Clinical Pharmacology in Psychiatry

Neuroleptic and Antidepressant Research

Edited by

Earl Usd n, Svein G. Dahl,
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CLINICAL PHARMACOLOGY IN PSYCHIATRY

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NEUROLEPTIC AND ANTIDEPRESSANT RESEARCH

Edited by

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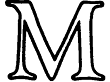

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Preface

The second International Meeting on Clinical Pharmacology in Psychiatry was held on June 20–21, 1980 at the northernmost university in the world: the University of Tromsø. The 24 hours per day of sunlight allowed for long, pleasant and productive sessions. Confining the coverage of this meeting to areas of neuroleptic and antidepressant research allowed greater in-depth coverage. The rapidity of developments in the field of clinical pharmacology in psychiatry is evidenced by the relatively short interval between the first and second international meetings. The common objectives of the experimental pharmacologists, the clinical pharmacologists, and the clinicians who contributed to the meeting and to this volume are improvements in the utilization of neuroleptic and antidepressant drugs and, ultimately, the better management of psychiatric patients.

Rockville, Tromsø and Odense, 1981

E.U.
S.G.D.
L.F.G.
O.L.

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Abbreviations

AAG	α_1-acid glycoprotein	FNM	flunitrazepam
ADTN	2-amino-6, 7-dihydroxy-1,2,3,4-tetrahydroxy-naphthalene	FPZ	fluphenazine
AMI	amitriptyline	GABA	γ-aminobutyric acid
AMP	adenosine monophosphate	GABA-T	GABA transaminase
AMPT	α-methyl-*p*-tyrosine	GAG	γ-acetylenic GABA
AUC	area under the curve	GLC	gas–liquid chromatography
BP	blood pressure	HBE	His bundle electrocardiography
BPRS	Brief Psychiatric Rating Scale	HDRS	Hamilton Depression Rating Scale
cAMP	cyclic AMP	5-HIAA	5-hydroxyindole acetic acid
CI	chlorimipramine	HPLC	high performance liquid chromatography
CNS	central nervous system	HRS	Hamilton Rating Scale
CPRS	Comprehensive Psychiatric Rating Scale	HSA	human serum albumin
CPZ	chlorpromazine	5-HT	serotonin
CPZ-NO	CPZ-*N*-oxide	HVA	homovanillic acid
CPZ-SO	CPZ-*S*-oxide	IMI	imipramine
CSF	cerebrospinal fluid	IU	International Units
DA	dopamine	Li	lithium (salts)
DMCI	demethylchlor-imipramine	LPH	lipotropin = lipoprotein hormone
DMI	desmethylimipramine	LSD	lysergic acid diethylamide
Dopa	dihydroxyphenylalanine	LVET	left ventricular ejection time
DOPAC	3,4-dihydroxyphenyl-acetic acid	MAO	monoamine oxidase
DTγE	destyrosine-γ-endorphin	MAOI	MAO inhibitor(s)
ECD	electron capture detector	MF	mass fragmentography
ECG	electrocardiogram	MHPG	3-methoxy-4-hydroxyphenylene glycol
EDTA	ethylene diamine tetraacetic acid	MOPEG	MHPG
EEG	electroencephalogram	MS	mass spectrometry
Eq	equivalents	NaP	sodium phosphate buffer
FAD	flavin adenine dinucleotide	NE	norepinephrine

NIAMDD	National Institute of Arthritis, Metabolism, and Digestive Disorders
NIMH	National Institute of Mental Health
NPA	*N*-*n*-propylnorapomorphine
NT	nortriptyline
OH-CPZ	hydroxy-CPZ
OH-DA	hydroxy-DA
OH-DMI	hydroxy-DMI
OH-IMI	hydroxy-IMI
OH-NT	hydroxy-NT
PEP	pre-ejection period
P/M	parent drug/metabolite (ratio)
PRL	prolactin
PRP	platelet-rich plasma
PSE	Present State Examination
RBC	erythrocyte
REM	rapid eye movement (sleep)
RIA	radioimmunoassay
RRA	radioreceptor assay
S.A.	specific activity
S.D.	standard deviation
SHAM	slopes, heights, area, first moment (of curves)
STI	systolic time intervals
$t_{1/2}$	half-life
TBEP	tris (2-butoxyethyl) phosphate
TCA	tricyclic antidepressant drug(s)
TD	tardive dyskinesia
TLC	thin layer chromatography
VMA	vanillylmandelic acid
VPD	ventricular premature depolarization

Section One
Recent Developments in Analytical Procedures of Psychoactive Drugs

Evaluation of existing methods for quantitation of neuroleptics in relation to clinical use

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INTRODUCTION

When Curry in 1968 published his first gas chromatographic method for the determination of chlorpromazine (Curry, 1968) many expected therapeutic monitoring of this drug to be just around the corner. Since then 12 years have elapsed, characterized by an enormous advance in analytical possibilities, but without much progress when evaluated from a clinical aspect (Cooper, 1978; May and Van Putten, 1978); some possible reasons for this will be sought. The present paper is divided into two main parts: first, general considerations concerning some practical and pharmacokinetic items; second, a review of six different analytical methods, including both their advantages and problems, evaluated from a clinical point of view.

GENERAL CONSIDERATIONS

Most assays for neuroleptics have been created by chemists lacking a background in psychiatry. As I see it, this problem is of particular significance, realizing that our efforts are to give patients the best possible medical care. This can, in my mind, be fully achieved only if the chemist and the psychiatrist work close together in monitoring therapeutic drugs and related investigations.

Clinical pharmacological investigations often start with estimating basic pharmacokinetic parameters, such as the elimination half-life, the total clearance and the distribution volume of the parent compound. A further step is a determination of the dose interval and a determination of the plasma concentration profile during constant medication in order to get an

idea about the fluctuations of the concentration within the dose interval. This will also give information about the proper time to draw the samples. Furthermore, this will render it possible to estimate the average concentration level from one dose interval to the next, what we usually call 'steady state'. These pharmacokinetic data should, of course, in each case be related to the clinical condition of the patient as evaluated by the psychiatrist. He, in turn, should report back to the chemist and tell him if the method fails to give information about clinically relevant parameters, such as non-compliance or metabolic innovations. The key issue for the chemist is, then, to adapt the method in order to fulfil all the requirements made by the psychiatrist.

This suggested sequence of events is necessary if improved medical treatment is to be achieved by monitoring plasma drug concentrations. Nevertheless, in my experience, the chemist and the psychiatrist communicate too little in these matters, and there results a situation where the analyst does not understand which kind of information the clinician really needs from the analytical method, and the psychiatrist often is unable to interpret fully the data he receives. The missing dialog may be a major reason for the poorly designed – not to say unsophisticated – clinical investigations which have been published, many of them with little relevance to the clinical situation. For example, only a few papers try to define how the investigators interpret and use the term 'steady state'. Thus, the failure to do so adequately may often cause an investigation to be terminated before a stable plasma level has been achieved or it may have resulted in plasma sampling at a wrong time in relation to the last dose administered.

In short, if pharmacokinetic information is not appreciated and understood, plasma concentration data will not be used properly either in clinical practice or clinical investigations.

Before I proceed to a review of the analytical methods, I want to demonstrate the plasma concentration profile of a neuroleptic, perphenazine, as it appeared in one patient. Some of my conclusions and ideas are based on, and will be exemplified with, results from the analytical experience with perphenazine.

This patient started an oral treatment of 12 mg perphenazine three times a day given with equidistant intervals and continued in this fashion for 56 days. No concomitant medication was given.

All plasma samples were drawn at the end of the dose interval and represent minimum concentrations. The fluctuations in plasma concentrations along the time axis are striking and appear in spite of unchanged medication. The final concentration level is unpredictable within the first 5 weeks of treatment. The quantitation of the metabolite, perphenazine sulfoxide, renders it unlikely that the fluctuations of the perphenazine concentration were due to lack of compliance. The concentration profile demonstrated here is not unique and therefore selected for this presentation. On the contrary, it seems to take 4–6 weeks to reach the final steady state level for about a third of the patients undergoing long term oral treatment with this drug.

In the light of this knowledge, I have drawn up some requirements for analytical methods which have to be fulfilled if optimal medical treatment is to be achieved:

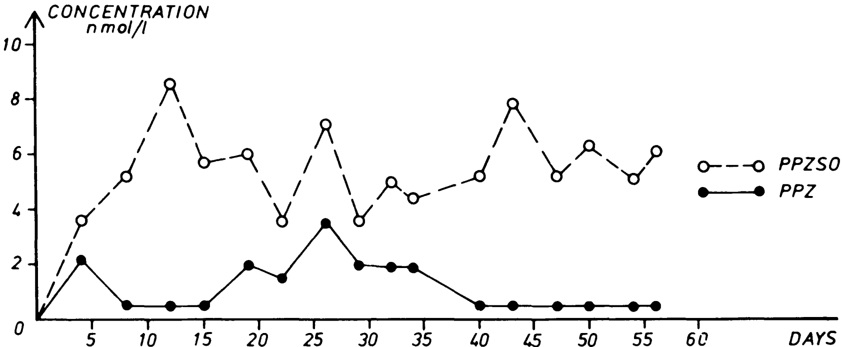

Figure 1 Plasma concentrations of perphenazine (PPZ) and of perphenazine sulfoxide (PPZSO) during a 56 day oral treatment with perphenazine. The drug was given three times a day with equidistant intervals. The daily dose was 36 mg. All samples were drawn immediately before a medication and represent minimum concentrations within the dose intervals.

(1) Specific measurement of the parent compound and metabolite(s) gives information about: (a) establishment of 'steady state', (b) change in metabolism, (c) non-compliance, (d) therapeutic level, (e) side effects level.

(2) Sensitivity down to 0.5 nmol l^{-1}.

(3) Precision must give no contribution to the biological variations.

(4) *In vitro* transformations should be avoided during the specimen sampling.

(1) Providing other neuroleptics behave similarly to perphenazine (and this is likely because of the uniformity in chemical structures), both the parent compound and at least one of the metabolites should be determined specifically by the assay. The selected metabolite(s) may be active or inactive. Monitoring its plasma concentration may reflect a change in metabolism of the parent compound. Thus, alterations in the ratio between metabolite and parent compound may make it possible to determine whether changes in steady levels could be due, for example, to change in the metabolism or to non-compliance.

(2) The sensitivity of the method should be of a magnitude allowing quantitation of the parent compound below the minimum concentration which is known or assumed to cause antipsychotic effect. For perphenazine this means a detection limit around 0.5 nmol l^{-1}; an equivalent value may be assumed for other low dose neuroleptics.

(3) The precision of the method should be such that the variation of the determination is moderate in relation to biological variations, giving no 'noise' at the steady state level. In practice this means a coefficient of variance not exceeding 10 percent in the lowest concentration range.

In general the capacity of the method should satisfy the daily requirement for analytical information. What that is depends on many things, but I want to stress the risk connected with the possibility of running a large number of samples giving much data. No compromise should ever be made between

specificity and capacity, and the justification of a method should not be carried out using capacity as a key argument.

(4) The last point concerns the procedure for drawing the blood samples – usually not given much attention. We have observed *in vitro* sulfoxidation of perphenazine in the presence of erythrocytes when samples were kept in a refrigerator overnight. Consequently, we have introduced the rule that the whole-blood sample must be centrifuged not later than half an hour after the venipuncture. Immediately after centrifugation the plasma shall be pipetted off and kept at −20 °C until analyzed.

REVIEW OF DIFFERENT METHODS

After these more general aspects, I shall turn to a review of six methodological principles which have been applied for the quantitation of neuroleptics within the last decade. A description of the methodological characteristics will be given – as estimated by practical values in clinical situations.

Thin layer chromatography (TLC) needs 3–5 ml of specimen for a single determination giving specific estimation of parent compound and metabolites (Chan and Gershon, 1973; Fredricson Overø, 1980; Zingales, 1969). The sensitivity has been reported to 5 $nmol\ l^{-1}$ using direct fluoroscense scanning of the plate or by scraping off the spots, followed by dansylation or colorimetric assay. The precision was reported to be ±10 percent with a capacity below 10 samples per day. The clinical relevance may be doubtful because of insufficient sensitivity for low-dose neuroleptics.

Gas–liquid chromatography (GLC) has been widely used and requires 1–5 ml of specimen for a single determination to give specific estimations of parent compound and metabolites (Cooper *et al.*, 1979; Curry, 1968; Larsen and Næstoft, 1975). The sensitivity has been reported to be 0.5 $nmol\ l^{-1}$ using electron capture detection. The precision was reported to be ±7 percent, with a capacity equal to 15–20 samples per day. The method fulfills all necessary demands to be classifiable as adequate for the clinical situation.

Mass fragmentography (MF) has been used by only a few groups (Hammer *et al.*, 1968; Hobbs *et al.*, 1974). The necessity for the large amount of specimen (5–10 ml) is due to extensive fragmentation giving decreased sensitivity for the selected fragments. Specificity and sensitivity (0.05 $nmol\ l^{-1}$) are excellent, and the method fulfills all necessary demands for the clinical situation.

High performance liquid chromatography (HPLC) needs 2–3 ml of specimen for a single determination to give specific estimation of parent compound and metabolites (Aaes-Jørgensen, 1980). The sensitivity has been reported to be 0.5 $nmol\ l^{-1}$ using ultra-violet absorption. The precision was reported to be ±6 percent. The methods fulfills all necessary demands for the clinical situation.

Radioimmuno assays (RIA) have been utilized for the determination of many drugs, and several kits are commercially available for specific quanti-

tation of many substances. Drugs which are only slightly metabolized in the body are particularly suitable for these methods. However, one should be very cautious in applying RIA assays to neuroleptics since these assays (Robinson and Risby, 1977; Wiles and Franklin, 1977), with one possible exception (Jørgensen, 1978), measure the sum of levels of parent compound plus metabolites. It is conceivable that this method does not give much relevant clinical information to the psychiatrist. Therapeutic monitoring of plasma concentrations to ensure antipsychotic effects without severe neurological side effects is not possible using this method. The only exception to this among the neuroleptics is flupenthixol (Jørgensen, 1978). In this case, the antibody was produced using 7-carboxyflupenthixol as a hapten, resulting in no co-determination of metabolites. The method estimates, therefore, the parent compound specifically. In spite of this chemical specificity, we have to conclude that this method, as other RIA, gives insufficient information to claim clinical relevance, since it does not give information on metabolic changes or non-compliance.

The radioreceptor assay (RRA) is a recently developed analytical principle which determines specifically the amount of dopamine receptor-active substances in a specimen. From a biological point of view, this principle seems perfect, but in terms of its relationship to the clinical situation, I doubt that it will bring any substantial contribution to the improved monitoring of neuroleptics.

The principle is based on an *in vitro* competition between active substances in the sample and an isotope-labelled neuroleptic for the binding sites in a suspension of dopamine receptors extracted from brain tissue of an animal (Creese and Snyder, 1977; Tune *et al.*, 1980). However, at least two criteria have to be fulfilled before the method can be claimed as an adequate alternative: first, the binding affinity must be similar to human receptors; second, the blocking *in vitro* must be equivalent to that *in vivo*. Furthermore, the estimate of total active substances in a serum sample does not necessarily give information on the ratio between parent compound and active metabolites. In the case of several active metabolites, this *in-vitro* estimate in serum will probably be a very poor indicator of the total amount of active substances at receptors *in vivo* since the ability of the parent compound and the metabolites, respectively, to penetrate from the vascular bed into the brain may be quite different. For neuroleptics which are mainly metabolized to inactive metabolites, results from the RRA will not give information as to whether a small active amount is due to rapid metabolism or to incomplete absorption or to non-compliance. Consequently, the clinical relevance of this method seems doubtful.

CONCLUSION

In conclusion, I will emphasize the necessity for a chromatographic system to precede the quantitation to ensure a separate estimate of parent compound and metabolites. The method must be sensitive enough to measure

the parent compound at the lowest therapeutic level with a precision that is unaffected by analytical 'noise'. Furthermore, I would strongly advise selection of analytical methods with a high degree of chemical specificity.

In my opinion the most suitable assays available for neuroleptics at the present time are based on GLC using electron capture detection and MF. However, liquid chromatographic methods for the determination of neuroleptics may soon be available. This analytical system seems to be technologically more robust and, in the years to come, it will eventually substitute for the GLC systems.

Finally I want to repeat the necessity of a careful design of the method, a design which takes its application to the clinical situation into account. However, this will not contribute to therapeutic advancement with neuroleptics unless the psychiatrist and the chemist expand their dialog both at the pharmacokinetic and at the clinical levels.

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Alternative Approaches

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TREATMENT APPROACHES

Why do we measure plasma concentrations? If we can answer this question clearly, the validity and feasibility of attaining that goal by other means can be evaluated. The practical value of measuring plasma concentrations is to optimize treatment, to attain the best balance in each particular patient between wanted clinical therapeutic effects and unwanted adverse and side effects (Richens and Warrington, 1979).

This ability to increase efficacy can be concurrent, that is, the treatment is monitored and the dosage changed as necessary, or predictive, when the results of a test dose can be used to calculate the appropriate dosage. The latter is generally more useful, especially when severely ill patients such as the suicidally depressed are involved, as it obviates the need for repeated dosage adjustment on a trial-and-error basis.

There are many reasons why the relationship between clinical response and plasma concentrations may be too weak to be clinically applicable – technical problems in measurement, tolerance, irreversible drug action, plasma binding complexities. I shall concentrate on the problem of defining and quantifying clinical response and then on other ways of optimizing dosage.

TYPES OF RESPONSE

Responses can be divided into wanted and unwanted effects and either or both may be evaluated for any relationship to plasma drug concentrations. Wanted effects include the following:

(1) The attainment of adequate concentrations for a minimally effective period of time, for example chemotherapeutic agents in the treatment of microorganismal infections. It is interesting that the first two

examples of monitoring of plasma concentrations were the sulfonamides (Marshall, 1940) and the antimalarials (Shannon, 1946). The response is the death or elimination of foreign microorganisms from the body. It is bacterial rather than human responses which are involved. Indeed, *in vitro* models such as plate sensitivities of bacterial cultures to antibiotics are usually valid and precise. The relationship between control of the infection and plasma concentrations is usually close except when the drug cannot diffuse readily to the infected area, for example in tuberculous meningitis.

(2) A commonly required response is suppression of symptoms. A good example in psychiatry is the use of the benzodiazepines to treat anxiety states symptomatically. The patient is 'titrated' against the dose until adequate symptom control is attained without undue sedation being induced. The less steep the dose–effect curve, the easier the attainment of the optimal dose. Correlations with plasma concentrations should be apparent, but apart from a few exceptions (see, for example, Lin and Friedel, 1979) are not extant in the literature. The problem may partly reflect the subjective nature of both the complaint and variations in the patients' tolerance of the symptom. What is vague uneasiness to one patient may be ineffably unbearable anxiety to another; one patient may insist that medication furnish beatific calm, another on merely having the edge of the symptom blunted.

Another example is the use of antipsychotic medication to suppress symptoms in patients with paranoid schizophrenic states supervening in later life: response is often very marked with undoubted relapse following withdrawal of medication. Unfortunately, no study to my knowledge has addressed itself specifically to examining response–concentration relationships in this context.

(3) Another response system concerns the use of drugs to improve function, for example L-Dopa in Parkinsonism, or perhaps the cerebral vasodilators or acetylcholine precursors in dementia (Yesavage *et al.*, 1979). Within limits, the greater the plasma concentration, the more complete should be the response. Drug effects can only be demonstrated when they are greater than the threshold sensitivity of the measures used to detect clinical response. A 'threshold' plasma concentration of drug will therefore be established.

(4) Many drugs are used to prevent or postpone episodes of illness. The problem of quantitating the response focuses around the time-scale. Thus, a long time is needed to establish the optimal clinical dosage in a patient having one epileptic fit every couple of months. Plasma concentrations provide a convenient predictive method for establishing an effective drug level while avoiding side effects.

Lithium (Li) is also used as a prophylactic, but the therapeutic concentrations are still not clearly established. Thus, some patients are apparently maintained in stable mood despite their serum Li concentrations being less than the generally accepted minimum of 0.8 mmol l^{-1}.

(5) Two major areas of psychotropic drug monitoring are associated with complex treatment responses. First, antidepressant drugs are used to

suppress the symptoms of depressive illness. However, maintenance therapy then typically proceeds at half the earlier dosage (Mindham *et al.*, 1973). This, together with clinical observations, suggests that a simple 'titration' of patient against dosage is not a sufficient explanation of the clinical type of action. Rather, symptom suppression is associated with a partial change in the underlying syndrome, perhaps decreased vulnerability to external events. Whatever the mechanism, the type of response is sufficiently complex that a close correlation with plasma concentrations is somewhat less likely than in the other examples outlined above (Risch *et al.*, 1979).

Even more complex is the use of antipsychotic medication to prevent relapse and to treat acute phases of schizophrenia. In many patients, drugs postpone rather than prevent relapse; social factors such as environmental events and the attitudes of close relations seem crucial (Vaughn and Leff, 1976). On admission of the patient to hospital after relapse, drugs are given to him but one overlooks the fact that hospital admission alters the social circumstances (or it should, if the old concept of 'asylum' is still valid). Thus, drug responses are set against the background of social interactions so complex that useful correlations with plasma concentrations may be unattainable (Lader, 1976).

(6) Plasma concentration monitoring can lessen the risks of toxicity. Anticonvulsants are one good example: Li is another, with the lowest therapeutic index of major drugs in psychopharmacology. Even so, clinical signs must be regarded as of prime importance: some patients show toxic effects despite normal plasma levels. This use of plasma concentrations is an old one. Serum bromide estimations were carried out routinely on admissions to many mental hospitals.

(7) Finally, patient rather than clinical responses can be monitored, that is, the use of plasma and urinary drug concentrations to check adherence to treatment regimens.

CLINICAL TECHNIQUES

Too much emphasis can be placed on laboratory methods for increasing the efficacy of a drug treatment, whether the monitoring techniques be plasma concentrations or other measures. In practical terms, the best monitoring technique is the clinical response of the patient. This can be refined using carefully standardized rating scales, the reliability of which are often compared to those of more 'objective' techniques, including plasma concentration estimations.

However, it is the prediction of therapeutic and unwanted effects which is more important, and here clinical rating techniques cannot be as helpful, especially as clinical response is often delayed in conditions such as depression. Even so, careful evaluation of the clinical features of each patient can be very illuminating. For example, daily ratings of depressed in-patients

treated with antidepressives confirmed the old clinical observation that insomnia is relieved very promptly and weight loss is arrested in patients who subsequently show a substantial antidepressant response in terms of psychomotor performance and mood.

BIOLOGICAL MONITORS

The biological alternatives to plasma level monitoring depend on the empirical establishment of a relationship between the putative monitor and the clinical response. However, these biological monitors all reflect effector systems and are therefore several steps beyond the plasma level estimation. Thus, the effector responses must be a product of the drug concentration at the receptor and not merely that of the total drug concentration in the plasma; it will also reflect receptor density and affinity in that individual and one should not assume that the interpatient variation in these factors is unimportant. Even so, the assumption is that receptor behavior underlying the biological monitor is akin to that underlying the clinical response. As the identity, location and properties of the latter receptors are usually unknown, the relationship must be established by direct correlative studies.

MAO activity

One of the most obvious examples of biological monitors is to assess MAO inhibition in body tissues as a correlate of the clinical action of the MAO inhibitors. In view of the lack of clear indications for these drugs and their hazardous drug and dietary interactions, they are used much less than the tricyclic antidepressants (TCA) and studies of MAO activity are few. An early measure was the estimation of urinary tryptamine, but this was not very useful. Platelet MAO activity has been examined by a few groups of workers, in particular by the Vermont group (Robinson *et al.*, 1978). They showed a significant association between percentage inhibition of platelet MAO at 4 weeks and clinical response. However, dosage and time factors are complex (Tyrer *et al.*, 1980). Problems even attend the choice of substrate for the MAO estimations. In view of the hazards of MAOI treatment and the need for high levels of inhibition, further studies with a view to the setting up of routine monitoring services are warranted.

Amine uptake

With respect to the TCA, biochemical tests have focused on the blockade of amine uptake. Both *in vivo* and *in vitro* tests are available. Tricyclics block the reuptake of amines such as norepinephrine and tyramine and this

property can be used to estimate the degree of blockade. The tyramine dose/pressor response test typically involves finding the amount of intravenous tyramine required to raise the blood pressure (BP) by 30 mm Hg. Tricyclics blocking amine uptake increase the tyramine dose required; antidepressants acting by different mechanisms do not (for example mianserin; Coppen *et al.*, 1978).

In vitro tests for blockade of serotonin (5-HT) uptake have involved mainly platelet studies. Both K_m values (representing the affinity of the uptake carrier) and V_{max} (reflecting its capacity) are altered by some tricyclic drugs. Again, relationship to clinical outcome with treatment has not been systematically evaluated. As platelet 5-HT transport mechanisms may be abnormal in depressed patients, this model might repay close examination.

Neuroendocrine measures

Many psychotropic drugs have neuroendocrine effects, some of which have been evaluated as monitors of drug action (Checkley, 1980). For example, stimulants such as methylphenidate increase hormone levels and these elevations relate closely to the induction of euphoria in normal subjects (Brown, 1977). In the clinical area, the most widely assessed measure has been prolactin. Dopamine-receptor antagonists such as the antipsychotic drugs are associated with marked prompt and sustained rises in plasma prolactin concentrations (see, for example, Nikitopoulou *et al.*, 1977). Nevertheless, evidence has accumulated that prolactin estimations are limited in predicting or monitoring clinical response because the prolactin system is too sensitive to drug action (Gruen *et al.*, 1978); that is, maximal prolactin rises may be attained at drug doses insufficient for clinical response. The mechanism underlying the differences in the dose–effect curves is unknown. In practical terms, the absence of a prolactin response would indicate that a clinical response is unlikely, but the presence of a prolactin response has no predictive value.

Physiological measures

These can be divided into two groups: central and peripheral. The most generally used central measure is the electroencephalogram (EEG), usually analyzed mathematically by filters or into Fourier spectra. An empirical relationship can then be established between changes in EEG patterns and clinical response. The relationship is entirely ‘empirical’ because the physiological basis of the EEG remains too obscure to allow the interpretation of drug effects in any more basic ways. We have reported modest but significant correlations (around 0.5) between EEG measures such as increase in fast-wave activity and clinical ratings in anxious patients treated with benzodiazepines (Bond *et al.*, 1974). Unfortunately, the techniques are too complex for routine use.

Autonomic and somatic peripheral measures have been widely evaluated.

Thus, pulse rate, blood pressure, sweat-gland activity, pupil size, salivary secretion and tremor are all useful indicators of drug action (see, for example, Bye *et al.*, 1979). However, they tend to be more use in evaluating unwanted effects of drugs rather than their therapeutic effects.

CONCLUSIONS

Validity, reliability and convenience are the major factors governing the use of monitoring techniques for drugs. Plasma concentration estimations are appropriate in instances where the drug effect is difficult to measure. Thus, plasma concentrations of antihypertensive agents or of anticoagulants are not measured routinely because the clinical effects are much more easily quantifiable. With psychotropic drugs, the clinical effects are not so easy to measure and, in particular, prediction of response is poor. Resort to estimations of plasma drug estimation thus appears logical but alternative approaches such as those outlined above should not be neglected.

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Radioimmunoassay of tricyclic antidepressants

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INTRODUCTION

The tricyclic antidepressant drugs (TCA) are widely used in the treatment of depression. Since there are marked interindividual differences in the steady state concentrations of a TCA in plasma, the monitoring of the plasma concentrations as a guide to effective dosage has been developed. There are indications that both high and low concentrations of nortriptyline (NT), for example, are associated with poor clinical responses (Åsberg, 1974; Kragh-Sørensen *et al.*, 1976), while the response may increase linearly with the plasma concentrations of imipramine and amitriptyline (AMI) (Gram, 1980).

A rapid and sensitive method for measuring plasma concentrations of TCAs is needed for routine clinical use. Although most academic research centers rely on gas–liquid chromatographic (GLC) or high pressure liquid chromatographic (HPLC) methods, these techniques may not be practical in smaller laboratories with less skilled personnel. The technique of radioimmunoassay (RIA) has been adapted to the measurement of plasma TCA levels and may be more easily applied in routine clinical chemistry laboratories.

With specific funding from the State of Michigan Department of Mental Health we have studied the RIA procedure developed by Brunswick *et al.* (1978). This method has performed very satisfactorily in our own laboratory and could be used by any general psychiatric hospital laboratory if the reagents are made available from a central source in the form of RIA kits.

METHOD

The three basic steps in the assay procedure are as follows: first, extraction of the drug from plasma; second, separation of the secondary and tertiary

amines; third, quantitation by RIA. Plasma samples are extracted into isoamyl alcohol–heptane containing sodium hydroxide. The organic extract is reacted with succinic anhydride to derivatize the secondary amines (for example desmethylimipramine, DMI). Sodium hydroxide is then added and the derivatized secondary amines enter the basic aqueous phase, while the tertiary amines (for example imipramine, IMI) remain in the organic phase. The tertiary amines are then extracted back into an acid aqueous phase with hydrochloric acid. This isolation of the secondary and tertiary amines by successive solvent extraction steps permits the separate quantitation of, for example, IMI and DMI in the plasma of patients being treated with IMI. The same extraction steps are used to separate NT and AMI when AMI is the drug being taken by the patient.

The mean *recovery* of IMI and DMI through the extraction steps in our laboratory is 96 and 97 percent, respectively.

Antibody

A single antibody is used for the measurement of all TCA drugs. The antibody is raised in rabbits against NT, following its conjugation to protein. The antibody cross-reacts with all TCA drugs (table 1), and this fact is used to advantage in clinical work since in virtually all cases the drug being taken by the patient is known. As shown in table 1, the cross-reactivity of the antibody ranges from 40 percent for doxepin to 200 percent for AMI. The antibody does not, however, react with the hydroxylated metabolites of IMI.

Table 1 Cross-reactivity of anti-NT antibody with TCA compounds and other drugs

Drug	Cross-reactivity with anti-NT (%)
Imipramine	100
Desipramine	60
Amitriptyline	200
Nortriptyline	100
Doxepin	40
2-OH-IMI	8
2-OH-DMI	2
10-OH-IMI	1
10-OH-DMI	1
Diazepam	0
Haloperidol	0
Thioridazine	1
Chlorpromazine	10
Perphenazine	9
Trifluoperazine	2

Data from Dr D. Brunswick.

With the exception of the structurally similar phenothiazines (chlorpromazine, perphenazine) other common psychotropic drugs (benzodiazepines, butyrophenones) do not cross-react with the antibody.

RIA quantitation

Aliquots of the aqueous extracts containing secondary or tertiary amines are reacted with dilute anti-NT antibody for1 h at 37 °C, in the presence of ^{3}H-IMI. Following this equilibration step the bound and free radioactivity are separated by the addition of dextran-coated charcoal. The ^{3}H-IMI not bound to the antibody is absorbed by the charcoal which is then precipitated by centrifugation. An aliquot of the supernatant is then counted by liquid scintillation to determine the bound radioactivity. Appropriate standards of the drug being measured are processed in the same way as the plasma samples. For quality control purposes we also assay prepared plasma pools containing known amounts of IMI and DMI at low, medium and high concentrations.

PERFORMANCE

This RIA method is particularly attractive for its simplicity, its rapidity and its capacity. One technician using prepared reagents can process 15–20 samples in duplicate, together with standards and quality control plasma pools, in one working day.

The reliability and precision of the assay are very acceptable, as determined by the inter-assay variability of quality control plasma pools (table 2). For both IMI and DMI the coefficients of variation calculated from 18 assays were $<$10 percent across the entire range of TCA concentrations studied (80–300 ng ml^{-1}).

Table 2 Inter-assay variability of tricyclic RIA (18 assays)

Quality control plasma pool	Concentration (ng ml^{-1})	Coefficient of variation (%)
Imipramine	80	5.3
	150	6.1
	300	7.3
Desipramine	80	9.9
	150	7.5
	300	6.1

The validity of the assay has been studied by comparing the results obtained in our laboratory with those found for the same samples by a GLC method. The GLC assays were performed by Mr Thomas Cooper at

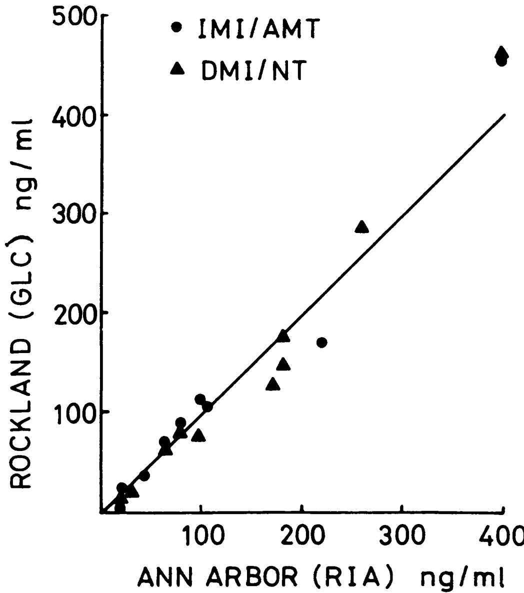

Figure 1 Comparison of TCA results obtained by RIA in the authors' laboratory and by GLC at Rockland Research Institute in Mr Thomas Cooper's laboratory.

Rockland Research Institute, New York. Figure 1 shows that the results found in the two laboratories were in good agreement ($r = 0.95$) and that the absolute values reported by RIA were equivalent to those found by GLC.

In 45 patients treated with IMI who had reached steady state conditions the ratio of IMI to DMI in plasma was examined. The distribution of IMI/DMI ratios is shown in figure 2. A skewed unimodal distribution is seen, with a median ratio of 0.90. These results are generally similar to those reported by Gram *et al.* (1977).

α_1-ACID GLYCOPROTEIN CONCENTRATIONS

Following the initial reports of Kragh-Sørensen (1980) and Gram (1980) that α_1-acidic glycoprotein elevations associated with infection could lead to very high plasma TCA concentrations, we have begun to examine this variable in our own patients. The glycoprotein was determined by radial immunodiffusion (Calbiochem). Our preliminary results indicate that a statistically significant increase of the glycoprotein concentration occurs in patients being treated with IMI (figure 3). The physiologic and pharmacokinetic importance of this finding is not yet clear. The pretreatment glycoprotein concentrations of the same patients were slightly above control values obtained in our laboratory. Further studies are in progress with fixed

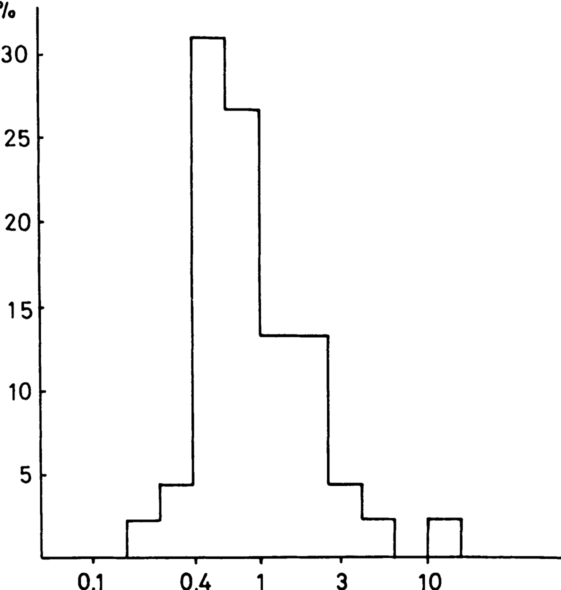

Figure 2 Steady state ratio of IMI to DMI in plasma of 45 patients treated with imipramine. Median = 0.90.

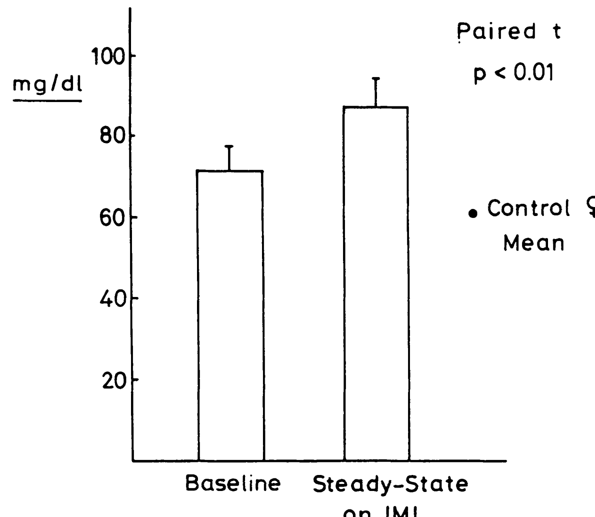

Figure 3 α-Acid glycoprotein concentrations in plasma of five depressed women before treatment (baseline) and at steady state during treatment with IMI. Bars indicate mean values + standard deviation.

doses of IMI to determine the correlation of glycoprotein concentrations with steady state TCA concentrations.

CONCLUSIONS

The RIA method described by Brunswick *et al.* (1978) for measuring plasma TCA concentrations has been examined with a view to its use in general clinical chemistry laboratories. In our own hands it gives excellent recovery and separation of tertiary and secondary amine TCA compounds by solvent extraction. The reliability and precision of the assay are very good across a wide range of TCA concentrations. The values found by RIA agreed closely with those obtained by GLC. The ratios of IMI to DMI observed in 45 patients were comparable to those reported by others. A preliminary study of α-acid glycoprotein concentrations revealed a significant increase of this protein in patients during treatment with IMI. The importance of this finding for TCA pharmacokinetics needs to be studied further.

Overall, the RIA method has many advantages for the quantitation of plasma TCA concentrations by small hospital laboratories.

ACKNOWLEDGEMENTS

Dr David Brunswick acted generously as consultant to our laboratory for setting up the RIA and kindly provided an initial supply of antibody. Mr Thomas Cooper collaborated enthusiastically in the measurement of TCA concentrations by GLC. Nancy Barber, RN, skillfully collected the TCA blood samples. Walelign Wagaw and Madeleine Cylkowski assisted with the TCA and glycoprotein assays. This work was supported by funds from the State of Michigan Department of Mental Health.

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Biological assay systems for tricyclic antidepressants

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During long term drug treatment, dosage adjustment can, in principle, be based on three different kinds of data:

(1) Therapeutic effect or side effects. This is still the common method for neuroleptics, benzodiazepines and – partly – antidepressants.
(2) Plasma or tissue concentration of the active drug, and possibly active metabolites as well. This is the common method for lithium, and partly for antidepressants.
(3) Assessment of some pharmacodynamic effects. There are two possibilities:

(a) Direct assessment of the therapeutic effect (such as lowering of blood pressure in hypertension).
(b) Assessment of some other biological effect of the drug; this effect may or may not be therapeutically relevant. If it is relevant, so much the better; if it is not (or probably not), the assay should be considered a way to obtain an indirect measure of the plasma (or tissue) concentration of the active drug and active metabolites.

Biological assay systems *may*, under certain circumstances, offer the following advantages in relation to drug level assessment:

(1) The assay may be more sensitive and reliable.
(2) Since only the effect of the free, non-protein-bound fraction is measured, the problem with individual variations in protein binding is avoided.
(3) The effects of all active metabolites are measured together. However, this is an advantage only if the measured effect is therapeutically relevant, or at least is correlated with the therapeutic effect for each of the active metabolites. It is disturbing to have the assessment influenced by a therapeutically non-relevant effect from otherwise inactive metabolites.

Only empirical data can show whether some of these possible advantages are actually present for a given method, for example by demonstrating that

the results obtained with such a method correlate better with clinical improvement than does the measurement of plasma drug concentration.

There are three main methods that have been used to measure the biological activity of tricyclic antidepressants (TCA) *in vivo*: (1) the 'tyramine test'; (2) assessment of the inhibitory effect of the patient's plasma on norepinephrine (NE) uptake in a suitable tissue (such as rat iris or brain slices); (3) assessment of the inhibitory effect of the patient's plasma on serotonin (5-HT) uptake in brain slices or blood platelets.

The tyramine test is, in principle, an assessment of the i.v. dose of tyramine required to give a defined increase in blood pressure (for example 30 mm in systolic blood pressure). Since TCA with an inhibitory effect on the 'NE pump' inhibit the neuronal uptake, and thereby the adrenergic effect, of tyramine, higher doses of tyramine will be required to give a defined increase in blood pressure in patients treated with such drugs. This assay has been shown to correlate well with, for instance, plasma levels of nortriptyline (NT) (Freyschuss *et al.*, 1970), but the test is rather cumbersome, and has not been demonstrated to offer advantages in relation to drug level measurement.

Plasma from patients treated with such drugs as NT or clomipramine has an inhibitory effect on the uptake of NE and/or 5-HT in brain slices (Hamberger and Tuck, 1973) and on NE uptake in rat iris (Tuck *et al.*, 1972). As would be expected, NT has the stronger effect on NE uptake, and clomipramine on 5-HT uptake. Results obtained with these methods also correlate well with plasma drug level. However, the measurements are time-consuming and expensive, and seem to be of more theoretical than practical value.

It is easier and more convenient to measure the inhibition of uptake in platelets, but this can be done only for 5-HT, not for NE or dopamine.

In patients on TCA, 5-HT uptake inhibition *in vivo* gives reduced platelet 5-HT, and this reduction can be used as an indirect measure of uptake inhibition. However, it is probably not a very reliable measure compared to a direct measurement of uptake rate *in vitro*, and there will be an appreciable 'time lag' from maximal uptake inhibition to maximal 5-HT depletion.

The measurement of platelet 5-HT uptake rate is most conveniently performed with the patient's own platelets, but this has the drawback that one has to use fresh blood samples – platelets are not viable for more than a few hours after blood samples are drawn. Before presenting some results obtained with this method, however, I want to mention the possibility of sending *platelet-free* plasma from the patient to the laboratory, in which fresh platelets from an untreated person are incubated in the patient's plasma.

Now I would like to present some of my own results with assessment of platelet 5-HT uptake inhibition in patients treated with clomipramine. The method is described in detail in a recent paper (Lingjærde, 1979), in which the results from 14 clomipramine-treated and 20 amitriptyline-treated patients were presented. The clomipramine-treated group has recently been increased to comprise a total of 33 patients, with ages ranging from 24 to 76 years. As for the method, suffice it to say here that I measure the initial

uptake rate at four different concentrations of ^{14}C-5-HT in platelet-rich plasma diluted with an equal amount of buffer, using EDTA as anticoagulant. A complete kinetic analysis is performed on each set of data, with calculation of the kinetic parameters K_m and V_{max}. In the results to be presented here, inhibition of uptake is expressed as increase in the kinetic parameter K_m or, in other words, decrease in affinity of the uptake receptor for 5-HT (which is the effect of a competitive uptake inhibitor). Although it is somewhat time-consuming to measure K_m (and V_{max}) rather than the uptake at one single concentration of 5-HT, it offers some important advantages: for instance that the increase in K_m is linearly related to inhibitor concentration (see Lingjærde, 1979).

I have not yet had the opportunity to measure plasma concentration of clomipramine and metabolites in my patient group, nor to assess the clinical improvement in such a way that it would be meaningful to correlate it to uptake inhibition. Therefore, I will discuss here only how the uptake inhibition is related to such factors as daily dose, age, duration of treatment and concomitant use of other drugs.

Figure 1 shows the 5-HT uptake inhibition in relation to daily dose in 27 depressed patients on clomipramine; none of them were treated with other drugs interfering with uptake (see below). To the lower left is shown the

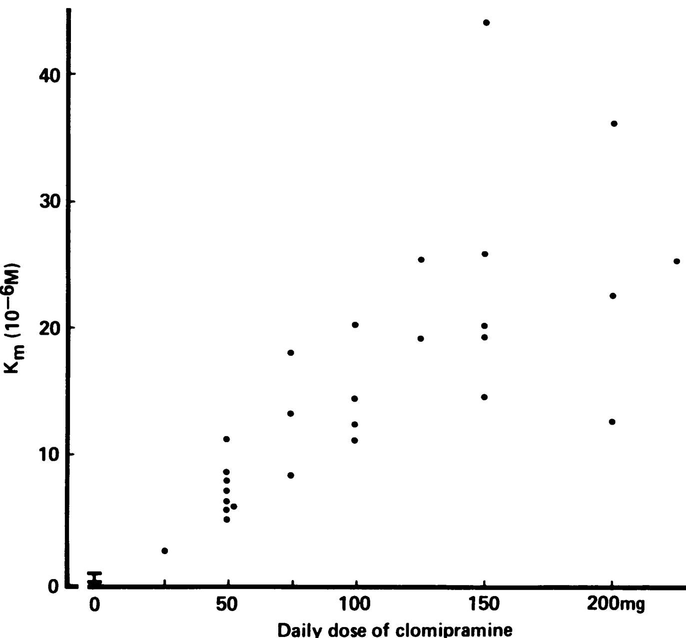

Figure 1 Inhibition of platelet 5-HT uptake in 27 depressed patients treated with clomipramine, expressed as increase in apparent K_m, in relation to daily dose. The range of K_m in a control group of 21 unmedicated depressed patients is shown at daily dose '0'. (For details of methods, see Lingjærde, 1979.)

range of K_m values in 21 untreated depressed patients. (Since I have found no abnormalities of K_m in untreated depressed patients of any kind, I find it unnecessary to give the exact diagnoses of my patients – most of them were suffering from unipolar endogenous depression.)

The results shown in figure 1 are in good accordance with a linear relationship between daily dose and uptake inhibition. Given this linear relationship, the inhibition can also be expressed relative to dose, as an 'inhibition index' which is simply the increase in K_m divided by daily dose. The ratio between the highest and the lowest inhibition index in this patient group is 4.8, which is remarkably small compared to the common ratio of about 10 to 1 in steady state plasma level of TCA after a given dose. Thus, the individual variation in 5-HT uptake inhibition, measured as increase in K_m, seems to be less than the individual variation in plasma level of such drugs.

In this patient group there is no significant difference in inhibition index between men and women, nor between smokers and non-smokers. Figure 2 shows that there is a slight trend toward an increased inhibition index with age, but it is not significant. Figure 3 shows the inhibition index in relation to duration of treatment; included here are some patients investigated after varying periods of treatment. It is seen that the 5-HT uptake inhibition, in relation to dosage, tends to remain at the same level even after years of continuous treatment.

What about interaction with other drugs? Figure 4 shows the inhibition index in various groups of patients according to concomitant treatment with

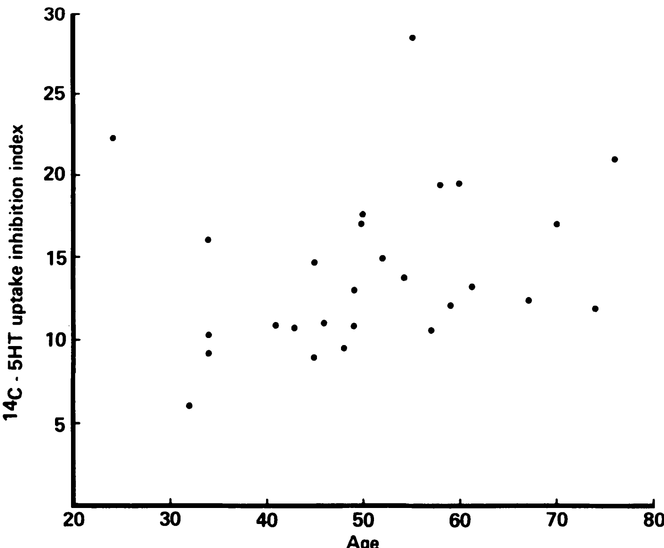

Figure 2 Inhibition of platelet 5-HT uptake in the same patients as shown in figure 1, in relation to age. Inhibition is expressed as the 'inhibition index', that is the ratio between increase in K_m and daily dose of clomipramine.

