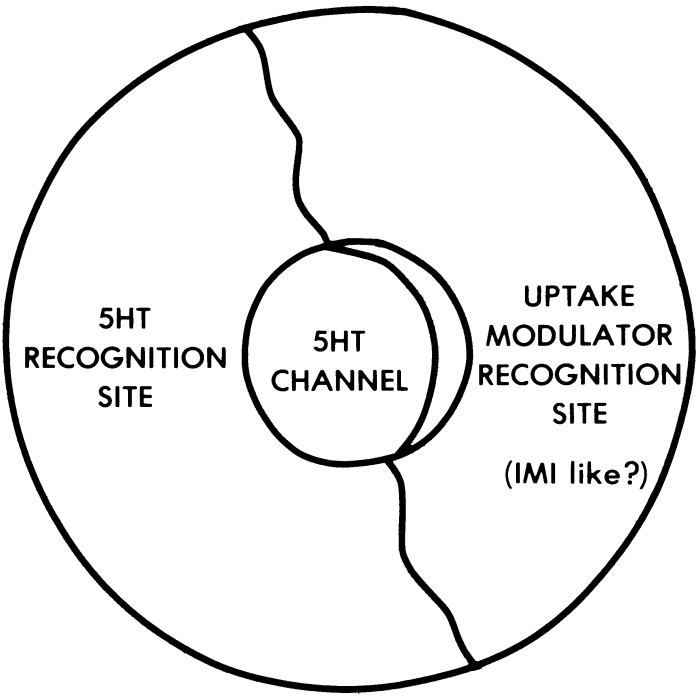


**Figure 1.** Schematic representation of the recognition sites at the level of the transporter for serotonin. Two different recognition sites appear to be present for the transporter of serotonin at the level of the nerve terminals. One is the substrate recognition site for serotonin and the second, where $^{3}$H-imipramine binds with high affinity, may be a site which modulates the uptake of serotonin. The $^{3}$H-imipramine binding site may be activated by an endogenous ligand which could be different from serotonin.

## CHARACTERIZATION OF THE $^{3}$H-DESIPRAMINE BINDING SITE

Following the discovery that $^{3}$H-imipramine binds with high affinity to sites located on 5-HT nerve terminals and associated with the uptake mechanism for 5-HT (for review, see Langer *et al.*, 1981c) the question arose as to whether antidepressants which are selective for the inhibition of neuronal uptake of NE, like desipramine, could bind with high affinity to specific sites on noradrenergic nerve endings, possibly associated with the neuronal uptake of NE.

As shown in table 3, the high-affinity binding site for $^{3}$H-desipramine is present in the peripheral and the central nervous

Table 3. Localization and density of $^3$H-imipramine and $^3$H-desipramine binding sites in the rat

| | $^3$H-Imipramine binding | | $^3$H-Desipramine binding | |
|---|---|---|---|---|
| | $K_d$ (nM) | $B_{max}$ (fmol/mg prot.) | $K_d$ (nM) | $B_{max}$ (fmol/mg prot.) |
| Hypothalamus | 5.2 | 279 | 2.6 | 156 |
| Cerebral cortex | 4.0 | 249 | 2.4 | 101 |
| Striatum | 2.8 | 164 | 3.4 | 29 |
| Cerebellum | 8.0 | 60 | 1.5 | 80 |
| Heart | | N.D. | 1.5 | 63 |
| Submaxillary gland | | N.D. | 2.3 | 308 |
| Vas deferens | | N.D. | 2.6 | 1015 |
| Platelets | 2.3 | 1750 | | N.D. |

$K_d$ and $B_{max}$ values for $^3$H-imipramine and $^3$H-desipramine binding were determined with the method described by Raisman *et al.* (1980) and Langer *et al.* (1981b). N.D. = non-detectable.

system (CNS) with a tissue distribution which follows the density of noradrenergic innervation. Table 3 also shows the differences between the distribution of $^3$H-imipramine and $^3$H-desipramine binding sites: in peripheral tissues with a dense noradrenergic innervation like the vas deferens, the heart and salivary glands $^3$H-desipramine binding is present while $^3$H-imipramine binding is non-detectable. On the other hand, in platelets, which have an active transport mechanism for 5-HT, but not for NE, there are $^3$H-imipramine binding sites but no $^3$H-desipramine binding sites (table 3). In the CNS the lowest density of $^3$H-desipramine binding sites is in the striatum, which lacks noradrenergic innervation, while for $^3$H-imipramine binding the cerebellum is the region with the lowest density of binding sites (table 3). The density of both $^3$H-imipramine and $^3$H-desipramine binding sites are high in the hypothalamus and cerebral cortex. While chemical sympathectomy with 6-hydroxydopamine leads to a reduction of $^3$H-desipramine binding sites in the CNS (Hrdina *et al.*, 1981) it does not affect the density of $^3$H-imipramine binding sites in the brain (Gross *et al.*, 1981). In the periphery, surgical sympathetic denervation of the rat heart or salivary glands leads to a pronounced decrease in the $B_{max}$ of $^3$H-desipramine binding (Langer *et al.*, 1981b; Raisman *et al.*, 1982b). These results support the view that the $^3$H-desipramine binding sites are localized on noradrenergic nerve endings.

The profile of inhibition of $^3$H-desipramine binding by drugs is also different from that of $^3$H-imipramine (compare tables 1 and 4). Compounds known to act at classical neurotransmitter receptors are generally inactive at inhibiting $^3$H-desipramine binding (Raisman *et al.*, 1982b). Drugs which inhibit neuronal uptake of

**Table 4.** Inhibition of $^3$H-desipramine binding in membranes of the rat vas deferens

| | $IC_{50}$ (nM) |
|---|---|
| Desipramine | 5 |
| Nisoxetine | 8 |
| Imipramine | 117 |
| D-Imafen | 80 |
| L-Imafen | 400 |
| Fluoxetine | 4600 |
| (-)-Norepinephrine | 112000 |
| Metaraminol | 34500 |

$^3$H-Desipramine binding was measured in membranes of the rat vas deferens at 2.5 nM $^3$H-desipramine in the presence of various concentrations of the drugs. IC50's were calculated from semilog plots. Each value was obtained from 8 to 10 points which were repeated at least three times.

NE all inhibit $^3$H-desipramine binding with high affinity (table 4). As shown for $^3$H-imipramine binding, the inhibition of $^3$H-desipramine binding is stereoselective when the two optical isomers of oxaprotiline are compared (Raisman *et al.*, 1982b). Similar results were obtained with the two optical isomers of imafen (table 4). It should be noted that D-imafen is the isomer active at inhibiting $^3$H-NE uptake (Laduron *et al.*, 1982) and it was 5 times more potent than the inactive isomer, L-imafen, at inhibiting $^3$H-desipramine binding (table 4).

These results are at variance with those of Laduron *et al.* (1982), who failed to find a stereospecific inhibition of $^3$H-desipramine binding with the L- and D-enantiomers of imafen in the rat cerebral cortex. The discrepancy between our results (table 4) and those of Laduron *et al.* (1982) may reflect either tissue or methodological differences.

Comparison of the potency of a number of drugs on the inhibition of $^3$H-desipramine binding and the inhibition of the uptake of NE gives a highly significant correlation (Raisman *et al.*, 1982b). While 5-HT is weakly active at the $^3$H-imipramine binding site (table 1), the natural substrate for the NE uptake mechanism is inactive at inhibiting $^3$H-desipramine binding (table 4). Similar results were obtained for other substrates of the NE uptake mechanism such as metaraminol (table 4), dopamine and tyramine (Raisman *et al.*, 1982b).

While denervation experiments clearly indicate that the $^3$H-desipramine binding sites are localized on noradrenergic nerve terminals, the failure of pre-treatment with reserpine to change either the $K_d$ or the $B_{max}$ of $^3$H-desipramine binding indicates that the binding site is not associated with the granular storage sites for norepinephrine (Raisman *et al.*, 1982b). Consequently, it

appears that $^3$H-desipramine binds to a presynaptic site, different from the uptake-substrate recognition site for NE but associated with the neuronal uptake of NE. Since neuronal uptake is the main inactivating mechanism for released NE, the modulation of neurotransmitter uptake may play an important role in the regulation of neurotransmission.

## CONCLUSIONS

The high-affinity binding sites for $^3$H-imipramine and $^3$H-desipramine label different sites with pharmacological profiles and tissue distribution corresponding to an association with the neuronal uptake mechanisms for 5-HT and NE respectively. Both binding sites have most of the characteristics of the recognition site of a pharmacological receptor or a site of drug action (Langer and Briley, 1981).

It appears that the $^3$H-imipramine binding site may represent a new biological marker in depression. Studies carried out in platelets from severely depressed untreated patients have revealed a significant decrease in $B_{max}$ of $^3$H-imipramine, without changes in $K_d$ (Langer *et al.*, 1980d, 1981c, 1982; Briley *et al.*, 1980; Raisman *et al.*, 1981). These observations have recently been confirmed by other groups (Asarch *et al.*, 1981; Paul *et al.*, 1981). It is of interest that a recent study carried out in *post-mortem* brains of suicide victims revealed a significant decrease in $B_{max}$ of $^3$H-imipramine binding in the frontal cortex, when compared with matched control brains (Stanley *et al.*, 1982). These results would suggest that the changes in $^3$H-imipramine binding observed in the platelets of depressed patients may indeed reflect similar changes in the brain, as previously suggested (Langer *et al.*, 1981c, 1982). In support of this view, it was recently reported that chronic treatment of cats with imipramine produces a down-regulation of $^3$H-imipramine binding in the brain as well as in platelets (Briley *et al.*, 1982).

It is of interest to note that the decrease in $B_{max}$ of $^3$H-imipramine binding observed in the platelets of severely depressed untreated patients co-exists with a decrease in $V_{max}$ of uptake of $^3$H-5-HT (Tuomisto *et al.*, 1979; Raisman *et al.*, 1982a). The existence in plasma of a substance which inhibits both $^3$H-5-HT uptake and $^3$H-imipramine binding, IDF, might be associated with the above-mentioned findings in depressed patients. Although this suggestion is highly speculative, it is possible that IDF may turn out to be an endogenous substance which plays a significant role in the biochemical changes linked to affective disorders.

The $^3$H-desipramine binding site is likely to become a useful tool for studies related to the neuronal uptake of NE. This binding site is, however, not present in platelets, red blood cells or lymphocytes, making its determination in human tissues less easily available.

Both for human and animal experimental studies in affective disorders, the $^{3}$H-imipramine and $^{3}$H-desipramine binding sites represent new biochemical tools in relation to the role of the neuronal uptake of 5-HT and NE. Finally, we cannot exclude the possibility that endogenous ligands may exist which act on these high-affinity binding sites to modulate the neuronal uptake of both monoamines.

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# Characterization of high-affinity antidepressant binding to rat and human brain

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## INTRODUCTION

Studies examining the interaction of various radiolabeled psychotropic agents with neuronal membranes have played an important role in elucidating the central sites of action of many of these compounds. The benzodiazepines and opiate alkaloids, for example, have been demonstrated to interact with a high degree of specificity at recognition sites on neuronal membranes following pharmacologically relevant doses. In contrast, antidepressants interact with many membrane sites with varying degrees of specificity and affinity, including both pre- (Koe, 1976) and postsynaptic (Snyder and Yamamura, 1977) re-uptake and/or receptor sites, and perhaps other, as yet unidentified, binding or recognition sites (Biegon and Samuel, 1979). Therefore, the *in vitro* conditions employed in studying the binding of radiolabeled antidepressants should be clearly defined and optimized for selective and specific 'labeling' of a given membrane site. In this report, we will review studies from our laboratories on the binding of [$^3$H]-imipramine and [$^3$H]-desipramine to membrane preparations from rat and human brain as well as platelets, emphasizing the conditions under which these ligands will selectively label the serotonin and norepinephrine transport sites, respectively. The clinical implications of these findings will also be discussed.