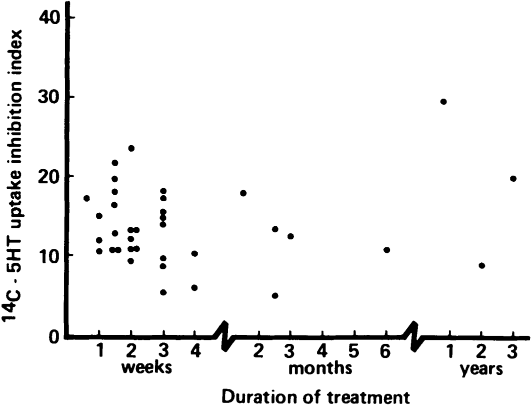

Figure 3 Inhibition of platelet 5-HT uptake in patients on clomipramine, expressed as the 'inhibition index', in relation to duration of treatment. Some of the patients (N=27) have been assayed more than once.

other drugs. Additional use of benzodiazepines or neuroleptics (or lithium; not shown on this figure) does not seem to interfere with the effect of clomipramine (these patients, therefore, are included in the material presented in figures 1, 2 and 3). Some patients on diuretics, on the other hand, have an unexpectedly high inhibition index; the highest index, which is more than twice as high as in any other patient without diuretics, was found in a woman of 58 years who was treated with hydrochlorothiazide, propranolol and 1-Dopa in addition to clomipramine. At present, I cannot say what is the mechanism behind the increased effect of clomipramine in patients who are

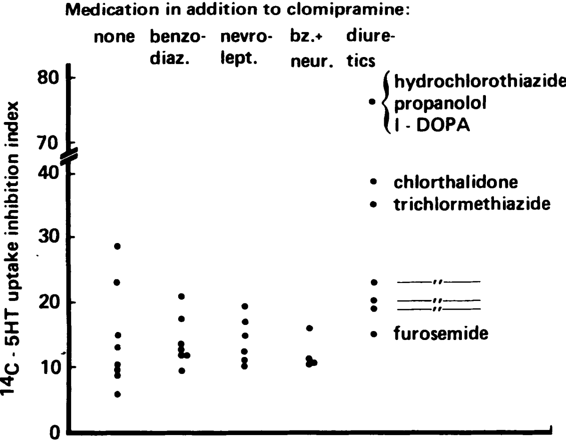

Figure 4 Inhibition of platelet 5-HT uptake in patients on clomipramine, expressed as the 'inhibition index', in groups according to concomitant use of other drugs. ('bz.+neur.'=benzodiazepines and neuroleptics.)

treated with diuretics. In addition to the diuretic as such, the underlying somatic disease, be it hypertension or heart failure, may have influenced the result. However that may be, one should probably take into account that such patients may be abnormally sensitive to a drug like clomipramine.

Taken as a whole, I believe that my results demonstrate that the measurement of platelet 5-HT uptake inhibition may be a useful tool in assessment of the effects of some TCA *in vivo*, although it remains to be seen whether it offers special advantages in relation to the measurement of plasma drug level.

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Section Two
Receptor Binding

Review on neuroleptic receptors: specificity and multiplicity of *in vitro* binding related to pharmacological activity

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INTRODUCTION

The *in vitro* biochemical investigation of the binding of pharmacological agents and endogenous substances such as neurotransmitters to specific binding sites on membranes was made possible by the availability of highly radioactively labeled compounds (> 10 Ci $mmol^{-1}$), allowing experimentation at nanomolar concentrations.

The empirical finding that labeled membranes were retained by adsorption on glass fiber filters after a vacuum filtration procedure provided a simple and fast technique for separating membrane-bound and free radioactivity from incubation mixtures of the membranes with the labeled ligands (Pert and Snyder, 1973).

Several criteria have been imposed to assess the binding specificity of the labeled sites to permit relating them with a pharmacologically defined 'receptor': (1) saturability of the sites at low concentrations (nanomolar order) of the labeled ligand; (2) selective inhibition of the binding by pharmacologically active unlabeled substances and, where possible, stereospecific inhibition by the pharmacologically active form of a pair of enantiomers (Goldstein *et al.*, 1971); (3) regional distribution of the density of the sites in the brain or periphery in accordance with the presumed function or distribution of neurotransmitter density; (4) correlation between binding affinities of compounds for the sites and pharmacological, physiological or clinical potencies.

In this way, several *in vitro* binding models for neurotransmitters and drug receptors have been described.

Once a model has been defined, the multiplicity or selectivity of an

envisaged receptor can be studied using various experimental approaches. Several labeled ligands, both antagonists and agonists, have to be investigated and the binding characteristics in various tissue preparations compared. Multiple sites can be classified according to various criteria:

(1) Pharmacology; differential binding affinities of pharmacological agents.
(2) Binding kinetics; deviations from normal mass-action interactions on a single site; curved Scatchard plots, biphasic dissociation curves, irregular inhibition patterns such as shallow or biphasic curves and slopes different from unity in Hill plots.
(3) Localization on different cell types; differential modification by various types of cell or neuronal lesions in the animal.
(4) Different regulation in binding *in vitro*; different sensitivity to ions or cofactors such as nucleotides; different ontogenetic development of various binding sites; differential variations after acute or chronic drug treatment of animals; differential occurrence in diseased states.
(5) Different chemical stability or composition of the macromolecules which constitute the binding sites.

In the last 5 years the various aspects of dopaminergic receptors have been extensively studied. However, many items remain unclear or controversial and the puzzle of the multiplicity of dopamine (DA) receptors is far from being solved.

The present review will focus primarily on the major reports concerned with the pharmacology of the specific binding sites for neuroleptics (as DA antagonists) in comparison with the binding properties of sites labeled by DA agonists in various tissues from different animal species. Kinetic binding properties will only be mentioned to illustrate the advantages or disadvantages of using certain labeled ligands. For a list of 29 clinically used neuroleptics, literature data on binding affinities measured in various dopaminergic receptor binding models are summarized in a table, which is supplemented with original data from our own research. In addition, a receptor-binding profile of these drugs comprising original K_i values measured in six different neurotransmitter receptor-binding models *in vitro* is reported.

METHODOLOGY IN *IN VITRO* RECEPTOR BINDING

Tissue preparation

Brain tissue, fresh or frozen, is used primarily. It is homogenized in a hypotonic medium for preparation of a total membrane fraction, or in an isotonic medium for separation of subcellular fractions. Membranes are washed by successive centrifugations and occasionally preincubated to remove endogenous ligands. Washed membranes are suspended in an

incubation buffer of optimum pH (7.2–7.7) and ionic composition in an appropriate dilution usually between 20 and 2 mg original wet weight of tissue per milliliter.

Binding assay

Membrane suspensions (0.5–2 ml) are incubated at an appropriate temperature (between 0 and 37 °C) in the presence of low concentrations (nanomolar order) of a ^{3}H-labeled ligand, in the absence or presence of unlabeled drugs, for a sufficient period of time (10–30 min) to reach binding equilibrium. To separate membrane-bound and free radioactivity, the suspensions are filtered rapidly through glass fiber filters and rinsed. The radioactivity on the filter, counted in a liquid scintillation spectrometer, is a measure of the binding of the ligand to membranes: total binding in the absence of unlabeled drugs together with residual non-specific binding (blank value) in the presence of excess (micromolar order) of an unlabeled competitor for the specific receptor sites.

Relative binding affinities of drugs

Unlabeled drugs are added in various concentrations to incubation mixtures with the labeled ligand; active compounds compete with the labeled ligand for the receptor site and reduce the labeling of the membranes. After subtracting the blank value from all assays, the drug concentration (IC_{50} value) inhibiting 50 percent of the specific binding of the labeled ligand is assessed by log-probit analysis.

Apparent equilibrium inhibition constants are calculated according to the relationship

$$K_i = \frac{IC_{50}}{1 + C/K_D}$$

(Cheng and Prusoff, 1973), where C is the concentration and K_D is the equilibrium dissociation constant of the labeled ligand – the latter being derived from linear transformations of the law of mass action, such as Scatchard plots (see Segel, 1975).

^{3}H-DOPAMINE ANTAGONIST BINDING IN STRIATAL TISSUE

^{3}H-Haloperidol

Dopaminergic receptors were first labeled and characterized in *in vitro* binding studies using ^{3}H-haloperidol and calf striatal tissue by Snyder and co-workers (Snyder *et al.*, 1975; Creese *et al.*, 1975; Burt *et al.*, 1976; Creese *et al.*, 1976). The binding was measured at 2 nM ^{3}H-haloperidol and

Table 1 Equilibrium inhibition constants (K_i values, nM) of neuroleptics and dopamine agonists in various binding models for dopaminergic receptors

	Calf caudate			Rat striatum			Human caudate
	Haloperidol (Burt *et al.*, 1976) (A)	Spiperone (Creese *et al.*, 1979*b*) (B)	Dopamine (Burt *et al.*, 1976) (C)	Haloperidol (original) (D)	Spiperone (Leysen *et al.*, 1978*a*) (E)	Apomorphine (original) (F)	Spiperone (Creese *et al.*, 1979*b*) (G)
Spiperone	0.25		1400	0.16	0.07	0.25	
Benperidol	0.33		4100	0.35	0.26	0.18	
Droperidol	1.0		880	0.8	0.46	0.4	
Pimozide	0.81		5300	1.2	0.92	1.4	
Haloperidol	1.4	5.8	920	1.2	1.18	1.3	2.8
Piflutixol	0.9		67	1.4	—	3.5	
Thioperidone	—		—	1.8	—	3.5	
Thiothixene	1.5		540	2.5	—	2.2	
Prochlorperazine	—		—	3.1	—	4.0	
Milenperone	—		—	3.9	—	1.3	
Propericiazine	—		—	3.9	0.73	0.8	
Trifluperazine	2.1		740	3.9	—	2.2	
Methithepine	1.4		210	3.9	0.58	3	
Perphenazine	—		—	3.9	—	3.6	
Fluphenazine	0.88	2.8	230	6.2	2.25	2.2	1.7
Levomepromazine	—		—	7.9	—	7.9	
Penfluridol	5.6		1600	9.9	—	8.9	
Chlorprothixene	4.4		250	11	3.26	5.6	
Loxapine	—		—	11	—	3.8	
Thioridazine	15	30	1800	15.7	13	8.9	19
Clothiapine	—		—	15.7	4.6	5.6	
Sulpiride	—	3400	—	31.3	50.5	44.7	330
Molindone	68	690	19000	25.3	—	24	

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Alimemazine	—		—	31.3	—	11.2		
Chlorpromazine	10.2	13	900	49.6	8.17	4.5	5	
Promazine	72	160	7100	99	—	38	160	
Pipamperone	31		4900	124	73	16		
Clozapine	120	210	2050	156	73	56	170	
Perlapine	—		—	462	—	125		
Correlation between the tests:	B & A	C & A	D & A	E & D	F & D	G & B	G & E	
Correlation coefficient	$r=0.893$	$r=0.396$	$r=0.917$	$r=0.955$	$r=0.892$	$r=1.000$	$r=0.886$	
(Spearman rank)	$n=7$	$n=18$	$n=18$	$n=15$	$n=29$	$n=7$	$n=6$	
Student's *t*-test	$p=<0.01$	$p=>0.05$	$p=<0.01$	$p=<0.01$	$p=<0.01$	$p=<0.01$	$p=<0.05$	

stereospecific binding was assessed by adding 0.1 μM (+)-butaclamol, a pharmacologically active enantiomer with neuroleptic activity. The pharmacologically inactive (−)-enantiomer did not inhibit the binding. The highest density of stereospecific ^{3}H-haloperidol sites in calf brain was found in caudal and rostral caudate, where the density was about two times higher than in the globus pallidus, the anterior putamen and the limbic areas. A small number of sites was present in the amygdala and midbrain, whereas in other brain areas specific binding was not detectable. K_i values of neuroleptics measured by Burt *et al.* (1976) are shown in table 1. Highly significant correlations were observed between the relative binding affinities of compounds (IC_{50} values or K_i values) and potencies in *in vivo* pharmacological tests for assessing antidopaminergic activity (such as antagonism of apomorphine- or amphetamine-induced stereotypy in the rat, and antagonism of apomorphine-induced emesis in the dog). Binding affinities also correlated significantly with average clinical dosages of drugs. Such significant correlations were not found between potencies of drugs for inhibiting DA-sensitive adenylate cyclase and the pharmacological or clinical potencies. DA-sensitive adenylate cyclase occurring in dopaminergic brain areas was previously proposed as a potential model of a dopaminergic receptor (Kebabian *et al.*, 1972). In calf striatal membranes, stereospecific ^{3}H-haloperidol binding represented about 30 percent of the total binding; it was saturable at nanomolar concentrations and straight Scatchard plots indicated involvement of a single high affinity binding site with an equilibrium dissociation constant K_D=3.3 nM at 37 °C and a receptor density of 17 pmol per gram wet weight tissue. Rates of association and dissociation at 37 °C were very fast (k_1=0.3 nM^{-1} min^{-1}, k_{-1}=1 min^{-1}). It was observed that inhibition curves of neuroleptics were steeper (slope in Hill plots about unity) than those of DA agonists (slope of Hill plots less than unity) (Burt *et al.*, 1976; Titeler *et al.*, 1978). Hence the interaction between antagonists and agonists appeared to be irregular, and not to follow single-site mass-action kinetics.

In addition to studies in calf brain, ^{3}H-haloperidol binding was investigated in rat (Seeman *et al.*, 1975; Leysen and Laduron, 1977*a*; Leysen *et al.*, 1977) and human brain (Seeman *et al.*, 1976). Similar stereospecific inhibition of ^{3}H-haloperidol (2 nM) binding by (+)-butaclamol (1 μM) was found; the sites were similarly distributed in human (Seeman *et al.*, 1976) and rat (Leysen and Laduron, 1977*a*) and calf brain (*vide supra*) with the highest enrichment in the caudate or striatum, followed by the limbic areas. In rats, stereospecific ^{3}H-haloperidol binding was also detected in the hypophysis (Brown *et al.*, 1976; Leysen and Laduron, 1977*a*). In rat striatum, binding affinities of drugs for the stereospecific haloperidol-labeled sites were of the same order of magnitude as in the calf striatum (see table 1) and correlated significantly with average clinical doses of neuroleptics (Seeman *et al.*, 1975) and with drug potencies to antagonize apomorphine-induced emesis in the dog (Leysen *et al.*, 1977). In rat striatum, the subcellular distribution of stereospecific ^{3}H-haloperidol binding sites, enriched in the microsomal fraction, was different from that of DA-sensitive adenylate cyclase, enriched in the mitochondrial fraction (Leysen and Laduron, 1977*a*). This indicated that both presumed DA receptor models are different macromolecular

entities occurring in different cell fractions. Moreover, there was no correlation between inhibition constants of drugs in both tests such as shown in figure 1. It was demonstrated that stereospecific ^{3}H-haloperidol binding

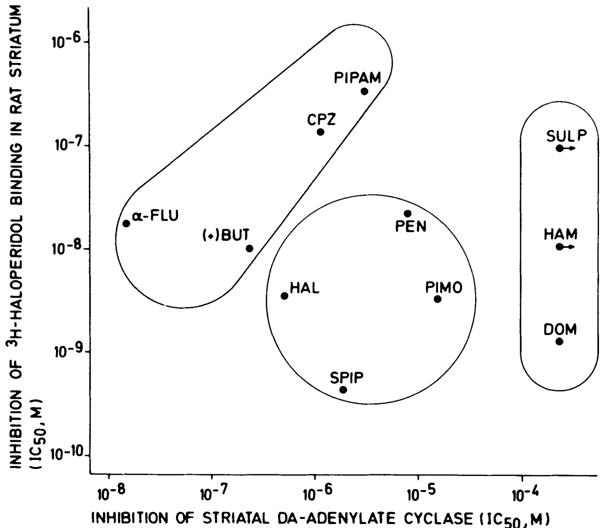

Figure 1 Affinities of drugs: α-flupenthixol (α-FLU), (+)-butaclamol ((+)-BUT), chlorpromazine (CPZ), pipamperone (PIPAM), haloperidol (HAL), spiperone (SPIP), penfluridol (PEN), pimozide (PIMO), domperidone (DOM), halopemide (HAM) and sulpiride (SULP) for binding to stereospecific sites labeled by haloperidol (Leysen *et al.*, 1977) and for inhibiting dopamine-sensitive adenylate cyclase (Leysen and Laduron, 1977*a*) in rat striatal tissue *in vitro.*

sites were completely distinct from opiate receptors, stereospecifically labeled with ^{3}H-fentanyl or ^{3}H-naloxone, in rat striatum. The regional distribution in the brain was different (Leysen and Laduron, 1977*a*) and compounds were recognized in the *in vitro* receptor binding models respectively, strictly according to their pharmacological properties (Leysen *et al.*, 1977). Table 2 shows examples of three structurally very similar benzimidazolone derivatives which are differentially recognized by the neuroleptic and opiate binding sites in striatal membranes. The diphenylbutyl derivative, that is the neuroleptic pimozide, binds with high affinity only to the ^{3}H-haloperidol-labeled sites, whereas the diphenylpropyl derivatives, which are potent morphinomimetics lacking neuroleptic activity *in vivo*, bind to the opiate receptor only.

^{3}H-Dihydroergocryptine

The ergot alkaloid ^{3}H-dihydroergocryptine was also used to label stereospecific binding sites in calf caudate. When excess phentolamine was added in all assays to prevent binding of the labeled ligand to α-adrenergic

Table 2 Differential binding of chemically related compounds with different pharmacological activities

X (in X—N spiro-piperidinyl benzimidazolone: H, O, N, NH)	IC_{50} (M)					Pharmacological activity
	Rat striatum (particulate cell fraction)				Rat fore-brain (cyto-plasmic cell fraction)	
	Tris-HCl+salts* ^3H-Haloperidol	Tris-HCl+salts* ^3H-Naloxone	Tris-HCl+Na** ^3H-Naloxone	Tris-HCl ^3H-Naloxone	Tris-HCl ^3H-Fentanyl	
$F-C_6H_4-CH(C_6H_4F)-(CH_2)_3-$	6.62×10^{-9}	10^{-5}	10^{-5}	8.5×10^{-7}	5.43×10^{-7}	Neuroleptic
$C_6H_5-CH(C_6H_5)-(CH_2)_2-$	1.90×10^{-6}	7.12×10^{-8}	7.5×10^{-8}	1.2×10^{-8}	3.98×10^{-9}	Morphino-mimetic
$C_6H_5-C(C_6H_5)(C(=O)C_2H_5)-(CH_2)_2-$	10^{-6}	8×10^{-10}	4.5×10^{-10}	3.8×10^{-10}	3.0×10^{-10}	Morphino-mimetic

Buffer: Tris-HCl 50 mM, pH 7.6 with added salts: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$* or 100 mM NaCl.**

receptors, the remaining sites could be stereospecifically inhibited by (+)-butaclamol. These sites showed similar binding properties as the ^{3}H-haloperidol-labeled sites in calf caudate (Titeler *et al.*, 1977).

^{3}H-Spiperone

In 1977 ^{3}H-spiperone (also called ^{3}H-spiroperidol) was introduced to label brain dopaminergic receptors (Laduron and Leysen, 1977; Fields *et al.*, 1977; Creese *et al.*, 1977) and because of its advantageous *in vitro* binding properties (Leysen *et al.*, 1978*a*) and usefulness for studying *in vivo* receptor binding (Laduron and Leysen, 1977; Laduron *et al.*, 1978), it was proposed as the ligand of choice for neuroleptic receptors.

As is the case for ^{3}H-haloperidol, ^{3}H-spiperone binding was inhibited stereospecifically by (+)-butaclamol. In human (Fields *et al.*, 1977) and rat brain (Fields *et al.*, 1977; Laduron *et al.*, 1978), the density of stereospecific ^{3}H-spiperone binding sites was highest in the striatum, followed by the limbic areas (as observed for the ^{3}H-haloperidol binding distribution). However, the high density in the frontal cortex and other cortical areas was different, as was the detection of significant amounts of binding in several subcortical brain areas.

In rat striatum, the pharmacological specificity of stereospecific ^{3}H-spiperone and ^{3}H-haloperidol binding sites appeared to be identical. Binding affinities of DA antagonists and agonists were of similar order of magnitude in both tests and correlated highly significantly (Leysen *et al.*, 1978*a*). A highly significant correlation was also found between the relative binding affinities of neuroleptics for the striatal stereospecific ^{3}H-spiperone sites and the potencies of the drugs to antagonize apomorphine-induced stereotypy in rats (figure 2). In contrast, no significant correlation existed between the binding affinities and drug potencies to antagonize tryptamine-induced clonic seizures in rats (the latter being a measurement for antiserotonergic activity (Leysen *et al.*, 1978*c*)).

As shown in table 1, inhibition constants (K_i values) of drugs for ^{3}H-spiperone binding in human, rat and calf caudate are nearly identical for neuroleptics, although some compounds appeared somewhat less potent with calf tissue. Catechol-like DA agonists are about four times better inhibitors in the rat striatal tissue than in the calf or human striatal tissue (Creese *et al.*, 1979*b*). The superiority of spiperone as a ligand for labeling dopaminergic receptors is attributed to the more favorable ratio between stereospecific versus non-specific binding, its higher binding affinity and its slower dissociation rate (Leysen *et al.*, 1978*a*; Howlett and Nahorski, 1978). Although analysis of binding equilibrium in Scatchard plots indicated a single high affinity binding component (Fields *et al.*, 1977; Leysen *et al.*, 1978*a*), first-order plots of dissociation rates were biphasic. This was subsequently found to be due to cooperative binding effects (Leysen and Gommeren, 1978). As observed with ^{3}H-haloperidol binding, inhibition curves of ^{3}H-spiperone binding in striatum were steep for DA antagonists but shallow or even multi-phasic for catechol-like DA agonists (Fields *et al.*, 1977; Creese *et al.*, 1979*b*).

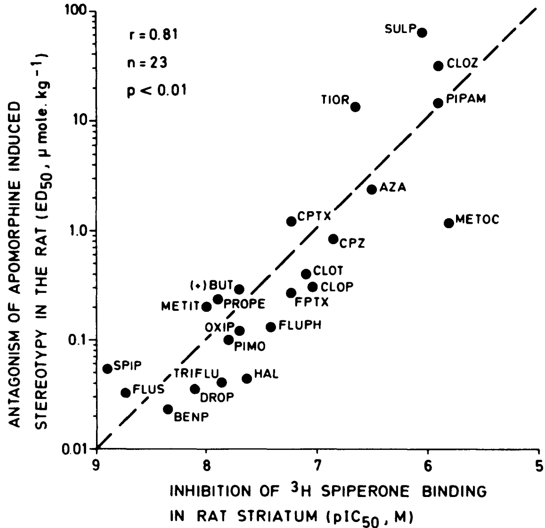

Figure 2 Correlation between relative binding affinity of drugs for sites labeled by ^{3}H-spiperone in rat striatal tissue *in vitro* (Leysen *et al.*, 1978*a*) and potencies of drugs to antagonize apomorphine-induced stereotypy in rats (Niemegeers and Janssen, 1979). Benperidol (BENP), fluspiperone (FLUS), spiperone (SPIP), droperidol (DROP), trifluperidol (TRIFLU), haloperidol (HAL), pimozide (PIMO), oxiperomide (OXIP), fluphenazine (FLUPH), methithepine (METIT), propericiazine (PROPE), (+)-butaclamol ((+)-BUT), flupenthixol (FPTX), clopimozide (CLOP), clothiapine (CLOT), chlorpromazine (CPZ), chlorprothixene (CPTX), metoclopramide (METOC), azaperone (AZA), thioridazine (TIOR), pipamperone (PIPAM), clozapine (CLOZ), sulpiride (SULP).

^{3}H-Domperidone

Domperidone is a potent antiemetic, lacking central antidopaminergic activity *in vivo*, since it does not readily cross the blood–brain barrier. *In vitro*, stereospecific ^{3}H-domperidone binding showed similar regional distribution (Laduron and Leysen, 1979) to that of ^{3}H-haloperidol binding, and in striatum a similar pharmacological specificity was found (Martres *et al.*, 1978; Baudry *et al.*, 1979).

^{3}H-Flupenthixol

Stereospecific ^{3}H-*cis*-(**Z**)-flupenthixol binding, defined by inhibition of binding with cold (+)-butaclamol, was found in rat brain in decreasing order of density in the following tissues: striatum, olfactory tubercles, frontal cortex and thalamus. Binding affinities of drugs for rat striatal ^{3}H-flupenthixol-labeled sites correlated with the potencies of the drugs for inhibiting DA-sensitive adenylate cyclase, but not with drug binding af-

finities for ^{3}H-haloperidol-labeled sites. Moreover, flupenthixol labeled about three times as many sites as haloperidol. The pharmacological significance of the ^{3}H-flupenthixol sites is not clear (Hyttel, 1978, 1980).

^{3}H-Clozapine

In rat brain ^{3}H-clozapine apparently binds to muscarinic cholinergic receptors, which can be inhibited by hyoscyamine. The binding component which could be inhibited by (+)-butaclamol seemed not to be related to a dopaminergic receptor (Hauser and Closse, 1978).

^{3}H-Sulpiride

Using a centrifugation technique and cold sulpiride to define specific binding, a moderately high affinity binding, K_D=27 nM of ^{3}H-sulpiride, could be detected in rat striatum. A small stereospecific difference was found between inhibitory potencies of (+) and (−)-sulpiride and (+) and (−)-butaclamol. *Cis-* and *trans-*flupenthixol could not inhibit the binding and various compounds showed a different maximal inhibition of the total binding. The pharmacological significance of the binding has not yet been assessed (Theodorou *et al.*, 1979).

Conclusion

In striatal membranes from calf, rat or human, stereospecific binding sites can be labeled at nanomolar concentrations of ^{3}H-haloperidol, ^{3}H-spiperone, ^{3}H-dihydroergocryptine+excess phentolamine and ^{3}H-domperidone. Using all these ligands and animal species, the binding sites show similar pharmacological properties and appear to be related to dopaminergic receptors. The biochemically assessed binding affinities of DA antagonists for these sites are significantly correlated with several *in vivo* pharmacological antidopaminergic potencies and with clinical dosages. Exceptions can be encountered mostly because of pharmacokinetic or metabolic effects *in vivo.* Compounds belonging to pharmacological classes other than DA agonists or antagonists do not display high affinity binding for these sites. The *in vitro* labeled dopaminergic binding sites are clearly distinct from the enzyme: DA-sensitive adenylate cyclase. The pharmacological significance of the latter is not yet clear. Since the enzyme was detected first it is called the DA_1 receptor and the dopaminergic binding sites are called DA_2 receptors (Kebabian and Calne, 1979). ^{3}H-Spiperone displayed the most favorable *in vitro* binding kinetic properties to label the DA_2 receptors. ^{3}H-Flupenthixol, ^{3}H-clozapine and ^{3}H-sulpiride do not apparently label the DA_2 receptors, but do label sites for which a pharmacological function has not yet been ascribed.

^{3}H-DOPAMINE AGONIST BINDING IN STRIATAL TISSUE

^{3}H-Dopamine

The early studies of ^{3}H-DA binding in calf striatum (Burt *et al.*, 1975, 1976; Creese *et al.*, 1975) and in rat striatum (Seeman *et al.*, 1975) were performed and reported in parallel with the ^{3}H-haloperidol binding studies. Specific ^{3}H-DA binding was assessed at a concentration of 5 nM with 10 μM (+)-butaclamol or 1 μM DA as blank. The regional distribution in calf brain was completely similar to that of ^{3}H-haloperidol and matched the regional variations in density of dopaminergic nerve endings. Similar numbers of sites were detected with both ligands (Burt *et al.*, 1976). However, in calf striatum relative binding affinities of DA antagonists for the ^{3}H-DA labeled sites differed considerably and were much lower (K_i values of micromolar order) than those for the ^{3}H-haloperidol labeled sites (K_i values of nanomolar order) (table 1). No significant correlation between drug K_i values measured in both assays was found (Creese *et al.*, 1975; Burt *et al.*, 1976). K_i values for the ^{3}H-dopamine-labeled sites did not correlate with any *in vivo* pharmacological antidopaminergic potency or average clinical dosages of the drugs (Creese *et al.*, 1975). In contrast to the DA antagonists, catechol-like DA agonists showed higher binding affinity (lower K_i values) for the ^{3}H-DA than for the ^{3}H-haloperidol sites (Burt *et al.*, 1976).

Inhibition of ^{3}H-DA with agonists yielded regular and steep curves while curves of antagonists were shallow. Scatchard analysis of binding equilibrium indicated a single high affinity binding component with $K_D = 17$ nM at 37 °C and a maximal binding site density of 31 pmol per gram of tissue, which is about twice the maximal density of the ^{3}H-haloperidol sites. Association and dissociation rates of ^{3}H-DA binding at 37 °C were extremely fast (Burt *et al.*, 1976).

In rat striatum, binding affinities of DA agonists for ^{3}H-DA sites were lower than in the calf striatum and binding affinities of antagonists were higher (Burt *et al.*, 1975; Seeman *et al.*, 1975). It seemed that in rat striatum the ^{3}H-DA sites were more similar to the ^{3}H-haloperidol sites than in calf striatum. However, Titeler *et al.* (1979) recently reported to have measured high affinity ^{3}H-DA binding in rat striatum showing similar binding properties as the ^{3}H-DA binding in calf caudate. In that study, specific ^{3}H-DA binding was defined by adding a large excess of cold DA (500–10 000 nM). In both calf caudate and rat striatum Scatchard plots were straight and revealed K_D values of about 2.5 nM. Titeler *et al.* designated these high affinity DA binding sites which showed low affinity for DA antagonists as DA_3 receptors. The pharmacological or physiological significance of these sites has not yet been elucidated.

^{3}H-Apomorphine binding

^{3}H-Apomorphine was introduced as a better agonistic ligand to label DA

receptors (Seeman *et al.*, 1976). In calf striatum the binding was stereospecifically inhibited by (+)-butaclamol. In human brain stereospecific ^{3}H-apomorphine binding was enriched in dopaminergic brain areas (*vide supra*). In a small series of compounds, similar relative binding affinities were found for ^{3}H-apomorphine and ^{3}H-DA sites in calf striatum, which were hence not correlated with binding affinities for ^{3}H-haloperidol-labeled sites (Seeman *et al.*, 1976; Thal *et al.*, 1978).

Linear Scatchard plots indicated a single high affinity binding site with a K_D value of 3.5 nM. However, inhibition curves of antagonists for ^{3}H-apomorphine binding were biphasic, which was interpreted as indicating multiple binding sites (Titeler *et al.*, 1978).

Creese *et al.* (1978, 1979*b*) reported species differences in ^{3}H-apomorphine binding; drug binding affinities for ^{3}H-apomorphine sites in rat striatum were unlike those in calf striatum, and were similar to binding affinities for ^{3}H-butyrophenone sites.

In optimal assay conditions, where stereospecific ^{3}H-apomorphine binding in rat striatum was reproducibly measured (Leysen, 1980*a*) (*vide infra*), we were able to substantiate further the observations of Creese. In rat striatum high affinity ^{3}H-apomorphine binding was inhibited stereospecifically by (+)-butaclamol, and this binding showed a K_D value of 1.7 nM at 25 °C and a maximal number of binding sites of 13.3 pmol per gram wet weight tissue. In our studies, the maximal number of stereospecific ^{3}H-apomorphine sites in rat striatum was two to three times lower than the maximal number of both ^{3}H-haloperidol and ^{3}H-spiperone sites (30.4 pmol per gram wet weight tissue) (Leysen, 1979*a*). Both DA agonists and antagonists bound with high affinity to these sites and IC_{50} values for ^{3}H-apomorphine binding correlated significantly with IC_{50} values for ^{3}H-haloperidol or ^{3}H-spiperone binding (Leysen, 1979*a*, 1980*a*). K_i values are shown in table 1. However inhibition of ^{3}H-apomorphine binding by antagonists still revealed shallow curves whereas curves of agonists were steep (Creese *et al.*, 1979*b*; Leysen, 1979*a*, 1980*b*).

^{3}H-2-Amino-6,7-dihydroxy-1,2,3,4-tetrahydroxynaphthalene (ADTN)

The conformationally restricted DA analog, ^{3}H-ADTN (Roberts *et al.*, 1977) appeared to label sites similar to ^{3}H-apomorphine and ^{3}H-DA in calf striatum (Seeman *et al.*, 1979), that is sites different from those of the ^{3}H-butyrophenones.

In experiments by Creese *et al.* (1978) using rat striatum, ^{3}H-ADTN-labeled sites were similar to the ^{3}H-apomorphine sites, and were thus more comparable to the ^{3}H-butyrophenone sites.

^{3}H-*N*-*n*-Propylnorapomorphine (NPA)

^{3}H-*N*-*n*-Propylnorapomorphine labeled stereospecific sites, defined with

(+)-butaclamol, in calf striatum which had similar binding properties as the ^{3}H-butyrophenone sites (Creese *et al.*, 1979*a*). Hence these sites should also resemble the sites which were labeled with ^{3}H-butyrophenones in rat and human striatum and with ^{3}H-apomorphine in rat striatum in our studies (Leysen, 1979*a*, 1980*a*, *b*). It appeared that specific ^{3}H-NPA binding was easier to detect and less problematic than the binding of previously used ^{3}H-agonists (Creese *et al.*, 1979*a*). Titeler and Seeman (1979) claimed that ^{3}H-NPA labeled both high affinity agonist and high affinity antagonist sites in calf striatum. This would become apparent by selectively blocking one of the sites with 100 nM DA or 50 nM spiperone respectively.

^{3}H-Lisuride

The ergot derivative, lisuride, which behaves as a DA agonist, was shown to label with high affinity, stereospecific sites in rat striatum, which displayed similar binding properties as the sites labeled by ^{3}H-spiperone, ^{3}H-haloperidol or ^{3}H-dihydroergocryptine. However, unlike ^{3}H-dihydroergocryptine, at nanomolar concentrations ^{3}H-lisuride seemed not to interact with α-receptors, but might possibly label a small amount of 5-HT receptors (Fujita *et al.*, 1979).

Considerations of anomalies between ^{3}H-antagonist and ^{3}H-agonist binding

In recent reports (Leysen 1979*a*, 1980*a*, *b*), we have discussed thoroughly anomalies which might influence *in vitro* receptor binding, especially with regard to chemically unstable catechol derivatives. Assay conditions and the composition of the medium are very critical for a clear detection of specific binding. As such, it was observed that addition of metal chelating agents could reduce the large non-specific binding of catechols, presumably by preventing complexing of the catechol moiety with membrane-bound transition metals. Without this precaution, it was difficult to distinguish stereospecific binding. Catechol-like compounds, as well as all metal chelating agents, competed with high affinity for the non-specific complexing of the ^{3}H-ligand. This rendered accurate blank assessment very crucial. Moreover, it is advisable to use sufficiently large volumes and sufficient amounts of tissue, first to prevent an excessive loss of free drug (unlabeled or labeled) through adsorption to laboratory ware and, second, to harvest enough counts of membrane-bound radioactivity. It appeared that in most agonist receptor binding studies these criteria were not adequately considered. Furthermore, it has been noted that in the studies where ^{3}H-agonist binding differed appreciately from ^{3}H-antagonist binding, such as with calf and human tissue and with rat tissue (Titeler *et al.*, 1979), frozen tissue was always used. In contrast, fresh rat brain tissue was used in the other studies and this resulted in a more similar drug specificity between ^{3}H-agonist and ^{3}H-antagonist labeled sites. It has recently been reported that ^{3}H-agonist

binding is much more rapidly abolished by noxious tissue pretreatment than ^{3}H-antagonist binding (Lew and Goldstein, 1979). These observations do not necessarily indicate that agonists and antagonists bind to completely different receptors. It might well be that both bind to a somewhat different region of the receptor complex (see general discussion). It is not uncommon that such different parts of the receptor complex have different physical stabilities. Methodological problems as described above are possible causes for the discrepancies in the experimental findings in the various tissues and between various investigators.

In trying to find an explanation for the apparently irregular inhibition patterns for interactions between agonist and antagonists we have pointed out the importance of physicochemical surface phenomena in receptor binding studies (Leysen, 1980*b*) such as discussed by Blank for pharmacology (Blank, 1979). Surface phenomena occur when there are interactions between membrane micelles and lipophilic compounds, such as DA antagonists, which have highly surfactant properties and form concentrated monolayers around the micelles, in contrast to the catechol-like agonists, which are usually more water soluble. Surface phenomena are not taken into account in the law of mass action and they might cause apparently irregular binding patterns. Therefore the shallow curves for inhibition of agonist binding by antagonists or vice versa are not necessarily an indication for the involvement of distinct multiple sites.

Conclusion

Regional distribution in the brain of stereospecific binding sites for both ^{3}H-DA agonist and ^{3}H-DA antagonist is in accordance with the density of dopaminergic nerve terminals and is similar in calf, rat and human.

The binding sites labeled in calf and human striatum by ^{3}H-DA, ^{3}H-apomorphine and ^{3}H-ADTN are apparently different from the DA_2 receptors labeled by DA antagonists. Furthermore, there is no apparent relationship between these agonist sites and the DA_1 receptor, DA-sensitive adenylate cyclase. The pharmacological or physiological significance of these sites is not clear. It has been discussed that methodological and physicochemical problems might account in part for the anomalies between agonist and antagonist binding. However, the more potent or less unstable DA agonists, ^{3}H-NPA and ^{3}H-lisuride, labeled stereospecific sites which were similar to the sites labeled with ^{3}H-DA antagonists in various species. Such sites could also be detected with the other catechol agonists in freshly prepared rat striatal tissue. Thus, the pharmacological properties of these sites resembled more those of DA_2 receptors.

The observation that binding of catechol DA agonists is reduced by high concentrations of guanyl nucleotides, whereas the binding of ergot-like agonists and of antagonists is not influenced, has not been reviewed here. This topic has been investigated by Creese and co-workers (see references in Creese and Sibley, 1979).

DOPAMINERGIC RECEPTORS IN THE PITUITARY

In regional distribution studies, it was shown that ^{3}H-haloperidol (Brown *et al.*, 1976; Leysen and Laduron, 1977*a*) and 3H-DA (Brown *et al.*, 1976; Cronin *et al.*, 1978) labeled stereospecific binding sites in rat pituitary.

In bovine anterior pituitary both ^{3}H-spiperone and ^{3}H-NPA labeled, at nanomolar concentrations, stereospecific sites which showed identical drug binding affinities as the DA_2 sites labeled by ^{3}H-spiperone in the striatum (Creese *et al.*, 1977; Creese and Sibley, 1979). However, ^{3}H-NPA labeled only about half the number of sites which were labeled by ^{3}H-spiperone in the pituitary. Sites with binding properties of DA_2 receptors were also labeled by ^{3}H-dihydroergocryptine in lamb anterior pituitary (Cronin *et al.*, 1978). These authors claimed that similar sites were detectable in the sheep stalk median eminence, although Brown *et al.* (1976) could not detect them in rat hypothalamus. Denef demonstrated that potencies of DA antagonists for disinhibiting apomorphine-suppressed prolactin release from cultured pituitary cells, correlated significantly with the relative binding affinities of the drugs for ^{3}H-haloperidol sites in rat striatum (Denef and Follebouckt, 1978). Hence in various species DA_2 receptors could be detected in the pituitary using various ^{3}H-ligands. The drug binding affinities of the DA receptors correlated with the potencies of drugs in activating prolactin release.

DOPAMINERGIC RECEPTORS IN THE LIMBIC AREAS

In the limbic areas from various species (calf, rat, human) stereospecific binding sites were labeled with ^{3}H-haloperidol, ^{3}H-spiperone, ^{3}H-DA and ^{3}H-apomorphine and the binding site density was about the density in the striatum (Burt *et al.*, 1976; Seeman *et al.*, 1976; Leysen and Laduron, 1977*a*; Laduron *et al.*, 1978). Burt *et al.* (1976) showed that K_i values of DA antagonists and agonists for ^{3}H-haloperidol binding in calf striatum, olfactory tubercle and nucleus accumbens were identical, which indicated the occurrence of similar DA_2 receptors in calf mesolimbic and striatal areas.

In rat tuberculum olfactorium+nucleus accumbens, ^{3}H-spiperone, used at nanomolar concentrations, labeled both dopaminergic and serotonergic sites to an equal extent. Binding to both sites was stereospecifically inhibited by (+)-butaclamol. As shown in figure 3, the labeling of the serotonergic sites could be prevented by adding 100 nM R 43448, a compound with potent antiserotonergic and antihistaminic properties but lacking antidopaminergic activity. Figure 3 also shows that, using ^{3}H-haloperidol, the major part of the stereospecific sites are dopaminergic (Leysen *et al.*, 1979*a*).

In rats, the dopaminergic binding sites labeled in the limbic brain areas

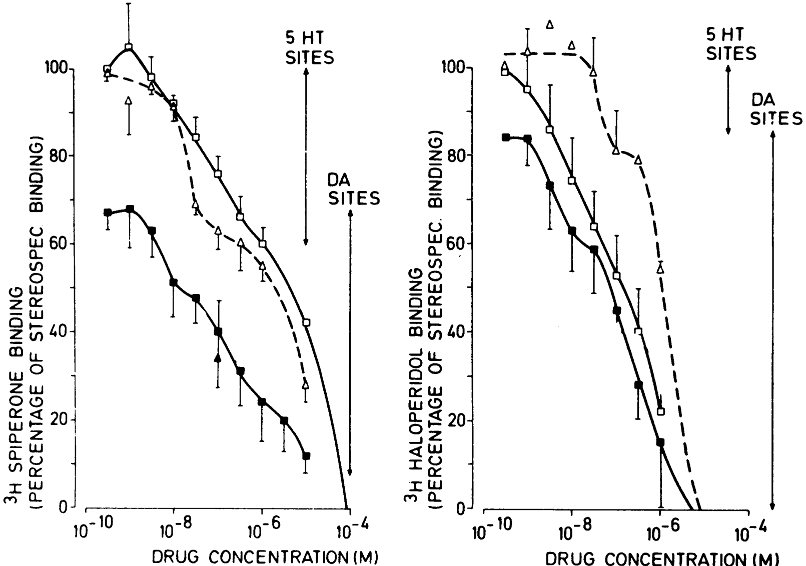

Figure 3 Inhibition curves of R 43448 (△- -△) and inhibition curves of 2-(*N*, *N*-dipropyl)amino-5, 6-dihydroxytetralin in the absence (□—□) and in the presence (■—■) of 10^{-7} M R 43448 for stereospecific ^{3}H-spiperone (2 nM) and ^{3}H-haloperidol (2 nM) binding to a total membrane fraction of rat nucleus accumbens+tuberculum olfactorium (values are means of four independent determinations in duplicate).

with ^{3}H-haloperidol and ^{3}H-spiperone also displayed the same binding properties as the DA_2 receptors in the striatum: similar K_i values of drugs as shown by the correlation in figure 4; equal K_D values of the labeled ligands; regular inhibition curves with dopamine antagonists but irregular curves with agonists (Leysen, 1979*b*).

It can be concluded that, as yet, no differentiation exists between drug binding properties of dopaminergic sites in striatal and limbic brain areas.

DOPAMINERGIC RECEPTORS IN OTHER BRAIN AREAS AND THE RETINA

Small amounts of dopaminergic receptors labeled by ^{3}H-spiperone were detected in rat substantia nigra (Quick *et al.*, 1979; Reisne *et al.*, 1979) and in rat anterior cerebral cortex (Marchais *et al.*, 1980).

Recently, stereospecific ^{3}H-spiperone binding sites were detected in the

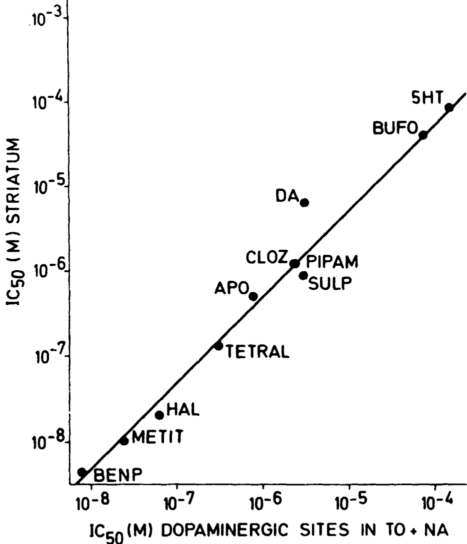

Figure 4 Correlation between relative binding affinities (IC_{50} values) of drugs for stereospecific sites labeled by ^{3}H-spiperone (2 nM) in the striatum and by ^{3}H-spiperone (2 nM) in the presence of 10^{-7} M R 43448 in rat nucleus accumbens+tuberculum olfactorium. Abbreviations are as in legend to figure 2 and apomorphine (APO), 2-(*N*, *N*-dipropyl)amino-5, 6-dihydroxytetralin (TETRAL), dopamine (DA), bufotenin (BUFO).

bovine retina. The sites showed the same binding properties as brain DA_2 receptors (Magistretti and Schorderet, 1980).

INTERACTION OF PUTATIVE ENDOGENOUS PEPTIDES WITH DOPAMINERGIC RECEPTORS

In brain extracts, endogenous macromolecular materials were detected which could inhibit receptor binding of labeled DA agonists and antagonists (Leysen *et al.*, 1978*b*; Lahti *et al.*, 1978; Leysen *et al.*, 1979*b*). This material, however, did not show specificity towards dopaminergic receptors but interfered with several neurotransmitter receptors (Leysen *et al.*, 1978*b*).

Opioid peptides appear not to bind to dopaminergic receptors (Leysen *et al.*, 1978*b*; Van Ree *et al.*, 1978; Członkowski *et al.*, 1978; Meltzer and So, 1979), hence their effect on prolactin secretion in the pituitary was interpreted as an indirect effect (Meltzer and So, 1979).

Destyrosine-γ-endorphin, which has been described as a peptide with antipsychotic activity, does not bind to dopaminergic receptors at concentrations below 10 μM (Van Ree *et al.*, 1978; Leysen *et al.*, 1978*b*).

^{3}H-SPIPERONE BINDING TO SEROTONERGIC RECEPTORS

In the early studies using ^{3}H-spiperone (Fields *et al.*, 1977; Laduron and Leysen, 1977; Laduron *et al.*, 1978) it was observed that this ligand, unlike ^{3}H-haloperidol, labeled a substantial amount of stereospecific binding sites in rat cortical brain areas. The stereospecific ^{3}H-spiperone binding sites in rat frontal cortex were found to be of serotonergic nature (Leysen *et al.*, 1978*c*). 5-HT agonists had higher binding affinities than DA agonists for these sites and 5-HT antagonists had very high binding affinities. Relative binding affinities of drugs correlated significantly with relative binding affinities for sites labeled by ^{3}H-LSD. A significant correlation was also found between binding affinities in the frontal cortex and drug potencies to antagonize tryptamine-induced clonic seizures *in vivo*, but not at all with *in vivo* antidopaminergic drug potencies (Leysen *et al.*, 1978*c*). Presently, no relationship is apparent between occupation of serotonergic receptors and the antischizophrenic properties of the drugs. Clinically it is observed that a compound such as pipamperone, which shows higher binding affinity for serotonergic than for dopaminergic receptors, is only weakly active in hallucinations or delusions, but it is known for its anti-agitation effects and is particularly useful for regulation of disturbed sleep–wake rhythms in psychiatric patients (Asoms *et al.*, 1977). The serotonergic sites labeled with ^{3}H-spiperone in the frontal cortex were completely distinct from the dopaminergic sites in the striatum in regard to both pharmacological and binding kinetic properties (Leysen and Gommeren, 1978). Similar serotonergic sites were detected with ^{3}H-spiperone in the limbic brain areas (Leysen *et al.*, 1979*a*, *b*) and in the hippocampus (Creese and Snyder, 1978). Recently Peroutka showed that the serotonergic spiperone binding sites which have high binding affinities for 5-HT antagonists, are different from sites labeled by ^{3}H-5-HT, for which agonists have higher, but antagonists much lower, binding affinities. ^{3}H-LSD should label both the ^{3}H-spiperone and the ^{3}H-serotonin sites (Peroutka and Snyder, 1979).