## [$^3$H]-IMIPRAMINE AND [$^3$H]-DESIPRAMINE BINDING: METHODOLOGIC ISSUES

Langer and coworkers (Raisman *et al.*, 1979) demonstrated the presence of high-affinity and saturable binding sites for [$^3$H]-imipramine to membrane preparations of rat brain. These authors

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speculated that this binding site might be a specific 'receptor' which mediates the antidepressant actions of tricyclic and other 'atypical' antidepressants (Langer *et al.*, 1980b). This conclusion was based on the correlation between the potencies of a series of antidepressants in displacing [$^3$H]-imipramine binding *in vitro* and their corresponding behavioral and/or clinical potencies in treating depression. In contrast, we observed a pharmacological profile for [$^3$H]-imipramine binding that differed from that initially reported by Langer *et al.* (1980a) which led us to conclude that the [$^3$H]-imipramine binding site was associated with a serotonin uptake or transport site (Paul *et al.*, 1980). This hypothesis has been confirmed by more extensive studies in our laboratory (Paul *et al.*, 1981a) as well others (Langer *et al.*, 1980a) using a variety of different techniques. However, neither the exact structural nor functional relationship between the [$^3$H]-imipramine binding site and the serotonin transport complex is completely understood.

In their initial report on [$^3$H]-imipramine binding, Langer and coworkers employed a prefiltration-dilution method in which an aliquot of the incubation reaction was quickly diluted more than 100-fold in ice-cold buffer immediately prior to filtration through glass fiber filters (Raisman *et al.*, 1979). In attempting to circumvent this somewhat cumbersome prefiltration procedure, we observed that elimination of this predilution step resulted in a significant amount of [$^3$H]-imipramine bound to filters in the absence of tissue (figure 1; cf. Paul *et al.*, 1981a). Furthermore, a significant amount of the binding of [$^3$H]-imipramine to filters was displaceable by an excess of non-radioactive antidepressant, resulting in spurious 'specific' binding of [$^3$H]-imipramine to filters. Further characterization of this 'filter binding' indicated that it was of low affinity, and the potencies of various tricyclic antidepressants in displacing this 'binding' were in the μM range. In contrast, rapid dilution of an aliquot of the incubation mixture resulted in almost totally eliminating the low-affinity 'binding' of [$^3$H]-imipramine to glass fiber filters. 'Low-affinity' binding of [$^3$H]-imipramine was also observed in denatured brain and peripheral tissues as well as in purified myelin and nuclear fractions of brain. Table 1 illustrates a temperature-inactivation curve for [$^3$H]-imipramine binding to brain membranes. Specific binding was totally inhibited when membranes were pre-incubated at 56°C for 15 min prior to assay. However, higher pre-incubation temperatures resulted in an increase in 'specific' [$^3$H]-imipramine binding to values approximately fourfold higher than observed in unheated tissue. However, characterization of [$^3$H]-imipramine binding in heat-treated membranes revealed that it was of low affinity and pharmacologically distinct from that observed in unheated membranes (table 1). Therefore, it appears that utilization of a prefiltration-dilution step is critical for the study of 'high-affinity' binding of tricyclic antidepressants to defined presynaptic sites.

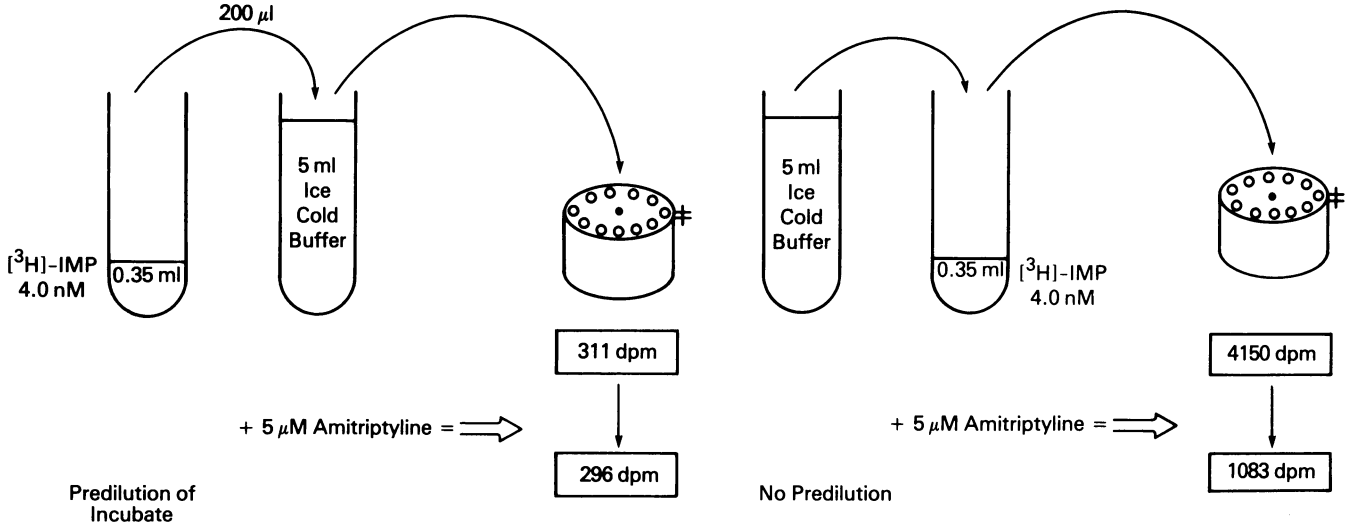


**Figure 1.** [$^3$H]-Imipramine binding to filters: effects of pre-dilution. [$^3$H]-Imipramine (4 nM) was incubated in 50 mM Tris-HCl buffer (pH 7.4) containing 120 mM NaCl and 5 mM KCl in a total volume of 0.35 ml. Incubations were performed in glass tubes at 0-4°C in the presence and absence of 5 µM amitriptyline. After 60 min of incubation, the reactions were terminated in two ways: (a) Aliquots (0.2 ml) of the incubation mixture were quickly diluted in 5 ml of ice-cold buffer and immediately filtered over GF/F filters under vacuum. Filters were then washed with three 5 ml aliquots of buffer. (b) After 60 min the entire incubation mixture was quickly diluted with 5 ml ice-cold buffer and filtered immediately. The tube was washed once more with 5 ml buffer and the filter washed twice with 5 ml aliquots of buffer.

Yet another important characteristic of the high-affinity binding of [$^3$H]-imipramine to brain and platelet membranes is the absolute requirement for sodium ions. The use of sodium-free and sodium-enriched buffers permitted the differentiation of specific 'high-affinity' binding of either [$^3$H]-imipramine or [$^3$H]-desipramine to brain and/or platelet membranes and the binding to 'low affinity' sites in these same tissues (Rehavi *et al.*, 1983). Figure 2 illustrates the effects of sodium ions on the binding of [$^3$H]-imipramine to the synaptosomal, myelin and nuclear fractions of rat brain. Only the binding to synaptosomal fractions was enhanced by sodium ions. The sodium ion dependency of both [$^3$H]-imipramine and [$^3$H]-desipramine binding is an important 'marker' for the high-affinity binding of tricyclic antidepressants to presynaptic neurotransmitter uptake sites.

## RELATIONSHIP OF [$^3$H]-IMIPRAMINE AND [$^3$H]-DESIPRAMINE BINDING TO BIOGENIC AMINE UPTAKE PROCESSES

Our initial report describing [$^3$H]-imipramine binding (Paul *et al.*, 1980) demonstrated the presence of high-affinity and saturable binding sites on human platelet membranes. Preliminary

Table 1. Effects of temperature on 'specific' [$^3$H]-imipramine binding to rat brain membranes

| Temperature (°C) | 'Specific' binding (DPM) | Specific binding ($T$) / Specific binding (0°C) |
|---|---|---|
| 0 | 470 | 1.0 |
| 56 | 0 | 0 |
| 65 | 404 | 0.9 |
| 76 | 940 | 2.0 |
| 90 | 1836 | 3.9 |
| 100 | 1843 | 3.9 |

| Temperature of pre-incubation (°C) | $IC_{50}$ for chlorimipramine (nM) |
|---|---|
| 0 | 3 |
| 90 | >100 |

Brain membranes were prepared as previously described (Raisman et al., 1979). Membranes were pre-incubated at various temperatures for 15 min, and then cooled on ice (0-4°C) prior to assay. A concentration of [$^3$H]-imipramine of approximately 1.0 nM was used and the assay was carried out as described previously (Rehavi et al., 1980). Membranes pre-incubated at 90°C for 15 min were examined for the displacement of 'specific' [$^3$H]-imipramine binding by chlorimipramine. The 'specific' binding of [$^3$H]-imipramine in the denatured membranes was of low affinity and only weakly inhibited by chlorimipramine.

studies in our laboratory suggested that tertiary amine antidepressants such as chlorimipramine, imipramine and amitryptyline were more potent in displacing [$^3$H]-imipramine binding from rat and human brain membranes than the corresponding secondary amine derivatives (i.e. desmethylchlorimipramine, desipramine and nortryptyline) (Paul *et al.*, unpublished data; Rehavi *et al.*, 1980). This pharmacologic profile was identical to that reported for the inhibition of serotonin uptake into synaptosomes (Koe, 1976), and prompted an examination of the binding of [$^3$H]-imipramine to platelets, since the latter has a well characterized uptake mechanism for serotonin. More extensive structure-activity studies demonstrated an excellent correlation between the affinities of a

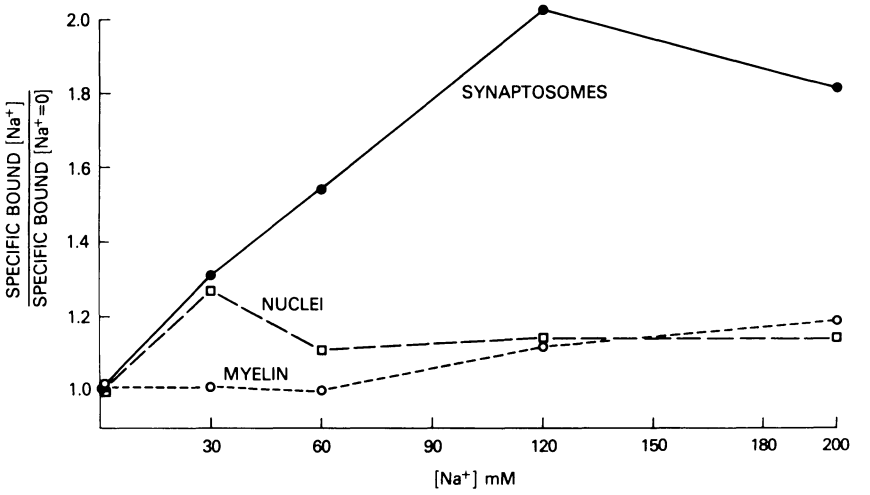


**Figure 2.** [$Na^+$] dependence of [$^3$H]-imipramine binding to subcellular fractions of rat brain. Rat forebrain was homogenized in 10 volumes of 0.32 M sucrose and the nuclei, synaptosomal and myelin fractions isolated according to the method of Whittaker and Barker (1972). The fractions were then disrupted with a Polytron (Brinkmann Instruments, setting 7, 20 s) in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM KCl and centrifuged at 30000*g* for 15 min. The disruption/centrifugation procedure was repeated three times. Binding of [$^3$H]-imipramine (4 nM) to membranes was performed as previously described (Raisman *et al.*, 1979). For each concentration of [$Na^+$], the binding in the presence or absence of 10 μM desipramine was measured. This is a representative experiment repeated twice with similar results.

large series of drugs for the imipramine binding site in platelets and their potencies as inhibitors of serotonin uptake (Paul *et al.*, 1980, 1981a). Moreover, if both the uptake and binding studies were performed under identical conditions (including a protein-free physiological medium), the concentrations of the various drugs as inhibitors of [$^3$H]-imipramine binding and serotonin uptake were almost identical (Paul *et al.*, 1981a). The correlation coefficients obtained between the potencies of various drugs as inhibitors of [$^3$H]-imipramine binding and serotonin uptake have now been reported by several laboratories to be between 0.89 and 0.98, similar to values reported for many drug receptor-effector interactions. The absence of a significant correlation between the potencies of various drugs at displacing [$^3$H]-imipramine binding and their potencies at other neurotransmitter uptake or receptor sites supports the selectivity of [$^3$H]-imipramine for the serotonin uptake site. The findings that there is a similar pharmacologic profile between the brain and platelet binding site (Rehavi *et al.*, 1980), and the lack of [$^3$H]-imipramine binding sites in peripheral tissue other than in platelets, further support this hypothesis.