When using frontal cortex tissue (Leysen and Gommeren, 1978) and limbic brain tissue (Howlett *et al.*, 1979) it has been emphasized that special attention should be paid to a precise assessment of stereospecific ^{3}H-spiperone binding in order to define receptor-linked binding. Indeed, in these brain areas a substantial part of the ^{3}H-spiperone binding is non-stereospecific, although saturable and of high affinity (displaced by low concentrations of unlabeled spiperone). The latter binding appeared to be related to the spirodecanone moiety and was of no known pharmacological or physiological significance.

Conclusion

Certain neuroleptics can label with high affinity more than one neurotransmitter receptor. Careful control of the nature and binding properties of the

sites is necessary, particularly since certain saturable non-receptor-linked sites can be labeled as well as various receptors.

RECEPTOR BINDING PROFILES OF NEUROLEPTICS

From the foregoing, it appeared that the therapeutic effect of neuroleptics in schizophrenia is related to the binding affinity of the drugs for dopaminergic receptors, hence their ability to block these receptors. However, drugs classified as neuroleptics belong to many different chemical classes of compounds and many of these compounds display different pharmacological profiles (Niemegeers and Janssen, 1979). Since, at present, *in vitro* receptor binding models have been described for several neurotransmitter receptors, the receptor binding profile of drugs can be assessed. Such a receptor binding profile gives direct information about the *in vitro* binding affinity of the compound for the receptors. Of course, the *in vitro* assessed activity of the compound does not take into account pharmacokinetic problems and *in vivo* certain compounds may never reach the receptors although they have the capability to occupy them. Neither is information obtained about the duration of action of the compounds. This may be very short acting, and hence the *in vivo* pharmacological effect might be of no significance, or very long acting, so that the importance of a receptor occupation might become much more prominent. However, receptor binding profiles might be very helpful in the interpretation of complex *in vivo* pharmacological effects. They may also give insight into the possible causes of clinically observed side effects of the compounds. Receptor binding also provides a possibility to classify drugs according to selectivity towards various neurotransmitter receptors. A number of *in vitro* receptor binding models are summarized in table 3. Receptor binding affinities of 29 neuroleptics, representing most clinically used drugs, measured using these models are given in table 1 for the dopaminergic receptor (^{3}H-haloperidol and ^{3}H-apomorphine, original data) and in table 4 for the other neurotransmitter receptors. It appears that certain compounds are very selective for dopaminergic receptors (for example penfluridol and pimozide). Sulpiride and molindone are also selective, but they have a much weaker affinity for the dopaminergic receptors. In contrast to these, many other compounds show high binding affinity for many different neurotransmitter receptors and often their binding affinity for dopaminergic receptors is weaker than that for the other receptors (for example chlorpromazine and clozapine).

Finally, receptor profiles are very useful for biochemical research. From tables 1 and 4 it is apparent that the choice of a labeled ligand for studying a certain neurotransmitter receptor is very critical. Compounds which display K_i values of nanomolar order for several receptors will act as labeled ligands at nanomolar concentrations, and label all these receptors if they are present in the tissue preparation. An example is ^{3}H-spiperone, which labels dopaminergic and serotonergic receptors, but with selectivity according to

Table 3 Receptor binding models

Receptor	^{3}H-Ligand (K_D, nM)	Specific inhibitor	Tissue	Method reference
Dopaminergic	^{3}H-Haloperidol (1.3)	(+)-Butaclamol	Rat striatum	Leysen *et al.*, 1977
	^{3}H-Apomorphine (2.2)	(+)-Butaclamol	Rat striatum	Leysen, 1980*a*
Serotonergic: Antagonist	^{3}H-Spiperone (0.97)	(+)-Butaclamol	Rat frontal cortex	Leysen *et al.*, 1978*c*
Serotonergic: Agonist	^{3}H-Serotonin (11)	LSD	Rat hippocampus	Bennett and Snyder, 1975
Adrenergic: α_1	^{3}H-WB 4101 (0.29)	Norepinephrine	Rat forebrain	Greenberg *et al.*, 1976
Adrenergic: α_2	^{3}H-Clonidine (2)	Norepinephrine	Rat cortex	Greenberg *et al.*, 1976
Histaminergic H_1	^{3}H-Pyrilamine (4.8)	Astemizole	Guinea pig cerebellum	Chang *et al.*, 1978
Cholinergic muscarinic	^{3}H-Dexetimide (0.65)	Dexetimide	Rat striatum	Laduron *et al.*, 1979

Table 4 Equilibrium inhibition constants (K_i values, nM) of neuroleptics in various neurotransmitter receptor models

	Serotonergic		Histaminergic	Adrenergic		Cholinergic
	Antagonist Spiperone	Agonist Serotonin	H_1 Pyrilamine	α_1 WB 4101	α_2 Clonidine	Muscarinic Dexetimide
Spiperone	1.2	160	>10000	10	>10000	3460
Benperidol	6.6	227	1727	2.3	2575	774
Droperidol	4.1	312	2174	0.8	2575	3872
Pimozide	32.8	211	>10000	41	>10000	1022
Haloperidol	48	7860	4390	8.1	>10000	4370
Piflutixol	1.3	62.5	29.3	1.3	25.8	>10000
Thioperidone	52	8.8	233	0.5	95	>10000
Thiothixene	9.2	843	25.6	12.9	324	>10000
Prochlorperazine	30.6	>10000	17.3	16.2	2403	>10000
Milenperone	9.2	786	64	6.8	>10000	>10000
Propericiazine	2.6	5000	36.9	1.8	816	>10000
Trifluperazine	41.2	>10000	51	20.4	>10000	>10000
Methithepine	1.8	49.7	5.5	0.4	32.4	>10000
Perphenazine	33	3937	7.7	91.3	437	>10000
Fluphenazine	32.8	>10000	27.4	8.9	1624	>10000
Levomepromazine	5.2	>10000	2.7	1.0	514	182
Penfluridol	232	1355	>10000	363	>10000	>10000
Chlorprothixene	3.3	3127	6.1	1	162	38.8
Loxapine	8.2	>10000	7.7	10.2	3242	489
Thioridazine	36	>10000	40.5	3.2	1383	77.5
Clothiapine	6	>10000	15.4	14.4	>10000	245
Sulpiride	26043	>10000	>10000	>1000	>10000	>10000
Molindone	3279	>10000	>10000	1818	>10000	>10000
Alimemazine	10.4	>10000	3.5	11.5	645	123
Chlorpromazine	20.2	3127	6.9	1.7	1024	162
Promazine	328	>10000	4.3	2.5	408	174
Pipamperone	5	5000	>10000	46	604	2450
Clozapine	15.7	3127	4.3	7.3	120	31
Perlapine	44.2	>10000	7.7	129	2575	74

the brain preparation. The sites labeled by ^{3}H-clozapine could not be defined, but it seems that this compound would be able to label at least H_1-histaminergic, α_1-adrenergic, serotonergic antagonist, and muscarinic cholinergic receptors, but only slightly dopaminergic receptors.

Amongst the labeled ligands thus far used, domperidone and haloperidol are the most selective for the dopaminergic receptors. Labeled pimozide and penfluridol were tried in our laboratory. As they are extremely lipophilic, the non-specific membrane adsorption of these compounds exceeds by several-fold the specific receptor binding, even at subnanomolar concentrations (Leysen and Laduron, 1977*b*). Therefore these compounds are unsuitable as ligands. This demonstrates that several factors have to be considered in order to design a good receptor binding model.

GENERAL CONCLUSIONS

The present review has focused exclusively on the pharmacological identification of neuroleptic receptors. According to the binding specificity of drugs, only one DA receptor has been pharmacologically characterized. These are the binding sites which have been called DA_2 receptors and which can be labeled by several DA antagonists and agonists, provided that experimental conditions are conscientiously controlled. The anomalies which have been encountered in the ^{3}H-DA agonist binding might possibly be due to experimental pitfalls such as noxious pretreatment of tissue, impurity or instability of ^{3}H-ligands, non-optimal assay conditions or inaccurate definition of specific binding.

The failure of some ^{3}H-DA antagonists to be useful ligands (for example ^{3}H-clozapine and ^{3}H-sulpiride) might be ascribed either to their interference with too many neurotransmitter receptors or to their weak binding affinity.

The fact that receptors constitute parts of membranes, and that binding studies are conducted with membrane suspensions, greatly hampers the analysis and interpretation of binding data. It is not usually taken into account that surface phenomena may play a very important role and might cause apparently irregular binding patterns.

Dopaminergic receptors detected using *in vitro* binding techniques, in various tissues, brain areas and species, all display a similar drug selectivity and thus probably constitute a common recognition site. However, it should not be overlooked that the binding site is just the start of a large processing and regulatory system which results in the final physiological effect. So, where binding studies measure the start, pharmacologists and physiologists only see the end effect. Little biochemical insight is yet available to tell us what is happening in between. When discussing membrane-bound receptors, the constitution of the membrane (Capaldi, 1974) should also be kept in mind. A receptor should not be looked at as a 'hole' in a membrane where certain structures fit. Therefore, we should like to give here a speculative but

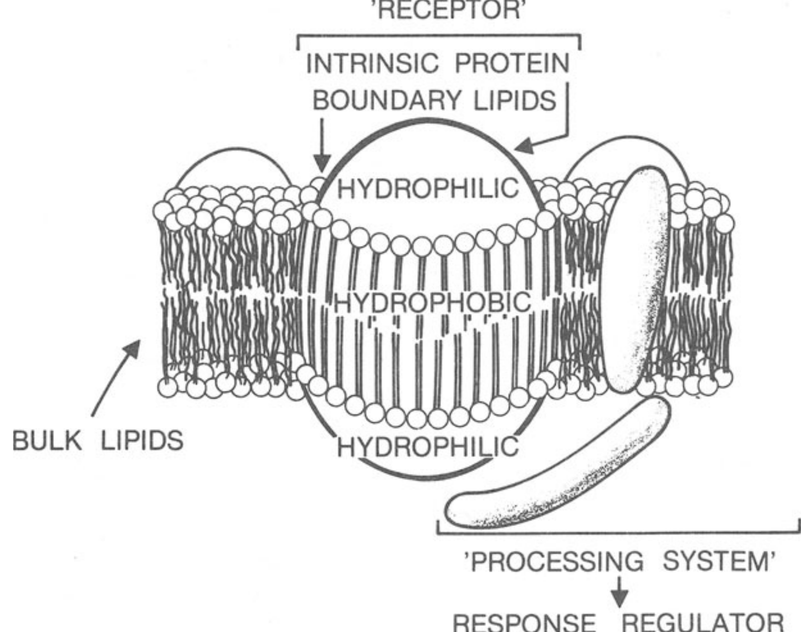

Figure 5 A speculative representation of a receptor-complex such as might be incorporated in a membrane. It is assumed that the receptor complex is composed of an intrinsic protein + the boundary phospholipids which keep each other in a defined conformation. The parts of the protein sticking out of the membrane are composed of hydrophilic amino acids and might form a binding area for hydrophilic compounds (for example agonists). The center part of the protein and the phospholipids are hydrophobic and might contain several binding sites for hydrophobic drugs. Mutual inhibition for binding to the several binding site areas would be caused by partial overlapping of the occupied sites or by conformationally induced changes upon occupation.

perhaps a more rational representation of what a receptor might look like (figure 5). The receptor or binding site area is probably formed by an intrinsic protein together with its boundary lipids which keep each other in a tight conformation. Conformational changes in the receptor complex might activate or block the processing system to which they are linked. Since agonists and antagonists are compounds of different chemical natures, with different chemical properties, one could speculate that more hydrophilic agonists would bind preferentially toward the hydrophilic part of the protein and initiate conformational changes, whereas the highly lipophilic antagonists would be immersed in the lipophilic area of the boundary lipids. A mutual inhibition of agonists and antagonists could be caused by partial overlap in occupation of binding areas or conformational changes. Such a working hypothesis might, perhaps, be helpful in the interpretation of certain binding data.

For several reasons, this review was restricted to the pharmacology of the

receptors and consideration of other criteria for classification of multiple receptors was omitted: first, because of the clinical orientation of the meeting; second, because of the lack of time and space to cover the vast amount of literature. But, mostly, because of my own personal feeling that receptor research is still too immature to give a clear-cut view on what multiple sites really mean. '*Qui nimium probat nihil probat*'.

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High affinity ^3H-imipramine binding: a new tool in biological psychiatry

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INTRODUCTION

During the last 20 years many studies were carried out in an attempt to find biological markers in affective disorders. The results obtained are often contradictory, and the existence of two types of depressions related either to norepinephrine (NE) or to serotonin (5-HT) remains an open question (Bunney and Davis, 1965; Schildkraut and Kety, 1967; van Praag, 1977).

Monoamine metabolites have been evaluated in different physiological fluids in man. With respect to NE its modifications have been explored by measuring 3-methoxy-4-hydroxyphenylglycol (MOPEG) levels in the cerebrospinal fluid (CSF) or in urine. The results differ widely according to the authors as either normal or decreased levels have been reported in endogenous depression.

Conflicting results have been reported for the catabolite of 5-HT 5-hydroxyindole acetic acid (5-HIAA) (Garver and Davis, 1979). Of considerable interest is the bimodal distribution of 5-HIAA in the CSF reported by Åsberg and Bertilsson (1979), suggesting the existence of a subgroup of depressed patients with a disturbance of 5-HT function. The low levels of 5-HIAA in CSF are associated with an increased tendency to attempt suicide (Åsberg and Bertilsson, 1979).

Evidence for a decrease in 5-HT uptake by blood platelets of depressed patients has been reported (Tuomisto and Tukiainen, 1976; Tuomisto *et al.*, 1979; Scott *et al.*, 1979).

Several reports indicate that the modulation of the release of growth hormone is altered in endogenously depressed patients. A second endocrinological parameter which appears to change in depressed patients is the pattern of release of cortisol. The inhibitory effect of dexamethasone on plasma cortisol levels appears to be considerably reduced in patients with endogenous depression (Nuller and Ostroumova, 1980; Brown *et al.*, 1980).

Tricyclic antidepressant drugs (TCA) are considered to be the most

effective pharmacological therapy of affective disorders. Although drugs like imipramine (IMI), desipramine (DMI), amytriptiline (AMI) and chloripramine have been used for more than 15 years, there is not as yet convincing evidence concerning the mechanism of action for their clinical effectiveness in depression.

Several biochemical effects of TCA have been suggested to be associated with their clinical effectiveness in affective disorders:

(1) Inhibition of neuronal uptake of monoamines – particularly of NE and 5-HT, dopamine (DA) only to a lesser extent.

(2) TCA were shown to interact with high affinity with a number of well-known neurotransmitter receptors: α_1- and α_2-adrenoceptors, histamine H_1-receptors, muscarinic cholinoceptors and 5-HT receptors. The high affinity of some TCA for H_1-receptors, muscarinic receptors and α-adrenoceptors appears to be linked to some of their side effects (for example sedation and atropine-like effects) rather than to their primary mechanism of action as antidepressants.

(3) Although TCA have a very low affinity for β-adrenoceptors, under conditions of chronic treatment with DMI or IMI there is a decrease in maximal binding to β_1-adrenoceptors in the central nervous system (CNS) (Banerjee *et al.*, 1977; Wolfe *et al.*, 1978), and subsensitivity of the NE-sensitive adenylate cyclase (Vetulani *et al.*, 1976).

While the inhibition by TCA of neuronal uptake of NE tends to support the catecholamine hypothesis of depression, their effects on 5-HT uptake would correlate well with an indoleamine hypothesis for affective disorders. Yet TCA have also been shown to produce postsynaptic effects at the level of responses of central neurons to NE, 5-HT and acetylcholine (Jones and Roberts, 1978, 1979). These effects appear to be independent from the inhibition of neuronal uptake of monoamines.

Another development that has challenged the existing hypothesis for the mechanism of action of TCA is the appearance of drugs like mianserine (a very weak inhibitor of monoamine uptake) and iprindol and viloxazine (which do not inhibit the uptake of monoamines and yet appear to be clinically effective antidepressants) (Rosloff and Davis, 1974; Bevan *et al.*, 1975; Lippman and Pugsley, 1976; Baumann and Maître, 1977).

HIGH AFFINITY ^{3}H-IMIPRAMINE BINDING SITES IN THE BRAIN

The use of receptor binding techniques has provided a powerful tool in establishing the affinity of drugs for certain receptors in the CNS, where the postsynaptic effects mediated by these receptors are more difficult to study than in the peripheral nervous system.

The use of radiolabeled drugs for binding studies at known receptor sites

(α- and β-adrenoceptors, DA receptors, muscarinic cholinoceptors, serotoninergic receptors) provided powerful tools to study these receptors in the CNS. The success of this methodology in the study of the well-known receptors was followed by the discovery of specific high affinity binding sites for ^{3}H-diazepam, one of the most potent benzodiazepines (Squires and Braestrup, 1977; Mohler and Okada, 1977). The binding of ^{3}H-diazepam fulfils most of the criteria for its identification as a receptor site which appears to mediate the pharmacological effects of benzodiazepines. In an attempt to find a specific high affinity binding site which could be related to the site of action of antidepressant drugs we employed a similar experimental approach when ^{3}H-IMI of high specific activity became available.

The presence of a specific binding site for ^{3}H-IMI was first reported in the rat brain (Raisman *et al.*, 1979*a*, *b*). This binding site for ^{3}H-IMI is of high affinity and the K_d value is in the nanomolar range. The binding of ^{3}H-IMI is saturable and gives a linear Scatchard plot, indicating a single homogenous population of non-interacting binding sites (Raisman *et al.*, 1980*a*).

The mean apparent equilibrium dissociation constant, K_d, in rat cerebral cortex was 4.04 ± 0.52 nM ($n = 13$) and the maximal binding 249 ± 23 fmol (mg protein)$^{-1}$ ($n = 13$). Hill analysis gave a Hill number of 0.97, indicating a lack of cooperativity. Experiments on association and dissociation of ^{3}H-IMI binding indicate that equilibrium occurs within 30 min ($t_{1/2} = 5$ min) with 2.5 nM ^{3}H-IMI at 0 °C. The binding is rapidly reversible: addition of 10 μM DMI displaces the specific binding completely in less than 45 min at 0 °C ($t = 5$ min). The dissociation affinity constant (K_d) derived from the kinetic constants of association and dissociation is very similar to that obtained from equilibrium experiments by Scatchard analysis (Raisman *et al.*, 1980*a*).

Recent studies carried out with the (**Z**) and (**E**) isomers of zimelidine, norzimelidine and the 10-hydroxymetabolites of nortriptyline (NT) and AMI have shown the stereoselectivity of the high affinity binding of ^{3}H-IMI in the rat brain (Langer *et al.*, 1980*a*).

The specific binding of ^{3}H-IMI is unevenly distributed in the rat brain: the highest density of binding sites was found in the hypothalamus and the lowest in the cerebellum (table 1). Specific ^{3}H-IMI binding was not found under our experimental conditions in peripheral organs like the heart and the vas deferens which possess a rich noradrenergic innervation. A more detailed study carried out in 23 regions of the rat brain shows that the hypothalamus and the amygdala are the richest areas for ^{3}H-IMI binding. The values of ^{3}H-IMI binding and endogenous 5-HT levels in some of these regions are shown in table 2. The results of table 2 indicate that there is a significant correlation between the density of serotoninergic innervation and the number of ^{3}H-IMI binding sites in the areas of the brain studied so far.

A large number of drugs were tested for their ability to inhibit ^{3}H-IMI binding. Certain drugs like phentolamine, chlorpromazine and pyrilamine were relatively potent in inhibiting ^{3}H-IMI binding. However, no single group of drugs acting on the classical neurotransmitter receptors either as agonists or as antagonists inhibited consistently the high affinity binding of ^{3}H-IMI (Raisman *et al.*, 1980*a*).

Table 1 Distribution of specific ^{3}H-imipramine binding in the rat brain

Region	n	K_d (nM)	B_{max} (fmol (mg protein)$^{-1}$)
Cerebellum	3	8.0±3.0	60±11
Hippocampus	3	3.3±0.2	143±26
Midbrain	3	2.4±0.7	156±35
Corpus striatum	7	2.8±0.5	164±11
Spinal cord	3	4.0±1.0	199±63
Cerebral cortex	13	4.0±0.5	249±23
Hypothalamus	6	4.1±0.8	317±50

The values are taken from Raisman *et al.* (1980*a*) and unpublished results. K_d and B_{max} of ^{3}H-IMI binding were calculated by Scatchard analysis. n=number of separate Scatchard plots each of 8–10 concentrations determined in duplicate.

Table 2 Regional distribution of ^{3}H-imipramine binding and serotonin concentration in some areas of the rat brain

Region	^{3}H-IMI binding (fmol (mg protein)$^{-1}$)	5-HT levels (ng (mg protein)$^{-1}$)
Cingulate cortex	58.7± 3.6	5.8
Septum	57.4±11.3	10.9
Globus pallidum	148.7±22.0	19.0
Substantia nigra	121.4±20.5	20.8
Anteromedial hypothalamus	147.0±25.6	15.2
Medial basal hypothalamus	136.5± 8.0	20.1
Lateral hypothalamus	203.7±26.2	25.8
Centromedial amygdala	113.5±17.6	18.4
Laterobasal amygdala	197.2±34.1	21.1
Medullary reticular formation	53.3±10.5	5.8

The values are taken from Palkovits *et al.* (1980). ^{3}H-IMI binding was determined at a single subsaturating concentration: 3.5 to 4.0 nM.

Most TCA inhibit ^{3}H-IMI binding in the nanomolar range (table 3). On the other hand, the non-tricyclic antidepressants, also referred to as 'atypical antidepressants', are much less potent or practically inactive in inhibiting ^{3}H-IMI binding (table 3).

It is of interest to note that while drugs that inhibit neuronal uptake of either NE of DA are not very potent in inhibiting ^{3}H-IMI binding (Raisman *et al.*, 1980*a*; Langer *et al.*, 1980*b*), those that preferentially inhibit neuronal uptake of 5-HT are consistently active in inhibiting ^{3}H-IMI binding in rather low concentrations. In a recent study the IC_{50} values for the inhibition of

Table 3 Inhibition of specific ^{3}H-imipramine binding by tricyclic and atypical antidepressant drugs

Drugs	IC_{50} (nM)
Tricyclic antidepressants	
Imipramine	15
Protriptyline	20
Clomipramine	25
Amitriptyline	25
Nortriptyline	200
Doxepine	300
Amoxapine	500
Non-tricyclic antidepressants	
Zimelidine	1200
Trazadone	1850
Nomifensine	3200
Iprindol	5500
Viloxazine	11500
Mianserine	20000
Bupropione	20000

The inhibition of ^{3}H-IMI binding was determined in membranes from the rat cerebral cortex at 2 nM ^{3}H-IMI. IC_{50} is the concentration of the drug required to inhibit by 50 percent the specific binding of ^{3}H-IMI.

Data taken from Raisman *et al.* (1980*a*).

^{3}H-IMI binding in the rat hypothalamus were determined for 17 antidepressant and non-antidepressant drugs and compared with their potencies in inhibiting neuronal uptake of NE and 5-HT in hypothalamic slices (Langer *et al.*, 1980*b*). A significant correlation was found between the potencies for inhibition of 5-HT uptake and ^{3}H-IMI binding while no such correlation was obtained when the potencies for inhibition of NE uptake were taken into consideration (Langer *et al.*, 1980*b*).

In further support of the view that the high affinity ^{3}H-IMI binding site is linked to the neuronal uptake process for 5-HT we have recently found that the maximal number of binding sites for ^{3}H-IMI binding in the rat hypothalamus is decreased by approximately 50 percent after electrolytic lesions of the raphe dorsalis which result in a 60 percent reduction of the endogenous 5-HT levels in the hypothalamus (Langer, Sette, Raisman, Leroux and Briley, unpublished observations).

It therefore appears that the high affinity ^{3}H-IMI binding site may label either the recognition site or a protein linked to the neuronal uptake complex in serotonergic nerve terminals. Consequently there are at least four different recognition sites for 5-HT at the level of the synapses in the CNS: (1) postsynaptic 5-HT_1 receptors, linked predominantly to adenylate

cyclase and labeled with ^{3}H-5-HT; (2) postsynaptic 5-HT$_2$ receptors, probably linked to the behavioral head twitches response and labeled with ^{3}H-spiroperidol; (3) presynaptic inhibitory 5-HT receptors which modulate the stimulation-evoked release of the neurotransmitter; and (4) neuronal uptake sites for 5-HT linked to the active transport of the transmitter in the nerve endings.

It should be noted that although the neuronal uptake sites for neurotransmitters are not usually referred to as receptors they should be expected to behave like pharmacological receptors in binding studies, because: (1) there is a recognition site for the corresponding transmitter which is usually stereoselective and for which there is a clear-cut structure–activity relationship for close analogs of the neurotransmitter; (2) activation of this recognition site by the neurotransmitter is somehow coupled with the active transport of the substrate inside the nerve terminal; (3) neuronal uptake of transmitters is inhibited by specific drugs which usually are different from the antagonists that block the postsynaptic receptor that mediates the response or the presynaptic receptor that modulates the release of the neurotransmitter.

It therefore appears that recognition sites for neuronal uptake of neurotransmitters can be incorporated into the growing list of neurotransmitter receptors that can be studied with receptor binding techniques. The fact that ^{3}H-IMI appears to label the recognition site for the neuronal uptake of 5-HT may contribute to our understanding of the physiology and pharmacology of these neuronal uptake sites linked to the process of inactivation of the transmitter which therefore regulate the concentration of the neurotransmitter in the synaptic cleft.

It is possible that the high affinity ^{3}H-IMI binding site is related to the site of the pharmacological action of some TCA. In support of this view, we have found a significant correlation for 10 antidepressant drugs between their IC_{50} values for inhibition of ^{3}H-IMI binding and their mean daily clinical dose as antidepressants (Raisman *et al.*, 1980*b*). When a similar analysis was tried for the non-tricyclic antidepressant drugs, there was no significant correlation between these two parameters, indicating that the non-tricyclic antidepressants exert their effects through a mechanism of action which does not seem to involve the high affinity ^{3}H-IMI binding site.

It should be noted, however, that some TCA and their metabolites inhibit preferentially the neuronal uptake of NE and this effect is likely to be involved in the mechanism of the antidepressant effects of these compounds. Yet, as already discussed, the high affinity binding site for ^{3}H-IMI appears to be unrelated to noradrenergic nerve endings.

The presence of ^{3}H-IMI binding sites has also been reported in the human brain (Agid, Raisman, Briley and Langer, unpublished observations; Rehavi *et al.*, 1980). The binding parameters for ^{3}H-IMI in post-mortem samples of the human brain as well as the profile of inhibition of ^{3}H-IMI binding in the human cortex, hippocampus, hypothalamus and caudate nucleus by a variety of different drugs is identical to that described in the rat brain.

PRESENCE OF HIGH AFFINITY ^{3}H-IMIPRAMINE BINDING SITES IN HUMAN PLATELETS

The possible association of ^{3}H-IMI binding with the neuronal uptake of 5-HT and the fact that platelets have been used extensively as a model resembling 5-HT nerve terminals prompted the search for high affinity binding sites in human platelets.

The presence of high affinity binding sites for ^{3}H-IMI in human platelets was first reported by Briley *et al.* (1979) and Langer *et al.* (1980*c*). These findings were recently confirmed by Rehavi *et al.* (1980).

^{3}H-IMI binding in human platelets appears to have properties identical to those reported in the rat and human brain. In a recent study the IC_{50} values for inhibition of ^{3}H-IMI binding in rat brain and human platelets were determined for 25 drugs. A highly significant correlation was obtained in this study ($r=0.81$, $p<0.001$) with a slope of approximately unity (Langer *et al.*, 1980*c*). In a similar study in which the IC_{50} on ^{3}H-IMI binding in human brain and human platelets was determined for a series of clinically active TCA the correlation was also highly significant ($r=0.97$; $p<0.001$) (Rehavi *et al.*, 1980). It therefore appears that the ^{3}H-IMI binding site in human platelets is identical with that originally reported in the rat and human brain.

In a recent study carried out in 35 healthy volunteers, no differences were found between the sexes in either the maximal binding (B_{max}) or the apparent affinity constant (K_d) for ^{3}H-IMI binding to platelet membranes. In addition, the K_d and B_{max} values for ^{3}H-IMI binding were found to be remarkably stable for each individual when determinations were made at 3–4 week intervals (Langer *et al.*, 1980*c*). In this study no changes were observed between either K_d or B_{max} values when female and male normal volunteers of the same age were compared. These authors found a decrease in B_{max} values with increasing age over the range between 17 and 97 years, although there were no changes in K_d values for ^{3}H-IMI binding as a function of age (Langer *et al.*, 1980*c*).

In view of the fact that ^{3}H-IMI binding can be determined repeatedly in human platelets, it was considered of interest to compare the binding parameters of the platelets from severely depressed untreated patients and those of age- and sex-matched control volunteers. While the control population consisted of healthy volunteers receiving no medication, the group of severely depressed patients were free of antidepressant drugs for 4 weeks and without any medication during the 24 h preceding the blood sampling.

As shown in table 4, the mean B_{max} value for the depressed patients was significantly lower than that of the corresponding controls while the K_d values did not differ between the two groups. The presence of a significant difference in the B_{max} for ^{3}H-IMI binding between controls and untreated severely depressed patients (table 4) supports the view that the ^{3}H-IMI binding site might be involved in biochemical changes related to affective disorders. At present we cannot exclude the possibility that the decrease in B_{max} for ^{3}H-IMI binding observed in the platelets of depressed patients

Table 4 Specific ^{3}H-imipramine binding in platelets from untreated depressed patients and control volunteers

Experimental group	n	K_d (nM)	B_{max} (fmol (mg protein)$^{-1}$)
Controls	38	2.4±0.3	584±38
Depressed	27	2.8±0.3	285±22*

Patients as well as normal volunteers were of both sexes, aged 20–65 (mean age in both groups, 44 years). Depressed patients (mono- or bipolar endogenous depression or reactive depression) were free of antidepressant treatment for at least 4 weeks. Hamilton depression rating scales (NIMH, 1967, 25 items) were all higher than 39, the value for the 27 depressed patients being 51±2 (mean ±S.E.M.). Binding parameters were determined by Scatchard analysis using a minimum of six concentrations of ^{3}H-IMI between 0.4 and 8 nM, each point being determined in duplicate.
Data taken from Raisman *et al.* (1980*b*).
*$p < 0.001$ when compared with the control group.

reflects compensatory mechanisms that develop in response to the depressive states.

It is of interest to note that the group of patients studied included both reactional as well as mono- or bipolar endogenous types of depression. With the number of depressed patients studied so far (27) there were no differences between the parameters of ^{3}H-IMI binding when reactional and endogenous depressions were compared. In a similar study carried out at the NIMH, Bethesda, USA, a 40 percent decrease in the B_{max} of ^{3}H-IMI binding in platelets was found in severely depressed patients when compared with age- and sex-matched normal volunteers (S. M. Paul, personal communication). Additional studies with a larger number of depressed patients as well as psychiatric non-depressed patients are required before the biological significance and the specificity of the present findings can be fully established.

In our study there was no correlation between the severity of the depression, determined by the Hamilton Depression Rating Scale (HDRS) and the B_{max} values of ^{3}H-IMI binding in the platelets of depressed untreated patients. Although the number of patients may be too small to reach a definite conclusion, it would appear that the level of ^{3}H-IMI binding cannot be used as an index of the severity of the depression. In support of this view, in a group of 17 patients that were treated for 9 days with TCA, and at a time when the HDRS indicated a significant clinical improvement, the B_{max} values for ^{3}H-IMI binding remained essentially unchanged when compared with the initial values prior to the beginning of the treatment. A third determination was carried out when these patients were still under antidepressant medication but had already recovered to the extent of having HDRS values within the normal range. In this group the B_{max} of ^{3}H-IMI binding was practically the same as those in the previous two determinations. There was, however, in the patients treated with TCA, a small but significant increase in

the K_d values for ^{3}H-IMI binding. Whether the increase in K_d values observed during treatment with TCA represents an artifact due to the residual antidepressant drug still present in the platelet membranes during the binding assay, or whether this change in apparent affinity constant is related to the clinical improvement of depression, is still an open question.

We do not have as yet information on ^{3}H-IMI binding parameters (K_d and B_{max}) in platelets of the patients shown in table 4 at a time when they are clinically recovered from their depression and are no longer on antidepressant medication. These results may help to clarify the question as to whether the low level of ^{3}H-IMI binding indicates a susceptibility or biological predisposition to depression or reflects changes in mood.

As discussed below, chronic treatment with TCA in cats produces a decrease in B_{max} for ^{3}H-IMI binding in the brain as well as in platelets (Arbilla *et al.*, 1980). Such an effect elicited by the treatment with antidepressant drugs could mask the increase in the B_{max} values in platelets from depressed patients who undergo clinical improvement to the extent of reaching normal values in their HDRS.

While the results shown in table 4 support the view that the high affinity ^{3}H-IMI binding may represent a useful biological marker in affective disorders, several major questions remain open. The first one concerns the possibility that the changes in ^{3}H-IMI binding in platelets observed in severely depressed patients might reflect similar differences in the brain. Studies of ^{3}H-IMI and other receptor binding assays in post-mortem brain samples from depressed patients as well as suicides are currently under way. Age- and sex-matched samples from accident victims with no history of mental disorders are determined simultaneously. These studies are long term projects and a clear answer to this question may require several years of research.

As already mentioned, in cats treated chronically with IMI there is a parallel decrease in B_{max} of ^{3}H-IMI binding in the hypothalamus and in platelets (Arbilla *et al.*, 1980). It is tempting to extrapolate these parallel changes in ^{3}H-IMI binding in the hypothalamus and platelets of cats to suggest a similar parallelism for the decrease in ^{3}H-IMI binding in platelets observed in depressed untreated patients. If these findings reflect similar changes in the brain, then the second question is the following: how does the message or a certain signal from the brain reach the platelets? In other words, through which mechanism is the information regarding the biochemical changes at the level of ^{3}H-IMI binding in the brain able to reach the circulating platelets? In trying to answer this question, it is tempting to speculate that a humoral factor may be involved and that a 'mood-regulating' substance may be present in the circulation. This endogenous mood-regulating substance could be different from the neurotransmitter involved in the site identified for ^{3}H-IMI binding in nerve terminals, namely 5-HT.

The third question is whether the decrease in B_{max} for ^{3}H-IMI binding observed in platelets from untreated severely depressed patients reflects the cause of depression or may in fact represent an unsuccessful compensatory attempt to overcome the depressive state. The paradoxical observation that

chronic treatment with TCA as well as the untreated severe depression both result in lower levels of ^{3}H-IMI binding in platelets may be indirect evidence in support of the view that we may be dealing with an insufficient compensatory response. While this question is still open it is of interest to note that there are many examples of homeostatic mechanisms which operate under physiological as well as physiopathological conditions (for example the baroreceptor reflexes which control the level of blood pressure). Similarly, one can speculate about biochemical changes which are geared to 'mood improvement' in the depressive state. At present there is no satisfactory answer to this highly speculative question, but it is possible that research in this area may prove to be both challenging and rewarding.

An analysis of the B_{max} values of ^{3}H-IMI binding in the normal population suggests a possible bimodal distribution (Raisman *et al.*, 1980*b*) while the distribution of the B_{max} values in the depressed population corresponds approximately to the low B_{max} population in the controls. Although a larger study is required to confirm these results, one possible interpretation of these data would be that the B_{max} of ^{3}H-IMI binding in platelets represents a biological marker for the susceptibility to depression. Interestingly enough, a bimodal distribution of CSF levels of the 5-HT catabolite 5-HIAA has been reported (Åsberg *et al.*, 1976). In addition severe depression as well as suicide attempts were reported to be correlated with the subpopulation of low 5-HIAA levels in CSF (Åsberg *et al.*, 1976; Åsberg and Bertilsson, 1979).

EFFECTS OF CHRONIC TREATMENTS ON ^{3}H-IMIPRAMINE BINDING

An important clinical observation concerns the fact that TCA produce their clinical therapeutic effects only after they are administered for at least 10–15 days. Nevertheless, some of the biochemical and pharmacological effects of TCA (for example inhibition of neuronal uptake of monoamines and their effects on paradoxical sleep) are already present after the first day of medication.

Therefore, considerable attention has been devoted to the biochemical and pharmacological effects of the chronic administration of TCA in an attempt to clarify the reasons for this latency period in the appearance of therapeutic action.

In rats or cats treated chronically with TCA a decrease in B_{max} for β-adrenoceptor binding has been demonstrated in various brain regions (Banerjee *et al.*, 1977; Wolfe *et al.*, 1978). Chronic treatment with TCA also reduced the B_{max} for ^{3}H-IMI binding in the rat and cat brain (Raisman *et al.*, 1980*a*; Kinnier *et al.*, 1980; Arbilla *et al.*, 1980). Both for β-adrenoceptor and for ^{3}H-IMI binding, the changes were restricted to the B_{max} while the corresponding K_d values remained unchanged. The 'down regulation' of β-adrenoceptors and the ^{3}H-IMI binding site observed during the chronic

administration of TCA do not seem to be interrelated because after chronic treatment with IMI, β-adrenoceptor binding decreased in the cerebellum while ^{3}H-IMI binding remained unchanged in this region (Kinnier *et al.*, 1980). When rats were treated chronically with a non-tricyclic antidepressant, iprindol, there were no decreases in ^{3}H-IMI binding in the rat brain (Kinnier *et al.*, 1980).

The down regulation of β-adrenoceptors observed in the CNS after the chronic administration of TCA is paralleled with decreases in sensitivity in the NE-sensitive adenylate cyclase (Vetulani and Sulser, 1975).

Recently it was reported that adding an α-adrenoceptor blocking agent to the TCA treatment accelerates the development of β-adrenoceptor subsensitivity in the rat brain (Paul and Crews, 1980). Since α-adrenoceptor blocking agents enhance the stimulation-evoked release of NE by acting on presynaptic inhibitory α_2-adrenoceptors (Langer 1974, 1977) and since the down regulation of β-adrenoceptors by TCA requires the presence of noradrenergic nerve endings (Schweitzer *et al.*, 1979), these results further support the view that chronic administration of TCA decreases β-adrenoceptor density through the long-lasting increase in the concentration of NE in the synaptic cleft. This increase in the concentration of the noradrenergic transmitter at the level of the synapse may also be responsible for the subsensitivity of presynaptic inhibitory α_2-adrenoceptors after the chronic, but not the acute, administration of DMI (Crews and Smith, 1978; Langer, 1978).

Chronic electroconvulsive shock in rats, which also mimicks antidepressant therapy, is known to decrease the density of β-adrenoceptors in the CNS (Bergstrom and Kellar, 1979*a*; Pandley *et al.*, 1979) and also the sensitivity of the NE-dependent adenylate cyclase (Vetulani and Sulser, 1975). More recently we have found that chronic electroconvulsive shock therapy in rats leads also to a decrease in B_{max} for ^{3}H-IMI binding in the rat cerebral cortex (Briley, Green and Langer, unpublished observations).

A decrease in the latency period for the onset of paradoxical (REM) sleep is a consistent observation in endogenous depression. In a recent study in which rats were subjected to paradoxical sleep deprivation for 72 h, there were significant decreases in the B_{max} for both β-adrenoceptor and ^{3}H-IMI binding in the cerebral cortex and the hypothalamus (Mogilnika *et al.*, 1980). These results are of particular interest because total sleep deprivation or paradoxical sleep deprivation alone and in association with TCA has been reported to be useful in the therapy of depression.

The fact that several treatments that are known to be effective in the therapy of depression lead to decreases in B_{max} of ^{3}H-IMI binding in the brain support the view that the TCA binding site appears to be pharmacologically relevant and it may be related to the site of action of antidepressant drugs. The ^{3}H-IMI binding site may also in some way be linked to the biochemical changes that occur in affective disorders.

In spite of the fact that the ^{3}H-IMI binding site appears to be related to the neuronal uptake mechanism for 5-HT, there is as yet no clear pattern for the effects of chronic administration of TCA on 5-HT receptor binding. Some groups reported a decrease in ^{3}H-5-HT binding after the chronic admini-

stration of TCA (Segawa *et al.*, 1979) while others failed to report such changes (Bergstrom and Kellar, 1979*b*; Savage *et al.*, 1980). It appears that chronic treatment with TCA produces a selective down regulation of 5-HT_2 but not of 5-HT_1 receptors in the rat brain (S. H. Snyder, personal communication).

Concerning the studies on the effects of chronic treatments with antidepressants on the binding parameters for various receptors, a word of caution appears to be appropriate. To start with, most studies employ rather high doses of TCA and it cannot be excluded that one is looking at the effect of subtoxic rather than therapeutic doses of these drugs. In addition, it is not clear as to whether the down regulation of various receptors after chronic antidepressant treatment represents an adaptative change unrelated to the therapeutic effects of these drugs or if it is directly related to the mechanism of action of these antidepressant drugs.

In spite of these critical comments there is no doubt that studies based on the chronic administration of antidepressant drugs represent a useful model to clarify their mechanism of action. The latency of approximately 2 weeks for the development of the antidepressant effects of the tricyclic drugs in man continues to be an important problem in the field of the pharmacotherapy of depression.

CONCLUDING REMARKS

The high affinity ^{3}H-IMI binding site fulfills most of the criteria required for the binding of a ligand to a receptor site. The evidence available so far is compatible with the view that ^{3}H-IMI binds with high affinity to the neuronal uptake site for 5-HT. Although it is not yet clear if ^{3}H-IMI binds to the recognition site, an associated protein or the actual transport mechanism for 5-HT on 5-HT nerve terminals, it is likely that ^{3}H-IMI binding is somehow associated with the site of neuronal uptake of this neurotransmitter. It follows from this proposal that serotoninergic neurotransmission may be related to the pathogenis of depression and/or the mechanism of action of some antidepressant drugs.

It is likely that ^{3}H-IMI binding may prove to be a useful tool for studies at the biochemical and molecular level of the physiopathology and clinical pharmacology of affective disorders. Studies of the effects of drugs on ^{3}H-IMI binding may also provide an insight on the mechanism of action of antidepressant drugs as well as becoming a useful screening method in the search for new antidepressant drugs.

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A radioreceptor assay for neuroleptic drugs

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INTRODUCTION

The routine monitoring of blood levels of drugs such as digoxin, gentamicin, lithium and anticonvulsants has been recognized as a valuable and necessary aid to clinical management. A similar approach to the control of therapy with antipsychotic, neuroleptic drugs has been complicated by (1) the large number of drugs presently in use, (2) their even larger number of active and inactive metabolites, (3) the lack of satisfactory assay procedures, and (4) a general lack of recognition of the toxic effects which may result from high dose levels.

Recent evidence indicates that long term neuroleptic treatment may be accompanied by adverse effects, such as persistent tardive dyskinesia (Baldessarini and Tarsy, 1979). Although the symptoms of acute over-dosage with neuroleptics, that is extrapyramidal Parkinsonian-like symptoms, are easily discernable, it now appears that tardive dyskinesia, a serious and sometimes irreversible sequella of neuroleptic therapy, may be associated not only with long term, high dose treatment (Klawans, 1973) but also with high blood levels which can occur even at a 'recommended' dose level (Jeste *et al.*, 1979). Thus it is important to determine whether patients who are not improving with therapy are on a potentially detrimental dose schedule. Because there are such large interindividual differences in the bioavailability of neuroleptics at a given dose due to differences in absorption, intestinal microsomal oxidation, first-pass hepatic metabolism, and plasma protein

binding, the recommended dosages may be either too low to achieve optimal therapeutic results or be unnecessarily high. Thus serum neuroleptic concentration may provide a more sensitive and safe indicator of optimum dose regimens.

Although it is tempting to believe that plasma levels may provide a precise way of monitoring doses of drugs to achieve optimal clinical treatment, evidence to date to support this concept as regards neuroleptic drugs is tenuous (Lader, 1976; Cooper *et al.*, 1976; Cooper, 1978). This, in part, results from the techniques of measurement which have been difficult and not easily reproducible. The drugs themselves undergo extensive metabolism and the metabolic fate of any particular drug varies considerably between subjects. Some of these metabolites are biologically inert; however, many are even more potent than the parent compound (Creese *et al.*, 1978*b*). Thus measurement of the parent compound alone may be of relatively little value and, in some cases, quite misleading.

The assay techniques previously available have all had a number of inadequacies (Usdin, 1971): the fluorimetric procedures were often poorly reproducible, lacked sensitivity and could both be interfered with by, or not measure, active and inactive metabolites of the parent compound. The recent technical developments of mass spectrometry, high performance liquid chromatography and gas chromatography have markedly improved both the specificity and the sensitivity of assays for the measurement of neuroleptic drugs. However, these methods are both complex and time-consuming, allowing measurements of only a small number of samples per day, and, being highly selective, the methods must be modified to measure different compounds. Furthermore, they require expensive equipment and highly educated and experienced personnel. Another major problem is the inability to ascertain all the potential active metabolites that may be present, and thus to quantify them. A number of radioimmunoassays (RIA) are presently available for some neuroleptic drugs and their development has certainly enabled a marked increase in analytical capacity. However, RIA suffer from similar inadequacies of both the fluorimetric and spectrometric/chromatographic techniques: active metabolites may not be measured while inactive metabolites may also have substantial affinity for the antibody.

Thus it is not surprising that it has proved difficult to demonstrate a direct relationship between plasma concentrations of neuroleptic drugs and their clinical efficacy. Such clinical studies have further been complicated by the problems of diagnosis, lack of homogeneity in clinical populations, non-compliance and spontaneous remissions. That the kinetics and metabolism of so few neuroleptics have been well characterized attests to the inherent complexity of these studies.

Many of these problems have been solved with the development of a radioreceptor binding assay (RRA) for the measurement of blood levels of neuroleptics (Creese and Snyder, 1977). In several respects the RRA technique resembles a RIA. However, instead of antibodies it utilizes tissue-bound dopamine (DA) receptors with specific high affinity for neuroleptic drugs. The method is adequately sensitive and has a very high

analytical capacity. A major improvement over the RIA method is that it is chemically non-specific. Any drug present in the blood which has DA receptor blocking activity (and thus possesses neuroleptic activity) is measured, irrespective of its chemical structure. Thus the RRA is pharmacologically specific in that, when properly performed, it measures the DA receptor blocking activity of all substances present in the blood regardless of whether they are the actual drug administered or active metabolites produced *in vivo*. Thus, it is not even necessary to have previously identified potentially active metabolites. The RRA will measure the combined DA receptor blocking activity of all administered drugs and their active metabolites, although not determining their concentrations separately. Of course the utility of this assay depends on the demonstration that neuroleptic drugs do indeed possess their clinical efficacy through blocking DA receptors in the brain. We will now briefly review the evidence that the biochemical mechanism of action of neuroleptic drugs is the blockade of CNS DA receptors.

IDENTIFICATION OF 'NEUROLEPTIC' DOPAMINE RECEPTORS IN THE BRAIN

Much biochemical and behavioral evidence suggests that neuroleptic drugs alleviate schizophrenic symptoms and induce Parkinsonian-like extrapyramidal side effects by blocking DA receptors in the brain (Snyder *et al.*, 1974). While molecular modeling indicates how phenothiazines can assume the preferred conformation of DA, the conformation of butyrophenones at their receptor sites is unclear from such analyses (Feinberg and Snyder, 1975; Koch, 1974). Nevertheless, in animal tests both phenothiazines and butyrophenones block DA-mediated behaviors induced by DA agonists in proportion to their clinical potencies (Janssen and van Bever, 1978).