More direct evidence for a structural relationship between the [$^3$H]-imipramine binding site and the presynaptic uptake mechanism for serotonin derives from two sources. Electrolytic or chemical lesions of the ascending serotonergic pathways from the raphe nuclei to various forebrain regions have been reported by several groups to destroy selectively high-affinity [$^3$H]-imipramine binding sites (cf. Paul *et al.*, 1981a; Langer *et al.*, 1980). We have also observed that the decrease in [$^3$H]-imipramine binding in individual animals is directly proportional to the decrease in serotonin uptake produced by these lesions (Paul *et al.*, 1981a). Since lesions to the midbrain raphe nuclei have been shown to be highly specific for denervating serotonin-containing presynaptic terminals, these results support the structural localization of [$^3$H]-imipramine binding sites to such terminals. Second, incubation of synaptosomal or platelet membranes with 2-nitroimipramine, a specific, slowly dissociating, essentially irreversible ligand of the [$^3$H]-imipramine binding site, results in a dose- and time-dependent decrease in both serotonin uptake and [$^3$H]-imipramine binding (Rehavi *et al.*, 1981a). Furthermore, 2-nitroimipramine was without effect on norepinephrine or dopamine uptake in synaptosomes, as well as a number of neurotransmitter-receptor systems (e.g. muscarinic, cholinergic, benzodiazepine), suggesting that the inhibition was specific for serotonin uptake.

Since the secondary amine tricyclic antidepressants such as desipramine and nortriptyline are more potent as inhibitors of norepinephrine uptake, we explored the possibility that high-affinity binding sites for [$^3$H]-desipramine were also present in rat brain. Incubation of brain membranes with low (i.e. nM) concentrations of [$^3$H]-desipramine followed by a prefiltration-

dilution step as described for [$^3$H]-imipramine binding revealed the presence of high-affinity and saturable binding sites for [$^3$H]-desipramine (Rehavi *et al.*, 1981b, 1982a). In contrast to [$^3$H]-imipramine binding, the secondary amine tricyclic antidepressants were more potent than their tertiary amine derivatives as inhibitors of [$^3$H]-desipramine binding. Furthermore, there was no correlation between the potencies of a series of drugs in inhibiting [$^3$H]-desipramine and [$^3$H]-imipramine binding in cerebral cortical membranes, suggesting that each ligand labeled a different population of binding sites. Predictably, there was an excellent correlation between the potencies of a series of drugs in inhibiting [$^3$H]-desipramine binding and the inhibition of norepinephrine uptake into synaptosomes. Like [$^3$H]- imipramine binding, the binding of [$^3$H]-desipramine displayed heat sensitivity, dependency on sodium ions, and a regional distribution throughout the CNS. High concentrations of [$^3$H]-desipramine binding sites were found in the septum, cerebral cortex and hypothalamus (areas rich in norepinephrine-containing terminals), whereas lower densities of binding sites were observed in the medulla, cerebellum and corpus striatum. Direct evidence for a structural association between [$^3$H]-desipramine binding sites and presynaptic norepinephrine uptake was revealed by studying the effects of intraventricular 6-hydroxydopamine on both the binding of [$^3$H]-desipramine and norepinephrine uptake. A marked decrease in both norepinephrine uptake and [$^3$H]-desipramine binding was observed in cerebral cortical membranes prepared from 6-hydroxydopamine lesioned animals compared to vehicle-treated animals, while no significant alterations were observed in either serotonin uptake or [$^3$H]-imipramine binding (Rehavi *et al.*, 1982a). Similar results have now been reported by several groups (Lee and Snyder, 1981; Langer *et al.*, 1981) using a variety of techniques. Taken together with the characteristics of [$^3$H]-imipramine binding outlined in the preceding section, these studies strongly support the hypothesis that radiolabeled imipramine and desipramine can be used to label (and perhaps quantitate) the uptake or transport sites for serotonin and norepinephrine, respectively.

## DISCUSSION

High-affinity, sodium-dependent binding sites for [$^3$H]-imipramine and [$^3$H]-desipramine appear to be recognition sites which mediate the inhibition of serotonin and norepinephrine uptake elicited by tricyclic antidepressants and perhaps other chemically unrelated agents. The pharmacological significance of inhibiting biogenic amine uptake is unclear, particularly as it relates to antidepressant activity. Therefore, it is premature to refer to these binding sites as receptors for antidepressants. However, since these sites are functionally, and perhaps structurally, associated

with uptake sites for biogenic amines, the use of radiolabeled antidepressants for further characterization of these uptake mechanisms seems possible. Recent studies in our laboratory on the solubilization and purification of the [$^3$H]-imipramine binding site from platelet membranes suggest that this site is part of a larger, supramolecular transport complex (Rehavi *et al.*, 1982b). It should not be surprising, therefore, if a number of drugs (e.g. digitalis glycosides) will be found to inhibit biogenic amine uptake by affecting other sites on this transport complex (e.g. a $Na^+$-$K^+$ ATPase) rather than interacting directly with [$^3$H]-imipramine binding sites.

Although there is both direct and indirect evidence that [$^3$H]-imipramine and [$^3$H]-desipramine binding sites are structurally associated with serotonin- and norepinephrine-containing presynaptic terminals, not all investigators concur with these findings. Laduron *et al.* (1982) have recently reported that [$^3$H]-imipramine and [$^3$H]-desipramine binding sites are not associated with biogenic amine uptake because of the differential subcellular distribution of the binding and uptake mechanisms. In their report 'specific' [$^3$H]-imipramine and [$^3$H]-desipramine binding was associated with the nuclear fraction of rat brain while serotonin and norepinephrine uptake were associated with the mitochondrial fraction (which presumably contained synaptosomes). These investigators, however, failed to characterize fully the binding of radiolabeled antidepressants in the individual subcellular fractions. In similar experiments performed in our laboratory (Rehavi *et al.*, 1983) we have characterized the binding of [$^3$H]-imipramine and [$^3$H]-desipramine (as well as the uptake of serotonin and norepinephrine) in the various subcellular fractions. In contrast to the results of Laduron *et al.* (1982), we observed that high-affinity, sodium-ion-dependent binding of radiolabeled imipramine and desipramine was predominantly localized in the synaptosomal fraction, with virtually no 'specific' binding in the nuclear fraction. In our experiments, a very significant quantity of 'displaceable' binding was observed in the myelin, but this binding was of very low affinity and *not* affected by sodium ions. Such 'low affinity' binding sites for tricyclic antidepressants have been reported for a variety of tissue proteins (as well as glass fiber filters and denatured tissue). Thus, the methodologic caveats described in this chapter must be considered when interpreting the results of binding studies which employ only a large excess of unlabeled drug to define 'specific' binding.

Despite the lack of a behavioral correlation between the clinical efficacy of various tricyclic antidepressants and their potencies in displacing radiolabeled imipramine and desipramine binding *in vitro*, several lines of evidence suggest that these 'binding sites' may still prove to be clinically relevant. We have emphasized the biochemical and pharmacological similarities

between the high-affinity binding sites for [$^3$H]-imipramine in human brain and platelet membranes (cf. Rehavi *et al.*, 1980) and subsequently clinical studies have demonstrated that platelets from severely depressed patients possess fewer [$^3$H]-imipramine binding sites than platelets from age- and sex-matched controls Briley *et al.*, 1980; Paul *et al.*, 1981). Furthermore, we have recently examined the binding of both [$^3$H]-imipramine and [$^3$H]-desipramine in suicide victims and compared them to values obtained from specimens of matched controls who succumbed to accidents or trauma (Paul *et al.*, in preparation). A significant reduction in the number of [$^3$H]-imipramine, but not [$^3$H]-desipramine, binding sites was observed in the suicide brain specimens, and the magnitude of this reduction (∿30-40%) was similar to that observed in platelets from severely depressed patients. It is tempting to speculate that a decrease in the density of [$^3$H]-imipramine binding sites (and perhaps an alteration in presynaptic serotonin uptake) in brain may be involved in the pathogenesis or pathophysiology of severe affective disorders characterized by suicidal behavior.

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# Section Six

# Clinical Psychopharmacology of Peptides, Amino Acids and Related Compounds

# The human psychopharmacology of peptides related to ACTH and ∝-MSH

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## INTRODUCTION

There is considerable interest in the diverse actions of ACTH (adrenocorticotrophin) -like peptides on the central nervous system (CNS), and in their application to man. Much of the experimental literature has been reviewed quite recently, e.g. in relation to structure-activity relationships (Van Nispen and Greven, 1982), animal pharmacology (Beckwith and Sandman, 1978; Bohus and De Kloet, 1979; Rigter and Crabbe, 1979; De Kloet and De Wied, 1980; Rigter, 1982), biochemical mechanisms (Wiegant *et al.*, 1981) and basic and human studies (Pigache and Rigter, 1981; Pigache, 1982a, b; Rigter *et al.*, 1982)

The review that follows is meant to update those cited above, and in so doing will try to avoid repetition. The peptides to be dealt with comprise $ACTH_{1-39}$, $ACTH_{1-24}$, $ACTH_{11-24}$ and $ACTH_{1-10}$ and also fragments with sequences shared by ACTH and α-MSH: $ACTH_{4-10}$ and $ACTH_{4-7}$, and the orally active synthetic peptide [$Met(O_2)^4$, $D\text{-}Lys^8$, $Phe^9$] $ACTH_{4-9}$ (Org 2766). In rat, the sequence $ACTH_{4-7}$ is the shortest fragment possessing the full behavioral potency of $ACTH_{1-39}$ (or equally $ACTH_{1-24}$) in the pole-jump test (Greven and De Wied, 1973). The most extensively studied of these peptides in man, however, is Org 2766. Evidence that Org 2766 crosses the blood-brain barrier remains circumstantial (Pigache, 1982b), apart from data reported for rat (Verhoef and Witter, 1976; Verhoef *et al.*, 1977) which need replication with new high-performance liquid chromatography methods. Nonetheless, effects on the human electroencephalogram (EEG) following oral Org 2766 (Rockstroh *et al.*, 1981, 1982; Fehm-Wolfsdorf *et al.*, 1981), strongly support the assumption of central activity.

The origin of interest in the central effects of ACTH lies more than 30 years ago and is documented by the reviews of Lidz *et*

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*al.* (1952) and Quarton *et al.* (1955) who referred to 'euphorigenic' effects of the hormone, but could not dissociate these from the cortisol response or from the favorable therapeutic outcomes. During the next decade, however, De Wied (1969) was able to study the effects in rats of various ACTH fragments, e.g. $ACTH_{4-10}$, which had reduced corticotrophic activity. This led to the first study in man (Endroczi *et al.*, 1970) with such fragments, which are dealt with next.

## EFFECTS OF $ACTH_{1-24}$, $ACTH_{11-24}$ AND $ACTH_{1-10}$

Endroczi *et al.* (1970) gave acute doses (0.5 mg) intravenously (i.v.) of $ACTH_{1-24}$ (a peptide which possesses full corticotrophic activity) to volunteers practiced in making a handgrip response to a stimulus of 12 cycles per second (c.p.s.) (tone or flicker) and no response to a 4 c.p.s. stimulus. The stimuli were presented alternately, i.e. a 'go, no-go' alternation task. After 50 to 100 practice trials, the spontaneous EEG following either type of stimulus became synchronized, i.e. exhibited alpha activity. The synchronization represented a habituation of earlier orienting responses. The action of $ACTH_{1-24}$ (compared with placebo) was to desynchronize the EEG following the 12 c.p.s. stimulation, i.e. dishabituation. Curiously, however, this desynchronization was not apparent during the first 1-2 h after peptide administration, but only 24 h later. The effects of $ACTH_{1-10}$ (1 and 2 mg i.v.) were the same as $ACTH_{1-24}$, whereas $ACTH_{11-24}$ (1 and 2 mg i.v.) had no effect at all. Thus the dishabituating property of $ACTH_{1-24}$, appeared to reside in its 1-10 amino acid sequence. However, the study was only single-blind and no statistical analysis was reported. The findings require replication.

Miller *et al.* (1974) studied the effects of a single dose of $ACTH_{1-24}$ (0.5 mg i.v.) on spontaneous EEG, contingent negative variation, and other psychophysiological variables, in a double-blind comparison with placebo. Measurements were made 1 h after treatment during the acquisition of a 'go, no-go' task (fixed fore-period reaction-time paradigm). There were no EEG effects and none on the Benton Visual Retention Test or the Rod and Frame Test. This discrepancy with the Endroczi *et al.* (1970) result might be explained by procedural differences. A discrepancy with the results of Rockstroh *et al.* (1981) for Org 2766 (see later) should also be noted.

Any interpretation of the foregoing results is complicated by the possibility that the peptides would have stimulated cortisol release. Many behavioral effects of corticosterone in rat, e.g. facilitation of active-avoidance extinction and impairment of passive-avoidance, are contrary to those of ACTH fragments lacking corticotrophic activity (see review by Bohus and De Wied, 1980). Equivalent opposing effects of cortisol and certain ACTH-like fragments might occur also in man.

## EFFECTS OF $ACTH_{4-7}$

In contrast to the absence of effects at 1 h after $ACTH_{1-24}$ treatment described by Endroczi *et al.* (1970), a recent Russian report (Medvedev *et al.*, 1980) notes marked behavioral effects with the short $ACTH_{4-7}$ core fragment (devoid of adrenocorticotrophic activity) as soon as 45 min after intranasal (i.n.) administration (40-60 mg). [N.B. When given intranasally, much larger doses of peptide may be needed to elicit effects equivalent to an injected dose, as with synthetic $\alpha^{1-18}$-corticotropin (Geyer and Templ, 1976).] Medvedev *et al.* report five statistically significant effects of $ACTH_{4-7}$, in a double-blind comparison with placebo:

(i) decreased omission and commission errors, increased rate of information processing and decreased time to complete a proof-reading task;
(ii) increased immediate (but not delayed) recall of nonsense syllables;
(iii) increased digit span;
(iv) improved running addition of sequential digit pairings;
(v) improved immediate recall of geometric shapes.

These results for $ACTH_{4-7}$ contrast with the largely non-significant findings of numerous studies using similar cognitive tests (for reviews see Pigache and Rigter, 1981; Pigache 1982a, b) with $ACTH_{4-10}$ and Org 2766. They are reminiscent, however, of some of the earlier claims made for the peptides and certainly need replication. It is possible, of course, that the $ACTH_{4-7}$ results may be attributable to bias (not immediately apparent in the condensed and translated report) or to a property peculiar to the $ACTH_{4-7}$ sequence. The next section deals with examples of how ACTH peptides differ.

## DIFFERENCES BETWEEN ACTH-LIKE PEPTIDES

Differing structure-activity relationships between various ACTH-like peptides were first reported by Greven and De Wied (1973) and have been the subject of a recent review (Van Nispen and Greven, 1982). The present comments are restricted to the peptides under consideration.

Although $ACTH_{4-7}$ and $ACTH_{4-10}$ are similar (and similar to $ACTH_{1-24}$) in terms of delaying pole-jump extinction and facilitating passive-avoidance in rats (Greven and De Wied, 1973), they do differ in terms of certain opiate-like properties (table 1). While these differences might not be directly relevant to their possibly different activities in man, they allow the possibility that other differences might exist. Similarly, there are qualitative differences (table 2) between $ACTH_{4-10}$ (which resembles $ACTH_{1-24}$) and Org 2766.

Table 1. Differences between $ACTH_{4-7}$ and $ACTH_{4-10}$

| Test | Effects | | References |
|---|---|---|---|
| | $ACTH_{4-7}$ | $ACTH_{4-10}$ | |
| Inhibition of mouse vas deferens contraction | inactive | active | Plomp and Van Ree (1978) |
| Displacement of $^{125}I$-morphine from morphine antiserum | inactive | active | Plomp and Van Ree (1978) |
| Affinity for opiate binding sites | virtually inactive | active (weak) | Terenius *et al.* (1975) |
| Induction of excessive grooming | active | virtually inactive | Wiegant *et al.* (1977) |

Table 2. Differences between $ACTH_{4-10}$ and Org 2766

| Test | Effects | | References |
|---|---|---|---|
| | $ACTH_{4-10}$ | Org 2766 | |
| Social interaction (familiar environment) | decreased* | - | File and Vellucci (1978) |
| | decreased* | - | File (1979) |
| | - | increased*† | File (1981) |
| | decreased | increased § | Clarke and File (1981) |
| Social interaction (unfamiliar environment) | decreased* | increased* | Niesink and Van Ree (1982) |
| Affinity for opiate binding sites | active (weak) | inactive | Pigache (1982b) |

*Intraperitoneal
†Dose-related effect
Intraseptal route
§Also increased in a comparison with α-MSH (File, 1981).

Finally, it appears that in some situations Org 2766 has both agonist and antagonist activities in rats, depending on the dose given. In active-avoidance tests the dose-response relationship for Org 2766 is linear (Greven and De Wied, 1980), but in other tests (listed in table 3) the relationship is more like an inverted 'U'. To explain the downturn of effects with 'high' doses of the peptide ('high' and 'low' are relative terms, with absolute doses varying according to the task), Fekete and De Wied (1982a) have suggested that the major metabolite of Org 2766, i.e. the COOH-terminal tripeptide (Phe-D-Lys-Phe, otherwise PDLP), could be responsible. When PDLP was given intracerebroventricularly (i.c.v.) it acted just like 'high'-dose Org 2766 (table 3), leading the authors to suggest that 'high' doses of Org 2766 possibly generate enough PDLP to reverse the effects produced by lower doses of the parent peptide. We do not know if such reversals of response would occur in man, nor which doses should be considered 'high'.

The predictive value for man of experimental models in rat remains uncertain. However, differing properties of short peptides

**Table 3.** Effects of 'low-' and 'high'-dose Org 2766 and of 'PDLP' in rats

| Test | Effects | | | References |
|---|---|---|---|---|
| | Org 2766 | | PDLP (COOH-terminal tripeptide Org 2766) | |
| | 'Low' dose | 'High' dose | | |
| Active avoidance | delayed | - | delayed* | Van Nispen and Greven (1982) |
| (extinction) | - | delayed † | - | Greven and De Wied (1980) |
| Passive avoidance | facilitation ‡ (not blocked by naltrexone) | inhibition (blocked by naltrexone) | inhibition (blocked by naltrexone) | Fekete and De Wied (1982a) |
| Intracranial§ self-stimulation | facilitation | inhibition | unknown | Fekete et al. (1982) |
| Exploratory** behavior | increased | decreased | unknown | File (1981) |

*Potency one-tenth $ACTH_{4-10}$ (on weight basis)
†Dose-related effects
‡Potency x 1000 $ACTH_{4-10}$ (Fekete and De Wied, 1982b)
§Medial septal area
**Time spent head-dipping

related to ACTH and α-MSH justify caution in generalizing from one peptide to another. From another standpoint, behavioral differences between Org 2766 and its metabolites (mainly PDLP) must also complicate any question of whether a given clinical effect would represent 'substitution therapy' or 'direct pharmacological action' by the peptide.

## VIGILANCE AND EEG EFFECTS OF $ACTH_{4-10}$ AND ORG 2766

Both $ACTH_{4-10}$ and Org 2766 improve vigilance performance in man. This has been a constant effect following acute dosing with the peptides (for review see Pigache, 1982b). Thus a significant decrease in long reaction times during a 30 min serial reaction-time task was reported following subcutaneous (s.c.) $ACTH_{4-10}$ (30 mg) (Gaillard and Sanders, 1975), following each of three dose levels (5, 10 and 20 mg) of Org 2766 given per os (p.o.) (Gaillard and Varey, 1979), and almost significantly so ($p < 0.06$) following Org 2766 (10 mg p.o.) in hypophysectomized patients (Gaillard and Zwaan, 1978). A subsequent study by O'Hanlon (cited in Pigache, 1982b) showed that Org 2766 (40 mg p.o.) and dexamphetamine (10 mg p.o.) equally and significantly prevented a time-related decrement in performance during a long (105 min) vigilance task. These results were obtained in healthy young subjects tested 90 min after dosing. In a later study (cited in Pigache, 1982b), using somewhat different procedures, O'Hanlon failed to find a comparable effect of Org 2766 (40 mg p.o.) in healthy elderly subjects. Results in vigilance studies are highly sensitive to variations in the task parameters. In an attempt to reconcile the above discrepant findings, O'Hanlon *et al.* (1982) recently adopted a different procedure to permit analysis according to 'signal detection theory'. Twenty college students performed a TV monitoring task that involved a difficult discrimination. In successive trials (spaced every 2.5 s) they were shown either video noise or the noise plus a 'hidden' signal and were asked each time to indicate, at one of four levels of confidence, whether or not a signal had occurred. In comparison with the earlier task the new paradigm depended less on memory and more on the processes of perception, decision-making and response selection. It was also shorter (60 min) and was given just 15 min after Org 2766 (40 mg p.o.), or placebo, administration. A preliminary analysis indicates that the subjects performed better after Org 2766. Gross performance measures (e.g. percent signals detected) indicated a trend ($p < 0.1$, two-tail).

Interesting and sometimes stronger differences were seen with finer measures (e.g. a significant, $p < 0.02$, reduction under Org 2766, compared with placebo, of the signal distribution, but no change of the noise distribution). For these comparisons the necessary data were not forthcoming from every subject. The

results suggest that the peptide facilitated perceptual discrimination between the stimuli.

The above conclusion would seem to agree with an earlier statement of Rockstroh *et al.* (1981) that Org 2766 (40 mg p.o.) 'facilitates the fixation of a single and simple set of stimuli'. This referred to EEG evoked responses showing shorter N 100 latencies ($p < 0.05$), a tendency toward greater N 100 amplitudes and increased P 300 amplitudes ($p < 0.10$) in a fixed fore-period (6 s) reaction-time task, where Org 2766 (40 mg p.o.) was compared with placebo. These results have been replicated in a second, similar, study (Rockstroh *et al.*, 1982), performed and analyzed double-blind. Fehm-Wolfsdorf *et al.* (1981), in an almost identical experiment, except that subjects had to respond to each of two different stimuli presented in separate trials, found that Org 2766 (40 mg p.o.) impaired the switching of attention, as measured by the same EEG variables.

The foregoing effects of Org 2766 on various tests and measures related to attention are summarized in table 4. They require, however, some interpretation. The N 100 changes in the EEG evoked response are said to relate to 'detection/selection of simple cue characteristics' (Hillyard *et al.*, 1978; Parasuraman and Beatty, 1980) and the P 300 to better 'stimulus evaluation/categorization in relation to a neural template' (Hillyard *et al.*, 1978; Parasuraman and Beatty, 1980; McCarthy and Donchin, 1981). Both processes appeared to be improved by Org 2766 treatment.