The first direct biochemical evidence that neuroleptics affect postsynaptic DA receptors derived from studies of a DA-sensitive adenylate cyclase. Greengard and associates (Greengard, 1976) and Iversen and colleagues (Iversen, 1975) demonstrated an enzyme in homogenates of rat corpus striatum which caused a DA-stimulated accumulation of cyclic AMP (cAMP). Phenothiazines were effective inhibitors of the enzyme with influences which, in general, paralleled their pharmacological potencies in animals and man. However, there were marked discrepancies in the case of butyrophenones. Haloperidol, which clinically and pharmacologically is about 100 times more potent than chlorpromazine (CPZ), appeared weaker than, or at best equal to, CPZ in influence on the adenylate cyclase. Similarly, the most potent known butyrophenone, spiroperidol or spiperone, which is about five times more potent than haloperidol in intact animals, was weaker than haloperidol and CPZ on the adenylate cyclase. In fact, when considering a large number of neuroleptic drugs from different structural classes there is no correlation between their ability to block DA-stimulated cAMP production and their potencies in man as antischizophrenic agents (Snyder *et al.*, 1975).

These discrepancies could be contrued as a challenge to the hypothesis that antischizophrenic drugs produce their therapeutic effects by blocking postsynaptic DA receptors in the brain. However, in 1976 it became evident that like cholinergic, histaminergic and adrenergic receptors, there must be multiple types of DA receptors (Creese and Sibley, 1979). The clinical effects of neuroleptic drugs appear to be mediated through the blockade of DA receptors which are not associated with adenylate cyclase (Creese *et al.*, 1976; Seeman *et al.*, 1976). These DA receptors can be identified by their selective labeling with low concentrations of high specific activity ^{3}H-butyrophenones. Initial studies utilized ^{3}H-haloperidol but the availability of ^{3}H-spiperone, which has a higher affinity and specific activity, has superseded its use. The most important technical hurdle to overcome in demonstrating specific DA receptor binding is to minimize the amount of non-specific association of the ligand with membrane components, since such non-specific sites exceed, by a large margin, the number of DA receptors. Evidence that these ligands in fact label the physiologically relevant DA receptor derive from several sources (Creese *et al.*, 1978*a*). First of all, the regional distribution of ^{3}H-butyrophenone binding parallels regional variations in endogenous DA concentration, with highest levels of binding in the corpus striatum, somewhat lower levels in areas of the limbic system that possess DA pathways such as the nucleus accumbens and olfactory tubercle, and very little binding in other regions. Drug specificity for displacing ^{3}H-spiperone from receptors also provides important evidence. Among adrenergic agonists, DA is most potent, norepinephrine less so and isoproterenol weakest. The direst-acting DA agonist apomorphine is more potent than DA itself.

Most importantly, the affinities of neuroleptics of diverse structures for DA receptors labeled with ^{3}H-haloperidol or ^{3}H-spiperone correlate closely with their pharmacological actions in animals and man (Creese *et al.*, 1976) (table 1). The average clinical daily dose of numerous neuroleptics as antischizophrenic drugs correlates highly with their absolute affinities for ^{3}H-butyrophenone DA receptor binding sites ($r=0.87, p<0.001$). Because the clinical activity of drugs is difficult to quantify, the binding properties of these neuroleptics have been related to their acute pharmacological actions in animals. Apomorphine produces a stereotyped sniffing, licking and gnawing in rodents which derives from stimulation of postsynaptic DA receptors in the corpus striatum and nucleus accumbens. The ability of neuroleptics to block apomorphine-induced stereotyped behavior predicts the clinical potencies of drugs and has been used by the pharmaceutical industry as a screening test. Neuroleptic affinity correlates highly with antagonism of apomorphine stereotypy ($r=0.92$, $p<0.001$). Similarly, neuroleptic antagonism of amphetamine-induced stereotypy also is highly correlated with neuroleptic DA receptor affinity ($r=0.93$, $p<0.001$). Neuroleptics are also among the most effective antiemetics known, presumably blocking DA receptors in the chemoreceptor trigger zone in the brain stem. The ability of neuroleptics to prevent apomorphine-induced emesis also correlates closely with their affinity for ^{3}H-butyrophenone binding sites ($r=0.93$, $p<0.001$). Thus, affinity of an unknown compound for ^{3}H-

Table 1 Antischizophrenic drugs: comparison of affinities for ^{3}H-haloperidol binding sites with *in vivo* pharmacological potencies

Drug	Inhibition of ^{3}H-haloperidol binding, K_i (nM)	Inhibition of apomorphine stereotypy in rat, ID_{50} (μmol kg^{-1})	Inhibition of apomorphine-induced emesis in dog, PD_{50} (μmol kg^{-1})	Inhibition of amphetamine stereotypy in rat, ID_{50} (μmol kg^{-1})	Average clinical daily dose (μmol kg^{-1})
Spiroperidol	0.25±0.02 (4)	0.177	0.0006	0.051	0.058
Benperidol	0.33±0.02 (4)	0.118	0.0012	0.071	0.060
Clofluperol	0.50±0.03 (4)	0.198		0.117	0.077
(+)-Butaclamol	0.55±0.09 (8)		0.095		2.14
Fluspirilene	0.60±0.13 (4)				0.066
Pimozide	0.80±0.07 (4)	0.370	0.024	0.242	0.108
Trifluperidol	0.95±0.19 (3)	0.067	0.016	0.056	0.096
Droperidol	1.0 ±0.10 (4)	0.185	0.003	0.095	
α-Flupenthixol	1.1 ±0.22 (4)	0.867		0.650	0.099
Fluphenazine	1.2 ±0.12 (6)	0.255	0.012	0.196	0.168
Bromoperidol	1.4 ±0.15 (4)	0.324	0.038	0.126	0.153
cis-Thiothixene	1.4 ±0.11 (4)	1.42		0.803	0.393
Haloperidol	1.5 ±0.14 (9)	0.532	0.050	0.101	0.152
Moperone	1.9 ±0.26 (4)	0.638	0.050	0.059	0.802
Triflupromazine	2.1 ±0.12 (4)	4.62	0.50	0.746	4.59
Trifluoperazine	2.1 ±0.34 (4)	1.14	0.08	0.520	0.297
Fluanisone	3.8 ±0.80 (4)	6.17	0.40	0.757	3.44
Penfluridol	5.6 ±1.40 (7)				0.466
Azaperone	10.0 ±0.60 (4)	27.4		9.16	
Chlorpromazine	10.3 ±0.2 (5)	18.3	2.0	3.09	12.0
Thioridazine	14.0 ±0.2 (5)				12.6
Pipamperone	31.3 ±5.2 (4)	635	3.5	11.1	11.1
Promazine	71.5 ±3.2 (4)	>250	60	99.6	33.3
Clozapine	100 ±6 (6)				24.6
Promethazine	238 ±32 (4)				
Correlation with ^{3}H-haloperidol binding		$r = 0.94$ $P<0.001$	$r = 0.93$ $P<0.001$	$r = 0.92$ $P<0.001$	$r = 0.87$ $P<0.001$

Drugs are listed in order of affinity for ^{3}H-haloperidol binding sites of calf striatal membranes. For each drug competition of binding of both ligands was measured at two to four concentrations of drug, and 50 percent inhibitory concentrations, IC_{50}, were derived by log-probit analysis. These values were converted to apparent K_i values according to the equation $K_i = IC_{50}/(1 + C/K_D)$, where C is the concentration of radioactive ligand and K_D is its dissociation constant. Each value is the mean±standard error of the mean for three to ten determinations (N is given in parentheses). *In vivo* animal data and clinical potencies were calculated from published results; ID_{50} and PD_{50} are 50 percent inhibitory dose and 50 percent protective dose, respectively. (Modified from Creese *et al.*, 1976).

butyrophenone binding sites is highly suggestive that the compound will exhibit antidopaminergic and antischizophrenic activity *in vivo.*

That the DA receptor binding technique is capable of measuring the blocking activity of metabolites produced *in vivo* has been demonstrated by detailed studies of the influence of a variety of chlorpromazine (CPZ) metabolites and derivatives on the binding of ^{3}H-haloperidol to brain membranes (Creese *et al.*, 1978*b*) (tables 2 and 3). The various possible hydroxylations of the aromatic rings of CPZ produce interesting patterns of differing affinities for DA receptors. 7-Hydroxychlorpromazine (7-OH-CPZ) is only slightly less potent than CPZ in competing for ^{3}H-haloperidol binding, which accords with evidence in animal studies of its neuroleptic activity. Blood levels of 7-OH-CPZ are comparable to those of the parent drug CPZ and, in some studies, are better predictors of clinical response than levels of the parent drug (Sakalis *et al.*, 1973). 3-Hydroxylation increases potency, 3-OH-CPZ being about twice as potent as CPZ itself in competing for ^{3}H-haloperidol binding. The 3,7-diOH-CPZ derivative is similar in potency to CPZ itself. 8-Hydroxylation of CPZ essentially abolishes potency. However, addition of a 3-OH group appears to restore some activity, while adding a 7-OH group does not improve the activity of 8-OH-CPZ. Whereas 7-OH-CPZ possesses considerable potency, 7-methoxy-CPZ is less than 1 percent as potent as CPZ itself and 3,7-dimethoxy- and 7,8-dimethoxy-CPZ are totally inactive. Chlorpromazine-5-oxide (CPZ sulfoxide) is inactive in numerous pharmacological screens for neuroleptic activity and lacks activity in competing for ^{3}H-haloperidol binding. Moreover, CPZ-*N*-oxide and CPZ-5,*N*-dioxide are also inactive. Removal of one or both of the terminal *N*-methyl groups of CPZ also reduces potency in competing for ^{3}H-haloperidol binding sites.

Table 2 Dopamine receptor affinity of hydroxylated chlorpromazine metabolites and derivatives

Compound	IC_{50} (nM)	Relative potency (chlorpromazine=1)
3,7-Dihydroxychlorpromazine	2.8± 0.2 (4)	2.6
3-Hydroxychlorpromazine	3.2± 0.2 (5)	2.3
Chlorpromazine	7.2± 0.8 (11)	1.0
7-Hydroxychlorpromazine	10 ± 1 (5)	0.7
3,8-Dihydroxychlorpromazine	270 ± 40 (4)	0.03
3,7,8-Trihydroxychlorpromazine	890 ±100 (5)	0.01
8-Hydroxychlorpromazine	>1000 (3)	<0.01
7,8-Dihydroxychlorpromazine	>1000 (3)	<0.01

Rat striatal membranes were incubated in triplicate with 1.5 nM ^{3}H-haloperidol and three or more concentrations of the compound under study. IC_{50} values were determined from log probit plots of the displacement of specific ^{3}H-haloperidol binding. IC_{50} values are presented as the mean±S.E.M. for the number of experiments noted in parentheses and are the concentration of drug required to inhibit 50 percent of specific ^{3}H-haloperidol binding. (From Creese *et al.*, 1978*b*.)

Table 3 Dopamine receptor affinity of chlorpromazine metabolites and derivatives

Compound	IC_{50} (nM)	Relative potency (chlorpromazine=1)
3,7-Dihydroxychlorpromazine	2.8 ± 0.2 (4)	2.6
3-Hydroxychlorpromazine	3.2 ± 0.2 (5)	2.3
Chlorpromazine	7.2 ± 0.8 (11)	1.0
7-Hydroxychlorpromazine	10 ± 1 (5)	0.7
Desmethylchlorpromazine	20 ± 2 (3)	0.4
Didesmethylchlorpormazine	71 ± 12 (5)	0.1
2-Cl-10-(3-Dimethylamino-2-hydroxypropyl) phenothiazine	140 ± 10 (4)	0.05
3,8-Dihydroxychlorpromazine	270 ± 40 (4)	0.03
3,7,8-Trihydroxychlorpromazine	890 ±100 (5)	0.01
7-Methoxychlorpromazine	1000 ±140 (6)	<0.01
8-Hydroxychlorpromazine	>1000 (3)	<0.01

IC_{50}>1000 nM—three replications: 7,8-dihydroxychlorpromazine, 2-Cl-7,8-dihydroxy-10-(3-dimethylamino-2-hydroxypropyl)phenothiazine, 2-Cl-7,8-dioxo-10-(3-dimethylamino-2-hydroxylpropyl)phenothiazine, 3-(2-Cl-10-phenothiazinyl)propionic acid, chlorpromazine-5-*N*-dioxide, chlorpromazine-*N*-oxide, chlorpromazine-5-oxide (sulfoxide), desmethylchlorpromazine-5-oxide, didesmethylchlorpromazine-5-oxide, 3,7-dimethoxychlorpromazine, 7,8-dimethoxychlorpromazine, 7,8-dioxochlorpromazine. Experiments were conducted as detailed in table 2. Metabolites with IC_{50}>1000 nM are considered inactive. For example the weak or inactive phenothiazines, promazine and promethazine, have IC_{50} values of 140 and 500 nM respectively. (From Creese *et al.*, 1978*b*.)

The DA receptor binding affinities are in good agreement with *in vivo* pharmacological activity. For example, Meltzer *et al.* (1977) have investigated the effects of CPZ and its metabolites on prolactin levels in rats. Neuroleptics increase blood levels of prolactin by blocking a dopaminergic inhibitory influence on pituitary prolactin secretion (Clemens *et al.*, 1974). In agreement with the receptor affinities found in our binding studies, 7-OH-CPZ produced increases in plasma prolactin equivalent to those produced by CPZ. 8-Hydroxy-CPZ, 7,8-dihydroxy-CPZ, 7-methoxy-CPZ and CPZ-5-oxide, which are at least 100-fold weaker than CPZ in displacing ^{3}H-haloperidol binding, did not alter prolactin levels, even at doses up to five times higher than CPZ.

DEVELOPMENT OF THE NEUROLEPTIC RADIORECEPTOR ASSAY

In this assay (Creese and Snyder, 1977), serum is used without any extraction or other purification procedure. Minimal interference by serum is

apparent from experiments in which 15 μl of serum in a total assay volume of 1 ml reduced ^{3}H-haloperidol or ^{3}H-spiperone binding by only 10–15 percent. Increasing volumes of serum reduced binding linearly with about 30 percent inhibition occurring with 150 μl of serum. Small volumes of serum reduce both specific and non-specific ^{3}H-ligand binding to similar extents, suggesting that serum proteins bind the ^{3}H-ligand, making less available for interactions with brain membranes. The degree of inhibition of ^{3}H-ligand binding by control sera from several laboratory personnel was uniform. Specific binding of ^{3}H-haloperidol in the presence of 15 μl of serum from 18 different subjects was 91±1.6 percent (total binding 2 325 cts min^{-1} blank with 0.1 mM DA 953 cts min^{-1}) of control values while specific ^{3}H-spiperone binding was 85±1.2 percent (total binding 1 264 cts min^{-1} blank with 1 mM dopamine 191 cts min^{-1}) of control for 10 different subjects. This small degree of variability in inhibition of ^{3}H-ligand binding by control sera falls within the error for repeated determinations of the same sample.

Neuroleptic present in a serum sample reduces ^{3}H-ligand binding beyond the small reduction attributable to serum alone and the degree of inhibition of specific ^{3}H-ligand binding is proportional to the amount of neuroleptic present. By constructing a standard curve of inhibition of specific ^{3}H-ligand binding by known amounts of the drug the amount present in a serum sample can be easily determined.

This method requires that the presence or absence of neuroleptic in a serum sample will not affect the degree of non-specific (blank) ^{3}H-ligand binding. In 46 serum samples from patients on neuroleptics, blank ^{3}H-haloperidol binding was 887±6 cts min^{-1} while non-specific binding in the presence of five control sera was 894±17 cts min^{-1}. In six patients on haloperidol, non-specific ^{3}H-spiroperidol binding was 199±4 cts min^{-1} and 198±6 cts min^{-1} in the presence of six control serum samples. Thus specific binding in the presence of patient sera can be determined by subtracting non-specific binding values obtained with control sera from total binding with patient sera. The percentage inhibition of specific ^{3}H-ligand binding (in the presence of control serum) by the patient serum (containing drugs) is then calculated and compared to a standard displacement curve for determining actual neuroleptic content.

Up to 90–99 percent of neuroleptics may be bound to blood components (Curry, 1970; Forsman and Ohman, 1977). In this assay the neuroleptic bound to blood proteins dissociates during the incubation so that total neuroleptic levels are measured. In confirmation of this assumption, in one experiment 15 nM haloperidol was preincubated for 10 min at 37 °C with control serum to allow binding to serum proteins to occur. The serum containing haloperidol was then added to a standard binding assay which was at equilibrium and it progressively reduced ^{3}H-haloperidol binding with increasing durations of incubation, with a maximal lowering by 5–10 min. The time course and extent of reduction of binding was the same for haloperidol preincubated with serum or simply dissolved in water. The maximum percentage lowering of binding was equivalent to that produced by 15 nM haloperidol added directly to the assay with no serum present.

For the neuroleptic RRA fresh rat striatum was sonicated in 100 volumes (w/v) 50 mM Tris buffer, pH 7.7 at 25 °C with a Sonifier, setting 4, for 30 s. The homogenate was centrifuged twice at 50 000*g* for 10 min at 4 °C (Sorvall RC2-B) with rehomogenization of the intermediate pellet in fresh buffer. The final pellet (which may be stored frozen) was resuspended in 125 volumes, for assays using ^{3}H-haloperidol, or 285 volumes for ^{3}H-spiperone, of freshly prepared 50 mM Tris buffer containing 0.1 percent ascorbic acid, 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$ to give a final pH of 7.1 at 37 °C. ^{3}H-Haloperidol (15 Ci mmol^{-1}, New England Nuclear) or ^{3}H-spiperone (25 Ci mmol^{-1}, New England Nuclear or Radiochemical Centre, Amersham) was diluted to 10 nM or 1 nM, respectively, in fresh 0.1 percent ascorbic acid. Borosilicate glass, 12 mm × 75 mm incubation tubes, received, in order, 15–150 μl serum (on drug therapy or drug-free), 100 μl ^{3}H-ligand, 100 μl drug for standard curve or DA for blanks (10^{-4} M for ^{3}H-haloperidol and 10^{-3} M for ^{3}H-spiperone) or the drug solvent (0.1 percent ascorbic acid), and tissue suspension to 1 ml total volume. Final concentrations of ^{3}H-haloperidol or ^{3}H-spiperone were 1 nM and 0.1 nM, respectively. The tubes were incubated at 37 °C for 10 min (^{3}H-haloperidol) or 15 min (^{3}H-spiperone) and rapidly filtered under vacuum through Whatman GF/B filters with three 5 ml rinses of ice-cold 50 mM Tris buffer, pH 7.7 at 25 °C. ^{3}H-Ligand trapped on the filters was counted by liquid scintillation spectrometry after remaining overnight in scintillation vials containing NEN formula 947 fluor (New England Nuclear). A standard displacement curve for the drug under study was determined in the presence of equal volumes of control serum with final concentrations of the drug about one-third, three times and the same as its K_i value determined previously (Creese *et al.*, 1976). A log-probit plot was used to convert the sigmoid displacement curve to a straight line so that percentage inhibition of ^{3}H-ligand binding can be easily converted to molar drug concentration (figure 1). In the presence of serum the ratio of total to non-specific binding was about 2.4 for ^{3}H-haloperidol and 6.6 for ^{3}H-spiperone, so that the latter is the preferred ligand. Plasma can be used for assays if the KCl, $CaCl_2$ and $MgCl_2$ in the buffer are replaced by 5 mM sodium EDTA, which prevents the plasma from clotting with the brain homogenate.

To examine recovery, five neuroleptics were preincubated with serum for 10 min at 37 °C, to provide time for binding to serum proteins, and subsequently assayed for neuroleptic levels. Haloperidol, fluphenazine, trifluoperazine, CPZ and thioridazine were all fully recovered in the neuroleptic RRA. To examine the recovery of neuroleptics from the blood of patients treated *in vivo,* we measured blood levels in four patients receiving oral doses of haloperidol both after extraction into organic solvent (heptane–5 percent isoamyl alcohol, back extracted into 0.1 percent ascorbic acid) which should remove all drug bound to serum protein, and by adding serum directly to the binding assay as in the above method. Serum drug levels ranged between 10 and 250 nM and agreed within a mean of ±10 percent whether assays were conducted with or without extraction.

In general clinical practice, since varying degrees of metabolism of the parent compound will have occurred, the neuroleptic serum level of the

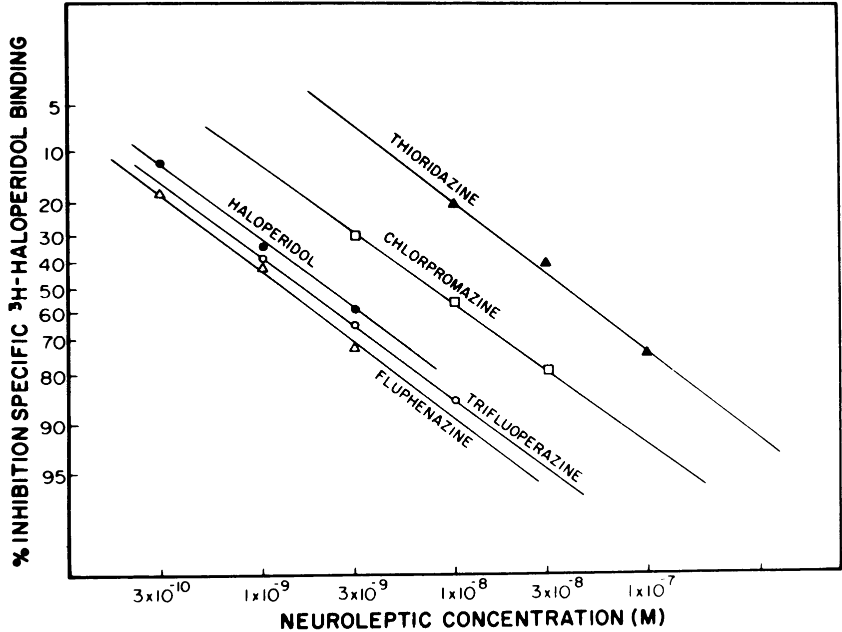

Figure 1 Log-probit plots of neuroleptic inhibition of specific ^{3}H-spiroperidol binding to rat striatal membranes. ▲—▲, Thioridazine; □—□, chlorpromazine; O—O, haloperidol; Δ—Δ, fluphenazine. (From Creese and Snyder, 1977.)

patient is determined from the standard displacement curve of inhibition of ^{3}H-spiperone binding by CPZ and is expressed as 'CPZ equivalents'. For example, the neuroleptic serum level of a patient treated with Haldol might be 50 ng ml^{-1} CPZ equivalents; that is the DA receptor blocking activity of the serum is equal to that of a serum containing 50 ng ml^{-1} added CPZ. If one were to convert this level to haloperidol equivalents, one needs to know that the IC_{50} of haloperidol is about sixfold lower than the IC_{50} of chlorpromazine. Thus 50 ng ml^{-1} CPZ is equivalent to about 8 ng ml^{-1} haloperidol. However, one would still need to express the blood level as 'haloperidol equivalents', because the DA receptor blocking activity could be due to both haloperidol and any active metabolites formed *in vivo.* The 'equivalent' scale was chosen because the assay does not measure a specific neuroleptic in the clinical situation, but rather total DA receptor blocking activity.

The sensitivity of the RRA is largely determined by the potency of a neuroleptic to displace ^{3}H-spiperone from its receptor sites. The IC_{50} value, or concentration of the drug required to displace 50 percent of the specifically bound ^{3}H-spiperone, is the crucial value in determining sensitivity. The lower the IC_{50}, the greater the sensitivity. The formula relating IC_{50} to receptor affinity is $IC_{50} = K_i\ (1 + [L]/K_D)$, where K_i is the inhibition constant of the receptor binding of the drug, [L] is a concentration of ^{3}H-spiperone and K_D is a dissociation constant of ^{3}H-spiperone from its receptor sites.

From this formula we can see that two determinants of increased sensitivity are (1) a low K_i or high affinity of the displacing drug for the receptor; and (2) utilization of a low concentration of ^{3}H-spiperone relative to its K_D. The assay is sensitive enough to measure the low blood levels of the most potent neuroleptics since they have a corresponding higher affinity for DA receptors. Another determinant of sensitivity is the dilution of the serum sample which occurs in the assay. Of course the greater the dilution the lower the sensitivity. Thus, increasing the volume of the serum sample and decreasing the total incubation volume increases sensitivity. However, this is limited practically by the extent to which serum can be added without drastically disturbing the receptor assay.

THE WELLCOME® NEUROLEPTIC ASSAY KIT

The Wellcome Reagents Division of Burroughs Wellcome Company has developed a neuroleptic assay kit for potential clinical use (Lader, 1980). The kit differs in a number of ways from the laboratory protocol detailed above. Calf or bovine caudate tissue is used instead of rat striatum. Furthermore, the receptor-rich tissue is lyophilized for ease of storage and transportation. For a typical batch of material 40 g of calf caudates are homogenized for 30 s in a Silverson homogenizer, Model L2R, in 500 ml, 200 mM phosphate buffer, pH 7.4. The ligand, ^{3}H-spiperone, is also added to the tissue preparation at 1.9 nmol, diluted into 200 ml phosphate buffer with the final volume of the caudate/ligand mixture adjusted to 1250 ml. The resulting suspension is mixed on a magnetic stirrer for at least 30 min at room temperature before being aliquoted and lyophilized. After reconstitution of the dried material with four times the original volume of deionized water, the concentration of caudate tissue is 8 mg ml^{-1} and tracer 0.38 pmol ml^{-1}. The freeze-dried tissue–tracer mixture is stored at 4 °C; after reconstitution it can be stored at 4 °C for up to 2 days or at −20 °C for up to 6 months. A set of six freeze-dried standards of 0, 15, 30, 100, 300 and 1000 nM haloperidol in serum are also included. The non-specific binding is measured in the presence of 100 μM haloperidol. The standards are prepared in pooled normal human serum so that the neuroleptic content of a serum sample can be read directly from the standard curve (which is also conducted in the presence of serum). Although this assay presently uses 'haloperidol equivalents', it will also be available with the more generally acceptable CPZ equivalent standard curve.

The assay is carried out in 12 mm×55 mm polypropylene tubes (Walter Sarstedt UK). Triplicate tubes are set up for measuring non-specific binding in the presence of 100 μM haloperidol, total binding, haloperidol standards and patient samples. The incubate consists of 100 μl standard or sample and 500 μl reconstituted tissue–tracer mixture. The final concentration of tracer in the incubate is 0.3 nM. After mixing, the tubes are incubated in a water bath at 37 °C for 30 min. Cols saline (2.5 ml at 4 °C) is then added to the

tubes which are centrifuged for 20 min at 4 °C at not less than 100**g**. The supernatant is decanted by aspiration and the tissue pellets suspended in 1.5 ml scintillation cocktail before counting by liquid scintillation spectrometry. Dopamine blocking activity of drug in serum samples is calculated from logit-log calibration curves and results expressed as neuroleptic units in terms of the haloperidol standards.

Table 4 shows the relative potencies of various neuroleptics and other psychotropic drugs in the Wellcome® Neuroleptic Assay Kit (Lader, 1980). As can be seen, the relative potencies of various neuroleptics are very similar to those previously demonstrated for fresh or frozen bovine striatal tissue. Lyophilization, *per se*, does not appear to change drastically the receptor recognition properties. However, it is apparent that the Wellcome® Neuroleptic Assay Kit is somewhat less sensitive than the laboratory pro-

Table 4 Relative potencies of neuroleptic and other psychotropic drugs

Drug		IC_{50}(nM)		Potency relative
		Mean	S.D.	to haloperidol
Neuroleptics				
Haloperidol	(9)	61.9	13.2	100
Spiroperidol	(4)	1.86	0.5	3330
Fluphenazine	(3)	17.5	1.9	357
Perphenazine	(3)	37.5	3.7	164
Trifluperazine	(2)	47.4	6.6	130
Pimozide	(5)	76.3	24.5	81.3
α-Flupenthixol	(4)	148.8	8.0	41.7
Chlorpromazine	(4)	338	81.4	18.3
β-Flupenthixol	(5)	499	151	12.4
Thioridazine	(4)	1361	217	4.5
Promazine	(1)	2357	—	2.6
Clozapine	(5)	3571	712	1.7
Reserpine	(1)	>4285	—	<1.4
Tricyclic antidepressants				
Amitriptyline	(2)	2072	303	2.2
Nortriptyline	(3)	6048	951	0.94
Imipramine	(1)	4286	—	1.4
Desmethyl-imipramime	(2)	6429	—	0.75
Miscellaneous drugs				
Metoclopramide	(4)	1332	271	4.7
Methysergide	(2)	1286	202	4.4
Cyclizine	(1)	15714	—	0.4

IC_{50} values (drug concentration, nM, displacing 50 percent of specific binding) were calculated from log-logit dose–response curves. Figures in parentheses indicate number of assays. This data was derived from assays using 8 mg caudate membranes per tube. (From Lader, 1980.)

tocol detailed above. The theoretical least concentration of haloperidol which can be distinguished from 'zero drug' was 10 nM (4 ng ml^{-1}). In general, if the patient sample produces less than 15 percent inhibition in the logit-log dose–response curve, one is close to a 'practical' limit of sensitivity. For haloperidol this is equivalent to 7 ng ml^{-1}. Equivalent doses of other neuroleptic drugs at 15 percent inhibition calculated from their potencies relative to haloperidol (table 4) were CPZ 105 nM (34 ng ml^{-1}), thioridazine 426 nM (170 ng ml^{-1}), and fluphenazine 5.3 nM (2.7 ng ml^{-1}).

Several tricyclic antidepressant drugs tested in the assay produced dose–response curves parallel to those of the neuroleptic drugs with potencies similar to some of them (table 4). However, no interference in the assay was seen with benzodiazepines or anticholinergic drugs (orphenadrine, procyclidine and benzhexol).

Within assay precision and between assay reproducibility were assessed by the inclusion of quality control samples in routine assays. The results are summarized in table 5. Precision and reproducibility with the laboratory method are better with coefficient of variations approaching 5 percent (Rosenblatt *et al.*, 1980).

The recovery of haloperidol and thioridazine was investigated and for the latter compared with values determined by gas chromatography (GLC). Known concentrations of haloperidol were added to pools of human serum and EDTA plasma and assayed on three separate occasions. The mean recovery of added drug was 93 percent and no significant differences were observed between recoveries from serum or plasma. Thioridazine demon-

Table 5 Precision of neuroleptic assay: quality control samples (16 assays)

	Coefficient of variation (%)	
	Within assay	Between assay
All values (nU l^{-1})	16.2	21.3
Values from 0 to 100	19.4	28.0
Values from 100 to 250	18.2	19.1
Values from 250 to 500	12.7	—
Values >500	10.2	19.0

Between assay reproducibility: results for individual quality control samples (16 assays)

Control serum	Mean content (nU l^{-1})	S.D.	Coefficient of variation (%)
1	125.6	23.5	18.7
2	591.4	105.4	17.8
3	42.3	11.8	28.0
4	161.4	31.4	19.5
5	502.6	99.8	19.9

strated recoveries in the range of 95–105 percent while recoveries as measured by GLC ranged from 88 to 110 percent.

A more complete comparison of the neuroleptic assay kit's determination of serum haloperidol levels and haloperidol serum levels measured by RIA was undertaken (haloperidol tracer and antiserum from IRE, Belgium). Sixty-six plasma samples from patients treated with haloperidol as the only neuroleptic drug were compared in the two assays using haloperidol in human serum as a standard for both. The correlation between the two methods was good ($r=0.75$, figure 2) but the slope of the regression line was 0.64, indicating that the values for the RRA were only two-thirds those of the RIA. This finding suggests that the unidentified metabolite mentioned in the product insert accompanying the RIA kit may be involved in the differences observed in the assay values, the compound being more immunologically than pharmacologically active. The two assays were further compared by measuring samples in which haloperidol alone was added to normal serum. There was excellent agreement between the two methods and recovery of added drug was close to 100 percent (table 6).

In conducting these studies it became clear that either serum or plasma (heparin or EDTA) may be used in the assays (table 7). Blood may be collected into glass or plastic syringes and transferred to glass or plastic tubes. Alternatively, samples may be collected into Royal Blue-stoppered Vacutainer® tubes (Becton Dickinson, plain or heparin). The use of other

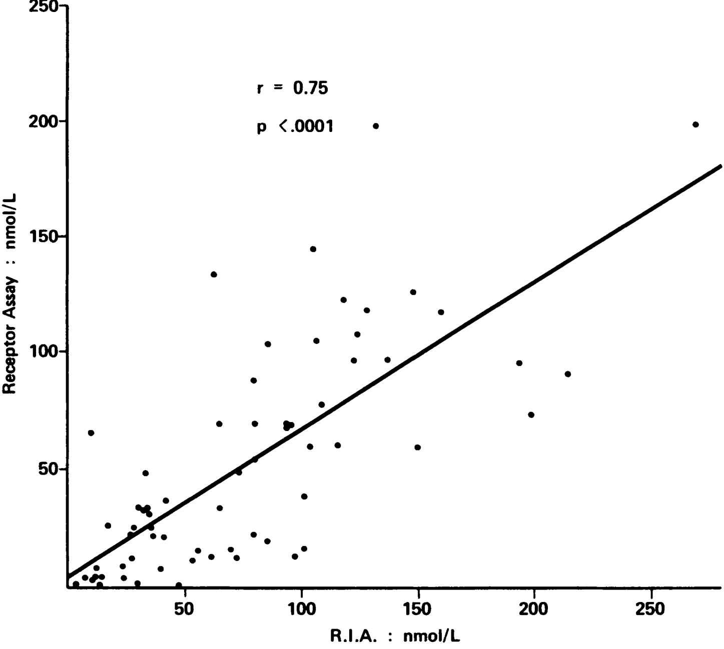

Figure 2 Correlation between radioimmunoassay and radioreceptor assay for haloperidol in clinical samples. Each point represents an individual serum sample from different patients. (From Lader, 1980.)

Table 6 Comparison of radioimmunoassay and radioreceptor assay for haloperidol added to serum *in vitro*

Expected value (nmol l^{-1})	Assayed value (nmol l^{-1})	
	RIA	RRA
134	132	142
107	116	116
100	123	120
60	67	55
54	56	59
45	47	39
27	26	26
Zero	2	3
Overall percent recovery	107	102

$r=0.991$; $p<0.0001$; slope 1.032; intercept −3.3.

Table 7 Recovery of haloperidol added to serum and plasma pools

Expected value (nM)	Assayed value (nM)								Mean
	Serum			EDTA plasma					
	n	Mean	S.D.	*n*	Mean	S.D.	*t*	*p*	recovery (%)
300	24	255	76	24	281	83	1.17	0.25	89
150	24	144	45	24	149	57	0.37	0.71	98
75	24	60.9	27	6	76.8	7.4	1.06	0.3	92

n= number of replicate determinations.

Vacutainer tubes *must* be avoided (Patzke *et al.*, 1980) as the plasticizer Tris (2-butoxyethyl) phosphate causes aberrant assay values, as has been observed in other systems (Fremstad and Bergerud, 1976; Brunswick and Mendels, 1977). Table 8 demonstrates that the Blue top serum tubes produce equivalent specific binding to the control glass syringe. Red top tubes consistently yield a higher value for the number of counts per minute bound and higher specific binding. The plasma samples collected into Green and Lavender tubes consistently also had higher binding of ^{3}H-spiperone than Blue top plasma tubes (table 8). It thus appears that the plasticizer component of the Red, Green and Lavender stoppers increases the binding of ^{3}H-spiperone for receptors in the presence of serum, probably by decreasing serum protein binding of the ^{3}H-ligand.

In preliminary studies it has also proved possible to use the RRA to measure neuroleptic activity in both urine and saliva samples from patients receiving therapeutic doses.

Table 8 Assay of normal human sera and plasma using the Wellcome Neuroleptic Assay Kit

	Collection system					
	Blue top serum	Blue top plasma	Red top serum	Glass serum	Lavender top plasma	Green plasma
Additive	None	Heparin	None	None	EDTA	Heparin
Mean specific binding	20.98	22.26	23.5	20.39	27.18	26.46
S.D.	1.43	1.54	1.95	1.56	0.81	1.96

CLINICAL STUDIES

Results are promising in the few clinical studies which have been conducted to date using the neuroleptic RRA. It is apparent that whereas neuroleptic dosage does not correlate with clinical efficacy, as has been previously demonstrated, blood levels of neuroleptic drug activity as determined by the RRA do correlate with clinical effects. In one study (Calil *et al.*, 1979) it was demonstrated that serum concentrations of haloperidol were related to the Brief Psychiatric Rating Scale (BPRS) total pathology score as well as the BPRS factor scores for thinking disturbance and paranoid disturbance (table 9). In this study serum concentrations of haloperidol were not different among patients with or without the presence of extrapyramidal symptoms or among patients receiving or not receiving concurrent anti-Parkinson medication (table 10). In another study (Rosenblatt *et al.*, 1980) it was demonstrated that co-treatment with benztropine mesylate led to lower total serum concentrations of neuroleptic activity. However, in

Table 9 Analysis of variance, general linear model, haloperidol group

Serum concentration of haloperidol versus		
Age	F=2.91	p=0.11
Sex	F=0.36	p=0.55
Total BPRS scores versus		
Serum concentration	F=4.77	p=0.05
Treatment duration	F=0.50	p=0.49
Age	F=0.33	p=0.58
Weight	F=0.03	p=0.87
Serum concentrations of haloperidol versus BPRS factors		
Thinking disturbance	F=7.51	p=0.02
Psychomotor disturbance	F=0.06	p=0.81
Paranoid disturbance	F=9.29	p=0.01
Depressive disturbance	F=3.46	p=0.09

From Calil *et al.* (1979).

Table 10 Haloperidol serum concentration

Variable	*N*	Serum concentrations			
		Mean	S.E.M.	Min. value	Max. value
EPS*					
Present	7	139.1†	53.1	0	399.0
Absent	10	80.4	29.8	0	261.0
Unknown	5	83.2	35.0	0	202.0
Anticholinergic‡					
Yes	17	90.9†	25.2	0	399.0
No	5	131.0	54.8	0	261.0

* EPS=extrapyramidal symptoms.
† Student's *t* test, not significant.
‡ Anticholinergic =concomitant treatment with anticholinergic drugs.
From Calil *et al.* (1979).

another study where the same patients were followed on and off anticholinergic medication, no change in blood level of neuroleptic was apparent (Burnett *et al.*, 1980).

Wyatt's group has reported two intriguing preliminary findings. In one series of experiments, patients who had tardive dyskinesia exhibited about fivefold higher blood levels of neuroleptic activity than a control group whose daily dose of neuroleptic was matched in terms of milligram equivalents of CPZ (Jeste *et al.*, 1979). This preliminary finding suggests that a high serum concentration of neuroleptic may contribute to the pathophysiology of tardive dyskinesia in at least a subgroup of patients. In another study, Wyatt's colleagues demonstrated that older patients (greater than 45 years of age), although receiving about a fourth of the daily neuroleptic dose of patients younger than 45 years of age, exhibited higher blood levels of neuroleptic activity (table 11, Rosenblatt *et al.*, 1980). This finding would, therefore, lend pharmacological support to the 'conventional clinical wisdom' that older patients require lower doses for optimal therapeutic response. Also, these findings raise a question of whether the greater frequen-

Table 11 Neuroleptic daily dosage and serum concentration of patients grouped by age

Age	*N*	Daily dose (mg CPZ-E)	Serum concentration (ng ml^{-1} CPZ-E)
<45	12	1407±149	243±42
45–60	6	436±121*	350±231
>60	8	350±118*	343±153

*$p<0.05$ compared to patients <45.
From Rosenblatt *et al.* (1980).

cy of confusional states and tardive dyskinesia in elderly patients treated with neuroleptic results in part from high serum neuroleptic concentrations rather than age-related changes in the CNS. If this is the case, measurement of serum neuroleptic concentrations may have value in the prophylactic treatment of elderly psychotic patients.

A detailed study of serum neuroleptic levels in 30 schizophrenic patients receiving one of six different neuroleptics was undertaken (Tune *et al.*, 1980*a*). Their clinical state was monitored with an abbreviated version of the Present State Examination (mini-PSE). There was a significant correlation between serum levels of neuroleptics and clinical status as monitored by the mini-PSE score (figure 3). Of the 13 patients with serum levels less than 50 ng ml^{-1} CPZ equivalents, eight had mini-PSE scores greater than 4, indicating considerable schizophrenic psychopathology. Three out of the nine patients with blood levels between 50 and 100 ng ml^{-1} also had mini-PSE scores greater than 4. Strikingly, none of the eight patients with blood levels between 100 and 200 ng ml^{-1} had mini-PSE scores greater than 4. The three different groups of patients (0–50, 50–100, 100–200 ng ml^{-1}) did not differ in duration of present illness prior to hospitalization, number of previous psychotic episodes and hospitalizations, or intervals between admission and blood collection for neuroleptic assay. These differences between groups were significant ($p<0.02$). Eighteen of the 30 patients were receiving haloperidol. There was no correlation between the absolute milligram dosage of haloperidol and PSE scores. Thus, neuroleptic serum

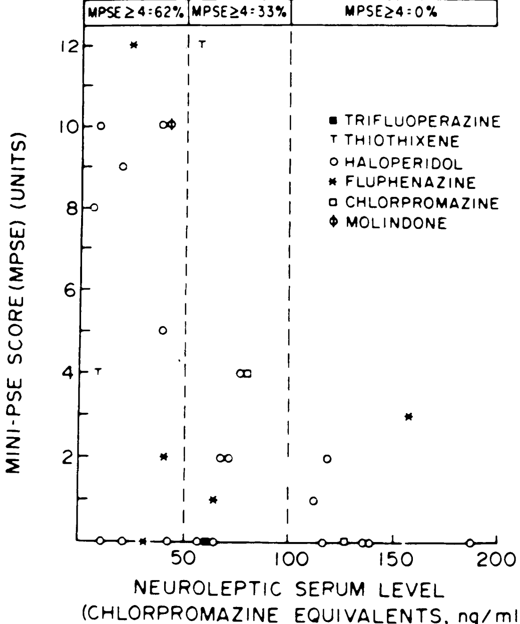

Figure 3 Relationship of serum neuroleptic levels with clinical response. (From Tune *et al.*, 1980*a*.)

levels were significantly correlated with clinical response as monitored by the PSE. By contrast, drug dosage did not predict clinical response. Moreover, dosage and blood levels of the neuroleptics were not significantly correlated.

In another study (Tune *et al.*, 1980*b*), patients who received injections of fluphenazine decanoate ranging from 10 to 75 mg were monitored for serum levels of fluphenazine. RRA levels detected were between 1 and 30 ng ml^{-1} CPZ equivalents (0.1–3 ng ml^{-1} absolute concentration of fluphenazine) (figure 4). This study indicated the feasibility of monitoring fluphenazine blood levels of patients who have been treated with fluphenazine decanoate, utilizing the laboratory RRA. Serum levels after fluphenazine decanoate treatment were more stable than serum levels of neuroleptics following daily treatment with oral medication. The extraordinary low levels of fluphenazine following administration of its decanoate reported by chemical assay previously (Curry *et al.*, 1978) might suggest that the therapeutically active agent is a metabolite. The present RRA demonstrates that this is not the case. How could such extraordinary low blood levels exhibit therapeutic responses? At present the best suggestion is that the constant low plasma levels occurring during fluphenazine decanoate treatment might be adequate for prophylaxis in these stabilized patients. According to this concept, adequate blood levels of neuroleptics, to maintain prophylaxis in stabilized patients may be substantially lower than levels required for inducing remission in acutely psychotic patients. Alternatively, the transient rise in fluphenazine levels within the first 3 days of injection (Curry *et al.*, 1978)

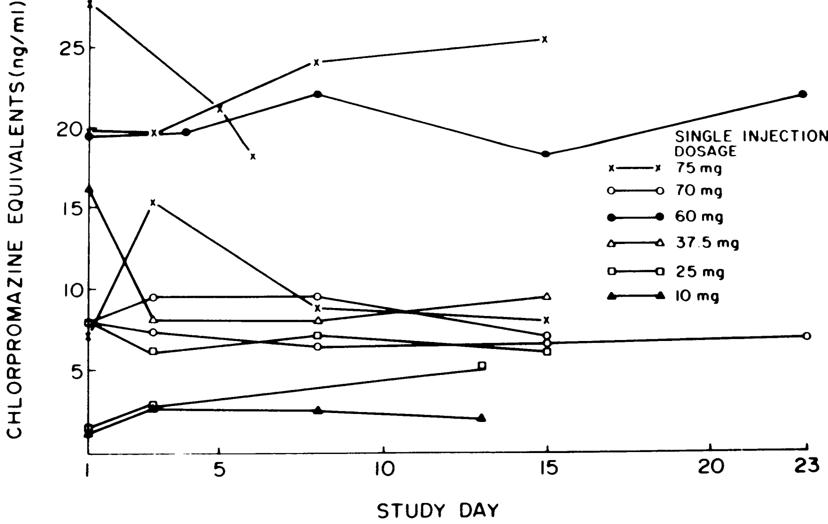

Figure 4 Fluphenazine serum levels of nine chronic schizophrenic patients after treatment with fluphenazine decanoate. Measurement of day 1 was done before injection and reflects levels from previous injection (given at 2–3 week intervals). One patient repeated the study, which accounts for 10 assessments rather than nine. (From Tune *et al.*, 1980*b*.)

may suffice to protect against relapse, despite the subsequent extremely low blood levels.

The unusually high blood levels of thioridazine and low levels of fluphenazine from depot injections suggests that results obtained from the RRA may have to be standardized for each drug (Cohen *et al.*, 1980). However, the study of Tune *et al.* (1980*a*) suggests that other neuroleptics might have similar therapeutic windows when blood levels are expressed in CPZ equivalents from the RRA. More clinical studies are required before a conclusion can be reached.

PLASMA PROTEIN BINDING AND THERAPEUTIC ACTIVITY

High proportions of total plasma neuroleptic content are bound to plasma proteins. For CPZ the percentage of drug bound to plasma protein can range between 90 and 99 percent in different individuals, so that with identical total plasma levels of the drug the concentration of free CPZ can vary tenfold (Curry, 1970). Recently McDevitt *et al.* (1976) showed that the extent of clinically evident β-noradrenergic receptor blockade elicited by the β-blocking drug propranolol did not correlate with total plasma levels of propranolol, was only modestly related to red cell levels of the drug, but closely paralleled free levels of propranolol in patient plasma. These findings confirm the supposition that only the free drug is available to target organs such as the heart or brain. In the RRA, as has been described above, all plasma-bound drug dissociates in the assay procedure owing to the serum dilution and the higher affinity of DA receptors for the drug compared to the protein binding sites.

Perhaps measuring total blood levels of neuroleptic activity may not be the most efficacious indicator of clinical response. Recently, we measured the amounts of free versus bound drug for a number of neuroleptics and their metabolites by dialyzing plasma samples against normal saline (Freedberg *et al.*, 1979). The percentage of free neuroleptic varies with concentration for certain of the drugs (figure 5, table 12). Thus with CPZ at respective concentrations of 8, 80 and 400 μM the free levels are 0.13, 0.24 and 0.79 percent. This pattern is also evident with *cis*-thiothixene and mesoridazine, somewhat less with thioridazine and promazine and not apparent for 7-OH-CPZ. The percentage of free drug at the lowest plasma concentration is probably most relevant to therapeutic practice, since therapeutic total plasma levels of CPZ (0.1–1.0 μM) are less than the lowest concentration of CPZ examined here. At 8 μM only about 0.13 percent of plasma CPZ is free, while more than 10 times as much 7-OH-CPZ is free. Even at the highest concentration examined, 400 μM, more than twice as much 7-OH-CPZ is free compared to CPZ.