The decrements in performance, as seen under placebo, in the vigilance tasks may be attributable to sensory adaptation, habituation or a loss of incentive-motivation. It is not easy to distinguish between these possibilities, empirically. Habituation (or 'reactive inhibition') occurs to both the non-specific (irrelevant) aspects of a task, with a resultant progressive loss of arousal, and to the 'critical' stimuli whenever these are not perceived as being different from the background (i.e. as salient). The ensuing decrease in arousal during a monotonous task decreases further any likelihood of detecting critical stimuli. Since acute doses of Org 2766 do not increase general arousal (Pigache, 1982b), or alter any sleep parameter (Nicholson and Stone, 1980), it would seem that Org 2766 attenuates a vigilance decrement by attenuating sensory adaptation, or impairing habituation (i.e. interference with the encoding of irrelevant stimulus characteristics), or increasing incentive-motivation. The first proposition is possible, the second seems fairly unlikely for a peptide which, so far, has failed to modify human memory. However, increased incentive-motivation, i.e. an increase in the subjective 'value' of a stimulus, has already been proposed as a mechanism underlying ACTH and Org 2766 effects (De Kloet and De Wied, 1980; Bohus, 1981; Pigache and Rigter, 1981). Increased incentive-motivation could also account for the Org 2766 improvement of stimulus detection and of evaluation/categorization. These pro-

Table 4. Profile of Org 2766 effects in man

| Subjects ($n$) | Daily dose (mg) | Treatment duration (days) | Effects | References |
|---|---|---|---|---|
| Acute dosing | | | | |
| Healthy students (26) | 5,10,20 | 1 | ↓ habituation* | Gaillard and Varey (1979) |
| (18) | 40 | 1 | ↓ habituation | O'Hanlon (cited in Pigache,(1982b) |
| (20) | 40 | 1 | ↑ discrimination | O'Hanlon et al. (1982) |
| (30) | 40 | 1 | ↑ stimulus detection/ selection† ↑ evaluation/ categorization† | Rockstroh et al. (1981) |
| (32) | 40 | 1 | ↓ switching of attention | Fehm-Wolfsdorf et al. (1981) |
| Subchronic dosing | | | | |
| 'Symptomatic' elderly (50) | 10,20 | 14 | ↓ anxiety ↓ depression ↑ competence ↑ attention ↑ energy | Ferris and Reisberg (1981) |
| (43) | 10-20 | 7 | ↓ anxiety | Willner (1981) |
| Mild-moderate dementia (mixed)(35) | 40 | 28 | ↓ withdrawal/apathy ↑ sociability | Braverman et al. (1981) |

↑ increase, ↓ decrease

* Or ↑ motivation

† Replicated in subsequent study (Rockstroh et al., 1982)

cesses make for the salience of relevant stimuli and belong to selective attention. The impairment which followed Org 2766, i.e. in switching attention between two relevant stimuli (Fehm-Wolfsdorf *et al.*, 1981), would not ordinarily be considered a counterpart to enhanced selective attention, but the stimuli concerned had different motivational loadings (one was always followed by an aversively loud tone). Perhaps the peptide caused attention to 'lock on' more firmly to both stimuli, though possibly unequally, and an impairment in switching attention was the consequence.

A number of theoretical and possibly semantic issues have been raised in this section. It is evident that further work must be done with Org 2766 to clarify the results. Moreover, this work

should develop from a model of attention that, potentially, would be able to integrate all the data. It may also be hoped that beneficial effects following acute Org 2766 administration will persist with chronic treatment.

## EFFECTS OF ORG 2766 IN THE ELDERLY

Effects of subchronic treatment with Org 2766 in elderly 'symptomatic' volunteers and in mildly demented patients are summarized in table 4. These findings, and others, have been extensively reviewed quite recently (Pigache and Rigter, 1981; Pigache 1982a, b). In addition, a study in 39 patients with moderate to severe primary degenerative dementia has just been completed by Martin *et al.* (1982). These researchers found no convincing effects of Org 2766 (40 mg p.o. for 1 month), but they suggested, however, that the patients might have been at the end-stage of the disease, with little capacity for improvement.

As Raskin (1981) remarked recently, the long-standing difficulty of translating geriatric rating scale scores into clinically meaningful measures of change persists. In the Braverman *et al.* (1981) study of Org 2766, changes with the Plutchik Geriatric Rating Scale in mildly demented patients (of mixed etiology) amounted to about 15% (more of course for the patients who responded) and was considered to be clinically significant. It may be hoped that the effects of Org 2766 will be especially useful in demented outpatients, perhaps enabling them to maintain their lives longer in the community.

A qualitative appraisal of the effects produced, so far, in aged and demented subjects, with subchronic Org 2766 treatment indicates that the peptide improved rated: competence, attention, energy, mood and sociability. These effects may be compared to the 'behavioral qualities' identified and grouped under two general classes of behavior (table 5) by Wittenborn (1981), representing 'loss of awareness' and 'distractibility'. Wittenborn (1981) adds, that, in some subjects, the two classes might be 'mutually dependent and parts of the same behavioural complex'. Before arriving at his classification Wittenborn (1981) analyzed the item content of 40 observer rating scales for geriatric subjects and grouped together semantically or behaviorally equivalent qualities, which appeared to change after treatment with various 'geriatric drugs' (cyclandelate, Hydergine®, meclofenoxate, naftidrofuryl, papaverine and piracetam). The behavioral qualities referred to in table 5 appeared to respond much more to these 'geriatric drugs' than to other psychotropic drugs, e.g. major or minor tranquilizers and antidepressants. However, the 'geriatric drugs' might have had some antidepressant actions which, by improving affective tone, could have increased alertness and awareness, too. Wittenborn (1981) proposes that 'some behavioural qual-

**Table 5.** Behavioral qualities common to geriatric rating scales (Wittenborn, 1981)

| 'Loss of awareness' (items) | 'Distractibility' (items) |
|---|---|
| disorientation | restlessness |
| loss of memory | emotional lability |
| loss of alertness | |
| incontinence | |

ities may be more susceptible to amelioration in elderly people than other qualities'. It would be interesting to know if there might be intrinsic limits to how much such qualities could change, but this might depend on the advent of drugs with sufficient impact.

Single doses of Org 2766 given to healthy young subjects produced effects (table 4) that have been ascribed to increased motivation (Gaillard and Varey, 1979). For the sake of parsimony, it was argued elsewhere (Pigache and Rigter, 1981; Pigache, 1982a,b) that increased motivation might underly the improved mood seen after subchronic dosing in the elderly (table 4). However, it is also possible that Org 2766 acted otherwise, on pharmacologically sensitive behaviors such as those identified by Wittenborn (1981) (table 5).

## ACUTE VERSUS CHRONIC ADMINISTRATION OF ORG 2766

Perhaps the acute dose effects of Org 2766 in young adults do not connect with those of subchronic dosing in the elderly. An example of acute and subchronic treatments having different effects was seen in rats, where acute doses of Org 2766 had no effect on glucose metabolism (Delanoy and Dunn, 1978) whereas subchronic treatment (10 days) significantly increased glucose utilization in limbic areas (McCulloch *et al.*, 1982). The same tests and treatment regimens will need to be applied at both ends of the human lifespan in order to clarify whether the duration of dosing would account for the different responses noted, or rather that the peptide should be considered to have multiple actions (Rigter *et al.*, 1982).

In the same vein, it is extremely interesting that very prolonged administration (8.5 months) of Org 2766 to rats, from 16 months of age, reduced neuronal loss and astrocyte reactivity in the hippocampus, which occurred in controls as a function of age (Landfield *et al.*, 1981). A behavioral concomitant of this apparent protection against brain aging was seen in the better acqui-

sition of reversal learning by Org 2766 treated rats. Reversal learning is a paradigm used to evaluate selective attention in rats (e.g. Mackintosh, 1969). It is impaired both by aging (Elias and Elias, 1976) and by hippocampal lesions (Niki, 1966). An effect of chronic Org 2766 treatment on attentional processes would also mirror the effects ascribed to acute treatments in rat (De Wied, 1974; Sandman and Kastin, 1977) and man (see earlier section). Further neurotropic action of Org 2766 (and other ACTH peptides) was seen following treatments lasting no more than 18 days, which facilitated the recovery of sensorimotor function in rats with sciatic nerve injuries (Bijlsma *et al.*, 1981). If the neurotropic actions of ACTH peptides in rat can be extrapolated to man, we may hope that prolonged treatment with Org 2766 will attenuate the gradual cerebral degeneration in dementia. Such an action would not have to relate to any of the effects reviewed earlier, for treatments given for one month or less.

## OTHER POSSIBLE INDICATIONS FOR ACTH-LIKE PEPTIDES

Although much of this paper has concentrated on the treatment of dementia, it may be that ACTH-like peptides would produce more favorable results in other patient groups. There are good reasons for extending Org 2766 treatment to younger patients (perhaps to Down's syndrome, but probably not to infantile epilepsy currently treated with ACTH (Willig and Lagenstein, 1980)), to degenerative nervous system diseases other than primary degenerative dementia, and to demyelinating diseases. It is not possible to do everything at once, however, and for the time being it seems wise to follow existing leads and to establish whether or not the peptide will be beneficial to the growing population of demented patients. This work is in progress.

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# Clinical psychopharmacology of endorphins

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## INTRODUCTION

One of the most active topics in current psychopharmacology is the investigation of CNS-active endogenous ligands of pharmacological agents, e.g. studies as to the possible existence of an endogenous diazepam-like material, endogenous neuroleptics, etc. Such developments result not only from the discovery of the endorphins (i.e. endogenous morphine-like substances) which opened a novel field of research, but were also associated with novel concepts of the operation of the CNS and the introduction of advanced pharmacological technology.

Historically, endorphins were discovered when specific opiate receptors were studied not only indirectly by pharmacological characterization but also directly by use of receptor binding studies (Pert and Snyder, 1973). These findings raised questions as to the possible biological significance of these receptors and as to the chemical identity of the natural ligands; as a consequence, Hughes *et al.* (1975) were able to isolate and characterize an opiate-active material in the brain containing the pentapeptides met-enkephalin and leu-enkephalin. Further biochemical research revealed a whole family of opiate-active peptides which may be classified as the transmitter-like pentapeptides (enkephalins) and larger molecules (e.g. β-endorphin, α-neo-endorphin, β-casomorphin, dynorphin; for review, see Emrich, 1981; Höllt *et al.*, 1981).

From the mode of action of endorphins, a spectrum of activity similar to that of morphine might be anticipated. Generally, it has been shown that this is indeed the case. In particular, it has been established that chronic administration of endorphins, in a fashion similar to opiates, results in the induction of tolerance and physical dependence; thus, a physiological analgesic and/

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or tranquilizing drug of therapeutic potential cannot be derived simply from an endorphin-like chemical structure. Differences between the actions of endorphins and of opiates depend mainly on pharmacokinetic and physicochemical differences. However, recent investigations (Childers *et al.*, 1979; Lord *et al.*, 1977; Robson and Kosterlitz, 1979; Wüster *et al.*, 1980) have demonstrated the specificity of certain opioid agonists and antagonists on different subtypes of opiate receptors (μ, δ, ε). The possible clinical implications of these findings cannot as yet be evaluated, since clinical experience in humans up to now is confined to the application of β-endorphin, the enkephalin analog FK 33-824 and the opiates. The primary effects of these substances are mediated by μ-receptors and, in the case of the psychotomimetic partial agonists, such as cyclazocine and nalorphine, their psychotogenic properties are apparently independent of their opiate-receptor affinity (Shearman and Herz, 1982). More specific agonists for e.g. δ-receptors, such as [D-Ala$^2$,D-Leu$^5$]-enkephalin, have not yet been examined clinically; therefore, the possible psychotropic action of these subtypes of opioids, e.g. psychic effects initiated by the activation of different subtypes of opiate receptors, is up to now completely a '*terra incognita*'.

From the analgesic and tranquilizing effects of opioid-peptides, a central functional role in psychic responses to stress may be predicted. In fact, a plethora of animal studies have shown that opioid peptides act as modulators of nociception, temperature control and motor behavior under conditions of stress (for review see Millan and Emrich, 1981). On the contrary, in human studies comparatively few investigations have as yet positively demonstrated an involvement of endorphinergic systems in stress phenomena, whereas a great number of studies showed negative results (for review see Emrich, 1981). The main reason underlying this discrepancy may be the fact that for ethical reasons in human studies the intensity of enforceable stress may, in many cases, be insufficient to activate an endorphinergic response.

The clinical psychopharmacology of opioids is confronted with problems concerning (a) possible beneficial effects of opiate-like substances in various types of psychiatric disorders, and (b) a possible mediation of the psychotropic effects of other classes of substances via an activation of endorphinergic systems. Furthermore, (c) the possible therapeutic and/or antitherapeutic actions of specific opioid antagonists (naloxone, naltrexone) demand consideration.

## PSYCHOTROPIC ACTIONS OF OPIOIDS

In response to the question as to the psychopharmacological profile of action of opioids, their euphorogenic and anxiolytic

properties are of pertinence; this prompts the hypothesis of a possible antidepressant property of opioids. Interestingly, endorphin research in the past has been undertaken in completely the opposite direction: based on the endorphin hypothesis of schizophrenia (Terenius *et al.*, 1976), schizophrenic patients were treated with the specific opiate antagonist naloxone. This approach evolved partially from the concept (Terenius) that some types of partial agonists (e.g. cyclazocine and nalorphine) exert their psychotogenic effects via a specific action on opiate receptors. This concept was derived from the contention that the psychotogenic effects of cyclazocine and nalorphine are naloxone-reversible (Jasinski *et al.*, 1967) which is apparently not the case (see also Shearman and Herz, 1982). The strategy to try to counteract schizophrenic symptoms by application of naloxone was furthermore based on experiments which detected elevated levels of endorphins (radioreceptor assay) in the CSF of schizophrenic patients (Terenius *et al.*, 1976). However, attempts to replicate these findings (radioimmunoassay: Emrich *et al.*, 1979a; radioreceptor assay: Naber *et al.*, 1981) have proven unsuccessful. Therefore, the essential theoretical basis for the naloxone treatment of schizophrenic patients is conspicuously weak, and an alternative explanation for certain interesting results acquired with this drug must possibly be constructed (see below).

Investigations as to a possible beneficial effect of β-endorphin in psychotic patients were initiated by Kline and coworkers in 1977. In a series of open studies, Kline *et al.* (1977) investigated possible effects of β-endorphin in different types of psychoses and neuroses. The profile of action of β-endorphin was described as tranquilizing and mood-elevating. In some cases hallucinatory effects were also observed. Subsequently, the clinical efficacy of β-endorphin was investigated under double-blind conditions by Catlin's group (Gerner *et al.*, 1980), by the NIMH group (Pickar *et al.*, 1981), and by Berger's group in Stanford (Berger *et al.*, 1980). From the present state of knowledge, an antidepressant effect of β-endorphin may be recognized as clear (Gerner *et al.*, 1980; Gorelick *et al.*, 1981), whereas its antischizophrenic properties are highly questionable

Investigations using the enkephalin-derivative FK 33-824 (Jørgensen *et al.*, 1979; Nedopil and Rüther, 1979) point to a weak antischizophrenic effect; its possible antidepressant efficacy has not as yet been evaluated satisfactorily.

The finding that endogenous opioids apparently exert antidepressant effects in patients suffering from primary depression renders inconceivable the idea that there may be a constitutional defect of endorphinergic systems in endogenous depression. This question has recently been discussed extensively, and in a synthesis of the body of information concerning biochemical and pharmacological data no substantial evidence pointing to such a hypothesis could be derived (Emrich, 1982). Nevertheless, although

there may not be a deficiency in endorphin function in depression, exogenous application of opiate-like material is possibly of therapeutic use (e.g. the application of buprenorphine; Emrich *et al.*, 1982).

## ACTIVATION OF ENDOGENOUS OPIOIDS BY ELECTROCONVULSIVE THERAPY

Although the interesting findings of Grahame-Smith's group (1978) demonstrated a mobilization of central monoamines by an exposure to electroconvulsive therapy (ECT), the neurochemical basis of the therapeutic action of this manipulation remains an open question. Holaday's group (Belenky and Holaday, 1979; Holaday *et al.*, 1981) recently obtained compelling evidence for an activation of central endorphinergic systems by ECT. In line with these results are the findings of Emrich *et al.* (1979a) in which an increase in plasma levels of β-endorphin immunoreactivity was evaluated 10 min following ECT in depressed patients, a result which has recently been reproduced by Inturrisi *et al.* (1982).

From these findings a possible endorphinergic component of the mode of action of ECT may be hypothesized.

## ACTIVATION OF ENDORPHINS BY NEUROLEPTIC DRUGS

According to the findings of Costa's group (Hong *et al.*, 1979) and of Höllt and Bergmann (1982), chronic treatment with haloperidol or other neuroleptic drugs results in an elevation in levels of endorphins in the striatum, pituitary and plasma. From these findings and, in particular, the observation (Höllt and Bergmann, 1982) that chronic haloperidol treatment exerts a specific facilitation of β-endorphin biosynthesis (via an activation of messenger RNA; cf. Höllt, 1981), the hypothesis may be raised that therapeutic actions of neuroleptic drugs may be mediated via a potentiation of the functional activity of endorphins.

Clinical investigations in this regard have focused on two types of variables: On the one hand, the activating effect of chronic neuroleptic treatment upon endorphinergic systems was studied at the level of determination of β-endorphin immunoreactivity in plasma; on the other hand, the question was raised whether the therapeutic effects of neuroleptics might be counteracted by naloxone treatment. Investigation of β-endorphin immunoreactivity in plasma of schizophrenic patients prior to and during long-term neuroleptic therapy showed a small but highly significant increase during the course of medication (Gramsch and Emrich, unpublished data). Interestingly, the opposite is true in treatment of schizophrenic patients with high-dosage diazepam (this study was performed in cooperation with Haas and Beckmann, at Mannheim; Haas *et al.*, 1982). This reduction of β-endorphin

immunoreactivity in plasma may be due to the antistress effect of diazepam (Millan and Duka, 1981). The naloxone trial, employing 2 x 20 mg per day for 2 days in schizophrenic patients revealing productive psychotic symptoms, was designed to counteract the beneficial effects of neuroleptic drugs. In comparison to a placebo control, no blockade by naloxone of the antipsychotic effect of neuroleptic drugs could be demonstrated. However, the interpretation of these observations is difficult since, probably owing to the short half-life of naloxone, endorphin-activating effects may develop during the course of this treatment (cf. Reker *et al.*, 1983).

## ACTION OF THE OPIATE-ANTAGONIST NALOXONE IN DIFFERENT TYPES OF PSYCHOSES

The rationale for the therapeutic administration of naloxone in schizophrenic patients, as discussed above, was that competitive inhibition of a hypothetical opioid hyperactivity in schizophrenia may thus be attained. From the sum of naloxone studies performed in recent years (see Emrich, 1981) it has to be concluded that the (low) doses sufficient to precipitate withdrawal symptoms in opiate addicts are ineffective in schizophrenic patients, whereas higher dosages (4.0-25.0 mg, i.v.) induce a relatively small but statistically significant antipsychotic effect (Emrich *et al.*, 1977, 1979b; Pickar *et al.*, 1982; Watson *et al.*, 1978). In several studies, this therapeutic effect exhibited a time lag of 2-3 h and it is presently and open question whether this therapeutic effect may be interpreted as reflecting opiate receptor blockade or possibly an endorphin-activating activity of naloxone as demonstrated by Reker *et al.* (1983).

## SIGNIFICANCE OF ENDORPHINS IN HEROIN ADDICTION

The neurochemical bases of heroin addiction is attributable to a modulation of processes at a hierarchy of organizational levels:

(a) adaptive processes at the receptor/second messenger level;
(b) adaptive processes concerning endorphins (for a summary see Herz, 1981).

According to a hypothesis formulated by Goldstein (1976) there might exist a constitutional basis for the development of heroin addiction, i.e. the existence of an endorphin deficiency in this (sub-) population. To address a part of this hypothesis, β-endorphin immunoreactivity in the plasma of seven heroin addicts was evaluated before and during heroin withdrawal. No abnormality of β-endorphin immunoreactivity in plasma could be observed. During heroin withdrawal a relatively small but highly significant in-

crease of β-endorphin immunoreactivity could be demonstrated. From these data, no convincing support for the endorphin deficit hypothesis of heroin addiction can be deduced.

## SUMMARY

Endorphins undoubtedly play a major role in physiological responses to stress, although in humans these effects cannot be demonstrated easily. The pharmacological profile of opioids resembles that of opiates, although at present specific actions on subtypes of opiate receptors differing from the μ-receptor cannot be evaluated. An antidepressant effect of opioid peptides in humans has clearly been demonstrated, whereas its effects in schizophrenic patients are questionable. Neuroleptic drugs exert an activating effect upon endorphinergic systems. However, it cannot as yet be demonstrated that their antipsychotic effects are mediated via an endorphinergic mode of action. A similar action of ECT has been found, and it has been hypothesized that the antidepressant effect of ECT reflects, at least partially, this endorphinergic mode of action. Furthermore, the role of endorphins in heroin addiction is discussed.

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# GABA receptor agonists: pharmacological spectrum and clinical actions

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## INTRODUCTION

Stimulation of GABA receptors has been reported to modify the activity of a variety of cerebral neurotransmitter systems (see below). As these systems are affected in various neuropsychiatric disorders, GABA receptor agonists represent a potential class of new drugs with a wide therapeutic spectrum.

This review focuses briefly on the pharmacological spectrum and the clinical action of progabide (SL 76 002; (4-{[(4-chlorophenyl) (5-fluoro-2-hydroxyphenyl)-methylene]amino} butanamide) (Kaplan *et al.*, 1980), the first apparently non-toxic, specific GABA receptor agonist available for clinical studies. Thus, the action of this compound on norepinephrine (NE), 5-hydroxytryptamine (5-HT, serotonin) and dopamine (DA) neurons will be emphasized in the light of the therapeutic results in affective disorders, dyskinesia and epilepsy.

(For details on the pharmacological actions of progabide, see Worms *et al.*, 1982; Lloyd *et al.*, 1982; Scatton *et al.*, 1982; Scatton and Bartholini, 1982.)

## NE AND 5-HT NEURONS - DEPRESSION

Progabide on acute administration increases dose-dependently the concentrations of 3,4-dihydroxyphenylethyleneglycol sulfate - the major cerebral metabolite of NE - in limbic forebrain areas of the rat without alteration of the NE levels (Scatton *et al.*, 1982). Also, the α-methyl-*p*-tyrosine-induced disappearance of the amine is accelerated by progabide (Scatton *et al.*, 1982). These effects indicate an enhancement of NE turnover and, as they are blocked by picrotoxin, result from a GABA-mediated mechanism (Scatton *et al.*,

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1982). As iontophoretic administration of progabide or other GABAergic agents on locus coeruleus cells reduces their firing rate (Guyenet and Aghajanian, 1979), the acceleration of NE turnover by GABA receptor stimulation probably occurs via an indirect mechanism, i.e. via changes of neuronal inputs on noradrenergic neurons. Whatever the mechanism, the increased NE turnover by progabide should lead to an enhanced noradrenergic transmission. Similarly, tricyclic antidepressants, by inhibition of the NE re-uptake, increase the availability of the amine at the receptor sites and therefore noradrenergic transmission (Schildkraut, 1965). Indeed, both progabide and antidepressants antagonize the reserpine-induced ptosis which results from a reduced availability of NE in the synaptic cleft.

Progabide administered to rats daily for 14 days leads to a reduction of the enhanced NE turnover observed after a single injection; this occurs in the absence of changes of postsynaptic β-antagonist binding or of NE-sensitive adenylate cyclase and is probably the result of a tolerance of the noradrenergic system which develops to GABA receptor agonists; it is likely that the absence of receptor changes is a consequence of this tolerance (Zivkovic *et al.*, 1982). Tricyclic antidepressants on repeated treatment lead, in contrast, to a down-sensitization of β- binding and of the NE-sensitive adenylate cyclase activity (Sulser *et al.*, 1978).

Serotonergic neurons are also affected by GABA receptor stimulants but in the opposite manner than noradrenergic cells. Thus, progabide on acute administration reduces the 5-HT turnover in the rat striatum as indicated by (a) the diminution of 5-HT disappearance after α-propyl-dopacetamide; (b) the decreased accumulation of 5-HTP after inhibition of aromatic L-amino acid decarboxylase by NSD 1015; (c) the accumulation of 5-HT after pargyline (Scatton *et al.*, 1982). This effect probably occurs by direct stimulation of GABA receptors localized on raphe cells which are inhibited by iontophoretically administered GABA (Gallager and Aghajanian, 1976). Repeated treatment with progabide accentuates the reduction in 5-HT turnover (Zivkovic *et al.*, 1982). This further reduction in 5-HT turnover is accompanied by the development of a supersensitivity of postsynaptic target cells as indicated by the increase in spiroperidol binding sites (Briley, personal communication). Under these conditions, as progabide antagonizes the 5-HTP-induced head twitches (Worms, personal communication), serotonergic transmission should be reduced - even if this reduction may be partially compensated by receptor up-regulation.

In contrast, tricyclic antidepressants on acute administration increase 5-HT transmission as a consequence of the 5-HT re-uptake inhibition (Schildkraut, 1965); however, this leads under repeated treatment to a down-regulation of postsynaptic receptors (Peroutka and Snyder, 1980). According to these data,

5-HT uptake blockers, 5-HT receptor antagonists (e.g. mianserin) and GABA receptor agonists (e.g. progabide) decrease 5-HT transmission under chronic treatment.