Table 12 Neuroleptic drug proportions not bound to plasma protein (percent free)

	CPZ	7-OH-CPZ	PROM	THIOR	MESO	*cis*-THIO	HALO	FLU	MOL	TRIFLUO
Plasma concentration (μM)	8	8	30	30	30	0.8	0.5	10	2	70
Proportion free (mean±S.E.M.)	0.13±0.04	1.5±0.69	1.5±0.49	0.16±0.04	8.95±2.3	0.22±0.07	9.0±0.68	0.24±0.10	24±4.1	0.036±0.006
Plasma concentration (μM)	80	80	300	300	300	8				
Proportion free (mean±S.E.M.)	0.24±0.04	1.1±0.09	1.6±0.25	0.13±0.05	14±1.3	0.65±0.16				
Plasma concentration (μM)	400	400	1500	1500	1500	40				
Proportion free (mean±S.E.M.)	0.79±0.16	1.6±0.12	6.9±0.64	0.38±0.08	25±1.8	1.6±0.26				

Neuroleptic drugs were incubated with fresh human plasma, dialyzed and concentrations of drug bound and not bound (free) to plasma protein were determined as described in the text. Values are of 3–6 independent determinations on different human subjects, each conducted in triplicate, and the percentage of free drug was calculated as described in the text.

Abbreviations: CPZ, chlorpromazine; 7-OH-CPZ, 7-hydroxychlorpromazine; PROM, promazine; THIOR, thioridazine; MESO, mesoridazine; *cis*-THIO, *cis*-thiothixene; HALO, haloperidol; FLU, fluphenazine; MOL, molindone; TRIFLUO, trifluoperazine.

From Freedberg *et al.* (1979).

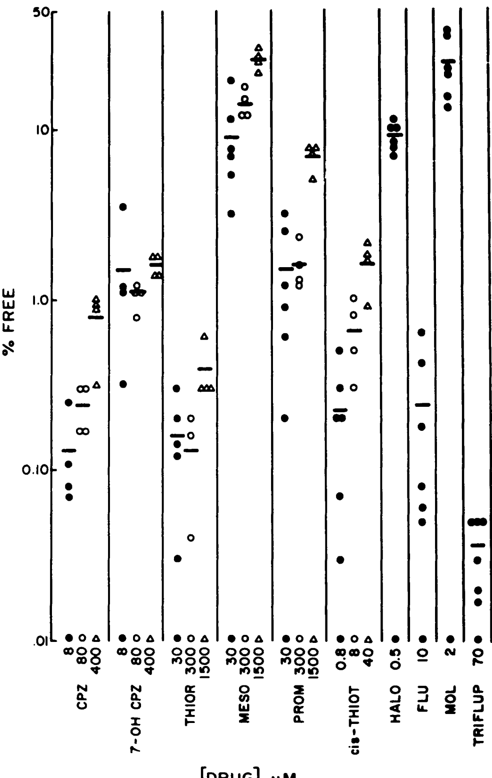

Figure 5 Plasma neuroleptics not bound to plasma protein (free). Fresh human plasma samples were incubated with neuroleptics, dialyzed, and bound and free drug concentration assayed as described in the text. Each point represents the mean value of triplicate assays from a separate human volunteer. Short horizontal bars indicate the means of the samples for each total drug concentration. Drug abbreviations are the same as in Table 12. (From Freedberg *et al.*, 1979.)

After chronic CPZ treatment, total plasma levels of 7-OH-CPZ are reported to be 20–120 percent of the CPZ levels (MacKay *et al.*, 1974; Phillipson *et al.*, 1977; Sakalis *et al.*, 1977; Alfredsson *et al.*, 1976). Assuming that the percentage of free drug at therapeutic doses corresponds to the values we obtained at 8 μM, then free 7-OH-CPZ will be two to 12 times the levels of CPZ. Thus after chronic treatment with CPZ the metabolite, 7-OH-CPZ, may be responsible for therapeutic effects, although with acute treatment with very low levels of 7-OH-CPZ, the parent drug may be the primary active agent. These observations may explain the better correlation of 7-OH-CPZ rather than CPZ plasma level with therapeutic response.

Mesoridazine, a metabolite of thioridazine, has about the same potency as the parent drug in blocking DA receptors and, after therapeutic doses of thioridazine, total plasma levels of mesoridazine are similar to those of thioridazine. At all free concentrations examined the percentage of free mesoridazine exceeds that of thioridazine by 50–100 times, confirming the findings of Nyberg *et al.* (1978), suggesting that during thioridazine treatment, mesoridazine is the major therapeutically active agent. Thus free levels of neuroleptic and active metabolites may provide better predictors of clinical responses than total plasma levels of parent drugs assayed in earlier studies. This is currently under investigation.

CONCLUSION

The development of the neuroleptic blood level RRA should greatly aid in the studies of the pharmacokinetics of neuroleptic drugs. The assay is both simple and inexpensive and has a large analytical capacity. Between one and two hundred samples can easily be assayed in a day. Recent studies suggest that the RRA may have great clinical utility in (1) ascertaining patient compliance, (2) determining if non-responders have adequate blood levels of a drug, (3) determining therapeutic windows, and (4) ensuring that patients (especially the elderly) are not over-medicated. Its use is likely to improve the present pharmacological management of schizophrenics.

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Radioreceptor binding technique as aid in treatment with neuroleptics

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INTRODUCTION

In all medicinal treatment efforts must be made to find the optimal dose for each patient separately. Both therapeutic effects and adverse effects may occur at doses differing widely from one individual to another. Furthermore, the use of a given dose of a drug may perhaps result in the blood concentration of the drug being anything up to 30 times higher in one patient than in another (Hammer and Sjöqvist, 1967; Forsman and Öhman, 1975; Axelsson and Mårtensson, 1976). It is possible to ascertain the optimal dosage level or range of several drugs, that is the dose producing a maximum effect with a minimum of side effects. By adjusting the dose of a drug according to its concentration in the serum, for example, it has proved possible to improve the effect of the drug and to minimize its side effects. But it has so far not been possible to establish a generally applicable concentration range for neuroleptics. This failure has been ascribed, first, to the fact that most neuroleptics are transformed to a series of active metabolites. One can therefore not *a priori* expect the clinical picture to vary solely with the blood concentration of the drug, which is only one of the active substances. Second, it has become increasingly clear that the response to a given concentration can vary from one patient to another. As far as neuroleptics are concerned, this variation may be due to a probably genetic variation of the susceptibility of the dopamine (DA) system. It might also be explained by differences in pathogenesis of apparently similar conditions requiring different doses of the drug. For example, the term schizophrenia does not designate any particular disease, but rather a group of mental illnesses with similar clinical pictures.

Solution of the two above-mentioned problems, namely active metabolites and interindividual variation of response to a given dose, requires accurate measuring methods. Efforts must be made to secure a measure of the available DA receptor blocking activity in a given case and, second, an

indicator of the response of the individual patient to such activity.

This paper surveys some recent advances in clinically applicable psychopharmacology in the hope that it may serve as a guide for psychiatrists interested in the treatment of psychoses.

MEASURING METHODS

Thanks to the quick strides made in the technical development of analytical methods in the last decade, it is now possible to measure the blood concentration of neuroleptics used in ordinary doses. The extremely precise methods now available for detecting and measuring even very low concentrations of drugs are listed below:

Measuring methods	*Properties*
Mass spectrometry (MS) High performance liquid chromatography (HPLC) Gas–liquid chromatography (GLC)	Chemically specific and very sensitive methods. Relatively low analytic capacity. Expensive.
Radioimmunologic assay (RIA)	Sensitive but somewhat uncertain concerning chemical specificity. Very high analytical capacity. Inexpensive.
Radioreceptor assay (RRA)	Chemically non-specific but pharmacologically specific. Measures DA receptor blocking potential. Good analytical capacity. Inexpensive.

Without the high specificity and sensitivity of the above methods, advanced pharmacokinetic research would not have been possible. However, the use of the chromatographic methods requires expensive equipment and highly educated and experienced personnel. This, together with their low analytical capacity, has limited their clinical use. But the RIA technique is very sensitive and at the same time has a high analytical capacity. However, in many situations it is not possible to be sure about its specificity. In other words, one is sometimes not certain whether one is measuring only the parent substance; metabolites and other drugs can interfere with the assay. Such interaction between the parent substance and metabolites need not depend on the pharmacologic activity of the metabolites.

In several respects, the RRA resembles the RIA assay. It uses DA receptors with specific affinity for neuroleptics instead of antibodies. The method is very sensitive and has a high analytical capacity, but, like the RIA, it is chemically non-specific. Yet it may be regarded as pharmacologically specific in that when properly performed it measures mainly DA receptor

blocking substance regardless of whether it is the actual drug used or an active metabolite or any other drug given at the same time. The method thus copies part of the reaction pattern in the brain and it is therefore not even necessary to know all the active metabolites. It is thus possible to ascertain the combined effect of all drugs given and their metabolites without determining the concentration of each substance separately. The RRA should therefore find wide clinical use for determining the neuroleptic activity in a given patient.

RADIORECEPTOR ASSAY AND PHARMACODYNAMICS

The RRA was developed by Snyder's group in Baltimore (Creese and Snyder, 1977). It may provide somewhat higher concentration values than a chemically specific method such as gas chromatography. This is due to the fact that RRA determines all active substances, which would be one of the advantages in clinical applications.

Even if RRA determine the total DA receptor blocking activity in serum, they alone cannot predict the individual reaction to this activity. Therefore an individual measure of the effect in each separate patient is needed. The effect of a drug can be objectively assessed by determination of acid transmitter metabolites in the cerebrospinal fluid (Fyrö *et al.*, 1974), quantitative EEG (Fink, 1978), or measurement of the prolactin concentration in serum (Öhman and Axelsson, 1978). At present interest is focused on the serum level of prolactin, which may reflect DA receptor blockade in the brain (Axelsson *et al.*, 1980).

CLINICAL STUDY

Application of RRA is briefly exemplified below with a clinical study. The clinical material consisted of 14 chronic schizophrenic men and women requiring high doses of neuroleptics. After receiving haloperidol alone in doses varying from 40 to 180 mg (mean 117 mg), a venous blood sample was drawn in the morning before the first dose of drug. The concentration of haloperidol and that of reduced haloperidol (a metabolite; Forsman and Larsson, 1978; Forsman *et al.*, 1980) were determined by a high performance liquid chromatographic method (HPLC), and the DA receptor blocking activity in serum was determined by means of RRA. Altogether 48 serum samples from 14 patients were analyzed in duplicate with each technique. The mean of the total concentration of haloperidol in serum was 61 ng ml^{-1} (HPLC) and the receptor blocking activity in serum corresponded to a haloperidol concentration of 70 ng ml^{-1} (RRA). The coefficient of correlation between the two methods was 0.8. The mean concentration of

reduced haloperidol as determined by HPLC was about 10 percent higher than that of haloperidol itself.

In a very recent study *in vitro* (to be published) the DA receptor blocking potential of reduced haloperidol was examined. It proved virtually inert in this respect and thus presumably without any appreciable neuroleptic activity. Reduced haloperidol reportedly raised the prolactin level in both animals and man (Hays *et al.*, 1980). This might be due to oxidation of reduced haloperidol back to haloperidol *in vivo.*

The higher concentration measured by RRA could thus not be explained by known active metabolites of haloperidol. The intake of medication by the patients during the trial was therefore rechecked and it was found that, contrary to instructions given before the study, some patients had also received other drugs during the study period. These consisted mainly of levomepromazine (Nozinan®) at bedtime. Fourteen test values in three such patients were then excluded and the mean concentration, as measured by HPLC, was then, on average, 57 ng ml^{-1}. The corresponding value for the RRA was 55 ng ml^{-1}. The correlation coefficient was 0.9. This extremely close correlation argues strongly against the occurrence of neuroleptic active metabolites of haloperidol.

CONCLUSIONS

(1) RRA measures the DA receptor blocking potential in serum regardless of the chemical substance responsible for the blocking effect. The method is thus pharmacologically, though not chemically, specific. The method measures the total effect of active metabolites as well as that of any other drugs administered and thereby gives the clinician an increased choice of therapeutic preparations without sacrificing the possibility of performing adequate serum determinations. It may prove advantageous to add a further step in the procedure to determine the dopaminergic blocking activity corresponding to the free fraction of drug and metabolites.

(2) RRA cannot replace determination of the response of an individual patient to a given available DA receptor blocking activity. Prolactin can, perhaps, be used as such a pharmacologic marker.

(3) Apparently well-controlled patients in hospitals may sometimes receive drugs other than those prescribed. Chemically specific measuring methods do not alone reveal such supplementary medication. It therefore appears that the additional use of a pharmacologically specific technique such as RRA can offer the clinician certain advantages.

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Preliminary assessment of a calf caudate radioreceptor assay for the estimation of neuroleptic drugs in plasma: comparison with other techniques

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INTRODUCTION

The measurement of neuroleptic activity in the plasma of patients by radioreceptor assay (RRA) was introduced by Creese and Snyder in 1977. It is based on an action common to these compounds, their ability to bind to dopamine (DA) receptors in the brain. The relative potency of neuroleptics in producing this effect has been shown to correlate significantly with their pharmacological potency as gauged by behavioral experiments in animals and their effective clinical doses in man (Creese *et al.* 1976; Seeman *et al.* 1976). A considerable practical advantage is that any drug having this action and its active metabolites can be measured by a single technique.

In order to make this technique more widely available to psychiatrists outside research centers, with less specialized laboratories, a simplified version of the RRA has been developed (Lader, 1980). This report describes the performance of the modified method in our laboratory both in the measurement of known concentrations of neuroleptics in plasma and in the assay of unknown specimens from patients receiving haloperidol treatment or a fluphenazine depot preparation.

Concentrations of neuroleptic activity obtained by RRA are compared with analyses made in the same samples using gas–liquid chromatography (GLC) and radioimmunoassay (RIA). Prolactin levels, also determined in the same samples, served as a guide to DA antagonism *in vivo*.

Here we focus on the estimation of multiple blood samples taken from comparatively few individuals in circumstances when the plasma drug level could be expected to be stable or changing in a predictable way.

METHODS

Analytical techniques

Figures for precision apply throughout the range of concentrations quoted in the results.

Radioreceptor assay (RRA) **(Lader, 1980)**

Materials kindly supplied by Dr S. Lader, Wellcome Reagents Ltd, Beckenham, Kent, UK. Precision (CV): intra-assay, ±15 percent; inter-assay, ± 25 percent.

Haloperidol, GLC **(Forsman and Ohman, 1974; modified by Franklin, 1980)**

Precision (CV): intra-assay, ±5 percent; inter-assay±12 percent.

Haloperidol, RIA **(Michiels *et al.*, 1976)**

Materials supplied by IRE, Fleurus, Belgium. Precision (CV): intra- and inter-assay, ±10 percent.

Fluphenazine (FPZ), RIA **(Wiles and Franklin, 1978)**

Precision (CV): intra-assay, ±10 percent; inter-assay, ±12.5 percent.

Prolactin (PRL), RIA **(McNeilly and Hagen, 1974)**

PRL standard MRC 75/504, results are expressed as International Units (IU) per liter. 1 IU l^{-1} is equivalent to 24 ng ml^{-1} NIAMDD PRL standard VLS H3. Precision (CV): intra-assay, ±8 percent; inter-assay, ±10 percent.

Blood specimens

Venous blood was taken into lithium heparin, plasma was separated by centrifugation and stored at −20 °C until assayed. Samples were exposed to light for the minimum time possible but they were frozen and thawed several times between the various assay procedures.

Subjects, drug dosage, blood sampling

Haloperidol

Five subjects (two women, three men) chronic schizophrenics (duration of illness >10 years) having residual symptoms. The dose was adjusted between 10 and 100 mg per day until an optimum was reached. Blood samples were taken at weekly intervals before and 2 h after the morning dose. The study period lasted 21 weeks in four patients and 8 weeks in one patient.

Fluphenazine decanoate

Six subjects (two women, four men) with chronic psychosis. Fixed doses of between 25 and 100 mg given at intervals of between 2 and 5 weeks had been established 6 months or longer. 11–21 blood samples were taken from each subject: several over the first 8 h post-injection and the remainder evenly spaced throughout an interval between injections.

Fluphenazine enanthate

Five subjects (all men) with a diagnosis of schizophrenia. Duration of illness – several years. They were studied during two 4-week periods of treatment with a 'low dose' (12.5 mg per week) and a 'high dose' (250 mg per week) of fluphenazine enanthate. (For more details see Dencker *et al.*, 1978.) Blood samples were taken weekly just before drug injection and daily during the first and fourth weeks of observation on each dose, when additional samples were also obtained at $\frac{1}{2}$, 1, 2 and 4 h post-injection.

RESULTS AND DISCUSSION

The radioreceptor assay

Binding characteristics

The percentage binding of ^{3}H-spiperone to calf caudate tissue in the absence of unlabeled drug was 26.4±1.5 (S.D.); $N=20$ assays. 'Non-specific' binding measured in the presence of excess unlabeled haloperidol was 7.4±0.7 percent. The proportion of 0.19 pmol ^{3}H-spiperone specifically associated with DA receptors was thus approximately 74 percent.

Specificity

The potencies of a number of neuroleptics and related compounds relative to haloperidol calculated from IC_{50} values are shown in table 1. They were found using solutions of these compounds in plasma; in general they follow the expected order of potency. Clinically inactive β-flupenthixol has about 10 percent of the activity of a α-flupenthixol. 7-OH-FPZ was more active than chlorpromazine (CPZ), 7-OH-CPZ also possessed some activity. The sulfoxides were inactive. An unexpected finding was that FPZ, enanthate was apparently more potent than haloperidol; however, preliminary experiments suggest that this was due to hydrolysis of the ester during incubation with the caudate homogenate to release fluphenazine. The decanoate was also affected in this way.

Table 1 Relative potencies of some neuroleptics and related compounds in the radioreceptor assay (RRA)

Compound	IC_{50} (nM)	CV	No. samples	Potency relative to haloperidol
Spiperone	8.17	9.3	3	2093
Fluphenazine	48	10.4	7	356
α-Flupenthixol	100.7	—	2	170
FPZ-enanthate	101.4	2.7	3	168
Pimozide	143.5	—	2	119
Haloperidol	171	10.5	4	100
Trifluoperazine	203	5.4	3	84
7-OH-Fluphenazine	315	16.1	3	54
Chlorpromazine	414	17.09	4	41
FPZ-decanoate	912	2.6	4	19
7-OH-Chlorpromazine	1057	3.3	3	16
β-Flupenthixol	1184.8	—	1	14
Thioridazine	1278	—	1	13

Inactive: fluphenazine sulfoxide, chlorpromazine sulfoxide.

Sensitivity

The lowest concentration of a drug in plasma which could be reliably measured (within the limits of precision quoted above) was that concentration producing a 20 percent decline in specific binding (IC_{20}). IC_{20} values for a number of commonly used neuroleptics are given in table 2. Although these IC_{20} values appear too high for the RRA to be used for routine drug measurement in subjects receiving conventional doses, drug concentrations obtained by RRA are invariably far higher than those found using GLC, which detects only the parent drug.

Table 2 Radioreceptor assay – lower limits of detection

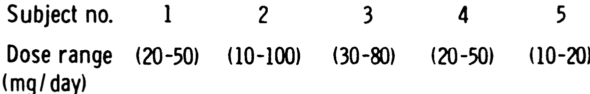
Compound	IC_{20} (ng ml^{-1}) Mean S.D.	*N*
Fluphenazine	5.0±0.6	3
α-Flupenthixol	10.3±2.5	2
Haloperidol	16.0±2.4	4
Trifluoperazine	16.7±0.6	3
Chlorpromazine	22.7±4.0	4
Pimozide	24.5±8.1	3

Analysis of samples from patients

Haloperidol

Comparison of drug levels obtained by RRA, RIA and GLC.

Haloperidol measurements (mean±S.D.) made using three techniques in samples from five subjects are shown in figure 1. The rank order of drug



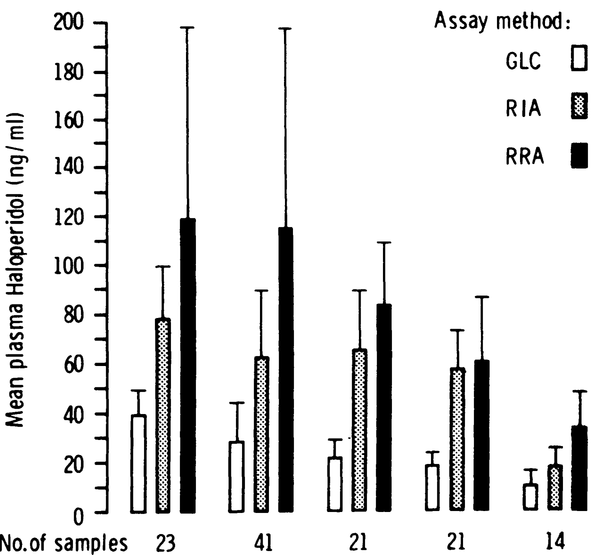


Figure 1 Haloperidol concentrations (mean±S.D.) in five subjects as measured by GLC, RIA and RRA.

concentrations by the three methods was RRA>RIA>GLC. This suggests that an active metabolite of haloperidol exists. The ratio of haloperidol concentration by RRA/GLC-determined levels was as great as 10 in individual samples and it varied from ×5 to ×9 within subjects. The ranges of haloperidol levels by RIA and RRA overlapped. An RIA using the same antiserum has previously been reported to give haloperidol values greater than those found by GLC (Van den Eeckhout *et al.*). Haloperidol determinations by RRA correlated significantly ($P<0.01$ to <0.001) with GLC in only one subject but with RIA in three out of five subjects. GLC and RIA determinations also correlated significantly in only one subject.

Relationship to dose

Haloperidol levels by RRA versus daily dose are shown in figure 2 for one subject. Note the wide range of drug levels at any particular dose. A significant correlation was found between dose and drug level by RRA for the 2 h post-dose specimens. Dose and plasma drug concentration correlated most strongly in the 2 h post-medication specimens (table 3) being significant for GLC in two, for RRA in three, and for RIA in five subjects.

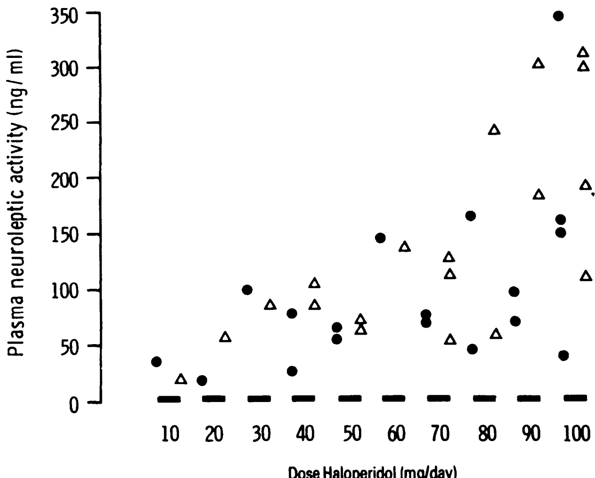


Figure 2 Plasma neuroleptic activity measured by RRA in one subject receiving haloperidol plotted against daily dose. Before morning dose (●), $r=0.5$; 2 h after morning dose (△), $r=0.72$.

Relationship to prolactin levels

Numbers of significant correlations between plasma PRL and drug levels by the three methods followed the same rank order as for dose, that is GLC none out of five; RRA two out of five; RIA three out of five subjects. This may be seen as further evidence that both RIA and RRA are responding to a pharmacologically active metabolite of haloperidol, undetected by GLC.

Table 3 Correlation between daily dose and plasma haloperidol level determined by three methods in 2 h post-medication samples

Subject	Dose range (mg per day)	Correlation coefficient RRA	GLC	RIA	*N*
1	20–50	0.3	0.2	0.8**	14
2	10–100	0.7**	0.8**	0.8**	19
3	30–80	0.0	0.2	0.9**	14
4	20–50	0.8**	0.7*	0.8**	13
5	10–20	0.9*	0.7	0.9*	8

*$p<0.01$; **$p<0.001$.

Fluphenazine

Comparison of drug levels by RRA and RIA in subjects receiving FPZ decanoate at fixed doses

Plasma drug levels by RRA were measurable in all samples. Average RRA levels for an interval between injections were higher than those found by RIA (table 4). The initial post-injection peak consistently obtained by RIA (Wiles and Gelder, 1979) was absent (figure 3). The RRA response clearly includes either a molecule or an activity not picked up by the RIA. It is tempting to suggest that hydrolysis of the decanoate present in the plasma sample occurs in the RIA, but no evidence of circulating FPZ decanoate in plasma of patients has hitherto been found (see, for example, Curry *et al.*, 1979). There was no correlation within subjects between FPZ levels obtained by the two methods. This could be explained by the generally rather narrow range of drug levels found during fixed dose depot treatment. There

Table 4 Plasma drug levels determined by RRA and RIA and their correlation during treatment with FPZ decanoate at fixed doses

Subject	Dose (mg wk^{-1})	RRA Mean (ng ml^{-1})	RRA CV (%)	RIA Mean (ng mlZW^{1})	RIA CV (%)	Correlation coefficient, *r*
1	50	24.8	41.6	19.7	23.0	0.14
2	50	51.1	26.5	9.4	27.4	0.46
3	37.5	162.4	83.4	16.2	32.6	0.17
4	6.3	11.9	14.2	4.2	72.5	−0.43
5	6.3	11.7	44.9	3.3	54.2	−0.31
6	5	10.4	29.4	1.4	115.4	0.12

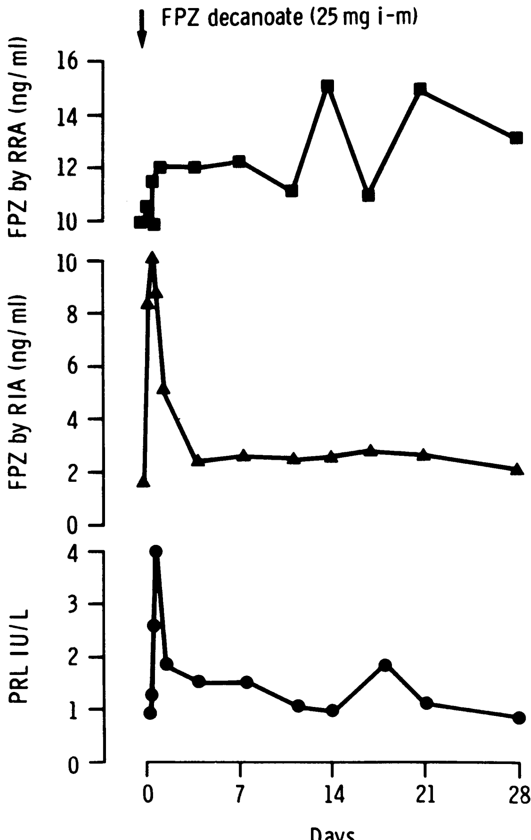


Figure 3 Plasma prolactin and drug levels by RIA and RRA during one interval between regular injections of FPZ decanoate (25 mg) given every four weeks in a male subject.

is a discrepancy between FPZ levels found in subjects receiving FPZ decanoate measured by this modified RRA and those published recently by Tune *et al.* (1980) who used the original rat caudate method of Creese and Snyder (1977). Levels obtained by the Lader method are about 20 times greater, suggesting important differences in the two caudate preparations. A comparison of these two RRA techniques is therefore necessary.

Comparison of plasma fluphenazine levels by RRA and RIA during a 20-fold change in dose of fluphenazine enanthate

When plasma drug levels from both 'high dose' and 'low dose' treatment periods were considered together, a significant correlation between RRA and RIA was found in three out of five subjects. The ratio of RRA/RIA ranged from 0.2 to 10 for all samples and varied from ×13 to ×23 among

individuals. When samples from a week closest to steady state conditions on each dose were examined separately (figure 4), either RIA or RRA detected with equal significance the 20-fold change in dose.

Drug concentrations and prolactin levels
Highly significant correlations between plasma FPZ levels by RIA were found in six out of seven subjects receiving treatment with FPZ esters, a significant correlation between RRA and PRL was found in only one of these. This may simply reflect the poorer precision of the RRA or indicate that it responds to substances inactive at pituitary DA receptors.

SUMMARY

In general

(1) The modified RRA is easier to perform than GLC or RIA but is less precise.
(2) It detects neuroleptics and their active metabolites but fails to respond to inactive ones.
(3) Fluphenazine esters were hydrolyzed in the RRA to release the parent drug.

During haloperidol treatment with varied doses

(1) Drug level by RRA>RIA>GLC.
(2) Both RRA and RIA measures correlated significantly with PRL on more occasions than those obtained by GLC.
(3) This suggests the presence of an active metabolite of haloperidol.
(4) Dose and plasma haloperidol level by RIA were significantly correlated in five out of five subjects in 2 h post-medication specimens.

In subjects receiving fluphenazine decanoate, at a fixed dose

(1) Drug concentrations by RRA>RIA.
(2) This modified RRA gave results about 20 times greater than obtained by the original Creese and Snyder (1977) method. A comparison is needed.

During a 20-fold change in the dose of fluphenazine enanthate

(1) Greater overlap between RIA and RRA measurements.
(2) Significant correlation between RIA and RRA in three out of five subjects.

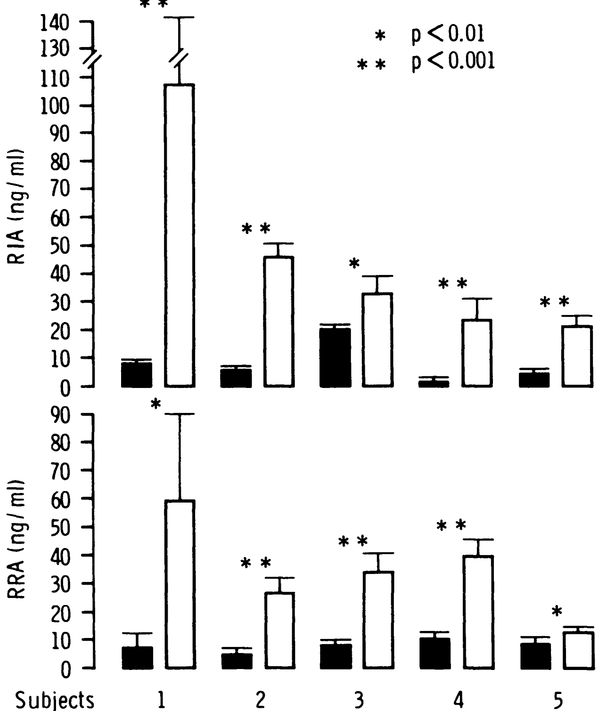


Figure 4 Plasma fluphenazine (FPZ) levels by RIA and RRA in five chronic schizophrenic subjects during treatment with FPZ-enanthate at dose rates of 12.5 mg per week (■) and 250 mg per week (□). Mean and S.D. from daily determinations during one week.

(3) Both RRA and RIA distinguished between 'high' and 'low' doses.
(4) Higher incidence of significant PRL correlations with RIA.

Further investigation is needed before this modified RRA is applied routinely.

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Section Three
Active Metabolites

Active metabolites of phenothiazine drugs

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INTRODUCTION

Many of the psychotropic drugs have one or more active metabolites which may significantly contribute to therapeutic and toxic effects in patients. The ratio between the blood concentrations of metabolites and the parent drug may vary largely between individuals and the active metabolites should, therefore, be measured (in addition to the parent drug) by blood level monitoring. This should be done also in studies of the relationship between blood drug concentrations and clinical effects.

Biological assay methods which measure the total amount of active compound in the sample require that the biological effect that is measured be closely related to the clinical effect of the drug. By application of a radioreceptor binding assay for neuroleptic drugs (Creese and Snyder, 1977), Tune *et al.* (1980) measured the plasma levels of six different neuroleptics in terms of chlorpromazine (CPZ) equivalents; they examined the relationship between the plasma levels and clinical ratings of the patients. The study is reviewed in more detail by Creese *et al.* (this volume). Besides its technical simplicity, this method offers the advantage that a wider number of suitable patients may be investigated in plasma level–effect studies than when only one drug is studied.

There are, however, examples of neuroleptic drugs having two or more active metabolites which are found in relatively high concentrations in plasma, where one metabolite is supposed to be responsible for certain side effects. The 7-hydroxy metabolite of CPZ (7-OH-CPZ) has, for instance, been suspected of causing photosensitive skin pigmentation and opacities in the eye (Perry *et al.*, 1964; Bolt and Forrest, 1967). It has also been reported that shortening of handwriting, which was regarded as a manifestation of subclinical hypokinesia, was associated with high levels of 7-OH-CPZ in psychiatric patients (Sakalis *et al.*, 1977). The side-chain sulfone of thioridazine apparently produces significantly more side effects than the

parent drug and the two other metabolites which are found in relatively high plasma concentrations after oral doses of thioridazine (Axelsson, 1977; Axelsson and Mårtensson, 1980).

Separate measurements of each active metabolite, therefore, seem to offer certain advantages over routine monitoring of parent drug. Specific assay methods (such as gas chromatography and mass fragmentography) require that the metabolites which are measured have previously been identified.

METABOLISM OF PHENOTHIAZINE DRUGS

The major routes of metabolism of the phenothiazine drugs in man are indicated in figure 1, together with their general molecular formula. The

S
N
R_1
R_2
I
O
II
HO
III
R—O
IV
$N-CH_3$
N—H
V
N
O

Figure 1 Major pathways of phenothiazine metabolism. The general molecular formula of the phenothiazine drugs is shown above. I. ring sulfoxidation; II, ring hydroxylation; III, conjugation; IV, *N*-demethylation; V, *N*-oxidation.

neuroleptic potency is strongly influenced by the electron-attracting or -donating properties of the ring substituent in the 2-position (R_2 in figure 1) (Gschwend, 1974; Fenner, 1974).

Dechlorination of CPZ, that is cleavage of the R_2 substituent from the phenothiazine nucleus, has recently been reported as another metabolic pathway of CPZ in man (Forrest *et al.*, 1980). It has also been reported that CPZ and promazine may be metabolized by cleavage of the side chain in the 10-position (R_1 in figure 1) of the phenothiazine nucleus (Fishman and Goldenberg, 1965).

The phenothiazine derivatives which have a piperazine ring at the side chain are also metabolized in man by *N*-dealkylation, whereby the terminal alkyl group is cleaved from the piperazine ring.

Thioridazine has a thiomethyl group as ring substituent in the 2-position, which is oxidized in man to the sulfoxide (Forrest *et al.*, 1965) and further to the sulfone (Gruenke and Craig, 1975; Mårtensson, *et al.*, 1975). The side-chain sulfoxide of thioridazine is identical to mesoridazine, which is used as an antipsychotic drug.

Levomepromazine (methotrimeprazine) has a methoxy group as the R_2-substituent, and it has been suggested that it may be metabolized by *O*-demethylation of the methoxy group (Usdin, 1978). This metabolite has only been tentatively identified in the rat (Afifi and Way, 1968).

The phenothiazine drugs may undergo several steps of biotransformation before they are finally excreted in the urine or feces. Several different metabolites may be formed by different combinations of the metabolic pathways, and CPZ is frequently mentioned as an example of a drug with a large number of different metabolites, many of them active.

WHICH METABOLITES CONTRIBUTE TO THE EFFECTS?

General criteria

In order to exert a clinical effect, a drug must have a certain intrinsic pharmacological activity, it must be given in an adequate dose, and have such pharmacokinetic properties that sufficiently high concentrations are obtained at the site of action. There is obviously no reason to believe that the same should not be the case for drug metabolites. It may, therefore, be assumed that in order to contribute significantly to the clinical effect or to the possible side effects of a drug, a metabolite must fulfill the following criteria: (1) possess a certain intrinsic pharmacological activity; (2) be formed in sufficient amounts after therapeutic doses of the drug; (3) have pharmacokinetic properties such that it reaches sufficiently high concentrations at the sites of action.

The pharmacologic activities of neuroleptic drugs have been studied in a number of different *in vitro* systems and animal models. The literature contains some reports on the biologic activity of different CPZ metabolites,

but surprisingly little about the activity of the metabolites of other neuroleptic drugs.

Administration of drug metabolites to patients

The amount of a drug metabolite that must be present in the body in order to produce a certain clinical effect cannot be determined without administration of the metabolite itself to humans. This is very rarely done because of ethical considerations.

Two CPZ metabolites have, however, been administered to psychiatric patients. Chlorpromazine sulfoxide (CPZ-SO) was given intravenously as a single dose of 100–200 mg (Davidson *et al.* 1957). A certain sedative effect was observed, but the sulfoxide, according to the authors, was four to eight times less active than CPZ in this respect. The 7-hydroxy metabolite of CPZ has been given to schizophrenic patients in oral doses of 1000 mg per day for 4 weeks, and it appeared to have an antipsychotic effectiveness which was equal to CPZ given in the same dose (Kleinmann *et al.*, 1980).

The side-chain sulfoxide (mesoridazine) of thioridazine and thioridazine side-chain sulfone have both been given as maintenance treatment to psychiatric patients (Axelsson, 1977). Both metabolites appeared to have antipsychotic properties, but a stronger tendency to give side effects than thioridazine itself.

Plasma, CSF and brain concentrations

For drug metabolites which have not been clinically examined, a fair assumption seems to be that when it has a pharmacological activity similar to the parent drug in relevant biological systems, a metabolite must reach about the same concentration at the site of action as is necessary for the parent drug in order to produce a certain effect.

Phenothiazine metabolites appear to be less bound to plasma proteins than the parent drugs (Kreiglstein *et al.*, 1972; Belpaire *et al.*, 1975; Freedberg *et al.*, 1979; Mårtensson *et al.*, 1980). This implies that they may reach CSF concentrations similar to those of the parent drug, even though their total concentration in plasma is lower than for the parent drug. This has recently been verified for 7-OH-CPZ, which showed levels in the CSF of the same order of magnitude as CPZ, although the total concentration in plasma was about five times as high for CPZ as for the metabolite (Sedvall *et al.*, this volume). Similar findings for thioridazine and its metabolites have been reported by Mårtensson *et al.* (1980). They measured the total and unbound concentrations of thioridazine and three of its metabolites in serum and CSF from psychiatric patients, and found that the total CSF to serum concentration ratio was generally higher for two of the metabolites than for thioridazine.

The plasma concentrations of most phenothiazine drugs are usually below 1 nM (300–400 ng ml^{-1}) after therapeutic doses, while the concentrations of the major metabolites in urine are in the microgram range. It is typical

that for CPZ, which is the neuroleptic drug which has been the subject of the largest number of pharmacological studies, a number of metabolites which have been identified in urine are not found in more than trace amounts in plasma (for review, *see* Usdin, 1971, 1978). Although the phenothiazine metabolites may have lower binding affinity to plasma proteins than the parent drugs, it does not seem likely that metabolites which are not found in measurable plasma concentrations could reach brain concentrations sufficiently high to give a significant contribution to the central effects of the drug.

Since the major part of the phenothiazine metabolites have never been administered to humans, the determination of which of these metabolites significantly contributes to the clinical effect of the drug must be done indirectly by inference from the available data on blood levels of the metabolites and information about their pharmacological activity.

METABOLITE CONCENTRATIONS IN PLASMA

From 10 to 18 different CPZ metabolites have been measured in human plasma or erythrocytes by assay methods based on thin-layer chromatography (TLC) (Kaul *et al.*, 1972; Turano *et al.*, 1972; Chan *et al.*, 1974; Schooler *et al.*, 1976). The specificity of these assay methods have, however, been questioned, as has been discussed by Larsen (this volume).

By application of methods based on gas chromatography or mass spectrometry, which are generally more sensitive and specific, it is mainly the metabolites formed by a single biotransformation step of the parent drug molecule, by one of the pathways (I, II, IV, V) indicated in figure 1 (plus side-chain sulfoxidation of thioridazine and mesoridazine), that are found in significant concentrations in plasma (Curry and Marshall, 1968; Hammar *et al.*, 1969; Sakurai *et al.*, 1975; Mårtensson *et al.*, 1975; Dahl and Garle, 1977). Didesmethyl chlorpromazine and thioridazine side-chain sulfone make two exceptions to this rule. Didesmethyl CPZ has been found in plasma concentrations up to 15 percent of the CPZ concentration (Curry and Marshall, 1968; Craig and Gruenke, 1980), and the plasma concentrations of thioridazine side-chain sulfone are usually 20–40 percent of the thioridazine concentration (Axelsson and Mårtensson, 1977; Axelsson, 1977).

Ring sulfoxidation

The ring sulfoxides are the metabolites which are generally found in the highest concentrations in plasma after oral phenothiazine dosage. It has been suggested that CPZ (Curry *et al.*, 1971; Gibaldi and Feldman, 1972) and levomepromazine (Dahl, 1976) undergo sulfoxidation by first-pass metabolism in the gut wall or the liver. The plasma levels of CPZ-SO relative to that of CPZ may vary greatly between patients, and high levels of the sulfoxide seem to be associated with a lack of response to CPZ treatment

(Lader, 1976; Dahl, 1979).

Patients who respond to treatment with CPZ usually have plasma concentrations of the sulfoxide in the order of 20–80 percent of the CPZ concentration (Mackay *et al.*, 1974; Sakurai *et al.*, 1975; Wiles *et al.*, 1976; Dahl and Strandjord, 1977). The plasma levels of the ring sulfoxide of thioridazine (Axelsson and Mårtensson, 1977; Axelsson, 1977) and levomepromazine (Dahl, 1976; Dahl *et al.*, 1977) are, on the other hand, generally one or two times higher than the levels of the parent drug after repeated oral doses in patients who are classified as responders to the treatment.

The plasma concentrations of perphenazine sulfoxide were similar to perphenazine concentrations in 21 patients after oral doses of the drug, while five other patients who were given perphenazine enanthate intramuscularly had significantly lower concentrations of the sulfoxide than of the parent drug (Hansen and Larsen, 1977; Hansen *et al.*, 1979).

The plasma concentration of promazine and its sulfoxide have been measured in one patient after repeated oral doses of promazine, by a GLC method. The sulfoxide concentration was about 20 percent of the concentration of promazine (Dahl and Jacobsen, 1976).

Ring hydroxylation

Chlorpromazine is hydroxylated mainly in the 7-position in man, and the plasma concentrations of the metabolite are usually 15–120 percent of the CPZ concentration after continuous oral treatment with CPZ (Sakalis *et al.*, 1973, 1977; Mackay *et al.*, 1974; Alfredsson *et al.*, 1976).

Hydroxylated metabolites of promazine (Fishman and Goldenberg, 1965) and of levomepromazine (Allgén *et al.*, 1963) have been found in urine but have not yet been measured in plasma from patients.

Hydroxylation in the 3-position has been reported as a minor metabolic pathway of CPZ (Fishman and Goldenberg, 1965). The 3-hydroxy and 3,7-dihydroxy metabolites of CPZ represent good examples of potentially active drug metabolites which probably do not significantly contribute to the effects of the drug, simply because they are formed in too small amounts. They have not been found in measurable concentrations in plasma.

Conjugation

Phenolic phenothiazine metabolites are mainly excreted in the urine as conjugates. Previous studies based on TLC have indicated that 7-OH-CPZ is also present, mainly in the form of a glucuronide in plasma (Chan *et al.*, 1974), while post-mortem tissues seem to contain a larger fraction of the metabolite in the unconjugated form (Bolt and Forrest, 1967).

Since glucuronides generally are considered to be inactive, less efforts have been put into the development of methods for their quantitation in plasma from patients.

N-Dealkylation

N-Demethylation appears to be a major metabolic pathway for the phenothiazine drugs that have an *N*-dimethylamino group at the end of the side chain, but of less importance for the derivatives which have a *N*-methylated piperidine ring in this position. In a study consisting of 11 patients who were treated with oral doses of thioridazine, *N*-demethylated metabolites were found only in low concentrations in urine and not in plasma (Mårtensson *et al.*, 1975).

The plasma concentrations of monodesmethyl CPZ are usually 10–50 percent of the CPZ concentration after oral treatment with CPZ (Curry and Marshall, 1968; Sakurai *et al.*, 1975; Alfredsson *et al.*, 1976). Monodesmethyl levomepromazine has been measured in plasma from five patients who had been treated with oral doses of levomepromazine, by a gas chromatographic assay method (Dahl, Bratlid and Lingjærde, unpublished results). The plasma concentrations of the monodesmethyl metabolite were higher than the concentrations of levomepromazine in four of the patients, and about 50 percent lower than the parent drug concentration in the fifth patient.

The dealkylated metabolite of perphenazine has been measured in plasma from 21 patients after oral doses of the drug (Hansen *et al.*, 1979). The plasma levels of this metabolite were similar to the levels of perphenazine in most of the patients, but two patients had between three and 10 times higher levels of *N*-dealkylperphenazine than of the parent drug.

N-Oxidation

A reliable quantitation of nanogram amounts of the *N*-oxides of phenothiazine drugs is technically difficult because of their chemical instability. Chan *et al.* (1974) reported that the plasma concentrations of chlorpromazine *N*-oxide (CPZ-NO), measured by a TLC method, were in the range of 50–100 percent of the CPZ concentrations in two patients. Craig and Gruenke (1980) reported that the plasma levels of CPZ-NO, measured by combined gas chromatography–mass spectrometry, were in the range of 30–50 percent of the CPZ levels in an unspecified number of patients.

PHARMACOLOGICAL ACTIVITY OF PHENOTHAZINE METABOLITES

Chlorpromazine metabolites

The activities of different CPZ metabolites have been examined by assessment of their effects on various biological systems, such as amphetamine-

induced stereotyped behavior in rats (Lal and Sourkes, 1972), monoamine metabolism in rat brain (Alfredsson *et al.*, 1977), prolactin secretion in the rat (Meltzer *et al.*, 1977), ^{3}H-haloperidol binding to rat striatal dopamine (DA) receptors (Creese *et al.*, 1978) and monoamine oxidase (MAO) activity in human brain (Roth *et al.*, 1979). Manian *et al.* (1965) examined the toxicity and activity of 7-OH-CPZ and nine other CPZ derivatives in a number of pharmacological tests. The relevance of the different test systems in predicting neuroleptic and other effects in man will not be discussed here. It seems, however, that with some exceptions, as mentioned below, the compounds which are active in one of the systems are generally active in the other systems as well. The general pattern that emerges from these studies is that 7-OH-CPZ and monodesmethyl CPZ both have an activity that is comparable, although slightly inferior, to CPZ itself, while CPZ-SO must be regarded as virtually inactive.

Didesmethyl CPZ had about the same inhibitory effect on human brain MAO activity as CPZ (Roth *et al.*, 1979), but only 10 percent of its binding affinity to striatal DA receptors (Creese *et al.*, 1978).

N-Oxidation is considered to be a pathway of oxidative *N*-dealkylation (Bickel, 1969). Although CPZ-NO must be regarded as a potentially active metabolite (Posner *et al.*, 1968; Lal and Sourkes, 1972; Alfredsson *et al.*, 1977), it is still unclear whether the molecule is active *per se* or not. The *N*-oxide was inactive in the DA binding test (Creese *et al.*, 1978), and Alfredsson *et al.* (1977) measured brain levels of CPZ and monodesmethyl CPZ that could account for the effect of the *N*-oxide on central monoamine metabolism in the rat. The assay method they used did not, however, include CPZ-NO. These latter reports both support the previous suggestion (Bickel, 1969) that CPZ-NO might act indirectly, after transformation to another active compound.

Metabolites of other phenothiazine drugs

The two thioridazine metabolites which are formed by sulfoxidation of the side chain are both pharmacologically active, as mentioned previously. Thioridazine ring sulfoxide is considered to be inactive, and high plasma levels of this metabolite appear to be associated with poor clinical response to thioridazine treatment (Sakalis *et al.*, 1980).

The EEG effects of promazine and monodesmethyl promazine have been examined in an isolated rat brain preparation (Rieger and Krieglstein, 1974). Both compounds were active and had similar effects in this system.

Cardiodepressive effects of monodesmethyl levomepromazine, levomepromazine sulfoxide and CPZ-SO have been studied in isolated rat atria (Dahl and Refsum, 1976; Passwal *et al.*, 1976). Monodesmethyl levomepromazine was active and had about the same potency as levomepromazine. An unexpected finding was that levomepromazine sulfoxide also appeared to be active in this system, while CPZ-SO was much less active.