Support for this view is provided by the fact that amitryptyline or mianserin antagonize 5-HT-mediated effects (Maj *et al.*, 1979; Ogren *et al.*, 1979) and progabide reduces the 5-HTP-induced head twitches. The three classes of compounds are effective in behavioral animal models predictive of antidepressant activity such as olfactory bulbectomy and learned helplessness (Broekkamp *et al.*, in preparation). In the case of progabide, this effect is blocked by bicuculline, indicating a GABA receptor-mediated event (Broekkamp *et al.*, in preparation).

In conclusion, progabide and probably other GABA receptor agonists represent a new class of antidepressant drugs; similarly to tricyclic compounds, progabide affects NE- and 5-HT-mediated transmission. However, the effects of progabide on monoamines differ from those of tricyclics (see above) and it is still unknown which common denominator is relevant to the antidepressant action. Also, the mechanism of progabide likely involves a primary change in NE and 5-HT cell firing rate whereas tricyclic antidepressants affect primarily synaptic events, i.e. monoamine re-uptake.

## Clinical Results in Depression

Data in man support the hypothesis that GABA-agonists have a therapeutic action in depressive syndromes.

In a preliminary open study on 15 depressive (11 endogenous and four reactive) patients, progabide administered at doses of 20-30 mg $kg^{-1}$ per day led within 8-15 days to a significant improvement in seven (six endogenous and one reactive) cases with mobilization of defenses, disappearance of death thoughts and culpability, reappearance of critical judgement and frank elevation of mood (Morselli *et al.*, 1980).

A second, more recent, 28-day double-blind controlled study was run against imipramine (1.6-3.3 mg $kg^{-1}$ per day) in patients suffering from major depressive episodes (DSM III) or depressive reactions. The antidepressant action of progabide (20-30 mg $kg^{-1}$ per day) was found to be similar to that of the tricyclic for the global clinical rating and the Hamilton rating scale for depression. The disappearance or significant reduction of depressive symptoms with resumption of daily activity was observed in eight out of 11 cases with progabide and nine out of 11 cases with imipramine.

In both studies, progabide was very well tolerated with a minimal incidence of minor and short-lasting side-effects such as light drowsiness, dry mouth, nausea (Morselli *et al.*, 1981, 1982).

These data confirm that GABA receptor agonists have a therapeutic action in major depression and support the hypothesis that

changes in GABA-mediated regulation of monoaminergic neurons play a role in the pathophysiology of the disease.

## DA NEURONS - DYSKINESIA/MANIA

Progabide, as well as other GABA receptor agonists such as muscimol, inhibits the activity of striatal and mesolimbic DA neurons in various animal species (rat, cat, etc.) (Scatton *et al.*, 1982). Thus, the synthesis of the neurotransmitter is diminished by progabide as indicated by the decreased tyrosine hydroxylase activity and the accumulation of dopa following inhibition of aromatic L-amino acid decarboxylase in the rat. Similarly, the release of DA is decreased as progabide reduces both the disappearance of the amine following α-methyl-*p*-tyrosine and the DA liberation from the cat caudate nucleus (perfused by means of the push-pull cannula). These effects of progabide are blocked by bicuculline, indicating mediation by GABA receptors. Finally, they are likely the consequence of the inhibition of firing rate of DA neurons and become more pronounced after their activation (e.g. after neuroleptic drugs, Scatton *et al.*, 1982).

The progabide-induced decrease in dopaminergic transmission, as well as in its enhancement by neuroleptics, is confirmed by behavioral results. Thus, progabide, although *per se* it does not induce catalepsy, potentiates the cataleptogenic action of neuroleptics (Lloyd *et al.*, 1979). This effect is explained by the diminution in DA neuron activity, DA release and the availability of the amine at the receptor sites for competing with neuroleptics.

Not only are the dopaminergic neurons affected by GABAergic stimulation but also their postsynaptic cells (Bartholini *et al.*, 1980). Thus, repeated co-administration of progabide and neuroleptics reduces (1) the tolerance which develops to the cataleptogenic action of the latter; (2) the supersensitivity to dopaminergic agents; and (3) the increase in DA receptors as indicated by the enhanced $B_{max}$ of $^{3}$H-spirone binding (Briley, personal communication); these are all events which occur following sustained neuroleptic treatment.

DA neurons are inhibited by GABA receptor agonists also in the limbic system. However, the doses of these drugs which decrease limbic DA turnover are higher than those effective in the extrapyramidal systems. This indicates that mesolimbic DA neurons are less sensitive to GABAergic inhibition than nigro-striatal cells (Bartholini *et al.*, 1979).

The inhibition of DA neuron activity by progabide and the prevention of the up-regulation of DA receptors following neuroleptics represent the biochemical basis for the therapeutic action of GABA receptor agonists in disorders which involve an absolute or relative increase in dopaminergic transmission. These disorders

include neuroleptic-induced dyskinesia, L-dopa-induced dyskinesia and mania. Results in monkeys with a ventrotegmental lesion show that small doses of progabide block the dyskinetic syndrome induced by dopamine receptor agonists such as piribidil or by L-dopa (Lloyd *et al.*, 1981a).

### Clinical Results in Dyskinesia

Clinical evidence has been provided for the effectiveness of progabide in various forms of dyskinesia. Thus, in a preliminary open study on eight patients suffering from neuroleptic-induced dyskinetic syndrome (as confirmed by electromyography), progabide was administered at doses of 10-30 mg $kg^{-1}$ per day for at least 6-8 weeks. An evident improvement was observed in six cases after two weeks whereas disappearance of abnormal movements took place in four cases after six weeks of treatment (Sevestre *et al.*, 1982).

A more recent double-blind controlled study on a larger number of subjects suffering from neuroleptic-induced dyskinesia confirmed these findings (Sevestre *et al.*, in preparation).

Similar results have been obtained in L-dopa-induced involuntary movements (Constantinidis, personal communication).

### Mania

The potential therapeutic action of GABA receptor agonists in mania is supported by the fact that valproate has been shown to be effective in preventing manic episodes in bipolar patients (Emrich *et al.*, 1981). No clinical data are available as yet with progabide in mania. Should the compound be effective, a single medication would thus be available for preventing and curing psychotic depression and mania.

## CELLULAR EXCITABILITY - EPILEPSY

GABA receptor agonists decrease cellular excitability and antagonize its enhancement induced by various mechanisms (Lloyd *et al.*, 1979; Worms *et al.*, 1982). In contrast to classical anticonvulsant agents such as ethosuccimide and diphenylidantoin - which are active in only some animal models of epilepsy - progabide and muscimol are effective in seizures which (1) involve changes in GABAergic transmission (bicuculline-, picrotoxin- or cortical penicilline-induced seizures) or (2) are apparently independent of GABA-related parameters (seizures induced by strychnine or ECS; audiogenic seizures). This wide spectrum of GABA receptor agonists in animal models parallels the efficacy of these compounds in

various forms of human epilepsy (see below). It is interesting to note that even in humans two populations of epileptic patients have been identified, one in which the focal and perifocal zones of the brain exhibit alterations of GABA-related parameters (GABA binding, GAD activity, GABA-T activity) and another in which no such changes are detectable (Lloyd *et al.*, 1981b). This points to different etiopathogenetic mechanisms involving or not involving GABAergic transmission.

Another advantage of GABA receptor agonists is the low incidence of side-effects. Thus, progabide exerts its anticonvulsant action at doses far lower than those inducing sedation and muscle relaxation. This differentiates GABA receptor agonists from other anticonvulsants including benzodiazepines which cause the above-mentioned side-effects at doses close to those effective in seizures (Lloyd *et al.*, 1979; Worms *et al.*, 1982).

## Clinical Results in Epilepsy

The anti-epileptic activity of progabide has been demonstrated in a series of clinical studies conducted mostly on severe epileptic patients poorly responding to the available anti-epileptic medication.

In a first preliminary open study on 36 epileptic patients suffering from partial and/or generalized seizures, administration of progabide (20-30 mg $kg^{-1}$ per day) for 4-8 weeks led to a significant reduction of seizure frequency in 47% of the cases (Baruzzi *et al.*, 1980). These preliminary observations have been successively confirmed by two controlled double-blind trials vs placebo. In both studies, progabide significantly reduced the incidence of complex partial seizures, showing in addition a therapeutic action on generalized seizures (Loiseau *et al.*, 1981; Van der Linden *et al.*, 1981); an evident clinical improvement was manifest in 59% and 45% of the patients, respectively.

Long-term studies (12-18 months) have given additional evidence for the anti-epileptic action of progabide; they have also shown that no tolerance develops to the therapeutic action of the compound administered as monotherapy or associated with other drugs (Martinez-Lage *et al.*, in preparation; Loiseau *et al.*, in preparation).

These studies have demonstrated that progabide, on long-term administration, is well tolerated, causing a minimal incidence of minor and transient side-effects such as drowsiness, nausea and hypotonia.

On the whole, it appears that progabide exerts a therapeutic action in 45-50% of the patients who respond poorly to 'classical' anti-epileptic drugs. This is in good agreement with the finding that 50-60% of patients undergoing neurosurgery because of poor responsiveness to pharmacological treatment have an alteration of GABA-mediated transmission (see above; Lloyd *et al.*, 1981b).

SUMMARY

Stimulation of GABA receptors (e.g. by progabide, a new GABA receptor agonist), affects noradrenergic and serotonergic transmission in animals and, on chronic administration, causes changes in 5-$HT_2$ receptors similar to those caused by ECS. Based on double-blind trials versus imipramine, it has been shown that progabide is effective in psychotic depression.

Progabide reduces DA turnover in animals and prevents supersensitivity caused by neuroleptic agents. This double mechanism on dopaminergic transmission appears to be the basis for the clinical action of the compound in various forms of dyskinesia (L-dopa- and neuroleptic-induced dyskinesia). It also suggests that progabide will be effective in mania.

Progabide decreases cellular excitability in animal brain and antagonizes seizures whatever their origin (GABA-mediated or GABA-unrelated mechanisms). Clinically, the compound is effective in various forms of epilepsy resistant to 'classical' medication in the absence of major side-effects such as sedation or muscle relaxation. The wide spectrum in animals seems to parallel the effects in humans. In this respect, it is interesting to note that, in epileptic patients, two populations have been distinguished, one with cerebral alteration of, and one with normal, GABA-related parameters in focal and perifocal brain areas.

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# Benzodiazepine receptor-mediated experimental 'anxiety' in rhesus monkeys after infusion of 3-carboethoxy-ß-carboline (ß-CCE)

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## INTRODUCTION

The presence of specific recognition (viz. receptor) sites for benzodiazepines in brain that are functionally (and perhaps structurally) coupled to both recognition sites for γ-aminobutyric acid (GABA) and a chloride ionophore has been firmly established (cf. Tallman *et al.*, 1980; Paul *et al.*, 1981; Skolnick and Paul, 1982). Both direct and indirect evidence suggest that this 'supramolecular complex' mediates the pharmacological actions of benzodiazepines as well as other structurally unrelated compounds which share common properties with the benzodiazepines (Paul *et al.*, 1981; Skolnick and Paul, 1982). Whether this supramolecular complex has a physiological role in the absence of an exogenous ligand (drug) has been a source of considerable speculation, particularly regarding its function in the pathophysiology of anxiety and related disorders (cf. Paul and Skolnick, 1981).

The report by Braestrup *et al.* (1980) that certain C-3 substituted β-carbolines possess very high affinities for the benzodiazepine receptor stimulated investigation of the pharmacologic actions of these compounds. In general, β-carbolines such as 3-carboethoxy-β-carboline (β-CCE) have been reported to antagonize the actions of benzodiazepines (Tenen and Hirsch, 1980; Cowen *et al.*, 1981; and others). However, more recent work suggests that subtle modification of the C-3 position of this compound results in dramatic changes in pharmacological activity with a spectrum from convulsant (Braestrup *et al.*, 1982; Schweri *et al.*, 1982) to benzodiazepine-like (Skolnick *et al.*, in prep-

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aration). These observations, supported by more detailed neurochemical findings (Skolnick *et al.*, 1982), suggest that the benzodiazepine receptor has several functional 'domains' (or subsites), and that binding of a ligand to a given domain on the receptor can result in opposite pharmacologic and behavioral effects.

Recently, we have examined this hypothesis by studying the behavioral and physiological actions of β-CCE in the rhesus monkey (Ninan *et al.*, submitted). Although most reports in rodents suggest that β-CCE antagonizes the pharmacologic actions of diazepam and related benzodiazepines with no marked behavioral actions by itself (however, see File, 1982), our data suggest that in primates β-CCE elicits a behavioral and physiological state of anxiety manifested by dramatic elevations in plasma cortisol and epinephrine, blood pressure and heart rate. Furthermore, both the behavioral and physiological effects of β-CCE are blocked by pretreatment with the benzodiazepine receptor antagonist, Ro 15-1788 (Hunkeler *et al.*, 1981). These results suggest that the benzodiazepine receptor may not only be involved in the anxiolytic actions of benzodiazepines and related compounds, but may also play a pivotal role in both the pathogenesis of 'anxiety' and its pathophysiologic sequelae in man.

## MATERIALS AND METHODS

Adult male rhesus monkeys (7-10 kg) were administered β-CCE i.v. (2.5 mg $kg^{-1}$) in 10 ml of 20% diluted Emulphor® - 80% 0.9% NaCl (cf. Skolnick *et al.*, 1980) during a 2 min interval. All animals were previously fitted with femoral venous catheters under ketamine anesthesia and placed in chairs at least 24 h prior to administration of drug or vehicle. The i.v. catheters were kept patent by administering a slow i.v. infusion of sterile 0.9% NaCl over the course of the experiment. The dose of β-CCE used (2.5 mg $kg^{-1}$) was empirically chosen based on the reported affinity of this compound for the benzodiazepine receptor ($\sim$1 nM) and previous studies demonstrating its very rapid metabolism when incubated with rodent plasma at 37°C (Mendelson *et al.*, 1982).

In this experimental protocol, animals were administered β-CCE or vehicle on the morning of day 1. On day 2, the same animals were administered Ro 15-1788 (5 mg $kg^{-1}$) 10 min prior to receiving an identical dose of β-CCE. In all studies, blood samples (1 ml) were collected at 20 min intervals beginning 40 min prior to the drug or vehicle infusion, and continuing for up to 4 h. Blood pressure and pulse rate were monitored automatically at 5 min intervals using a Dinamap Research Monitor (Model 1245) (Applied Medical Research). Plasma cortisol levels were measured by radioimmunoassay (New England Nuclear, Boston, MA). Behavior of the animals was videotaped during both vehicle and drug administration for subsequent rating by 'blind' investigators.

## RESULTS

Administration of β-CCE (2.5 mg $kg^{-1}$) elicited significant behavioral changes in all of the monkeys examined. Although both the time course and intensity of these changes varied among individual animals, they were easily distinguished from vehicle-treated animals. The behavioral effects of β-CCE included: increased rotation of the head and neck; hyperexcitability to external stimuli (e.g. hand clap); increased vocalizations; defecation and urination; hand clasping and hand wringing; intense scratching (sufficient to cause bleeding in some animals); and a heightened responsiveness to threatening stimuli. Many of these actions were apparent within 10 min after injection, and lasted for up to 2 h. In a number of animals, this increase in excitability was followed by a period of sedation, and in some cases sleep. Concomitant with the behavioral effects of β-CCE, there was a dramatic increase in heart rate (pulse) and mean arterial blood pressure. Figure 1 depicts the β-CCE-induced elevation of heart rate and blood pressure in a typical experiment. These changes are most likely caused by an increase in circulating plasma catecholamines observed following β-CCE administration (Ninan *et al.*, submitted). Since elevations of plasma cortisol can occur under stressful or anxiety-provoking situations in both laboratory animals and man, we examined the effects of β-CCE administration on plasma cortisol. A dramatic and highly significant elevation of plasma cortisol was observed following β-CCE administration (Figure 2). The elevations in plasma cortisol peaked at about 80 min and lasted for as long as 5-6 h (figure 2 and unpublished observations).

If the behavioral and physiological effects of β-CCE were mediated by a specific interaction with the benzodiazepine receptor, then pre-treatment with a benzodiazepine antagonist should prevent or blunt the response to β-CCE. Pre-treatment of monkeys with Ro 15-1788 (5 mg $kg^{-1}$) 10 min prior to injection of β-CCE completely reversed the elevation of plasma cortisol (figure 2) and greatly attenuated the elevation of both blood pressure and heart rate (data not shown). Ro 15-1788 had no significant behavioral or physiological actions when administered alone, although there was a slight trend toward decreased heart rate and blood pressure (unpublished observations).

## DISCUSSION

β-CCE binds to benzodiazepine receptors with high affinity and has been previously shown to antagonize some of the pharmacological actions of benzodiazepines in rodents (Tenen and Hirsch, 1980; Cowen *et al.*, 1981; and others), while eliciting no obvious behavioral changes when administered alone. However, a very recent

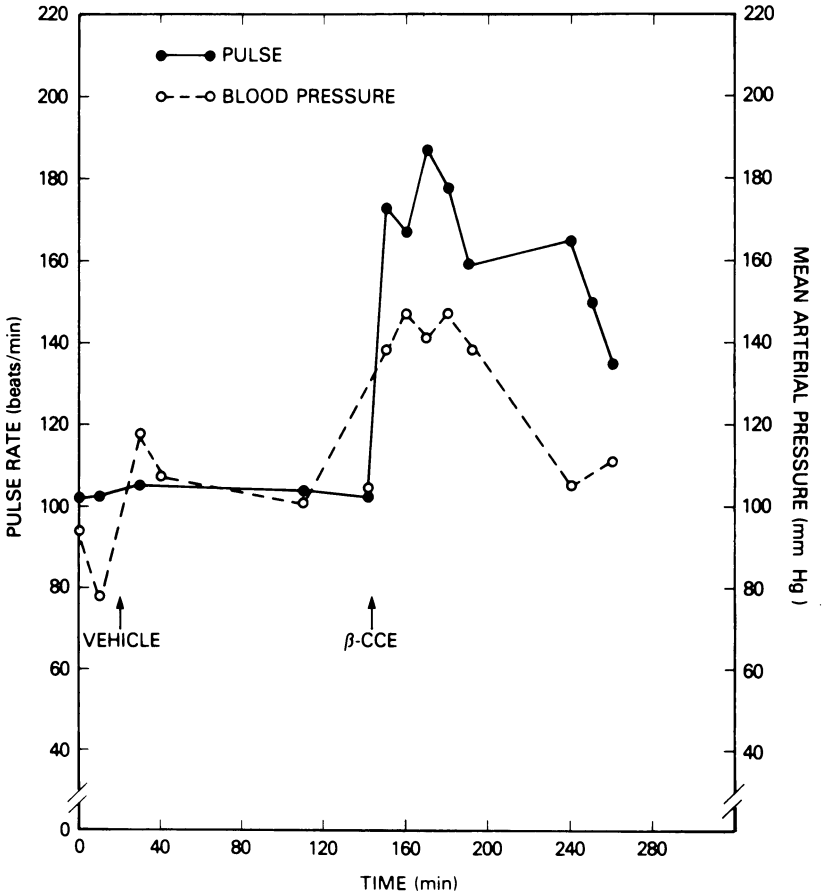


**Figure 1.** Effects of β-CCE on pulse rate and mean arterial blood pressure. In this representative experiment, a male rhesus monkey was injected with vehicle, and the pulse rate and mean arterial blood pressure monitored for 2 h. Following administration of β-CCE (2.5 mg $kg^{-1}$ i.v.), a marked rise in both blood pressure and pulse rate was observed.

report (File, 1982) suggests that, in a test of social interaction, this compound exerted an action 'opposite' to that of the benzodiazepines, which could be interpreted as 'anxiety'. However, to our knowledge, the physiological and behavioral actions of β-CCE have not been examined in primates.

In the rhesus monkey, β-CCE elicited a profound elevation in the circulating levels of stress-related hormones such as cortisol

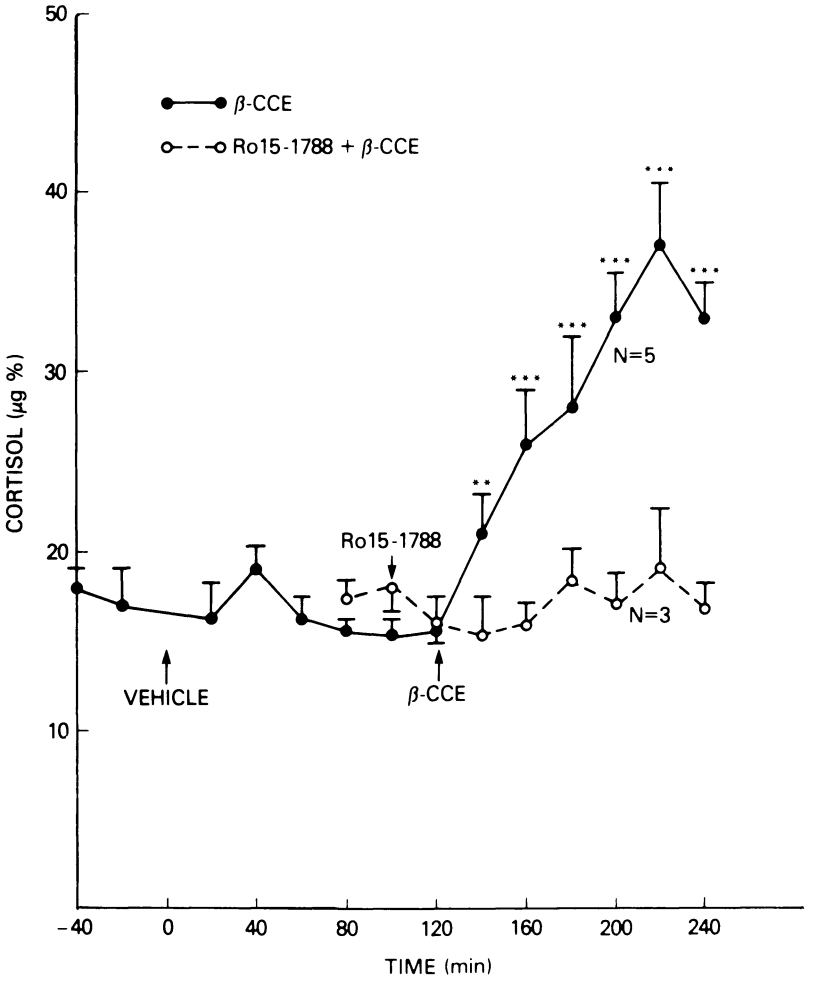


**Figure 2.** Effects of β-CCE and Ro 15-1788 on plasma cortisol levels. Rhesus monkeys were injected with vehicle, and the plasma cortisol levels were monitored by radioimmunoassay as described in the text. At the times indicated by arrows, β-CCE (2.5 mg $kg^{-1}$ i.v.) was administered alone (filled circles) or 10 min after treatment with Ro 15-1788 (5 mg $kg^{-1}$ i.v.) (open circles). Symbols: ** $p < 0.01$, *** $p < 0.001$ compared to a combination of Ro 15-1788 + β-CCE at the corresponding time point. These data were evaluated using Student's *t*-test.

and epinephrine. Concomitant with these endocrine changes, a dramatic increase in the somatic manifestation of 'anxious' behavior was also observed, reflected as an elevated heart rate and blood pressure. Furthermore, a wide range of behaviors were elicited by β-CCE which were not observed in vehicle-treated animals, many of which could be interpreted as 'anxious'.

The effects of β-CCE on the endocrine, somatic and behavioral parameters used in these studies are reminiscent of those typically observed in anxious patients and/or animals and humans exposed to anxiety-provoking or stressful situations. That these effects are mediated through the benzodiazepine receptor is supported by the blockade or muting of these effects by pre-treatment with the specific benzodiazepine receptor antagonist, Ro 15-1788. Taken together, these results suggest that the benzodiazepine-GABA receptor-chloride ionophore complex not only mediates the pharmacological actions of the benzodiazepines but may also subserve the affective and physiological expression of anxiety.

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# Contributors' index*



* Numbers indicate starting pages of chapters

# Subject index*



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