The binding affinity of levomepromazine, levomepromazine sulfoxide and monodesmethyl levomepromazine to rat striatal DA receptors have

been examined with ^{3}H-spiroperidol as tracer, and compared with the affinity of chlorpromazine (Dahl and Hall, 1981). Levomepromazine sulfoxide was inactive in this system. Levomepromazine and monodesmethyl levomepromazine were both active, and their potency was 0.6 and 0.4 respectively, relative to that of CPZ.

Perphenazine sulfoxide had 3–5 percent of the pharmacological activity of perphenazine in rodents, cats and dogs (Hotovy and Kapff-Walter, 1960).

Experimental data on the pharmacologic activity of the major metabolites of other phenothiazine drugs was not found in the literature.

CONCLUDING REMARKS

In spite of the large number of different phenothiazine metabolites that have been identified in urine, further progress in the understanding of the relationship between the plasma levels and effects of the phenothiazines seem to require that a smaller number of (relevant) metabolites be assayed by reliable methods in a larger number of patients.

More data are required about the identity and blood levels of the major metabolites of neuroleptics other than CPZ, and on the pharmacokinetic and pharmacodynamic properties of these metabolites.

The following may be deduced from the presently available data:

(1) Chlorpromazine has three metabolites that may significantly contribute to its effects in man, namely the 7-hydroxy-, the monodesmethyl- and the *N*-oxide metabolites. Didesmethyl CPZ appears to be of less importance.

(2) Levomepromazine has at least one active metabolite, monodesmethyl levomepromazine, which probably significantly contributes to the effects of the drug in man.

(3) Thioridazine has two metabolites which contribute to its effects, the side-chain sulfoxide and the side-chain sulfone. Significant amounts of thioridazine side-chain sulfone are also present in blood after administration of mesoridazine, which is identical to thioridazine side-chain sulfoxide.

(4) Ring sulfoxides of the phenothiazines are generally found in high concentrations in plasma after oral doses of the drug, but seem to be of less clinical importance because of their low intrinsic activity.

It would seem reasonable to suppose that phenothiazines other than CPZ produce relatively high blood levels of hydroxylated metabolites that may be active. Further, oral treatment with promazine results in significant blood levels of at least one active (monodemethylated) metabolite, and there are other as yet unidentified phenothiazine drug metabolites that give significant contributions to the clinical effects of the drug. All of this still has to be verified experimentally.

However, this does not rule out the necessity of comprehensive studies of

plasma level–effect relationships of neuroleptic drugs based on radioreceptor binding assays or other methods where the sum total of active compound in the sample is measured.

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Active metabolites of tricyclic antidepressants

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INTRODUCTION

Highly lipid-soluble psychoactive drugs which act on the central nervous system (CNS) are cleared primarily by metabolism since they are readily reabsorbed in the kidney. Consequently there is a high probability that biologically active metabolites will be formed in significant quantities – evidence for active metabolites of all classes of drugs acting on the CNS has been available for some time (Garattini *et al.*, 1975). A particularly dramatic example is provided by chlorpromazine (CPZ) which yields at least 35 identifiable metabolites in urine (Turano *et al.*, 1974), many of which have some degree of biological activity (Manian *et al.*, 1965). The tricyclic antidepressants (TCA) which are chemically closely related to CPZ produce a smaller but still substantial number of metabolites in the urine; for instance, as many as 16 metabolites can be identified in urine or tissues following administration of imipramine (IMI) (Crammer *et al.*, 1969; Christiansen and Gram, 1973). Many of these are conjugated metabolites (mainly glucuronides, on the evidence of hydrolysis after β-glucuronidase addition) that are not themselves active, but this still leaves seven identifiable metabolites that could be active (Crammer *et al.*, 1969).

Taking IMI as a model TCA, various types of biological activity of parent compound and metabolites can be identified (Potter and Calil, 1981). Once an activity is identified, a question arises concerning the relationship of this activity to either clinical or unwanted effects in patients. In order to perform a clinical investigation of the relationship between activities and effects one must have a measure of the active form(s) of drug at the site of action(s) — in other words the pharmacokinetic characteristics of both parent drug and active metabolites need to be known. What follows is therefore presented in three sections: (1) metabolites of TCA with biologic activity; (2) proposed relationships between these biologic activities and therapeutic or toxic effects and (3) pharmacokinetics of active TCA metabolites.

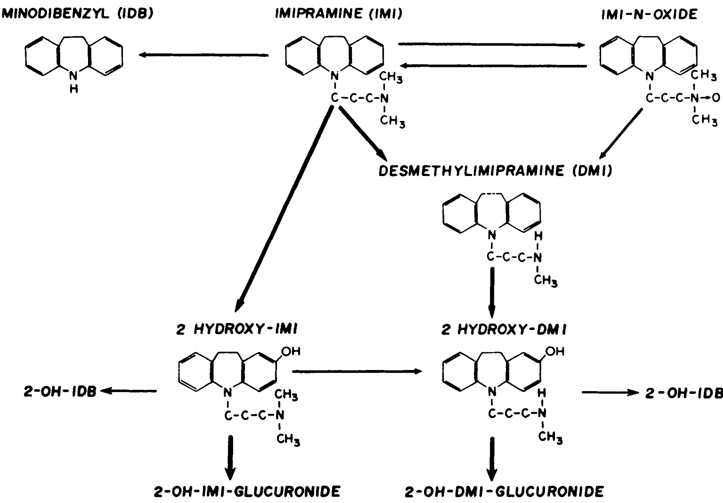

Figure 1 Major routes of the metabolism of imipramine in man.

METABOLITES OF TCA WITH BIOLOGIC ACTIVITY

Figure 1 shows the major metabolic routes of IMI in man. With regard to unconjugated hydroxydesipramine (OH-DMI), a current basic study suggests that a significant proportion is derived from the demethylation of hydroxyimipramine (OH-IMI) (T. J. Monks and J. R. Gillette, personal communication). Didesmethylimipramine and its hydroxylated forms are not shown since they represent such a small fraction of the detectable urinary metabolites (Crammer *et al.*, 1969). Similar overall metabolic schemes can be demonstrated for amitriptyline (AMI) and for chlorimipramine, the other two major tertiary amine TCA (figure 2).

Until recently, biologic activity has been assessed either on the basis of screening studies in animals (for review of techniques, see Sigg, 1968) or *in vitro* assessment of the ability of the compounds to influence uptake or release of radiolabeled norepinephrine (NE) or serotonin (5-HT) (Glowinski and Axelrod, 1964; Carlsson *et al.*, 1969; Randrup and Braestrup,

CHLORIMIPRAMINE — AMITRIPTYLINE

Figure 2 Structures of chlorimipramine and amitriptyline.

1977). More recently biologic or at least biochemical activity of TCA has been shown using *in vitro* displacement or blockade of various radioligands from histaminic (Green and Maayani, 1977; Richelson, 1978), and muscarinic (Richelson and Divinetz-Romero, 1977) 'receptors' as well as interactions with or actions on α- and β-adrenergic receptors (U'Prichard *et al.*, 1978; Vetulani *et al.*, 1976; Banerjee *et al.*, 1977).

It was quickly recognized that the major demethylated metabolites of tertiary TCA were active both in terms of animal screening experiments and *in vitro* effects on either NE or 5-HT uptake. Tertiary amines were more potent inhibitors of 5-HT uptake; secondary amines were found to be relatively specific for NE uptake inhibition (Maas, 1975; Randrup and Braestrup, 1977). Desmethylimipramine and nortriptyline (NT) were developed as antidepressants in their own right. Data suggest that desmethylchlorimipramine in and of itself would also be an effective antidepressant, at least in patients with 'high' pretreatment cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) (Traskman *et al.*, 1979).

More recently it has been shown that the hydroxylated metabolites of TCA have a similar spectrum of activity to their respective tertiary or secondary amine parent compounds (table 1, figure 3). The evidence in

Table 1 Inhibition of norepinephrine uptake by tricyclic antidepressants and their metabolites

	IC_{50} (nM)
Javaid *et al.* (1979)	
Imipramine (IMI)	24
2-OH-IMI	78
Desipramine (DMI)	2.4
2-OH-DMI	7.8
Potter *et al.* (1979)	
IMI	250
2-OH-IMI	180
DMI	55
2-OH-DMI	61
Bertilsson *et al.* (1979)	
Amitriptyline (AMI)	380
Nortriptyline (NT)	75
E-10-OH-AMI	670
Z-10-OH-AMI	720
E-10-OH-NT	160
Z-10-OH-NT	160
Desmethyl NT	1000
AMI-*N*-oxide	7300

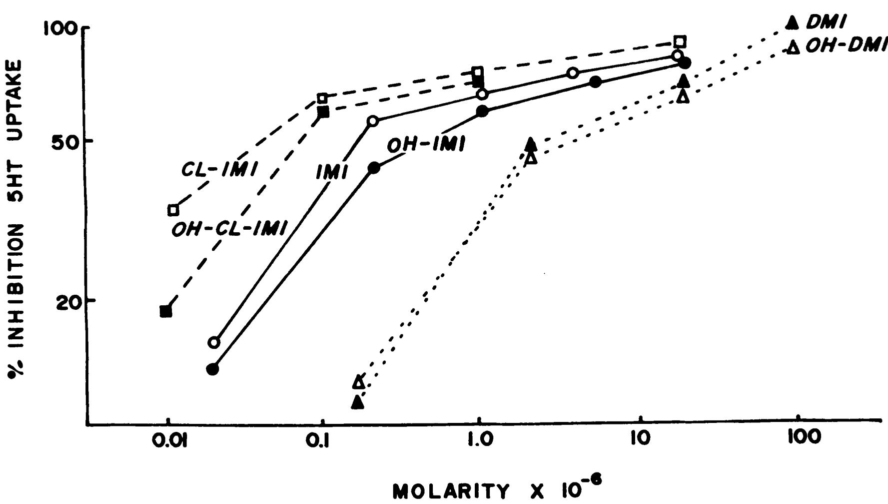

Figure 3 Comparison of effects of chlorimipramine (CL-IMI), imipramine (IMI), desipramine (DMI) and their hydroxylated metabolites on the uptake of ^{3}H-serotonin (5HT) into rat brain synaptosomes. Percentage inhibition plotted on log scale. (From Potter *et al.*, 1979.)

support of biologic activity of hydroxylated metabolites includes not only *in vitro* comparisons (Heikkila *et al.*, 1976; Javaid *et al.*, 1979; Bertilsson *et al.*, 1979; Potter *et al.*, 1979), but also *in vivo* administration to animals (Potter *et al.*, 1979; Potter and Calil, 1981).

Recently, it has also been demonstrated that tertiary and secondary amine TCA differ markedly in their potencies to interact with a variety of receptors (table 2). Interestingly, the tertiary amines are consistently the most potent in terms of blocking histaminic, α-adrenergic and muscarinic receptors, although there are also differences in potency between the different types of TCA. Most recently it has been shown that tertiary amine TCA bind preferentially to high affinity sites on platelets (from animals and man) demonstrated by a simple receptor – ligand displacement assay for tertiary TCA (Paul *et al.*, 1980). Moreover, the hydroxylated metabolites of IMI also bind to platelet sites with a similar affinity to the parent compound (Paul *et al.*, 1980).

As discussed elsewhere, *N*-oxides of TCA may also prove to have biologic activity; in the case of IMI administered as the *N*-oxide, this activity might be of biologic significance (Nagy and Hansen, 1978). With regard to the other known group of non-conjugated potentially active metabolites, the didesmethylated TCA, very little is known. Their amounts in urine following IMI or NT administration are very low (Crammer *et al.*, 1969; Alexanderson and Borgå, 1973). It would be of interest to see if these have some unexpected potency, although animal screening experiments do not suggest this to be the case at least for the didesmethyl metabolite of AMI (Bertilsson *et al.*, 1979).

Thus, on the basis of available data, active forms of TCA can be viewed

Table 2 Potencies of interaction of tertiary and secondary amine tricyclic antidepressants with histaminic, muscarinic and adrenergic receptors

	K_B (nM) for histamine$_1$ receptors		K_B (nM) for muscarinic receptors		K_i (nM) for α-adrenergic receptors
Tertiary amine					
Amitriptyline	0.10	0.13	25	100	24
Doxepin	2	0.03	42	300	23
Imipramine	5	10	100	400	58
Secondary amine					
Nortriptyline	6	7.14	250	1000	71
Protriptyline	30	34.4	63	2000	277
Desipramine	200	250	250	2000	148

Data in each column as follows: first from Richelson (1978), second and third calculated from Richelson (1979), fourth from Richelson and Divinetz-Romero (1977) and fifth column from U'Prichard *et al.* (1978).

broadly as falling into two different classes in terms of biologic activity: tertiary and secondary amines. In each class there are two active forms with similar potencies: the 'parent' and the hydroxylated form. Activities of other forms have not been convincingly demonstrated.

RELATIONSHIP BETWEEN BIOLOGIC ACTIVITY AND THERAPEUTIC OR TOXIC EFFECTS

None of the biologic activities identified in the preceding section has been proven to be the cause of an antidepressant or toxic effect. Proof is here defined as the *in vivo* demonstration of a quantitative relationship between an end effect, a biochemical alteration, and the concentration of drug. Nonetheless, there is indirect evidence that suggests several causal relationships, some of these will be briefly summarized (for review, see Richelson, 1979).

Anticholinergic effects of TCA

As shown in table 2, tertiary amine TCA have higher affinities for muscarinic acetylcholine receptors than do secondary amine TCA. Nonetheless, there may be overlap (for example, protriptyline versus IMI) and only in comparisons of the extremes (AMI versus DMI) does one find as much as an order of magnitude difference. Interestingly, AMI is clinically recognized as the most potent TCA in terms of sedation, dry mouth, and blurred vision — all compatible with anticholinergic effects. On the basis of the receptor

affinity data, DMI would be expected to have the least of these effects, a supposition which is supported by single dose clinical studies (Blackwell *et al.*, 1978; Peterson *et al.*, 1978). On the other hand, DMI is known to inhibit salivary secretion under clinical conditions (Watt *et al.*, 1972). It may be that even the least potent of the TCA in terms of interactions with muscarinic receptors might still achieve high enough steady state concentrations to inhibit salivation. Another possibility is that some TCA (for example AMI), even at very low clinical concentrations, may produce a maximum antimuscarinic effect so that increases in dose will not lead to an increase in side effects. More studies of plasma concentration versus antimuscarinic responses are necessary.

Anti-H_1 receptor properties

Again, it is the tertiary amine TCA that have high affinities for these classes of receptors, especially the H_1 (histamine) type (table 2). In one instance there is an overlap in potency between a tertiary and a secondary amine (IMI versus NT), in another there is a greater than three orders of magnitude difference in potencies (AMI versus DMI). As pointed out by Richelson (1979), since H_1 receptor antagonists can be used clinically as sedative hypnotics, this biochemical action might best explain the very potent sedative effects of TCA such as AMI. The wide *in vitro* differences suggest that a comparison of sedative potency between AMI and DMI in man might clarify whether antihistaminic or anticholinergic (see above) potency is most relevant.

α-Adrenergic receptor blockade

There is electrophysiological evidence for activation of presynaptic α-receptors at low TCA concentration and blockade as the concentration increases (Svensonn and Usdin, 1978). *In vitro* binding studies of brain homogenate show that TCA have a high affinity for α-noradrenergic receptor binding sites (U'Prichard *et al.*, 1978). Assuming that at steady state concentrations α-adrenergic receptors are blocked in man, the somewhat greater potency of tertiary amine TCA to do so (table 2) may be relevant to clinical studies: for example, IMI use is associated with a high incidence of orthostatic hypotension (Glassman *et al.*, 1979). The orthostatic effect could be mediated by either a central action or peripheral failure of vasoconstriction on standing. On the other hand, orthostatic hypotension is highly variable in patients and strongly correlated to its presence prior to treatment (Glassman *et al.*, 1979) — it is therefore a predictable drug effect only in special cases. Moreover, the differences in α-adrenergic blocking potency between, for instance, IMI and NT is not great (table 2). The latter TCA is reported not to influence the orthostatic blood pressure response (Freyschuss *et al.*, 1970). Caution must be exercised in extrapolating *in vitro* data to clinical situations.

Noradrenergic potency

In depressed adult patients (Freyschuss *et al.*, 1970), eneuretic boys (Lake *et al.*, 1979) and normal volunteers (Zavadil *et al.*, 1980) who are treated with NT, IMI or DMI, all potent NE uptake inhibitors *in vivo* (Maas, 1975), pulse and diastolic blood pressure increase. The pulse increase is by far the more marked and reproducible; the diastolic increase, however, is statistically significant. Both effects are consistent with a rise in plasma NE after drug treatment observed in the two current studies (Lake *et al.*, 1979; Zavadil *et al.*, 1980). It would be interesting to see if in the rare patients who do not have appreciable concentrations of secondary amine TCA following AMI or chlorimipramine administration the pulse and diastolic blood pressure response are absent. One might predict that NE uptake inhibitory potency will best correlate with these cardiovascular changes that can be most simply related to enhanced noradrenergic function in the periphery.

There are thus several biochemical and physiological parameters which are amenable to quantitative study *in vitro* and *in vivo*. In the future, clinical studies should be able to clarify which biochemical effects are most closely related to such 'side effects' as sedation, decreased salivary secretion, and cardiovascular changes. Concomitantly it should be possible to establish which active forms of TCA are most closely related to these effects. Such information will ultimately contribute to improved drug development.

The omission of further discussion concerning which biochemical parameter best relates to clinical effect is intentional—the problems in this area have been discussed recently (Potter and Calil, 1981).

PHARMACOKINETICS OF ACTIVE TCA METABOLITES

Both the single dose and steady state pharmacokinetics of the active secondary amine TCA have been well described, particularly in the case of NT (Alexanderson, 1972*a*, *b*). Tertiary and secondary amines TCA, in fact, have similar values of $t_{1/2}$ in the 20 h or longer range (for review, see Potter *et al.*, 1980*a*). Not surprisingly, when a secondary amine TCA is administered its mean steady state concentration is higher for a fixed dose than following administration of its related tertiary amine. Moreover, a given dose of a tertiary amine does not produce as high a concentration of itself as would a secondary amine (table 3). This latter finding is compatible with the well documented extensive demethylation that tertiary amine TCA undergo, whereas secondary amine TCA seem to be cleared primarily through hydroxylation. Only small amounts, for instance, of didesmethylimipramine or DMI appear in human urine following ^{14}C-IMI (Crammer *et al.*, 1969). With regard to steady state concentrations of unconjugated hydroxylated metabolites, only limited information is available, but it suggests that hydroxylated tertiary amine TCA per milligram dose will be low, whereas for secondary amines concentrations will be present in substantial amounts.

Table 3 Mean steady state plasma concentrations per 100 mg dose following administration of tertiary and secondary amine TCA

	Plasma concentration (ng ml^{-1})			
Administered drug	AMI	NT	IMI	DMI
Amitriptyline (AMI)	58	62		
Nortriptyline (NT)	—	110		
Imipramine (IMI)			39	55
Desipramine (DMI)			—	67

Calculated from data in Table 5 (Potter *et al.*, 1980*a*) by standardizing results from indicated reports to a 100 mg dose and taking the mean of the mean values from at least three independent studies.

On the average, OH-DMI concentrations are approximately 50 percent of those of DMI (Potter *et al.*, 1980*b*), whereas OH-NT concentrations are 140 percent those of NT (Bertilsson *et al.*, 1979) (table 4).

Working with the assumption that the steady state concentrations of active drug will ultimately be related to effect(s), it would be useful to understand the determinants of the relative ratios of TCA and their metabo-

Table 4 Concentration ratios of unconjugated hydroxylated metabolites to parent compounds in plasma*

Study	OH-IMI/IMI	OH-DMI/DMI	OH-NT/NT
Gram (1978) †	0.15	0.56	
Potter *et al.* (1980*b*)			
Young males	0.27	0.44	
Young males	—	0.44	
Adults	—	0.55	
DeVane and Jusko (1980)	0.27	0.56	
Kragh-Sørensen *et al.* (1977) (four patients)			2.35
Bertilsson *et al.* (1979)			1.40

*Abbreviation as follows: OH-IMI=2-hydroxyimipramine, IMI=imipramine, OH-DMI=2-hydroxydesipramine, DMI=desipramine, OH-NT=10-hydroxynortriptyline, NT=nortriptyline. All data at steady state except in outpatient study of DeVane and Jusko (1980), where conditions not always known. Dashes are for patients administered secondary amine only.

†Values derived from plot of individual data points on a figure since means and actual values not presented.

lites. Although one group has suggested that IMI is more extensively demethylated than AMI on the basis of steady state ratios of tertiary to secondary amine TCA (Gram *et al.*, 1977), a comparison of several studies does not provide support for such a clear distinction (Potter *et al.*, 1980*a*). The limited urinary data available show that the predominant IMI metabolite is 2-OH-DMI (free and conjugated) and that the next most prevalent is 2-OH-IMI (Crammer *et al.*, 1969). Since 2-OH-IMI can be converted to 2-OH-DMI, at least in rat microsomal preparations (T. J. Monks and J. R. Gillette, personal communication), this pathway may provide a major source of the latter hydroxylated and demethylated metabolite. Thus, high concentrations of 2-OH-DMI in urine do not necessarily support arguments that IMI is any more extensively demethylated to DMI than is AMI to NT.

What is needed is a better understanding of the rates of conversion along all major pathways for both IMI (figure 1) and AMI. Preliminary results of *in vitro* experiments using human liver microsomes do show that the rates of demethylation of AMI or 10-OH-AMI are higher than those for hydroxylation of AMI or NT (Bertilsson and Sjöqvist, this volume). On the other hand, there is no directly comparable evidence on the relative rates of glucuronidation of the hydroxylated metabolites. Following a single subcutaneous injection of 2-OH-DMI (5 $mg\ kg^{-1}$) to rats the total plasma clearance is 56.8 $ml\ min^{-1}\ kg^{-1}$ versus 44.0 $ml\ min^{-1}\ kg^{-1}$ for DMI (I. Kitanaka and W. Z. Potter, unpublished data). Knowing the clearance of 2-OH-DMI and its rate of formation should enable one to predict the steady state concentration of this compound following administration of DMI.

Since hydroxylated metabolites of TCA are not approved for administration to humans, there is no direct evidence on their clearance in man. The few studies which have followed the rate of disappearance of a parent TCA and hydroxylated metabolite have been on NT. Glucuronidation is evidently not rate limiting since the disappearance of 10-OH-NT parallels that of NT (Alexanderson and Borgå, 1973; Alván *et al.*, 1977; Nakano and Hollister, 1978). On the other hand, it may prove that 10-OH-NT is less rapidly cleared by glucuronidation than is 2-OH-DMI, which could explain the relative preponderance of 10-OH-NT at steady state (table 4). This possibility can not be distinguished from the alternative case, whereby hydroxylation of NT is more rapid than that of DMI. *In vitro* studies may be able to clarify whether this latter situation holds.

It has been shown that with another type of extensively hydroxylated drug, debrisoquine, there are rare individuals who appear to have a deficit in the ability to hydroxylate drug (Mahgoub *et al.*, 1977). Thus, instances of only trace amounts of hydroxylated metabolites in plasma following IMI (Potter *et al.*, 1980*b*), or NT administration (Bertilsson and Sjöqvist, this volume) may reflect a deficiency of TCA hydroxylation in some individuals. In order to demonstrate a true deficiency, however, it will need to be shown that the concentration of hydroxylated metabolites in urine is very low.

Whether or not true polymorphism in the hydroxylation of TCA is demonstrated, it is clear that the activity of this pathway varies dramatically between individuals as evidenced by the range of NT to OH-NT ratios and DMI to OH-DMI ratios (Bertilsson *et al.*, 1979; Potter *et al.*, 1980*b*). Since

hydroxylated metabolites are active, however, inhibition of hydroxylation and consequent increase of parent compound would not necessarily lead to excess active drug as has been suggested for inhibition of NT hydroxylation by perphenazine (Kragh-Sørenson *et al.*, 1977). In other words, it needs to be shown that total active drug, not just parent compound, is altered when metabolism of a single pathway is inhibited. As noted by Alexanderson and Borgå (1973) only about 50 percent of a NT dose is normally excreted as 10-OH-NT (free+conjugated). Similarly, even though hydroxylated metabolites and their conjugates are predominant after IMI, they still only account for 55–75 percent of administered ^{14}C-drug (Crammer *et al.*, 1969). Concomitant studies of plasma and urine concentrations of parent TCA and hydroxylated metabolites should provide answers to these questions.

There is another aspect of the pharmacokinetics of active TCA metabolites that requires attention in order to understand the relationship of concentration to effect. Since it is free drug which is pharmacologically active, the comparison of total steady state concentrations of more than one active form solely on the basis on *in vitro* potency may be misleading. It is the free concentrations that should be compared. In preliminary studies using CSF-to-plasma ratios as a measure of free drug, the hydroxylated metabolites of both DMI (Potter *et al.*, 1980*b*) and NT (Bertilsson *et al.*, 1979) have a modestly higher percentage free than do the parent TCA (table 5). On the other hand, in light of the somewhat weaker potencies of the hydroxylated metabolites (table 1), it may be practical to simply add the concentration of respective TCA and hydroxylated metabolite. As discussed in the previous section, it is the tertiary versus secondary distinction that has the most important implications in terms of biochemical activity.

Approaches to quantitating steady state concentrations of total unbound active TCA in plasma using a 'bioassay' in which irides are incubated in patient's plasma and the activity is measured in terms of extent of inhibition of ^{3}H-NE uptake (Borgå *et al.*, 1970) do not need to take into account

Table 5 Comparison of percentage of free parent TCA and free hydroxylated metabolities using cerebrospinal fluid-to-plasma concentration ratios

Drug	$\frac{\text{Conc. in cerebrospinal fluid}}{\text{Conc. in plasma}} \times 100$
Nortriptyline*	4.5 (3.7–5.2)
10-Hydroxytriptyline*	5.4 (5.1–5.8)
Desipramine†	12.5 (10.4–15.5)
2-Hydroxydesipramine†	17.7 (12.9–25.4)

*Mean and range of values in three patients (Bertilsson *et al.*, 1979)

†Mean and range of values in seven patients (W. Z. Potter, H. M. Calil and T. Sutfin, unpublished data).

multiple drug forms. As might be expected, using another technique, inhibition of binding of ^{3}H-IMI to high affinity platelet binding sites is related directly to the sum of IMI and OH-IMI in plasma rather than IMI alone (S. M. Paul and W. Z. Potter, unpublished data). The limitation of such techniques concerns the previously discussed issue (Potter *et al.*, 1980*a*) of what is relevant to measure. Ultimately, such 'bioassays' may simplify the task of dealing with multiple active drug forms. In the meantime, it is appropriate to quantitate all specific active drug forms by chemical methodologies in order to clarify what is the relevant activity.

A final practical clinical aspect of the pharmacokinetics of active hydroxylated metabolites merits mention: if the renal clearance of unconjugated hydroxylated metabolites is quantitatively important, as suggested for OH-NT (Kragh-Sørensen *et al.*, 1977), then impaired renal function may produce accumulation of active drug. For instance, since renal clearance decreases with aging, this could account for the disproportionately high concentration of 10-OH-NT for a given concentration of NT seen in elderly patients (Bertilsson *et al.*, 1979). This could have both clinical and toxicological implications (Potter *et al.*, 1980*a*).

OVERVIEW

Although it has been recognized for almost two decades that demethylated metabolites of TCA have biologic and clinical activity, the possibility that this is the case for hydroxylated metabolites has only recently been considered. Since the activities of the hydroxylated TCA appear to parallel those of the respective tertiary or secondary amine parent compound they do not really complicate pharmacodynamic studies. The question of what is the clinically relevant active TCA form has been the subject of a previous discussion (Potter *et al.*, 1980*a*). It was concluded that available clinical response data is unable to provide the precise measures of change which might directly relate to one of many biochemical activities.

In this paper a more modest pharmacodynamic task has been discussed. Side effects are more 'objectively' quantifiable along a continuum than antidepressant response, as are the numerous interactions of TCA with a variety of 'receptors'. Thus, it should be possible to at least identify the active TCA form(s) responsible for clear-cut side effects. When considered in detail, however, it becomes clear that the boundaries of moving from *in vitro* potency to a clinical situation have not been defined. Is an *in vitro* 10-fold difference in potency clinically significant? In the future, questions should be answerable if basic principles are taken into account. The important pharmacologic principle here would be to compare concentrations of TCA in man which are in a linear concentration–response range, not in the flat part of a curve where maximum effects are already present – this may, for example, be the case in some comparisons of the antisalivary effect of TCA.

In order to adhere to such basic pharmacological principles, active TCA must, of course, be quantitated. Therefore, an understanding of the pharmacokinetics of TCA and their metabolites is necessary. Some relevant points are as follows: (1) parent TCA concentrations partly predict those of the hydroxylated metabolite; (2) following administration of tertiary amine TCA and under steady state conditions there is likely to be a preponderance of total demethylated forms if the hydroxylated metabolite is included; (3) extreme concentrations of hydroxylated metabolite (high or low) are a function both of the rate of hydroxylation and the rate of glucuronidation; (4) variations in the preparation of unbound drug may be significant among different TCA metabolites and influence the 'weight' that each component be given if total (bound+free) concentrations are used; and (5) 'bioassays' of total active drug may prove useful but depend on the yet to be demonstrated clinical relevance of those that are available.

In attempting to relate TCA concentration to a variety of effects, improved results should be possible by quantitation of all active drug forms. As long as one is working with a secondary amine TCA and its similarly active hydroxylated metabolite there appear to be practical models for investigating the relationship to effect (Atkinson and Strong, 1977; Collste *et al.*, 1979). It is unlikely, however, that comparisons of sums, ratios, or weighted proportions of tertiary and secondary amine TCA plus hydroxylated metabolites with effect(s) will provide the precise demonstrations that are needed. Within these limitations, taking into account active metabolites of TCA should help to clarify concentration–response relationships and perhaps explain discrepancies in the literature.

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Preliminary studies on the effect of dothiepin and its metabolites on serotonin uptake by human blood platelets *in vitro*

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INTRODUCTION

It has been well established that tricyclic antidepressants (TCA) inhibit catecholamine and indoleamine uptake in the central nervous system (Carlsson *et al.*, 1966, 1968, 1969). While it is not possible to study human brain tissue directly, the blood platelet is readily available and is generally regarded as a good model of serotonergic neurons (Sneddon, 1973; Lingjærde, 1977). The effect of TCA on the uptake of serotonin (5-HT) by blood platelets has been studied for several drugs (Todrick and Tait, 1969; Waldmeier *et al.*, 1976; Lingjærde, 1976; Ehsanullah and Turner, 1977; Loonen and Soudijn, 1979; Lingjærde, 1979). Such studies have provided additional evidence for the mechanism of action of these compounds and antiserotonergic activity in the platelet system may indicate substances with potential antidepressant properties.

Dothiepin (Prothiaden, Boots) is a tricyclic compound structurally related to doxepin and amitriptyline (AMI) (see figure 1). Clinical trials have shown dothiepin to possess antidepressant properties (Lipsedge *et al.*, 1971). Dothiepin undergoes extensive metabolism in man to produce three major metabolites: northiaden (*N*-demethyldothiepin), dothiepin-*S*-oxide and northiaden-*S*-oxide (Crampton *et al.*, 1978). Recent studies have shown that certain tricyclic metabolites possess antiserotonergic activity (Potter *et al.*, 1979). It was of interest to study dothiepin and its metabolites for the ability to block 5-HT uptake by blood platelets as an indicator of possible antidepressant activity of these compounds. The kinetics of inhibition had not been studied for these substances and were determined in this investigation.

X

CH

CH_2

CH_2

N

H_3C CH_3

$X = CH_2$ - AMITRIPTYLINE

X = S - DOTHIEPIN

X = O - DOXEPIN

Figure 1 Structure of dothiepin in relation to doxepin and AMI.

METHODS

Blood (30 ml) was collected from healthy unmedicated volunteers by venipuncture from an antecubital vein and mixed with dipotassium EDTA to a final concentration of 4 mM. Platelet-rich plasma (PRP) was prepared by centrifugation at low speed (250*g*) for 15 min at room temperature. Platelets were isolated by centrifugation of the PRP at higher speeds (2000*g*) at room temperature for 15 min. The supernatant was discarded, the test tubes drained onto filter paper for a few minutes, and the remaining supernatant cautiously wiped off with filter paper. The platelets were resuspended in a protein-free medium (100 mM phosphate buffer pH 7.4, 70 mM NaCl, 7.5 mM KCl) to a concentration half that in PRP. Aliquots of 1 ml platelet suspension were mixed with a maximum of 50 μl of drug solution to give final concentrations ranging from 10^{-5} to 8×10^{-4} M. The samples were preincubated in a shaking water bath at 37 °C for 10 min and ^{14}C-5-HT (5-hydroxytryptamine-2-^{14}C-binoxalate from New England Nuclear, Boston, USA; specific activity adjusted to 11.4 μCi μmol^{-1}) was added in a volume not exceeding 20 μl, to final concentrations varying from 1.7×10^{-6} to 6.7×10^{-6} M. The reaction was stopped after 2 min by the addition of 1 ml of ice-cold phosphate buffer and transferring the tubes to ice. The platelets were isolated by centrifugation at 2000*g* at 4 °C for 25 min, the supernatant discarded and the tubes drained over filter paper as before. One milliliter of distilled water was added to each of the tubes, which were then frozen and thawed twice to rupture the platelet membrane and release the stored 5-HT. After further centrifugation, 0.6 ml of the supernatant was transferred to counting vials, mixed with 10 ml of Triton solution and the radioactivity measured in a Packard Tri-Carb model 3330 liquid scintillation spectrometer.

To correct for 5-HT trapped in the small volume of medium in the platelet pellet, and the small amount of 5-HT bound to the outer membrane of the platelets, blank values were obtained by adding ^{14}C-5-HT to samples kept in ice, with separate blanks obtained for each concentration of 5-HT used. Using this method it has been shown that no measurable passive uptake of 5-HT occurs, and that the initial rate of uptake obeys simple Michaelis–Menten kinetics up to a substrate concentration of 4 μM (Lingjærde, 1979). All assays were run in triplicate and mean values are reported.

Dothiepin, northiaden and their *S*-oxides were supplied by Boots Company (Australia) Pty Ltd, Sydney, and were used as received. Triton phosphor solution was prepared from Triton X-100 (500 ml), dimethyl POPOP (1.074 g) and PPO (10.74 g) dissolved in 1.5 l of toluene.

The number of platelets was assessed using a Thrombocounter Model C by adding a small volume of the platelet-rich suspension to isotone and counting. A yield of 10^8–3×10^8 platelets ml^{-1} was obtained by this method.

RESULTS

The compounds used in this study all have a marked inhibitory effect on 5-HT uptake by platelets in an artificial protein-free medium as shown in figure 2. A substrate concentration of 1.8×10^{-6} M was used in this experi-

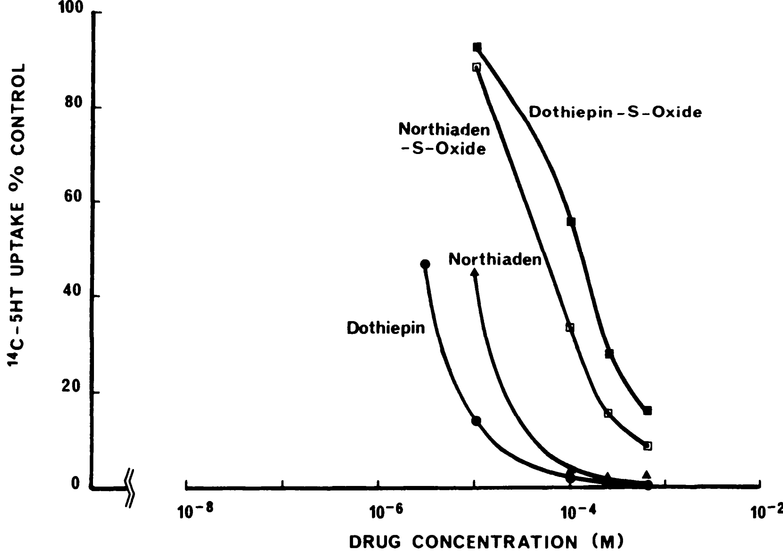

Figure 2 Inhibitory effect of dothiepin and its metabolites on 5-HT uptake in protein-free medium (see 'Methods'). Platelets were incubated with ^{14}C-5-HT for 5 min. Uptake (total radioactivity minus blank value) given as a percentage of controls without drugs added. Each point is the mean of three parallel samples.

ment, which is near to the apparent K_m for 5-HT uptake in platelets (Lingjærde, 1976). At this 5-HT concentration the IC_{50} values (the concentration for 50 percent inhibition of 5-HT uptake) of the four compounds showed the following order of relative potencies: dothiepin>northiaden>northiaden-*S*-oxide>dothiepin-*S*-oxide. Table 1 gives the IC_{50} values for the compounds studied.

Table 1 Concentrations for 50 percent inhibition of serotonin uptake by dothiepin and its metabolites

Compound	IC_{50} (μM)
Dothiepin	<5
Northiaden	<10
Northiaden sulfoxide	73
Dothiepin sulfoxide	198

The kinetics of the inhibitory effect of each of the drugs was determined by using the double-reciprocal plot of $1/v$ (in nanomoles of ^{14}C-5-HT per 10^8 platelets per minute) versus $1/s$ (5-HT concentration in micromolarities) (Lineweaver and Burk, 1934). Values of K_m and V_{max} were determined from the intercepts on the horizontal and vertical axes respectively. Figure 3 shows the inhibitory effect of dothiepin-*S*-oxide, with similar results being obtained for the other compounds investigated. As has been found for other tricyclic antidepressants, a competitive inhibition was observed.

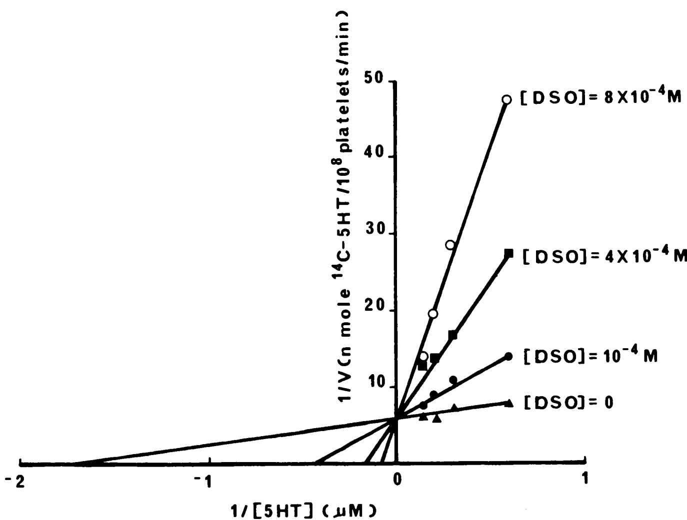

Figure 3 Double reciprocal plot of 5-HT uptake at varying concentrations of 5-HT and dothiepin-*S*-oxide in protein-free medium.

DISCUSSION

Dothiepin and its metabolites are all competitive inhibitors of 5-HT uptake by the blood platelet. In this respect they are similar to other TCA. Dothiepin most resembles doxepin in its chemical structure, which under similar experimental conditions, has been shown to have an IC_{50} of 2×10^{-7} M (Lingjærde, 1976) Dothiepin and its metabolites are weaker inhibitors than doxepin.

For all four compounds used in this study, the possibility of *cis–trans* isomerism exists. The relative proportion of each isomer in the compounds used in this study was not ascertained, nor was the inhibitory effect of one geometric isomer compared to the other. *Trans*-doxepin has a more potent inhibitory effect than *cis*-doxepin (Buczko *et al.*, 1974). Further studies are required to examine this effect for dothiepin and its metabolites.

The pharmacological activity of the sulfoxides in man merits further investigation. Both sulfoxides possess antiserotonergic activity and, after single oral doses, dothiepin-*S*-oxide reaches higher concentrations in blood than dothiepin or northiaden (Maguire *et al.*, 1980). This would suggest that after chronic dosing the sulfoxide would build up to higher concentrations at steady state than either dothiepin or northiaden.

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Active metabolites of antidepressants: novel aspects of hydroxylation and demethylation in man

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INTRODUCTION

The tricyclic antidepressant (TCA) tertiary amines imipramine (IMI) and amitriptyline (AMI) were found, soon after their introduction as drugs, to have active demethyl metabolites—desipramine (DMI) and nortriptyline (NT) (figure 1). These two secondary amines were later introduced as antidepressant drugs. Similarly the potent serotonin (5-HT) uptake inhibitor chlorimipramine (CI) is demethylated to an active secondary amine. In almost every patient the steady state plasma levels of demethylchlorimipramine (DMCI) exceeded those of the parent drug (Träskman *et al.*, 1979; Thorén *et al.*, 1980). As the demethyl metabolite is a potent inhibitor of norepinephrine (NE) uptake, demethylation changes the pharmacodynamic profile of the drug (Träskman *et al.*, 1979; Thorén *et al.*, 1980).

During the last few years it has also been shown that both DMI (Siwers *et al.*, 1977; Potter *et al.*, 1979) and NT (Bertilsson *et al.*, 1979) have hydroxy metabolites that are potent inhibitors of the neuronal uptake of NE. If the antidepressant effect of NT is related to NE uptake inhibition, 10-hydroxynortriptyline (10-OH-NT) in many cases (especially in the elderly) may contribute significantly to the clinical effect (Bertilsson *et al.*, 1979).

The two metabolic reactions, demethylation and hydroxylation, are thus important for both the qualitative and quantative effects of antidepressants in man. We have therefore investigated the regulation of these reactions in man in more detail using both *in vivo* and *in vitro* methods.

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AMI → NT

10-OH-AMI → 10-OH-NT

Figure 1 Main metabolic pathways of amitriptyline. (From Rollins *et al.*, 1980.)

DEMETHYLATION OF AMITRIPTYLINE AND CHLORIMIPRAMINE *IN VIVO*

The two structurally similar drugs AMI and CI were administered to 15 depressed patients in a cross-over study (Mellström *et al.*, 1979). The steady state plasma levels of the parent drugs and their demethyl metabolites were determined by high performance liquid chromatography (HPLC) (Mellström and Tybring, 1977; Mellström and Braithwaite, 1978). An interindividual variation of about fivefold was found in the plasma levels of all four compounds.

As the reciprocal plasma level during multiple dosing is proportional to the clearance of a compound, this parameter was used for linear regression analysis. In these 15 patients there was a significant correlation between the reciprocal plasma levels of CI and DMCI ($r=0.76$) and also between AMI and NT ($r=0.52$) (table 1). The levels of AMI and CI were closely correlated ($r=0.87$) and a similar correlation was found for the demethyl metabolites. These results indicate that similar factors control the plasma levels of AMI and CI during treatment of depression and that similar or identical enzymes may be involved in the metabolism of the two drugs.

In order to obtain an estimate of an individual's capacity to demethylate a drug, we have chosen AMI as a model compound, as the demethyl metabolite NT is a registered drug, which can easily be administered *per se*. To be

Table 1 Coefficients of linear regression analysis between reciprocal plasma levels of AMI and CI and their demethyl metabolites

Variables	Correlation coefficients	Significance, $p<$
CI vs. DMCI	0.76	0.001
AMI vs. NT	0.52	0.05
AMI vs. CI	0.87	0.001
NT vs. DMCI	0.77	0.001

From Mellström *et al.* (1979).

able to determine the degree of demethylation both the parent drug and the metabolite have to be administered (see below). In addition to demethylation, AMI is also metabolized by 10-hydroxylation (figure 1).

AMI and NT were administered to six healthy volunteers as single oral and intramuscular doses (Rollins *et al.*, 1980). The plasma level–time curves of AMI and/or NT are depicted for one subject in figure 2. The

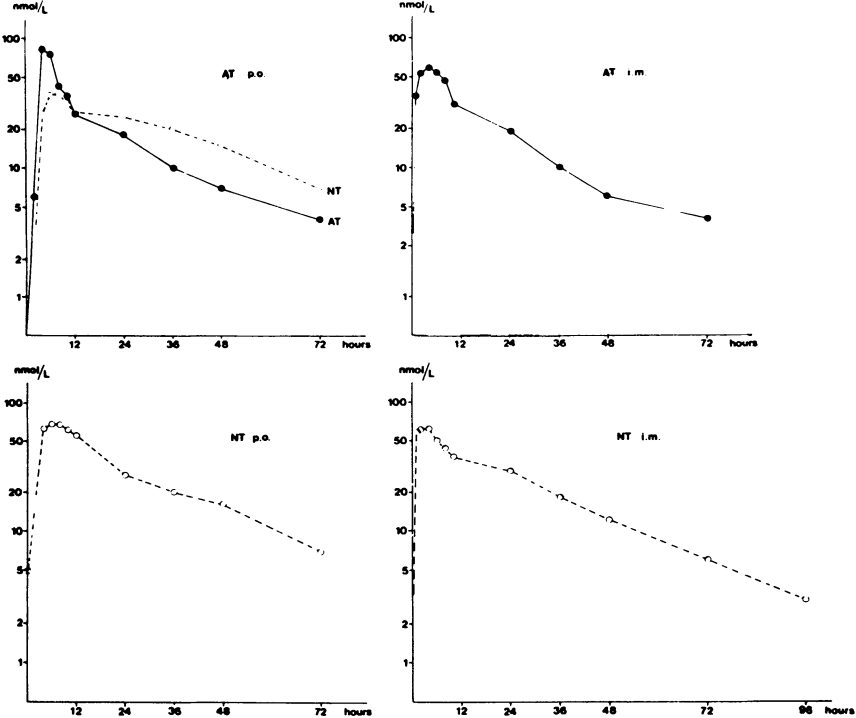

Figure 2 Plasma concentration–time curves of AMI (●—●) and NT (O---O) obtained in subject A.B. The doses were: AMI p.o. 50 mg; AMI i.m. 25 mg; NT p.o. 57 mg; NT i.m. 28.5 mg. (From Rollins *et al.*, 1980.)

plasma curves after a single oral dose of AMI shown in figure 2 are typical for five of the six subjects. NT rapidly reached its peak concentration and within 24 h the NT concentration exceeded that of AMI. In these five subjects, the terminal elimination half-life of NT was longer than that of AMI.

An equation was derived for the estimation of the fraction of an oral dose of AMI being demethylated to NT (f):

$$f = \frac{AUC_{NT\,(AMI)} / Dose_{AMI}}{AUC_{NT} / Dose_{NT}},$$

where $AUC_{NT\,(AMI)}$ is the area under the plasma concentration–time curve of NT found after an oral dose of AMI ($dose_{AMI}$). AUC_{NT} is the AUC of NT when given orally *per se* (Rollins *et al.*, 1980). Using this model, the fraction f of AMI demethylated to NT ranged from 0.25 to 0.89. The fraction f seemed to correlate with the estimated oral clearance of AMI ($r=0.66$), but this trend did not reach statistical significance in the six subjects investigated.

The overall data indicate that demethylation is of primary importance for the elimination of AMI at the doses studied.

METABOLISM OF ANTIDEPRESSANTS IN A PHARMACOGENETIC PERSPECTIVE

The late Dr Balzar Alexanderson showed in twin studies more than 10 years ago that the kinetics of NT are controlled by genetic factors (Alexanderson *et al.*, 1969; Alexanderson, 1973). From family studies it was implicated that a limited number of allelic genes are involved in controlling steady state plasma concentrations of NT (Åsberg *et al.*, 1971). However, in early studies Hammer and Sjöqvist (1967) had found an apparent bimodal distribution of the plasma concentration of TCA.

The elimination of NT depends on its rate of 10-hydroxylation, which varies markedly between healthy volunteers, with a minority showing very low hydroxylating capacity (Alexanderson, 1973; Alexanderson and Borgå, 1973). Recently, polymorphic *N*-oxidation of sparteine (Eichelbaum *et al.*, 1975, 1979) and *C*-oxidation of debrisoquine (Mahgoub *et al.*, 1977; Woolhouse *et al.*, 1979) (figure 3) were demonstrated. Thereby, new tools have become available to explore the regulation of drug oxidation in man. About 5 percent of a German sample of volunteers were phenotyped as sparteine non-metabolizers (Eichelbaum *et al.*, 1979). A similar incidence (about 8 percent) of defective debrisoquine hydroxylators (a urinary debrisoquine/4-hydroxydebrisoquine ratio exceeding 20) was found in a British population (Woolhouse *et al.*, 1979; upper panel in figure 4). The two metabolic reactions might be controlled by similar (the same?) genetic factors (Bertilsson *et al.*, 1980*a*; Inaba *et al.*, 1980). Of 118 healthy Swedish

subjects investigated so far only two (1.7 percent) were defective debrisoquine hydroxylators (a metabolic ratio exceeding 20) (figure 4).

Eight of the Swedish subjects, covering a wide range of ability to hydroxylate debrisoquine, were subsequently given single oral doses of both AMI and NT. The plasma clearance of NT in the eight subjects varied from 0.30 to 0.90 $l\,kg^{-1}\,h^{-1}$ and there was a fairly close relationship between the urinary debrisoquine/4-hydroxydebrisoquine ratio and NT clearance (Spearman's rank correlation coefficient $r_s=-0.83$; $p=0.01$) (Bertilsson *et al.*, 1980*b*). As the NT plasma clearance to a great extent is dependent on the rate of 10-hydroxylation (Alexanderson, 1973; Alexanderson and

Debrisoquine **Nortriptyline**

Figure 3 Formulae and benzylic hydroxylations of debrisoquine and nortriptyline.

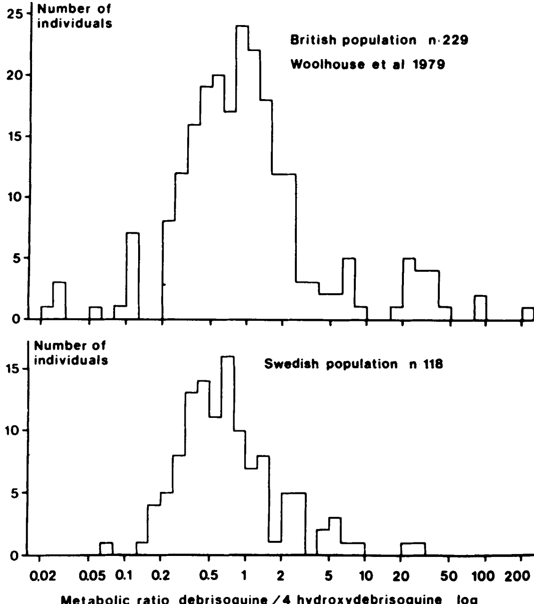

Figure 4 Distribution of the metabolic ratio of debrisoquine/4-hydroxydebrisoquine in urine after a single dose of debrisoquine in a British population (Woolhouse *et al.*, 1979) and in 118 Swedish healthy subjects.

Borgå, 1973), the benzylic hydroxylations of NT and debrisoquine (figure 3) seem to be controlled by similar genetic factors.

Recently we have also determined the ability to demethylate AMI in these eight individuals using the method of Rollins *et al.* (1980). AMI and NT were administered orally and the fraction of AMI being demethylated (f) was determined. f multiplied by the clearance of oral AMI gives an estimate of an individual's ability to demethylate AMI. This demethylation estimate was not significantly correlated to the debrisoquine metabolic ratio (Spearman $r_s = -0.38$; not significant).

These observations suggest that the 10-hydroxylation of NT but not the demethylation of AMI is related to the debrisoquine hydroxylation. Two different components of the P-450 system may be involved in the hydroxylation and demethylation. This finding is in line with the *in vitro* results discussed below.

METABOLISM OF ANTIDEPRESSANTS BY HUMAN LIVER MICROSOMES *IN VITRO*

To be able to interpret the pharmacokinetic data obtained with antidepressants in drug metabolic terms, we have studied their metabolism in human liver microsomes. Specimens of human adult livers were obtained within 20 min after stopping artificial respiration and life-supporting treatment of patients without cerebral activity, who were selected as kidney donors. Homogenization and subcellular fractionation was performed shortly thereafter (von Bahr *et al.*, 1980). The microsomes were stored at −80 °C until used.

When AMI was incubated with human liver microsomes the formation of NT and 10-OH-AMI was studied (figure 1). At an AMI concentration up to 250 μM, the demethylation showed no tendency to be saturated, but the hydroxylation did. In 13 livers studied the ratio between the rates of demethylation and hydroxylation was 6.7±2.7 (mean±S.D.) at an AMI concentration of 100 μM, while it was significantly lower (3.3±1.6; $p<0.001$) at a substrate concentration of 5 μM. This indicates that there is concentration-dependent metabolism of AMI in man.

When AMI was given orally, NT was present in plasma in fairly high concentrations (figure 2). On the other hand, when AMI was given intramuscularly to six healthy individuals, NT could never be detected in the plasma, although the HPLC assay may detect levels down to about 3 nmol l^{-1} (=1 ng ml^{-1}; Rollins *et al.*, 1980). This difference after oral and parenteral administration may be related to the finding of concentration-dependent metabolism of AMI. After an oral administration of AMI there will be an initial high concentration of the drug in the liver before entering the systemic circulation and the major metabolic reaction is then demethylation, not hydroxylation (which may approach saturation at high concentra-

tions). When AMI is given i.m. the drug is distributed in the body and fairly low concentrations of the drug reach the liver. At such low substrate concentrations hydroxylation may not be saturated and NT formed may be hydroxylated before reaching the systemic circulation. Alternatively AMI may be predominantly hydroxylated after an i.m. dose, a hypothesis being explored at present.

Another example where *in vitro* investigations may elucidate unexplainable findings *in vivo* is the fact that very little of a given oral AMI dose is excreted as 10-OH-AMI. Biggs *et al.* (1979) and unpublished results from our group have shown that only 3–5 percent of the dose is excreted as 10-OH-AMI and about 40 percent of the dose as 10-OH-NT. The rate of demethylation of 10-OH-AMI *in vitro* in human liver microsomes seems to be at least as fast as the 10-hydroxylation of AMI (table 2). The 10-OH-AMI formed *in vivo* from AMI may therefore be further metabolized to 10-OH-NT, which will be the major metabolite of AMI in body fluids.

Table 2 Metabolism of AMI and some of its metabolites *in vitro* in human liver microsomes. The incubations were performed at 37 °C for 10 min with a protein concentration of 1 mg ml^{-1} and a substrate concentration of 5 μM

Reaction		Rate of reaction (mean±S.D.) (pmol (mg protein)$^{-1}$ min^{-1})	*n*
AMI	→NT	26±12	14
AMI	→10-OH-AMI	9± 4	14
NT	→10-OH-NT	6± 3	5
10-OH-AMI	→10-OH-NT	11± 4	4

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Section Four
Protein Binding

Plasma and tissue binding of psychotropic drugs

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INTRODUCTION

Most pharmacological actions are the result of binding of drug molecules to specific receptors. However, only in a few instances are these receptors known entities which lend themselves to investigation. Thus, the large number of binding studies carried out in recent years rather reflects the fact that there is a multitude of additional drug binding sites, such as metabolic and transport sites, as well as a variety of non-specific sites. The latter are also called 'silent receptors' or 'sites of loss' since their occupation does not result in pharmacological actions. However, this kind of binding is believed to be a major determinant of pharmacokinetics which again modifies pharmacodynamics.

PLASMA BINDING

Plasma albumin has long been known to be a non-specific binder for a multitude of drugs. Many acidic drugs are strongly bound to albumin which often limits their volume of distribution. Binding studies with albumin have therefore been carried out for pharmacokinetic reasons, but also as a model of molecular interactions between drugs and biological macromolecules.

Most psychotropic drugs are basic lipophilic compounds. Like acidic drugs they bind to albumin, but their binding affinities are usually lower (Weder and Bickel, 1970; Jusko and Gretch, 1976). In addition to albumin, psychotropic drugs like chlorpromazine (CPZ) and imipramine (IMI) also bind to serum lipoproteins and to red cells (Bickel, 1975). In recent years, α-acid glycoprotein was found to be an additional binder of many basic lipophilic drugs, including IMI.

In spite of multiple plasma binding, psychotropic drugs are usually characterized by very high volumes of distribution, that is by tissue/blood concentration ratios of the order of 100. In the presumed absence of active transport processes for this type of compound, such a concentration gradient may be explained by tissue binding which over-compensates plasma binding and creates a sink effect. This hypothesis was one of the reasons for our tissue binding studies which will be briefly summarized.

TISSUE BINDING

Binding studies with tissue homogenates and similar preparations were carried out using conventional techniques such as equilibrium dialysis, ultracentrifuge sedimentation, or difference spectrophotometry (Bickel and Steele, 1974). Most of the tested psychotropic drugs revealed reversible, non-specific tissue binding to two classes of binding sites, neither of them being cytochrome P-450. Using subcellular fractions, the binding of CPZ and IMI could be traced to intracellular membrane fractions and finally to membrane phospholipids (Di Francesco and Bickel, 1977). Pure lecithin liposomes showed the same binding characteristics. The association constant of the high affinity site for CPZ was $3 \times 10^4\ \mathrm{M}^{-1}$, its binding capacity 580 nmol mg^{-1} phospholipid, and total capacity was 2.5 times as much. Thus, both affinity and capacity of tissue binding were higher than the corresponding values of albumin or other plasma binders.

Membrane binding of CPZ and IMI was further characterized by binding studies with liposomes consisting of chemically pure lipids of various head group charges, chain lengths, and number of double bonds, and by using various techniques and temperatures (Römer and Bickel, 1979). These studies lead to the following conclusions: Binding of the drugs is dependent on the physical state of the membrane lipids (gel or liquid crystalline) rather than on their chain length or degree of unsaturation. Binding of the drugs leads to isothermic fluidization of a rigid membrane. As revealed by the use of fluorescence markers, IMI binds near the polar surface of the membrane bilayer, whereas the more lipophilic and planar CPZ has additional binding sites in the non-polar core of the bilayer.

BINDING COMPETITION

The fact that psychotropic drugs are bound both to plasma and to tissue components, and that the latter binding is more pronounced in terms of affinity and capacity, suggests the existence of a binding competition operative between the intracellular and extracellular compartments. In order to

gain insight into the mechanism of binding competition, distribution dialysis was used as a simple *in vitro* model. In this model, each of the two chambers of an equilibrium dialysis device contains a binder which simulates intra- and extracellular phases, respectively, and a drug is then allowed to distribute in this system. The use of binders of known binding affinities and capacities in varying proportions clearly showed that a binding competition was operative in this system and that the distribution of drugs was a function of it. Using tissue homogenates and whole blood as binders, the distribution was comparable to the tissue/blood concentration ratios obtained *in vivo*. Thus, drug distribution is largely determined by a binding competition between tissue and blood sites.

Certain drugs, when given orally, largely disappear during their first passage through the liver, that is before reaching the systemic circulation. This first-pass effect is typical for many basic lipophilic drugs with a high volume of distribution, including psychotropic drugs. In a study with isolated rat livers perfused with IMI at therapeutic concentrations, 99 percent of the drug was taken up in the first passage; uptake rate was much higher than metabolism rate, and uptake was not significantly decreased by the presence of albumin or other binders in the perfusate (Stegmann and Bickel, 1977). Thus, intracellular binding produces a strong sink effect which results in a non-restrictive transport from blood to tissue. In other words, both free and plasma protein-bound drug are available for uptake, and binding competition is highly in favor of intracellular binding.

PSYCHOTROPIC DRUGS AS LYSOSOMOTROPIC AGENTS

Still another kind of intracellular binding was revealed by studies on interactions between psychotropic drugs and cellular receptors. Using cultured human fibroblasts, the uptake and intracellular localization of desipramine (DMI) was studied (Honegger *et al.*, 1980). Cellular uptake of this drug was rapid and led to cell/medium concentration ratios of about 1000. Uptake could be enhanced by a rising extracellular pH which is suggestive of ion trapping of the basic drug in organelles of acidic contents. In fibroblasts chronically treated with DMI, the lysosomal degradation of mucopolysaccharide sulfates and of membrane phospholipids was inhibited. These fibroblasts showed a typical granulation indicating storage of non-degraded material. These kinetic, functional, and morphological effects of DMI are indicative that this and possibly other antidepressant drugs are concentrated within lysosomes. This may not only contribute to pharmacokinetically important tissue binding but may also play a role in the mechanism of action of these drugs; a correlation may exist between antidepressant-induced changes of phospholipid patterns and the coupling of the adenylate cyclase to the β-adrenergic receptors which are known to be desensitized by these drugs.

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Protein binding and monitoring of psychotherapeutic drugs in plasma

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INTRODUCTION

Therapeutic drug monitoring is based upon determination of the *total* drug concentration in plasma or serum. This is, of course, acceptable only as long as the fraction of unbound drug remains virtually constant within the patient population and within each patient. However, under certain circumstances, protein binding may vary several-fold between subjects because of differences in the following: (1) primary structure of the binding proteins; (2) concentration of the binding proteins; (3) concentration of endogenous and exogenous binding inhibitors; (4) concentration of the drug to be measured. These factors (with the exception of (1)) may cause intraindividual as well as interindividual variability, since they may be influenced by, for example, age, physical activity, other drug therapy and diet. On the other hand, it appears that the primary structure of a binding protein is under genetic control (Wilding *et al.*, 1977). Furthermore, dramatically increased or decreased binding has been observed in several disease states, for example renal and liver disease (Piafsky, 1980).

The practising physician and the drug monitoring service laboratory should be concerned about how the variability in protein binding may affect the interpretation of the total drug level in plasma. There are probably several instances, as will be discussed below, where the accuracy of therapeutic monitoring will be greatly improved by the introduction of good clinical routine methods for assay of protein binding. This discussion will be quite general and not limited to psychotherapeutic drugs, although they may serve to illustrate the principles.

Another instance in therapeutic drug monitoring where protein binding plays a critical role is in the blood sampling procedure. Unsuitable blood

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collection material or procedures may affect drug protein binding, thereby affecting both the total and the unbound drug level in the plasma sample.

It may be relevant, at this moment, to point out that in general the importance of protein binding will be inversely related to the magnitude of the unbound fraction. Therefore, the problems associated with interindividual variability and different blood sampling procedures, as outlined above, will be most important for highly bound drugs, that is drugs bound to 90 percent or more in plasma.

THERAPEUTIC DRUG MONITORING – PRACTICAL CONSIDERATIONS RELATED TO PROTEIN BINDING

The steps involved in blood sampling and drug assay, and the important questions related to these steps, are examplified in figure 1.

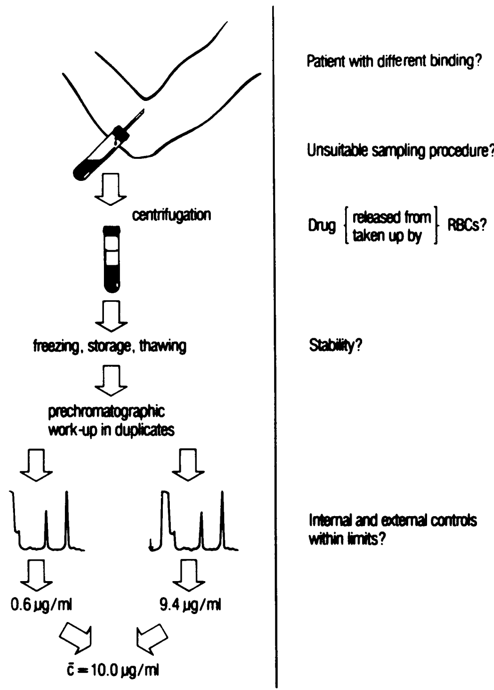


Figure 1 The determination of a drug concentration in plasma or serum involves several steps where errors may be introduced if unsuitable procedures or materials are used. Many laboratories seem unaware of pitfalls in the steps prior to the purely analytical ones.

Blood collection procedures – effect of temperature

Drugs that are lipophilic enough, as are most psychotherapeutic agents, will rapidly penetrate the red blood cell (RBC) membrane. An equilibrium will rapidly be established, the plasma/RBC ratio being determined by binding processes in plasma as well as in RBCs. Any factor disturbing these processes will alter the plasma/RBC ratio. As an example, plasma protein binding of phenytoin increases with decreasing temperature, while RBC binding is largely unaffected (Ehrnebo and Odar-Cederlöf, 1977). Thus, levels of phenytoin in plasma prepared by centrifugation of refrigerated blood samples were 10 percent higher than in plasma samples prepared at room temperature (Borgå *et al.*, 1980). Many other drugs should be expected to behave similarly but data are still insufficient.

The use of an indwelling heparinized cannula, the so-called heparin lock, has been demonstrated to increase the unbound fraction of propranolol (Wood *et al.*, 1979) and quinidine (Kessler *et al.*, 1979). The above observations emphasize the need of careful standardization of blood sampling procedures.

Blood collection material

Erroneously low plasma levels of propranolol were noted by Cotham and Shand (1975) when using Vacutainer® tubes. This was shown by them to be caused by the presence of some agent in the rubber stoppers of these tubes that was able to decrease propranolol protein binding. Thus, the plasma/RBC ratio was altered *in vitro*, and plasma levels of propranolol were drastically reduced. The displacing agent has been identified as tris(2-butoxyethyl)phosphate (TBEP) and its action shown to be limited to drugs bound to α_1-acid glycoprotein (AAG) (Borgå *et al.*, 1977). Lowered plasma levels when using Vacutainers have been observed for a number of lipophilic basic drugs, as shown in table 1, which is restricted only to psychotherapeutic agents. It thus may appear that all drugs in table 1 are to some extent bound to AAG. However, it remains to be proved that the above mechanism is the only explanation to the decreased plasma level for several of the drugs in the table, since adsorption to the rubber material is another theoretical possibility.

VARIANCE IN PROTEIN BINDING VERSUS VARIANCE IN DRUG ASSAY

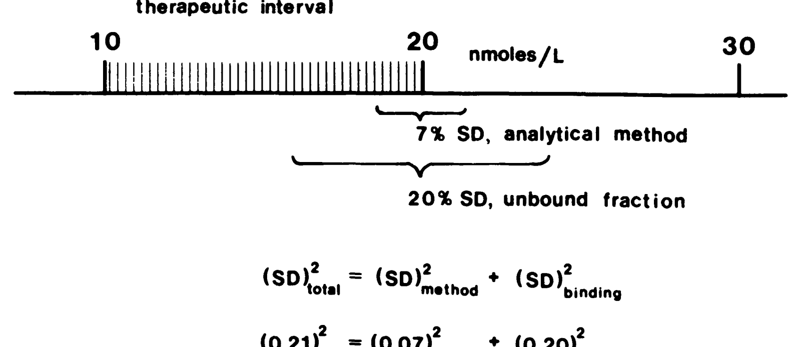
For drugs where the intersubject variation in unbound fraction is large, there is an obvious analogy between the determination of the total plasma level and the use of a drug assay of poor precision. However, while most laboratories would try to improve a poor assay method, probably very few

Table 1 Effect of Vacutainers on binding and plasma levels (C_p)*

Drug	Effect	Reference
Amitriptyline, Nortriptyline	Decreased C_p	Cochran *et al.* (1978), Veith *et al.* (1978)
Chlorimipramine, Demethylchlorimipramine	Decreased C_p	Mellström and Tybring (1977)
Doxepin	Decreased C_p	Veith *et al.* (1978)
Imipramine	Decreased binding Decreased C_p	Borgå *et al.* (1977) Brunswick and Mendels (1977)
Desmethylimipramine	Decreased C_p	Cochran *et al.* (1978), Veith *et al.* (1978)
Zimelidine, Norzimelidine	Decreased C_p	Westerlund *et al.* (1979)
Neuroleptics	Decreased binding	Freedberg *et al.* (1979)

*Slightly modified and extended from Piafsky (1980).

would feel motivated to introduce determination of unbound drug levels. However, a large biological variability in protein binding may be equally deleterious to the final result as a large variability in the analytical step, at least from the viewpoint of the physician using the monitoring service. Figure 2 is an attempt to illustrate this with a purely hypothetical case. In the example, the variability in protein binding of the drug is 20 percent (relative S.D.) while that of the analytical method is only 7 percent. The overall variability in the estimation of the underlying pharmacologically interesting



$$(SD)^2_{total} = (SD)^2_{method} + (SD)^2_{binding}$$

$$(0.21)^2 = (0.07)^2 + (0.20)^2$$

Figure 2 When total concentration in plasma is used as an index of unbound (that is pharmacologically active) drug then the total variance will be the sum of the variance in the analytical procedure and of interindividual variance in free fraction in plasma.

(that is the unbound) drug concentration will be 21 percent (expressed as a relative S.D.), in other words mainly governed by the biological variability in binding. The way to improve the situation is to determine each individual's drug binding, not to improve the analytical assay.

In the case in figure 2 the therapeutic interval is from 10 to 20 nmol l^{-1}. With an overall S.D. of 21 percent, a borderline concentration such as 20 nmol l^{-1} would represent a 'true' value somewhere within the range 16–24 nmol l^{-1} (that is 20±1 S.D.) in 68 cases out of 100 and within the range 12–28 nmol l^{-1} (20±2 S.D.) in 96 cases out of 100. This illustrates that a total level of 20 nmol l^{-1} may be a therapeutic concentration in one individual and produce dose-related side effects in another, yet the explanation in the latter case has nothing to do with increased sensitivity.

The previous reasoning was based upon the assumption that the therapeutic range was valid for an 'average' individual with an average binding. However, the width of the range may, at least partly, be a *result* of differences in binding, since it has been based upon total drug level data. This reasoning would imply that it would be possible to define a more narrow therapeutic window based upon unbound drug levels.

In conclusion, monitoring of unbound drug would be important for drugs having large variations in binding within the patient population. Factors responsible for such variations will be discussed below.

PROTEINS RESPONSIBLE FOR BINDING OF BASIC DRUGS

Many drugs which are weak acids are bound mainly to albumin in plasma. Not until recently has it become evident that basic drugs – and most psychotherapeutic drugs are bases – are bound with low affinity to albumin (Piafsky, 1980).

Other proteins must therefore be responsible for very extensive binding in plasma. Recent investigations have shown that AAG binds a number of mainly lipophylic basic drugs (see review by Piafsky, 1980). Among psychotherapeutic drugs, imipramine (IMI) and chlorpromazine (CPZ) are extensively bound to lipoproteins in addition to AAG (Bickel, 1975; Borgå *et al.*, 1977). The binding pattern of tricyclic antidepressants in plasma may in fact be even more complex, since studies using equilibrium gel filtration indicate the presence of four binding proteins of desmethylimipramine (DMI) (figure 3) (Borgå, 1973). In the light of recent studies, binding in the first peak (19 S) may be assigned to lipoproteins (Danon and Chen, 1979), while the third peak (4 S) represents binding to AAG and (to a minor extent) albumin (Borgå *et al.*, 1977). The second peak (7 S) must therefore represent binding to a so far unidentified protein. Binding to lipoproteins may be a more general phenomenon than appreciated at the moment, since so far very few drugs have been studied in this respect (Vallner and Chen, 1977).

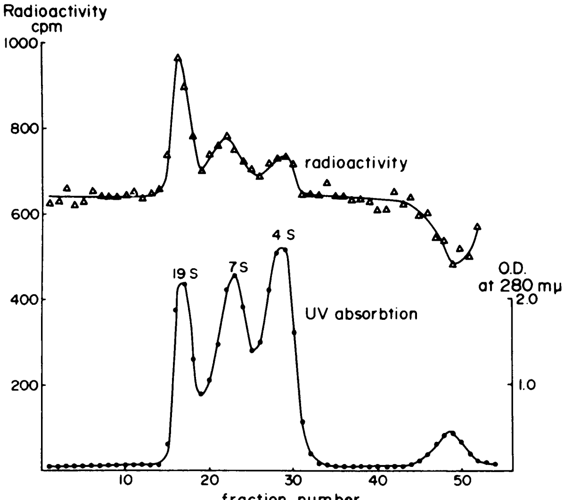


Figure 3 Binding of desmethylimipramine to plasma components using the technique of Hummel and Dreyer (1962). Heparinized plasma (2 ml) containing 5 percent glucose to increase the density was applied to a Sephadex G200 column (180 cm×1.5 cm, i.d.) pre-equilibrated with ^{3}H-desmethylimipramine (S.A. 14.7 mCi mmol^{-1}, concentration 0.3 μM). The proteins were eluted at 20 °C with a 0.1 M phosphate buffer (pH 7.4) containing 0.3 μM of the labeled drug. The radioactivity and optical density at 280 nm were determined in 5 ml fractions of the eluate (Borgå, 1973).

EXTENT OF BINDING AS A FUNCTION OF PROTEIN CONCENTRATION

At low enough drug concentrations the so-called binding ratio (bound/unbound) of the drug is linearly related to the concentration (P) of the binding protein (Odar-Cederlöf and Borgå, 1976):

$$\frac{\text{Bound fraction}}{\text{Unbound fraction}} = nK_a P,$$

where n is the number of binding sites and K_a the association constant. For drugs where nK_a is large enough, the bound fraction is close to unity. Thus (after rearrangement),

$$\text{Unbound fraction} = \frac{1}{nK_a} \frac{1}{P} = \text{Constant} \times \frac{1}{P}.$$

Thus an x-fold increase in P can, at the most, result in an x-fold decrease in unbound fraction. As an example, in a study in 60 subjects, both healthy and

with various diseases, the variation in AAG concentration was approximately sixfold (table 2) (Piafsky *et al.*, 1978). Unbound CPZ varied fivefold (0.57–2.74 percent) as a function of this variation. Contribution of lipoprotein binding was not investigated. Patients with Crohn's disease and inflammatory arthritis had the highest levels of AAG while the healthy controls varied from 0.50 to 1.07 g l^{-1}. However, even among subjects thought to be healthy, a fivefold variation in AAG may occur (Alván *et al.*, 1981; Sager *et al.*, 1979). Such variations will render predictions on whether a particular individual has a normal or altered protein binding impossible.

In healthy subjects, unbound IMI was negatively correlated to AAG levels (Piafsky and Borgå, 1977). In healthy subjects and patients with hyperlipoproteinemia Danon and Chen (1979) found higher binding of IMI in the latter. The differences were significantly correlated to plasma cholesterol and triglyceride levels.

QUALITATIVE FACTORS INFLUENCING BINDING

Among patients with vascular disease treated simultaneously with warfarin and other drugs, the unbound fraction of warfarin varied fourfold and quite independent of the relatively moderate differences in plasma albumin, the main binding protein of warfarin (Yacobi *et al.*, 1976). This may be explained by the presence of endogenous binding inhibitors (Sjöholm *et al.*, 1976), or shall we call them binding modulators since some of them cause increased rather than decreased binding (Nilsen *et al.*, 1977)? These modulators may then be present in concentrations that vary between subjects. Another explanation is genetic differences in the structure of albumin as indicated in a study in twins (Wilding *et al.*, 1977). It is too early to speculate whether similar mechanisms are operating in drug binding to lipoproteins or AAG. However, in a selected group of patients with chronic renal failure and no other concurrent disease, the unbound fractions of propanolol and CPZ were significantly correlated to increasing concentration of serum creatinine (Piafsky *et al.*, 1978). Also, basic drugs may compete for common binding sites; for example, quinidine added to plasma in concentrations between 15 and 154 nmol l^{-1} displaces propanolol, probably from a common site on AAG (Borgå and Stiko, unpublished). Various basic drugs such as prazocine, dipyridamole and CPZ and the steroid hormones progesterone and testosterone are able to decrease propranolol binding to AAG (Jacobsen, 1980).

METHODOLOGY TO MEASURE PROTEIN BINDING

The two standard methods are equilibrium dialysis and ultrafiltration, and these are also the main methods that have been used in studies on

Table 2 Interindividual variation in unbound fraction of some basic drugs

Drug	Percentage unbound, range	Relative variation (S.D.) percentage of mean	Subjects	Method	Reference
Propranolol	3.2 –17.7 (n=78)		Healthy and patients*	Equilibrium dialysis, 37 °C	Piafsky *et al.*, 1978
Chlorpromazine	0.57– 2.74 (n=60)		Healthy and patients*	Equilibrium dialysis, 37 °C	Piafsky *et al.*, 1978
Desmethylimipramine	5.5 –12.5 (n=41)	15	Healthy	Ultrafiltration, 25 °C	Borgå *et al.*, 1969; Sjöqvist *et al.*, 1969
Imipramine	5.4 –23 (n=26)	21			Glassman *et al.*, 1973
	6.1 –11.1 (n=23)		Depressed patients Healthy†	Equilibrium dialysis, 37 °C	Piafsky and Borgå, 1977
Desmethylchlorimipramine	5.1 –10.2 (n=10)	17	Healthy	Equilibrium dialysis, 37 °C	Bertilsson *et al.*, 1979
	5.3 –10.4 (n=14)	20	Depressed patients‡	Equilibrium dialysis, 37 °C	Bertilsson *et al.*, 1979
	1.1 – 4.0 (n=18)	26	Depressed patients	CSF/plasma ratio	Bertilsson *et al.*, 1979
	2.2 – 4.7 (n=5)	26	Healthy	Ultrafiltration	Bertilsson *et al.*, 1979

*Range of AAG concentration in plasma, 0.4–2.6 g l^{-1}.
†Range of AAG concentration in plasma, 0.4–1.1 g l^{-1}.
‡Range of AAG concentration in plasma, 0.5–1.0 g l^{-1}.

psychotherapeutic drugs. These drugs may, however, present special problems in the two methods as will be discussed later. In general, protein binding determinations, in order to give results that can be extrapolated to the *in vivo* situation, must be applied with the following considerations:

(1) Human plasma (or serum) should be used rather than isolated protein fractions, and all precautions should be taken to preserve its integrity. If frozen samples have to be used, the effect of freezing and thawing on the binding should be investigated for each new drug.

(2) In general one should try to perform the study at drug concentrations well below the molar concentration of the binding protein (unless molar therapeutic drug levels are in fact similar to that of the protein). For AAG in plasma, normal levels may be as low as 10 $\mu mol\ l^{-1}$. Studies on highly bound drugs may then require sensitive analytical methods or access to radiolabeled drug of high specific activity.

(3) For addition of the drug to plasma or dialysis buffer, water solutions should be preferred since organic solvents are likely to alter binding. If an organic solvent has to be used, it may be evaporated off and the residue dissolved directly in the plasma. It should be noted that solubility in plasma may exceed the solubility in water by several orders of magnitude.

(4) If the aim of the study is to investigate interindividual variability in binding, then the overall variability of the binding assay should be carefully evaluated. One method preferred by this author is to include in each assay belonging to a particular study a reference sample taken from a stock of frozen aliquots of one single control plasma. The figure obtained on day-to-day variation of the assay is then particularly relevant to the complete study. Any trend or sudden change in the value of the control plasma will serve as an early warning system, indicating, for instance, increasing concentrations of radioactive impurities in a labeled drug.

Equilibrium dialysis

Several factors may be critical, for example the composition of the buffer, the control of pH and the length and temperature of incubation to achieve distribution equilibrium. Usual procedures where plasma is allowed to equilibriate with an equal volume of buffer of pH 7.3–7.4 in a small Perspex or Teflon chamber will cause a fairly rapid increase in pH with time. Thus, pH may increase 0.4–0.5 units in 1 h, probably due to a loss of CO_2 from the plasma, and in spite of the use of an apparently closed system. The pH in plasma can be kept at 7.35 by blowing 5 percent CO_2 in air over plasma and Krebs–Ringer bicarbonate buffer before incubation, and by placing the chambers and the shaking device used in the equilibration step in a closed box containing the same gas mixture (Nilsen *et al.*, 1977) and kept at the appropriate temperature by thermostating the gas (cupboards used in microbiology are very suitable).

If large volumes of buffer compared to plasma are used, the concentration of the drug being studied and of various endogenous compounds in plasma that are able to modify its binding will be diluted. Thus the buffer compart-

ment should be as small as practically possible, for example a fourth of the plasma compartment. (This will further necessitate good pH control by other means than the buffer.) Equilibration times longer than 6–8 h should be avoided if possible, since bacterial growth and protein denaturation may occur. It is generally believed that studies should be performed at 37 °C. However, comparative studies at 37 °C and 20 °C may show that the relative differences in binding between plasma samples from a group of subjects are independent of temperature (Yacobi and Levy, 1975), but this has to be investigated for each new drug being studied.

Ultrafiltration

In ultrafiltration, plasma water and its low molecular constituents are forced through the pores of the semi-permeable membrane. The necessary pressure can be accomplished, for example by centrifugation (Lunde *et al.*, 1970) or by suction (Ultra-Free, commercial equipment from Millipore). Unless the plasma stays in contact with an atmosphere containing CO_2 (5 percent in air is suitable), the pH will rapidly rise to 7.9–8.1. For some drugs like theophylline this may alter the binding dramatically (Vallner *et al.*, 1979).

The use of ultrafiltration is based upon the assumption that drug molecules are filtered at the same rate as water, which is not always the case, as shown with alprenolol and using certain types of membranes (Johansson *et al.*, 1974). In addition, some membranes are able to adsorb certain drugs more or less completely from a water solution passing through them. This is a problem that occurs frequently with psychotherapeutic drugs. Apart from this, ultrafiltration has several advantages in that it is rapid and causes no dilution of plasma components which makes it the method of choice in studies on, for example, drug displacement interactions.

In vivo methods

The actual *'in vivo'* binding may be calculated from the cerebrospinal fluid (CSF) to plasma concentration ratio provided that distribution equilibrium has been reached. Another prerequisite is that binding does not occur to the proteins present in low concentration in the CSF, as demonstrated with CPZ (Sedvall, this volume).

Saliva to plasma ratios give a rough indication of binding but are unsuitable to pick up interindividual differences in binding (Barth *et al.*, 1976). They are particularly unsuitable for most psychotherapeutic drugs due to the difference in pH between saliva and plasma that causes a several-fold concentrating effect of these basic drugs into the saliva (Mucklow *et al.*, 1978).

Ultracentrifugation

Little experience has been gained with this method so far. In theory it lacks several of the drawbacks of the other methods, since no membrane is necessary to separate bound and unbound drug and the integrity of the plasma sample is little affected. The separation of bound and unbound drug is based upon the principle that protein and protein-bound drug are migrating downwards in a field of high gravity while the unbound drug is unaffected and thus remains in the top layer. It therefore must be shown for each new drug of study that in fact the unbound drug is not migrating in this field too.

INTERINDIVIDUAL VARIATION IN BINDING OF PSYCHOTHERAPEUTIC DRUGS

The previous findings in a limited number of studies on this subject may be discussed in the light of recently obtained information regarding nature of binding proteins and methodological difficulties. The earliest study was performed with DMI using ultrafiltration of plasma from 41 healthy blood-donors (table 2). Membrane adsorption was negligible. Interindividual variation was approximately twofold. A similar variation was later found with nortriptyline in 17 sets of healthy twins using a plasma–plasma equilibrium dialysis technique at 4 °C against a reference plasma (Alexanderson and Borgå, 1972). It is not known whether the long equilibration time (168 h) used could have had a deleterious effect on the binding. A twofold variation of IMI binding as studied by equilibrium dialysis at 37 °C over 17 h was again observed by Piafsky and Borgå (1977), the unbound fraction being related to levels of AAG (table 2). The above studies were all performed in small numbers of healthy subjects and they may, therefore, have underestimated the variability in binding, since variations in AAG and lipoproteins are wider in larger unselected groups. Also, it has been questioned whether the equilibrium dialysis is a valid technique for IMI. This technique was used in a study by Glassman *et al.* (1973), showing a fourfold variability in unbound IMI in 26 patients who were treated with this drug for psychiatric disorders. This finding is difficult to evaluate since methodological details were not explicitly reported. However, it appears that fairly long (over 24 h) equilibration times at 37 °C were used.

Severe methodological problems were reported with desmethylchlorimipramine (Bertilsson *et al.*, 1979). Thus, the CSF/plasma ratio observed in patients treated with chlorimipramine indicated a fourfold variation between patients, but possible binding in CSF was not studied. Ultrafiltration indicated a similar variation, but the results were questionable because of extensive uptake of the drug in the membrane. Also equilibrium dialysis at 37 °C was questionable since no stable equilibrium was obtained. A minimum free fraction of 6–8 percent was obtained at 6 h,

but this was considerably higher than that observed with the two other procedures. It was speculated that this result could be explained by unstable lipoprotein binding (Bertilsson *et al.*, 1979).

As mentioned above, variability in unbound fraction of CPZ may be large (Piafsky *et al.*, 1978). The CSF/plasma ratio of this drug is an unreliable method to determine binding since variable binding in CSF affects this ratio considerably (Sedvall, this volume).

In conclusion, further studies are needed, preferably in patients being treated with the drug of interest. Such studies should, to be informative, try to pin-point the reason for the observed variation by examination of the subjects' protein patterns and the possible presence of binding modulators. Also, it would be of great value to know whether the obtained binding figure is reproducible within an individual from one time to another. Finally, the selected protein binding technique should be validated by a second technique based on a different physical principle.

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Plasma protein binding of haloperidol: influence of age and disease states

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INTRODUCTION

Interindividual differences in the plasma protein binding of psychotropic drugs might be an important factor in the determination of clinical response. This is because it is the free 'unbound' fraction of drug that is pharmacologically active. In the case of haloperidol there has previously been only a small number of investigations carried out on a relatively small number of subjects (table 1). These studies have indicated that the drug is approximately 90–92 percent bound to plasma proteins (table 1) but no data exist on the nature of these proteins or their affinity for haloperidol. Moreover, most of these earlier studies have concerned healthy volunteers rather than patients. Thus, the influence of age and disease on haloperidol binding has not been evaluated systematically.

This present report concerns some preliminary observations concerning the serum protein binding of haloperidol in both healthy volunteers, elderly subjects, cirrhotic patients and psychotic children. These studies were all carried out *in vitro* using equilibrium dialysis at 37°C with ^{3}H-labeled haloperidol. These observations are part of a larger program aimed at evaluating the influence of age and different pathological conditions on the clinical pharmacokinetics of haloperidol and the relationship between possible changes in pharmacokinetics and clinical response.

MATERIALS AND METHODS

All binding studies were carried out using tritiated haloperidol (S.A. = 12 mCi mM^{-1}) obtained from IRE (Fleurus, Belgium) with a

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Table 1 Previous data on plasma protein binding of haloperidol

Study	Investigation and number of subjects	Technique (time) (h)	Temperature (°C)	Drug conc. ($ng\ ml^{-1}$)	Percentage of free drug	
					Mean	Range
Ilett *et al.*, 1975	Healthy volunteers (7)	ED (18)	4	100	8.3	NS
	Healthy volunteers (9)	ED (1)	37	100	9.7	NS
Hughes *et al.*, 1976	Healthy volunteers (11)	ED	37	40	10.4	8.6–11.9
Forsman and Ohman, 1977	Healthy volunteers (4)	ED (4)	37	2.5–20	8.1	7.1– 9.7
	Healthy volunteers (4)	UF	Room temperature	2.5–20	8.0	6.7–10.8
	(A) Psychiatric patients (12)	UF	Room temperature	NS	8.0	NS
	Psychiatric patients (5)	UF	Room temperature	NS	8.8	NS

Abbreviations: A, monotherapy; ED, equilibrium dialysis; UF, ultrafiltration; NS, not specified.

radiochemical purity of greater than 98 percent. Labeled drug was dissolved in ethanol to give a final concentration of 1 mCi ml^{-1} and was stored at −20 °C. Crystallized lyophilized human serum albumin (HSA) was obtained from the Sigma Chemical Co. (St Louis, USA). Phosphate buffer (pH 7.4) was freshly prepared before use and had the following composition: $Na_2HPO_4.12\ H_2O$ (8.04 g), $NaH_2PO_4.H_2O$ (0.78 g), NaCl (2.25 g) dissolved in distilled water to a final volume of 1 liter.

For the determination of drug binding, 1 μl of the ethanolic ^3H-haloperidol solution was added to 1 ml of plasma or HSA solution and incubated for 60 min at 37 °C. Of this solution, 750 μl was then dialyzed for 12 h at 37 °C against an equal volume of phosphate buffer using Perspex dialysis chambers and Technicon membranes. Following equilibrium the resulting radioactivity in buffer and serum was determined by scintillation counting using 100 μl of sample and 19 ml of Packard Instagel scintillator.

The free fraction (percent) of haloperidol was calculated from the formula

$$\text{Percentage of free drug} = \frac{\text{Cts min}^{-1}\ \text{buffer}}{\text{Cts min}^{-1}\ \text{serum}} \times 100.$$

The affinity constant (k) for the binding of haloperidol to serum or HSA was calculated using a modified Scratchard type plot as described by Romer and Bickel (1979), according to the formula

$$\frac{L_b}{B_0} = kL_{b\,max} - kL_b,$$

where L_b is the concentration of bound ligand, B_0 is the total binder concentration and $L_{b\,max}$ is the maximum concentration of bound ligand.

Serum concentrations of albumin and α_1-acid glycoprotein (AAG) were measured by a radial immunodiffusion technique (Partigen, Behring). The measurement of total serum protein was carried out by a standard colorimetric analysis.

PATIENTS

Ten milliliters of clotted blood samples were obtained from volunteers, elderly patients, cirrhotic patients and pediatric patients. The resulting serum samples were used immediately (volunteers and children) or deep frozen and thawed once within one month, immediately prior to the determination of drug binding.

RESULTS

Preliminary studies indicated that the time to reach equilibrium for both serum and HSA was 6 h and all binding studies were carried out using this

incubation time. No difference was found between fresh and frozen serum for haloperidol binding. There was found to be no change in haloperidol free fraction using cold haloperidol concentrations of between 5 and 5000 ng ml^{-1}. The free fraction of haloperidol increased proportionally with dilution of either HSA or serum (table 2). However, it can be clearly seen that the binding of haloperidol to HSA was only about 66 percent of that found for serum, suggesting that serum proteins other than albumin play a role in determining haloperidol binding *in vivo*. A calculation of the affinity constants for both HSA and serum gave values of 6.2×10^{-3} M and 1.9×10^{-2} g l^{-1} respectively.

Table 2 Influence of protein dilution on haloperidol serum protein binding

Dilution factor	Percentage of free and bound haloperidol* (mean of three determinations)			
	Serum†		HSA‡	
	Free	Bound	Free	Bound
1:1	10.5	89.5	40.3	59.7
1:2	14.9	85.1	48.2	51.8
1:4	23.1	76.9	64.6	35.4
1:8	38.2	61.8	76.2	23.8
1:16	55.2	44.6	84.8	15.2

*Concentration 10 ng ml^{-1}.
†Plasma protein content 78 g l^{-1}.
‡HSA concentration 40 g l^{-1}.

Table 3 Effect of age and disease state on haloperidol serum protein binding

Subjects	Total protein (g l^{-1})	Albumin (g l^{-1})	α_1-Acid glycoprotein (g l^{-1})	Haloperidol (% free)
Healthy young volunteers (6)	77±2.0 (69–82)	38±1.7 (31–42)	0.74±0.01 (0.61–1.05)	11.6±0.5 (10.0–13.2)
Elderly (10)	57±3.8 (33–72)	27±1.3 (23–37)	0.96±0.03 (0.88–1.18)	8.5±0.3 (6.6–9.6)
Cirrhotic patients (7)	49±2.7 (40–62)	19±2.6 (10–26)	0.40±0.04 (0.20–0.40)	18.9±1.4 (12.4–23.6)
Children (2) (10 and 15 years)	69 and 61	34 and 29	1.12 and 1.45	7.3 and 7.9

Data are expressed as mean values±S.E.; the range of variability is given in parentheses.

The binding of haloperidol to serum obtained from both healthy volunteers and different patient groups is shown in table 3. In the groups of healthy volunteers the mean unbound fraction of haloperidol was 11.6 percent with only a small interindividual variation (10–13 percent). In elderly subjects who showed reduced albumin but increased AAG concentrations, the free fraction of haloperidol was significantly reduced (mean 8.5 percent, range 6.6–9.6 percent). On the other hand, for cirrhotic patients, who had both reduced albumin and reduced AAG concentrations, the free fraction of haloperidol was significantly increased compared to both healthy volunteers and elderly patients (mean 18.9 percent, range 12.4–23.6 percent). In other hepatic patients with normal or elevated AAG concentrations ($>0.6\ g\ l^{-1}$), the free fraction of haloperidol was only moderately increased (mean 13.1 percent). In the two children who were investigated, who had slightly reduced albumin but increased AAG concentrations, the free fraction of haloperidol appeared to be reduced (7.3 and 7.9 percent).

DISCUSSION

The preliminary findings of this study have shown that proteins other than albumin contribute to the binding of haloperidol in human serum. Albumin at physiological concentrations appears to contribute only about 66 percent of total binding capacity, with a relatively low affinity ($\sim 10^{-3}$ M). AAG, however, appears to play an important role in determining haloperidol binding.

The free fraction of haloperidol obtained in the present group of young healthy volunteers (mean 11.6 percent, range 10–13 percent) is similar but a little higher than that reported by other studies (table 1) where mean values of between 8 and 10 percent were obtained using similar techniques and conditions. Previous studies (table 1) indicated that there were only minor interindividual differences in haloperidol binding. However, the present study has shown a threefold interindividual difference in haloperidol free fraction (6.6–23.6 percent), partly due to individual differences in AAG. Increased AAG concentrations appear to cause a reduction in the free fraction of haloperidol, even in presence of reduced albumin concentrations, whereas reduced AAG concentrations cause a decrease in binding and an increase in the free fraction of drug. The apparent influence of AAG on haloperidol binding is similar to that reported previously by other workers for imipramine, propranolol and chlorpromazine (Piafsky and Borga, 1977; Piafsky *et al.*, 1978).

In conclusion, both age and concurrent disease states may have an influence in determining individual differences in haloperidol binding. Part of this variability may be mediated by AAG but other serum proteins may also be involved. In some patients treated with haloperidol the unbound concentration of drug may be more important in determining clinical effects

than total (bound+unbound) plasma concentrations. Further studies are in progress to clarify this situation.

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Section Five

Pharmacokinetics and Plasma Level/Effect Relationships

(A) Neuroleptics

Clinical significance of neuroleptic plasma level monitoring

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INTRODUCTION AND DEFINITION OF THE PROBLEM

Every time the clinical significance of neuroleptic plasma level monitoring is discussed in congresses or seminars, two opposite opinions are encountered. On the one side, there are physicians and scientists who think that neuroleptic plasma level monitoring is only an interesting, but expensive, intellectual exercise which has very little to do with the treatment or the management of the psychotic patient; on the other side, there are physicians and scientists who believe that neuroleptic plasma level monitoring may be a useful tool for a more rational and safer therapeutic approach to psychiatry. Such a situation has apparently crystallized for at least six years, with no real dialogue between the two parties and a growing scepticism towards the possible value of therapeutic drug monitoring in psychiatry, as documented by a recent editorial (Editorial, 1979). This fact is highly regrettable because the field of neuroleptics appears as the only one which has not yet taken advantage of the increased knowledge on drug utilization and prescription brought about by the development of clinical pharmacology.

Psychiatry appears today as one of the few medical branches where no real effort has been made to rationalize drug usage, where the process of defining either the correct dose or the correct therapeutic agent is still based on 'impressions' and empirism. While on the one side our awareness and concern for tardive adverse reaction and rate of relapse is increasing (Gelder and Kolakowska, 1979), on the other side nothing or very little appears to be done really to improve the management of the psychotic patient.

The causes of such a stagnation, of such a lack of transfer of a 'message' which has been usefully applied in other areas of medicine are numerous, as recently reviewed (Morselli, 1979; Dahl, 1979; Morselli and Zarifian, 1980). Beside the difficulty of a precise evaluation of the therapeutic results conditioned by the heterogeneity of the psychotic syndromes, in our opinion the reasons which more than others have contributed and still contribute to

such a stagnation of knowledge and to the present diatribe are as follows: firstly, the sceptical attitude of most psychiatrists towards drugs and, secondly, the fact that in the majority of the studies the methodological approach applied was not always the most adequate one.

The 'built-in' resistance of most psychiatrists to accept fully the validity of the pharmacological approach has surely an important effect. Too often drugs are still considered as 'magical' entities and very little effort is made to understand better their pharmacokinetic and pharmacodynamic profile. This leads frequently to improper use of psychotic drugs with consequent poor therapeutic results. Generally, the poor result reinforces the sceptical attitude towards drugs and all the blame is 'transferred' to the patient who is labeled either as 'unresponsive' or as 'responding paradoxically'. There is no real attempt to try to understand the causes of variability in therapeutic responses and therapeutic failures are accepted as an inevitable part of variability of human nature.

Such an attitude has been accompanied frequently by methodological problems at both analytical and clinical levels. If we consider the various reports available on the possible relationship between neuroleptic plasma or blood concentrations and clinical effects, we may find that in most instances the clinical definition of the patient is very poor, the observation period is remarkably short, the analytical methodology measures only one or a part of the active species present in biological fluids and the treatment of the data is inadequate. Several times the 'wrong' chemical species has been monitored or a very 'difficult' drug has been chosen as a starting or paradigmatic model.

In only few reports is it possible to find information on the individual patient, since in most cases data are reported as mean values or as statistical analysis of linear relationships between various parameters. We feel that we should not consider (as is usually done in most of the analyses) either the patient or the clinical results as a perfectly homogeneous set of data. We should not, on this basis, look for either linear or curvilinear relationships between parameters which are too often very poorly defined, very heterogenous and which, even if apparently similar, are the expressions of different pathological processes. Furthermore, we should consider chronic schizophrenics (with a long history of drug intake) separately from patients with a relatively recent drug history. We feel one should try to evaluate not the 'mean data' but, on the contrary, the possible existence of therapeutic and/or toxic thresholds and of relationships between drug plasma levels and effects *within each individual patient* monitored at regular intervals longitudinally over a period not of weeks but of several months (Morselli, 1976, 1978, 1979; Morselli and Zarifian, 1980).

In other words, we think that in most instances the problem has not been approached in the correct way and that failures to find significant relationships (mainly due to inadequate methodology) are used to deny the value of an approach which has contributed substantially to the improvement of therapy in other areas of medicine. An example of such a negativistic attitude is given by a recent report where (despite the evident association of disturbing adverse reactions and high haloperidol serum levels and a 10-fold variation in serum drug concentrations for the same daily dose) the authors

question the utility of neuroleptic plasma levels monitoring because 'among other reasons neuroleptics usually have metabolites with different antipsychotic properties', 'receptor sensitivities varies', and 'tolerance phenomena develop during long-term treatment'! (Bjørndal *et al.*, 1980). Such a statement sounds really surprising because these are exactly the reasons which in other areas of medicine have prompted people to monitor drug plasma levels during chronic treatment in order to understand better the relative influence of the various factors contributing to the inter- and intraindividual variability in drug response.

Therapeutic drug monitoring has permitted, in most of the cases where it has been applied, an optimization of the drug's therapeutic potential leading to considerable advantages for both the individual (best risk/benefit ratio) and society (lower costs).

On an objective basis, it is really difficult to understand why in psychiatry the approach of therapeutic drug monitoring is refused, in most cases, '*a priori*'.

RECENT EVIDENCE SUPPORTING THE VALIDITY OF NEUROLEPTIC PLASMA LEVEL MONITORING

If, until 1978, as reviewed during the 11th CINP Meeting (Morselli, 1979; Forsmann and Ohman, 1979; Dahl, 1979), the evidence in favor of the therapeutic monitoring of neuroleptic drugs was not so convincing, in the last 3 years a series of new results has become available on the topic. The new data strongly suggest that, as with other therapeutic classes, neuroleptic monitoring in body fluids may be of clinical significance for the therapeutic management of the psychiatric patient.

Relationships between clinical response and drug plasma concentration

As summarized in table 1, several reports in the last 3 years have presented evidence for a relationship between plasma concentrations of chlorpromazine (CPZ), butaperazine and haloperidol and their clinical effects (therapeutic and/or toxic). A point which appears worth stressing is that the data obtained by independent authors appear as remarkably consistent and in good agreement with each other.

Rivera-Calimlim *et al.* (1978) reported that in a group of adult schizophrenic patients admitted to the hospital because of acute relapse, the CPZ plasma levels associated with improvement in thought disorders and paranoid hostility were between 100 and 200 ng ml^{-1}, while levels <50 ng ml^{-1} were associated with lack of clinical effect. Similar findings have been reported by Wode-Helgodt *et al.* (1978), who could observe a lack of response for CPZ plasma levels below 40 ng ml^{-1} and a

Table 1 Recent papers suggesting that neuroleptic plasma level monitoring may be of clinical significance

Drug	Patients age group	Parameter evaluated	Proposed optimal level	Analytical method	Authors
CPZ	Adults	Therapeutic response	Pl=100–200 ng ml^{-1}	GLC (ECD)	Rivera-Calimlim *et al.*, 1978
CPZ	Adults	Therapeutic response	Pl>40 ng ml^{-1}	GLC (ECD)	Wode-Helgodt *et al.*, 1978
CPZ	Children	Therapeutic response +side effects	Pl=40–80 ng ml^{-1}	GLC (ECD)	Rivera-Calimlim *et al.*, 1978
BTPZ	Adults	Therapeutic response	Bl>150 ng ml^{-1}	FLUOROM.	Smith *et al.*, 1979
BTPZ	Adults	Therapeutic response	Pl=75–250 ng ml^{-1}; RBC=30–60 ng ml^{-1}	FLUOROM.	Casper *et al.*, 1980
CPZ, HP, TFPZ, TTX	Adults	Therapeutic response	S=100–200 ng ml^{-1} as CPZ equivalents	RRA	Tune *et al.*, 1980
HP	Children	Therapeutic response +side effects	Pl= { 3–6 ng ml^{-1}* ; 5–10 ng ml^{-1}† }	GLC (NPD)	Morselli *et al.*, 1979
HP	Adults	Therapeutic response	Pl=5–15 ng ml^{-1}	GLC (ECD)	Smith *et al.*, 1979
HP	Children	Therapeutic response	S=70–100 ng ml^{-1} as CPZ equivalents	RRA	Meyers *et al.*, 1980
HP	Adults and children	Therapeutic response +side effects	Pl=5–10 ng ml^{-1} (wors.>50 ng ml^{-1}	GLC (NPD)	Morselli *et al.*, 1980*a, b*; Morselli and Zarifian, 1980

Abbreviations: CPZ=chlorpromazine; HP=haloperidol; TFPZ=trifluoroperazine; TTX=thiothixene; BTPZ=butaperazine; GLC=gas liquid chromatography; ECD=electron capture detector; NPD=nitrogen phosphorus detector; RRA=radio receptor assay; FLUROM.=fluorometry; Pl=plasma; Bl=blood; RBC=red blood cells; S=serum.

*Gilles de la Tourette syndromes.

†Psychotic syndromes.

significant correlation between high CPZ concentrations and disturbing side effects. (The study is reported in more detail by Sedvall in this volume).

More recently, these data have been confirmed by Tune *et al.* (1980), who measured, by means of a radioreceptor assay (RRA), the total concentrations of active drugs in patients receiving haloperidol, CPZ or other neuroleptics. Expressing the drug concentrations in plasma as CPZ equivalents (CPZ Eq) these authors observed that the best clinical outcome was present at drug concentrations of 100–200 ng ml^{-1} CPZ Eq while levels below 50 ng ml^{-1} CPZ Eq were associated with poor clinical response.

In two consecutive studies, the group of Davis (Smith *et al.*, 1979; Casper *et al.*, 1980) showed that a positive therapeutic outcome was associated with butaperazine plasma concentrations of the order of 100–250 ng ml^{-1} while poorer therapeutic responses were observed in patients with plasma concentrations lower than 50 ng ml^{-1}. By evaluating the drug concentrations in red blood cells (RBC), the same group (Casper *et al.*, 1980) reported the presence of a curvilinear (inverted U-shaped) relationship between RBC concentration and clinical outcome, with a therapeutic optimum between 30 and 60 ng ml^{-1}.

In the case of haloperidol, we observed that in patients suffering from Gilles de la Tourette syndrome a therapeutic effect was present at haloperidol plasma concentrations of 3–6 ng ml^{-1} while disturbing side effects became evident only at plasma levels over 6 ng ml^{-1} (Morselli *et al.*, 1979). In psychotic patients, favorable clinical responses were associated with plasma levels of 5–10 ng ml^{-1}. Similar results have been reported by Smith *et al.* (1979) who observed, in patients with a recent drug history, an antipsychotic effect at haloperidol concentrations of 5–15 ng ml^{-1}, while lack of response was associated with concentrations below 5 ng ml^{-1}.

The incidence of extrapyramidal side effects and sedation appear to rise proportional to haloperidol plasma levels and at concentrations over 10 ng ml^{-1} about 90 percent of the population we studied presented extrapyramidal side effects (Morselli *et al.*, 1979). Side effects other than extrapyramidal syndrome-like heavy sedation, sleep disturbances, aggressive episodes and psychomotor agitation have been found associated with haloperidol plasma levels of 50–150 ng ml^{-1} (Bjørndal *et al.*, 1980; Morselli *et al.*, 1980*b*).

It may be worthwhile to mention also a recent case report described by Meyers *et al.* (1980) and which appears to be in good agreement with that described above. These authors, monitoring haloperidol plasma levels in a psychotic child by means of RRA over a period of 74 days, noticed a striking parallelism between the fluctuations of symptomatology and haloperidol plasma levels, the optimum concentrations being 60–100 ng ml^{-1} CPZ Eq. Another interesting report concerns the relationship between anticholinergic drugs' plasma levels and their clinical effects. Tune and Coyle (1980) in fact demonstrated by means of a RRA that a concentration of at least 0.7 pmol atropine Eq (200 μl serum) was necessary to exert a significant protective effect towards acute extrapyramidal side effects due to neuroleptics. It should also be remembered that, at commonly used doses, about 50 percent of the cases presented serum concentrations below 0.7 pmol per sample (200 μl serum).

High doses of neuroleptics in 'treatment-resistant' chronic schizophrenic patients

For years the possibility of poor drug bioavailability has been hypothesized as one of the causes behind the lack of therapeutic responses in 'treatment-resistant' chronic schizophrenics. Three recent reports seem to indicate that poor bioavailability is probably not the major cause. For either haloperidol or flupenthixol it has, in fact, been shown that high doses of the two drugs (up to 200 mg per day for haloperidol and 40 and 200 mg per day flupenthixol decanoate i.m.) were associated with a corresponding increase in drug plasma concentrations (Bjørndal *et al.*, 1980; McCreadie *et al.*, 1979; Morselli *et al.*, 1980*b*). In the case of flupenthixol, only a small subgroup of patients improved significantly with the high dose, while for the majority the high dose was no more effective than the standard dose. Furthermore, the extrapyramidal side effects worsened significantly in the high dose group (McCreadie *et al.*, 1979). The increase in haloperidol plasma levels was found to be associated with a response of both striatal and tubero-infundibular dopaminergic structures as indicated by the rise in CSF HVA and plasma prolactin (Morselli *et al.*, 1980*b*; Bjørndal *et al.*, 1980). However, no improvement in the psychotic symptoms could be observed by either set of authors; on the contrary, in several cases they noticed a worsening of the clinical picture with increased psychomotor agitation, hallucinations and aggressive episodes.

The observation that a response by two dopaminergic systems could be observed without concomitant improvement of the clinical picture could support the hypothesis that in 'chronic treatment-resistant' schizophrenic patients dopaminergic pathways do not play a determining role (Morselli and Zarifian, 1980).

Effects of age on neuroleptic disposition and therapeutic threshold

It is today well documented that age may have an important effect on drug disposition and that therapeutic and/or toxic thresholds in children may be different to those observed in adults (Morselli, 1977; Morselli *et al.*, 1980*a*). Recent data suggest that modification of kinetics and therapeutic thresholds in children may also be encountered with neuroleptic drugs.

In a recent report, Rivera-Calimlim *et al.* (1979) have documented that children dispose of CPZ at a faster rate than adults and that consequently doses two to three times higher are needed in children to obtain similar steady state plasma concentrations of CPZ. Furthermore, both therapeutic optimum plasma concentrations and toxic plasma thresholds appear to be lower in children. Clinical improvement of target symptoms was in fact observed in psychotic children at plasma CPZ concentrations of 60–80 ng ml^{-1}, while severe drowsiness was evident at 70–100 ng ml^{-1}. The authors also noticed a frequent decline in drug plasma concentrations with time and an inhibitory effect of imipramine on CPZ elimination. Because of these findings, the authors recommend monitoring of plasma CPZ levels in children.

An age-dependent increase in plasma level/dose ratio has been described by our group in children of 7–16 years of age as well as the appearance of drowsiness and sedation at haloperidol concentrations of 6–8 ng ml^{-1} (Morselli *et al.*, 1979). Preliminary data on apparent plasma half-life of haloperidol in children tend to suggest that the clearance of the drug is increased remarkably in patients of 8–12 years of age when compared to adults.

The previously mentioned report of Meyers *et al.* (1980) is in good agreement with both the lower therapeutic threshold in children as well as with the faster disposition rate, as indicated by the marked drop in haloperidol blood levels following a reduction in the drug daily dose.

Comments

The series of studies reported above indicates that therapeutic optimum plasma concentrations do probably exist for the compounds considered and that in all cases a clear relationship exists between high drug concentration and disturbing side effects. These findings, together with the fact that a variability of seven- to 10-fold in steady state plasma levels exists for all of the compounds studied, should be a motivation for therapeutic drug monitoring in order not to expose our patients to unnecessary side effects or to prolonged hospitalization because of underdosing. The problem is of greater importance in children where adverse effects such as heavy CNS depression, sedation and psychomotor impairment may have negative effects on school performances and developing personality (Morselli *et al.*, 1978).

Furthermore, therapeutic monitoring of neuroleptic plasma concentrations in chronic 'resistant' schizophrenics, associated with assay of plasma prolactin and CSF HVA, permits us to suggest on the one hand that poor drug bioavailability is not the major cause of the lack of response and on the other that we are probably facing subgroups of patients with different biochemical substrates.

DISCUSSION

Even if the data reported above appear as encouraging and point toward the usefulness of therapeutic drug monitoring in psychotic patients, there is still a long way to go before we can either accept or refuse it. For instance, we should be able to answer the following questions for each of the neuroleptics we use: Is there any relationship between the drug's daily dose and peak or steady state blood levels? Do blood levels fluctuate over time? If yes, how much and what are the causes of the fluctuations? Do active metabolites contribute to the clinical effects (therapeutic and/or toxic) of the drug? Does the compound interact with commonly associated pharmacological agents either pharmacokinetically or at receptor level? Is there any evidence for

toxic and/or therapeutic blood level thresholds? Do possible thresholds vary as a function of age or of the severity of the illness? In the case of poor therapeutic outcome, how important is the compliance of the patient and that of the paramedical personnel? Is a maintenance treatment really needed? Are we using the correct dose and the correct schedule? Is there any indication or suggestion that in cases of lack of response we are facing a diagnostic subgroup where another kind of treatment should be prescribed? I think it does not need to be stressed that for many of the currently used neuroleptics we are not in a position to give an answer to most of the above questions. The fact that we are in such a position of ignorance, of lack of knowledge, appears to me to provide a good motivation, a rationale for supporting increased efforts for more research on the clinical significance of neuroleptic plasma level monitoring and not for denying it.

It must be stressed, however, that in pursuing such an effort we should apply a correct procedure and not use methodologies which, even though they have been proven to be very useful and valuable for other purposes (that is definition of efficacy), are not adequate for the scope and aims of therapeutic drug monitoring. We feel that the three-step procedure described in table 2 should be followed. Today we have sufficient information on most neuroleptics for the items in step 1. Our knowledge is really limited as to steps 2 and 3; only recently, as described above, has evidence become available on some of the points described in steps 2 and 3 for haloperidol and CPZ.

The procedure described in table 2 is not new at all. It is exactly the same one which has been followed for other therapeutic agents (such as digoxin, antiarrhythmics, theophylline, antibiotics and antiepileptics). All these are examples where an intelligent use of therapeutic drug monitoring has permitted a higher percentage of positive therapeutic outcome together with a reduced incidence of adverse reactions. Furthermore, such an approach has led to a better knowledge of specific factors of variability, thus permitting a more rational usage of drugs.

Very often therapeutic drug monitoring is criticized because it does not follow the rigid criteria of controlled clinical trials. However, its primary aim is not the statistical definition of efficacy but, on the contrary, the evaluation of possible relationships, in each individual patient, between blood or plasma concentrations of a drug (whose efficacy has already been proven) and the fluctuation or the variation of clinical parameters as they are normally appreciated and evaluated by the attending physician, by the patient and by his entourage. After all, it is on this basis that hospitalizations and dismissals are decided in 90 percent of cases! The finding of a relationship between blood levels and variation in symptomatology or in occurrence of side effects should, thus, not be disregarded as 'non-scientific' but utilized as a tool for better therapy. It is also frequently claimed that psychic symptoms fluctuate more than those of 'organic' origin and that no appreciation of their modification can be made without a control period. The claim is probably correct; however, 'organic' symptoms also fluctuate and it is indeed in these situations that therapeutic monitoring has proven to be most useful. Furthermore, because of such fluctuations over time, longitudinal

Table 2 Procedures to be followed for the evaluation of the clinical significance of neuroleptic plasma levels monitoring

Steps	Information acquired and practical consequences
1. Definition of pharmacokinetic profile (healthy volunteers and patients)	(a) Definition of correct dosage form (b) Definition of correct dose regimen (c) Identification of possible time- or dose-dependent kinetic alterations.
2. Population studies (patients)	(a) Identification of various factors capable of modifying the drug's kinetic profile (age, pathology, food, associated treatment, etc.) (b) Identification of range of variability and fluctuations of drug blood levels in a natural situation (c) Identification of possible 'statistical' therapeutic and/or toxic thresholds (d) Quantification of interindividual variability in dose/level relationship (e) Possible reduction of risks of overdosing and/or underdosing
3. Individual longitudinal monitoring (patients)	(a) Control of patient's compliance (b) Identification of possible individual therapeutic and/or toxic thresholds (c) Identification of specific factors of variability so reducing for that given individual the risk of adverse reaction or of underdosing (d) Control of compliance of paramedical personnel (e) Possibility of improving the therapeutic impact by reducing the incidence of side effects (f) Optimization of the drug therapeutic potential with both advantages for the individual (reduced hospitalization time) and for the society (reduced hospitalization costs)

therapeutic drug monitoring, carried out over several months, may be of greater value and give more information than a perfectly controlled clinical trial where duration is limited to 4–6 weeks and is normally carried out in a situation which is very different from the 'naturalistic' one.

Psychiatric patients should not be considered, as unfortunately they are today, as pathological entities outside the normal world of medicine, escaping from all rules which have been proven valid for other diseases. The refusal to consider the patient as biologically ill has for years conditioned the position of psychiatry on the border of medical sciences. Refusing to allow the psychiatric patient to take advantage of the improvement in our knowledge in drug management and utilization means once again refusing the 'normality' of his disease and arbitrarily reducing his chances of therapy.

In conclusion, we feel that by an increased effort in therapeutic drug monitoring, by a closer cooperation among the various groups operating in the field with a critical but positive attitude, we will surely gain a better knowledge of the various factors conditioning the very wide interindividual variability in clinical response to psychotropic drugs and, probably, of the biological processes underlying psychopathological phenomena. Finally, and more important, we will be in a position to better treat our patients.

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The clinical significance of measuring perphenazine in plasma during oral antipsychotic treatment

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INTRODUCTION

A major aim in any neuroleptic therapy is to minimize unwanted effects, especially the neurological ones, but not at the expense of the antipsychotic effect. In clinical practice, the extensive usage of antiparkinsonian agents is an important tool to defeat undesirable neurological phenomena. Unfortunately this may create an excuse for not seeking alternative solutions, such as dose reduction, leading psychiatrists to administer overly large doses of neuroleptics. Consequently, there might be a higher risk for development of tardive dyskinesia (Ayd, 1967; Crane, 1974).

After analytical methods to determine plasma concentrations of certain neuroleptics became available, several trials were done to find a relation between plasma levels of these drugs and clinical parameters to improve therapy (Bergling *et al.*, 1975; Kolakowska *et al.*, 1979; Rivera-Calimlim *et al.*, 1973, 1978). However, many difficulties concerning insufficient analytical methods, appearance of active metabolites, interpretation of plasma concentrations, patient selection and clinical evaluation showed up and, so far, routine plasma concentration monitoring of neuroleptics has not gained a solid place in therapy (Cooper, 1978).

RESULTS

In our studies of perphenazine, we have tried to overcome the abovementioned difficulties. First of all, this means development of a very specific and sensitive analytical method (Larsen and Næstoft, 1975). Next, extensive

pharmacokinetic studies have been carried out (Hansen *et al.*, 1976, 1979; Hansen and Larsen, 1977). The most important kinetic data can be summarized as follows: (1) within the first 3–6 weeks of treatment, plasma concentrations may fluctuate significantly; (2) dose adjustments result in disproportional changes of plasma levels; (3) concomitant treatment with anticholinergic agents does not change the plasma level of perphenazine; (4) an average plasma half-life is about 9 h. The considerable fluctuations occasionally occurring during long term treatment may necessitate assay of a large number of blood samples to define a plasma level at any time, when seeking a relationship to clinical events.

The next step was to find out how the pharmacokinetic data obtained could be supplied to the clinical situation. In one of our studies, we have seen a tendency to a lower limit for neurological side effects in the region of 5 nmol perphenazine per liter plasma (Hansen and Larsen, 1977). To try to reproduce this limit and to get an indication of plasma levels giving antipsychotic effects, we have recently done a longitudinal investigation on acute schizophrenic psychotic patients who had not received neuroleptic

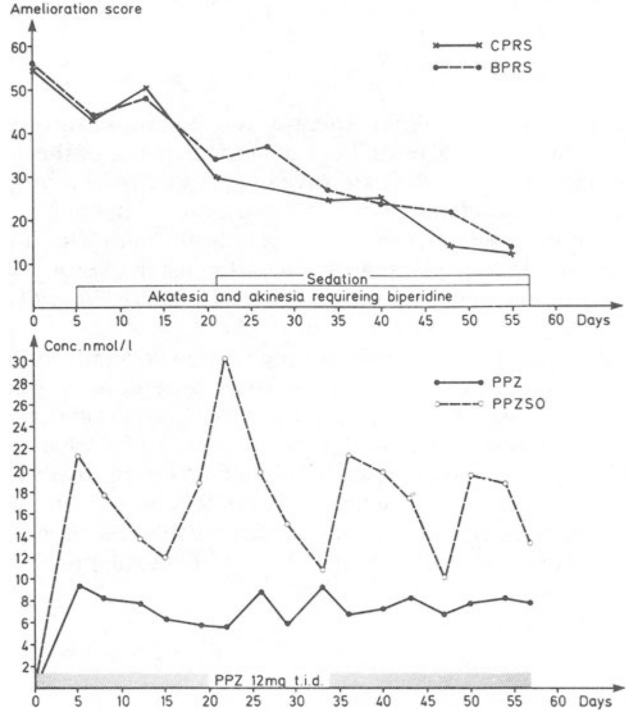

Figure 1 Plasma concentrations of perphenazine and its main metabolite plotted in relation to clinical effect and side effects during continuous oral treatment of a 22 year old schizophrenic female. CPRS=Comprehensive Psychiatric Rating Scale; BPRS=Brief Psychiatric Rating Scale; PPZ=perphenazine; PPZSO=perphenazine sulfoxide.

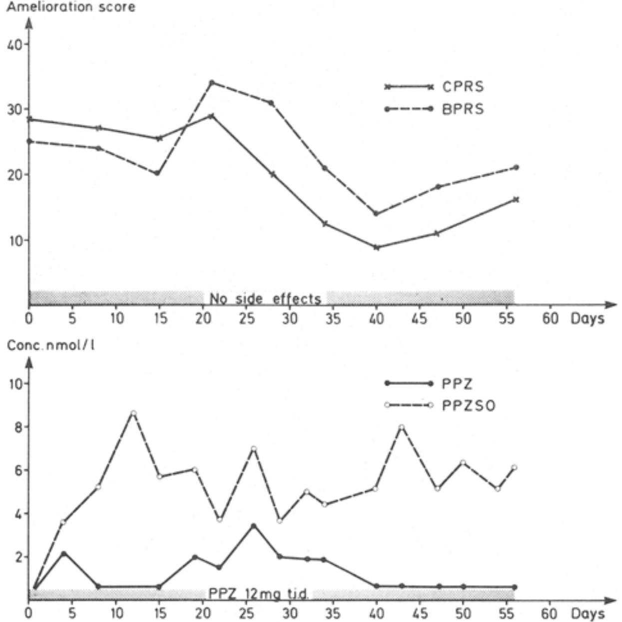

Figure 2 Plasma concentrations of perphenazine and its main metabolite plotted in relation to clinical effect and side effects during continuous oral treatment of a 30 year old schizophrenic female. Abbreviations as in figure 1.

therapy for the last two months prior to the study. Only patients with a minimum BPRS score of 25 points participated. Plasma concentrations (minimum values within an 8 h dose interval) were measured twice a week, and clinical effects and side effects were evaluated weekly for at least two months. The dose was deliberately kept constant as long as it seemed ethically justified; only a few patients had their dosage changed during the trial.

Three different types of plasma profiles were seen during the study. Figure 1 illustrates the most common plasma profile with a rapid increase to a final and rather stable level. As seen in this case, neurological side effects appeared immediately and the therapeutic outcome was excellent.

Figure 2 represents a rarer type, showing obvious fluctuations of the plasma concentration, which finally stabilized at a very low level, giving a plausible explanation for the relapse in therapeutic response. Measurable amounts of the inactive main metabolite concomitant with very low concentrations of the parent compound may rule out lack of patient compliance. No side effects appeared during the investigation period.

Figure 3 demonstrates another unusual profile. After an extended period with considerable fluctuations, the plasma concentrations suddenly increased to a much higher level, followed by a kind of stable situation. Clinically, the increasing plasma concentrations seem to provoke neurological side effects without enhancing the therapeutic effect.

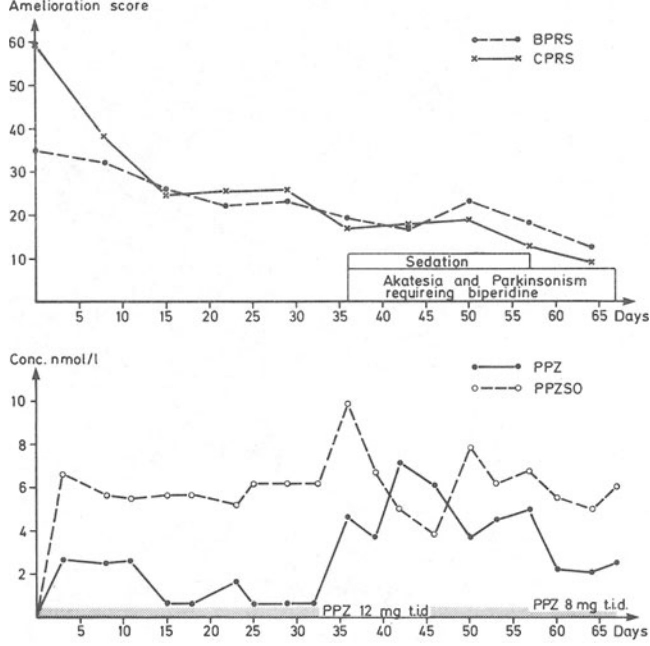

Figure 3 Plasma concentrations of perphenazine and its main metabolite plotted in relation to clinical effect and side effects during continuous oral treatment of a 49 year old schizophrenic male. Abbreviations as in figure 1.

In figure 4, steady state plasma concentrations of perphenazine from all patients (Hansen and Larsen, 1977) have been plotted in relation to absence or presence of neurological side effects. Our preliminary results seem to be confirmed, as the patients tend to fall into two groups: one with definite side effects and a plasma level above 4–5 nmol l^{-1}; the other free of side effects and a lower concentration of perphenazine.

If we disregard the few patients with a very low or zero plasma level, no significant difference in the therapeutic outcome could be detected between these two groups of patients. More controlled trials are, of course, needed to give confirmation of these findings. Nevertheless, in our daily administration of perphenazine to psychotic patients, the concomitant use of anticholinergic drugs has decreased substantially since dose adjustments are made based on plasma concentration monitoring.

CONCLUSION

As already stated, we find it of particular clinical significance that a threshold level for neurological side effects can be distinguished from a – not yet defined – lower level for antipsychotic effect.

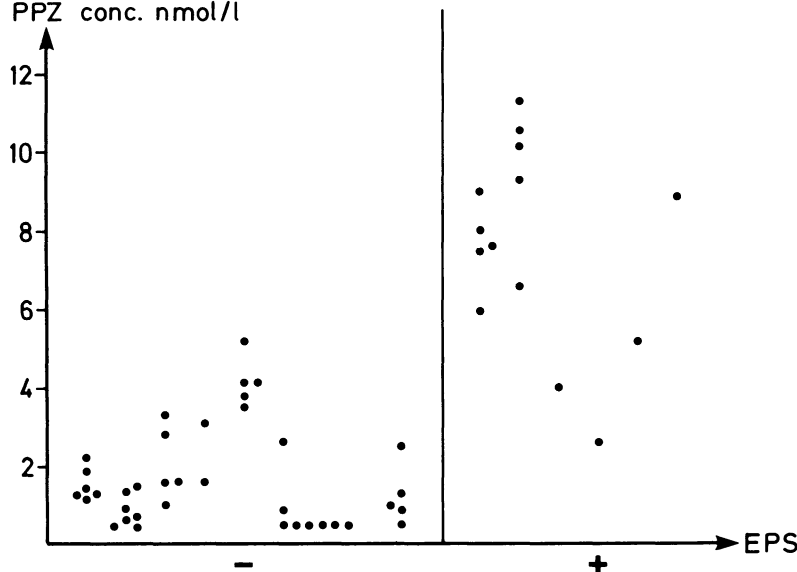

Figure 4 Steady state plasma levels of perphenazine plotted in relation to absence or presence of neurological side effects for all patients. PPZ=perphenazine; EPS=extrapyramidal side effects.

Furthermore, together with the fact that plasma concentrations may fluctuate considerably in spite of unchanged dosage and patient compliance, plasma concentration monitoring of perphenazine seems to be useful in the treatment of psychotic patients. However, this would be the case only where analytical and clinical pharmacological expertise and capacity are available.

We must admit that more work, particularly other controlled studies, are still necessary.

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Serum haloperidol determinations and their contribution to the treatment of schizophrenia

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INTRODUCTION

There are several analytical methods presently available for the quantitation of haloperidol concentrations in serum and plasma. Gas chromatographic techniques have been in use for almost a decade (Zingales, 1971; Marucci *et al.*, 1971; Forsman *et al.*, 1974; Bianchetti and Morselli, 1978; Hornbeck *et al.* 1979); these are quite specific for haloperidol and are sufficiently sensitive to measure circulating haloperidol levels in patients given the usual clinical drug doses. However, they require the extraction of a relatively large quantity of serum or plasma (at least 2 ml); relatively few samples per day can be analyzed; highly trained technicians and expensive and delicate equipment are needed, especially when gas chromatography is combined with mass spectrometry (Hornbeck *et al.*, 1979). Nevertheless, most of the available data on the pharmacokinetics of haloperidol and the relationship of clinical dose, blood levels, and psychotic symptomatology in treated patients have been developed with gas chromatographic assay techniques (Zingales, 1971; Forsman *et al.*, 1974; Bianchetti and Morselli, 1978; Hornbeck *et al.*, 1979; Forsman and Öhman, 1976, 1977; Mielke *et al.*, 1978; Ericksen *et al.*, 1978; Smith *et al.*, 1979).

Another more recently developed technique for quantitating circulating levels of haloperidol and other dopamine receptor blocking neuroleptics is the radioreceptor assay (RRA), which is based on displacement by the neuroleptic of a radiolabeled ligand from dopamine receptor-rich preparations of calf or rat caudate nucleus (Seeman *et al.*, 1975; Creese and Snyder, 1977; Calil *et al.*, 1979). The RRA is relatively rapid and straightforward, and the only major equipment required is for liquid scintillation counting of the radioactive tracer, which is usually tritiated spiroperidol. The RRA is relatively non-specific, measuring not only the parent dopamine receptor-

blocking compound, but also any metabolites with similar receptor-binding activity (Creese *et al.*, 1978). Thus, neuroleptic concentrations are reported not in absolute units but as 'chlorpromazine equivalents', which encompasses all dopamine receptor-binding species and assumes equipotent antipsychotic activity. Nevertheless, with the radioreceptor assay, positive relationships have been demonstrated in psychiatric patients among clinical haloperidol dose, serum haloperidol concentrations, and psychotic symptomatology (Calil *et al.*, 1979).

A third, also recently developed, laboratory technique for the measurement of circulating haloperidol levels is radioimmunoassay (RIA) (Michiels *et al.*, 1976; Clark *et al.*, 1977; Rubin *et al.*, 1980*a*). RIA offers the advantages of being generally more sensitive than the other two techniques, being relatively specific (to be discussed in detail below) (requiring only microliters of unextracted plasma or serum), being relatively inexpensive (requiring only the usual radioactive tracer counting equipment), and being so straightforward that hundreds of samples per day can be analyzed.

The sensitivity and accuracy of a RIA depends on several factors, including the affinity and specificity (cross-reactivity) of the antiserum. Two RIAs for haloperidol have been developed, both of which are more sensitive than the gas chromatographic and RRA techniques. Because the hapten–protein conjugates were made at different sites on the haloperidol molecule, the specificities and cross-reactivities of the resultant antihaloperidol sera differ accordingly. This paper briefly describes the development of these two RIAs and indicates their utility in the measurement of circulating haloperidol levels in treated psychiatric patients.

MATERIALS AND METHODS

Table 1 indicates the molecular structures of haloperidol, several related butyrophenones, and several metabolites of haloperidol. For the RIA of haloperidol developed at Janssen Laboratories (Michiels *et al.*, 1976), the drug was conjugated to bovine serum albumin (BSA) at the tertiary hydroxyl group of the piperidine ring. For the RIA developed in our laboratories (Clark *et al.*, 1977; Rubin *et al.*, 1980*a*), haloperidol was conjugated to BSA at the ketone group. These two different conjugation sites produced antibodies with quite different characteristics. Neither antibody cross-reacted with compounds structurally dissimilar from the butyrophenone class of compounds, such as chlorpromazine or thioridazine, but, as indicated in table 1, they did differ in their profiles of cross-reactivity with related butyrophenones and with metabolites of haloperidol. The Janssen antibody cross-reacted with other related butyrophenones, but it discriminated haloperidol completely from its acid and piperidinyl metabolites and from reduced haloperidol. The antibody produced in our laboratory discriminated haloperidol from certain related butyrophenones and from its acid metabolites, but it cross-reacted completely with reduced haloperidol, a

Table 1 Amounts of various butyrophenones and haloperidol metabolites required to reduce antibody binding of ^{3}H-haloperidol by 50 percent, relative to haloperidol itself (= 1.0 ng)

Compound number	Reference number	Structure F-⟨⟩-C(=O)-CH$_2$-CH$_2$-CH$_2$- ®	Generic name	Antiserum	
				Janssen	Clark, Tower, Rubin
1	R 1625	® = -N⟨⟩(OH)-⟨⟩-Cl	Haloperidol	1.0 ng	1.0 ng
2	R 4749	-N⟨⟩-N(C=O)N-H (benzimidazolone, fused ⟨⟩)	Droperidol	16 ng	>500 ng
3	R 5147	-N⟨⟩ spiro C(=O)-NH, N-⟨⟩	Spiperone	38 ng	> 500 ng
4	R 11 333	-N⟨⟩(OH)-⟨⟩-Br	Bromperidol	1.4 ng	1.2 ng
5	R 1658	-N⟨⟩(OH)-⟨⟩-CH$_3$	Moperone	1.7 ng	1.3 ng
6	R 2498	-N⟨⟩(OH)-⟨⟩-CF$_3$	Trifluperidol	2.9 ng	8.5 ng
7	R 11 302	F-⟨⟩-C(=O)-CH$_2$-CH$_2$-COOH	Acid Metabolite	> 5000 ng	> 500 ng
8	X 680	F-⟨⟩-CH$_2$-C(=O)-NH-CH$_2$-COOH	idem	> 5000 ng	> 500 ng
9	T 473	F-⟨⟩-CH$_2$-COOH	idem	> 5000 ng	385 ng
10	R 1515	H-N⟨⟩(OH)-⟨⟩-Cl	Metabolite (Piperidinyl Moiety)	> 5000 ng	12 ng
11	R 2572	F-⟨⟩-CH(OH)-CH$_2$-CH$_2$-CH$_2$-N⟨⟩(OH)-⟨⟩-Cl	Reduced Haloperidol	380 ng	1.0 ng

After Rubin *et al.* (1980*b*).

haloperidol metabolite with questionable activity (Hays *et al.*, 1980), present in quantity in the plasma of treated patients (Forsman and Larson, 1978). For both RIAs, the sensitivity ranged between 0.05 and 0.3 ng haloperidol per milliliter, and the intra- and interassay coefficients of

variation averaged 10 percent. Figure 1 shows mean serum haloperidol concentrations, ±1 S.E.M., for several hours after intramuscular and intravenous injection of a low, subclinical dose of haloperidol (0.5 mg) in seven normal adult men (Hays and Rubin, 1979), indicating the considerable sensitivity of the radioimmunoassay technique.

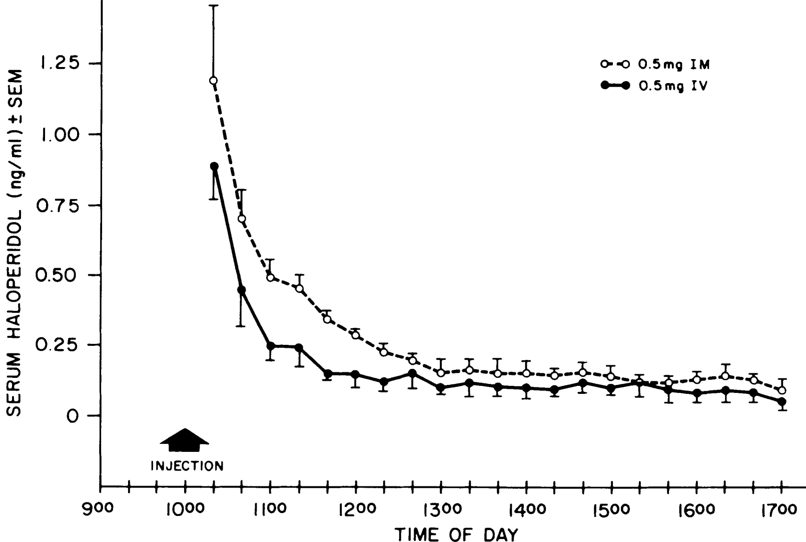

Figure 1 Average serum haloperidol concentrations, ±1 S.E.M., determined by RIA with our antibody, after intramuscular (IM) and intravenous (IV) injection of 0.5 mg haloperidol in seven normal men. (After Hays and Rubin, 1979.)

RESULTS

These RIAs have now been used to determine circulating haloperidol levels in several groups of schizophrenic patients. For the comparison of the RIA and gas chromatographic methods of haloperidol measurement, 21 serum samples collected from 11 schizophrenic patients chronically medicated with haloperidol were analyzed by gas chromatography and by RIA using the two different antibodies (Rubin *et al.*, 1980*b*). Figure 2 presents a comparison of total serum haloperidol concentrations determined by the two RIA methods with total haloperidol concentrations determined by gas chromatography. The Janssen antibody was used to assay the samples, under somewhat different conditions, both in our laboratory and at the Janssen laboratories in Belgium. Figure 2(B) shows RIA values related to gas chromatographic values between 10 and 70 ng ml^{-1}, and figure 2(A) shows similar data on an expanded scale for gas chromatography values

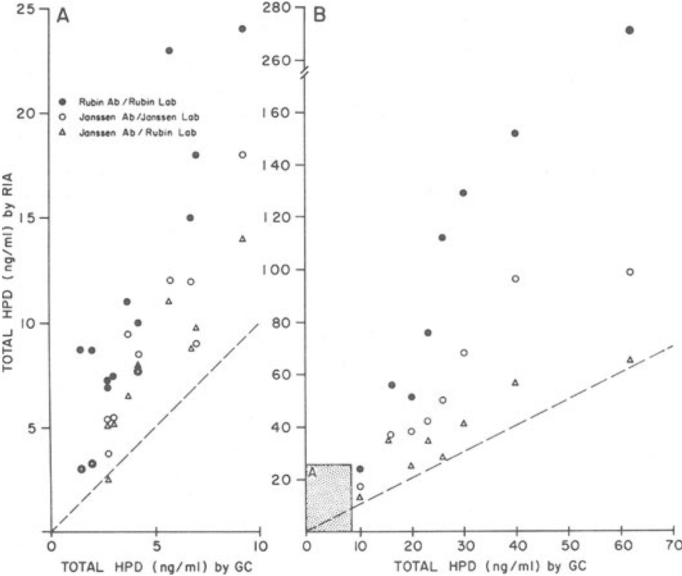

Figure 2 Serum haloperidol determined by three RIA systems, one using our antibody (in our laboratory) and two using the Janssen antibody (at Janssen Laboratories and in our laboratory), compared to serum haloperidol determined by gas chromatography. Twenty-one serum samples from 11 chronically medicated schizophrenic patients were analyzed. Figure 2(A) represents a magnification of the shaded area in figure 2(B). (After Rubin *et al.*, 1980*b*.)

between 0 and 10 ng ml^{-1}. The Janssen antibody produced a consistent, linear overestimation of haloperidol compared to gas chromatography values (average 54 percent), and our antibody produced a several-fold, non-linear overestimation of haloperidol compared to gas chromatographic values (average 223 percent). Since the gas chromatographic analysis is specific and the extraction is consistent over a wide range of serum haloperidol concentrations (Forsman *et al.*, 1974), and because the gas chromatographic values matched fairly closely those obtained with the Janssen antibody, it can be assumed that the gas chromatographic values are accurate. The large, non-linear overestimation of values by our antibody is most likely due to the relative non-specificity of this antibody which, as indicated earlier, binds the parent compound and its metabolite, reduced haloperidol, to the same extent (table 1).

The Janssen antibody thus has the requisite specificity for application to serum samples from patients chronically treated with haloperidol, who may have a significant build-up of metabolites. Our antibody has shown utility in measuring serum haloperidol concentrations acutely over a period of several hours in subjects given single, low parenteral drug doses, as indicated in figure 1. It also may be useful in assessing the build-up of reduced haloperidol and other metabolites over time, by a subtraction technique utilizing our antiserum for haloperidol and its metabolites and the Janssen

antiserum for haloperidol alone. Such a technique has been applied to other drug and hormone systems (Ratcliffe *et al.*, 1977; Rosenbloom and Fisher, 1974). Table 2 shows data from our laboratory on the steady state serum levels of haloperidol and haloperidol plus its metabolites (mainly reduced haloperidol) determined in this way in chronic schizophrenic patients. There is considerable variability in serum drug levels in patients receiving similar drug dosages, based on body weight. The levels of haloperidol plus its metabolites were consistently higher than haloperidol itself in all patients, and there is a high degree of intersubject variability in these values as well.

Table 2 Serum concentrations of haloperidol, determined with the Janssen antibody (Michiels *et al.*, 1976); haloperidol plus its metabolites (mainly reduced haloperidol), determined with our antibody (Clark *et al.*, 1977; Rubin *et al.*, 1980*a*); and percentage of metabolites, measured in blood samples from nine hospitalized schizophrenic patients under steady state conditions

Subject	Sex	Haloperidol dose ($mg\ kg^{-1}$ per day)	Serum haloperidol ($ng\ ml^{-1}$)	Serum haloperidol plus metabolites ($ng\ ml^{-1}$)	Percentage of metabolites (%)
1	M	0.05	6.5	7.0	7
2	M	0.08	4.8	8.1	41
3	F	0.16	31.1	48.9	36
4	F	0.51	22.5	30.0	25
5	F	0.51	47.0	76.0	38
6	F	0.69	168.0	173.0	3
7	M	0.98	164.0	260.0	37
8	M	1.10	56.0	79.0	29
9	F	1.15	170.0	300.0	43

DISCUSSION

Serum haloperidol levels determined by all three assay techniques (gas chromatography, RRA, RIA) have been related both to drug dosage and to clinical response in psychiatric patients (Mielke *et al.*, 1978; Ericksen *et al.*, 1978; Smith *et al.*, 1979; Calil *et al.*, 1979; Bjørndahl *et al.*, 1980). In general, over the clinical dose range of a few milligrams to 200 mg per day, there has been a very high correlation noted between daily drug dose and serum drug levels. Different patients on the same drug dosage may show quite different serum levels, but individual patients maintain fairly stable serum drug levels while on a constant drug dose.

On the other hand, the relationship between serum haloperidol levels and clinical response in schizophrenic patients has been variable. Smith *et al.*

(1979) studied 36 medication responders, treated with oral haloperidol at 15 mg per day, and six chronic non-responders, treated with oral haloperidol at 20–60 mg per day. The responders, who were treated with the lower haloperidol dosage, had a mean steady state plasma haloperidol level (determined by gas chromatography) of 15.3±2 (S.E.M.) ng ml^{-1} after 1 week of treatment and two-thirds that value after 3 weeks of treatment, whereas the non-responding patients, who were treated with much higher drug doses, had a mean steady state plasma haloperidol level of 4 ng ml^{-1}. Calil *et al.* (1979), in a study of 22 psychiatric patients on fixed haloperidol doses, found a very high correlation (+0.83) between drug dose and serum concentrations, as determined by RRA, and a statistically significant relationship between serum concentrations and total scores on the Brief Psychiatric Rating Scale (BPRS). The individual rating scale items that correlated best with serum haloperidol levels were thinking disturbance and paranoid disturbance.

In contrast, Ericksen *et al.* (1978) studied 42 acutely decompensated drug-free schizophrenic patients for 18 days by treating half the patients with a loading dose of haloperidol (60 mg per day intramuscularly) for 5 days, followed by gradually decreasing oral doses, and treating the other half with a standard drug dose (15 mg per day orally) throughout the study. As measured by gas chromatography, plasma haloperidol levels in the parenteral loading dose group averaged 25 ng ml^{-1}, which was about four times higher than the plasma levels of the standard oral dose group. The remission of psychotic symptomatology, as measured by the BPRS, however, was identical for the two groups. Finally, Bjørndahl *et al.* (1980) studied 23 chronic schizophrenics in a 12 week double-blind study of high dosage (average 103 mg per day) versus standard dosage (average 15 mg per day) haloperidol administration. The patients had been relative non-responders to previous neuroleptic treatment. There was a strong positive correlation (+0.90) between haloperidol dose corrected for body weight and serum haloperidol concentration as determined by RIA with the Janssen antibody. However, there was no relationship between serum haloperidol and clinical effect at the end of the 12 week study as determined by the BPRS total score.

In summary, it appears that all three analytic methods for determining circulating haloperidol levels (gas chromatography, RRA, RIA) are sufficiently developed to be reliable and useful. The relative advantages and disadvantages of each technique have been discussed above. In future studies, the choice of technique undoubtedly will rest not only on these advantages and disadvantages but also on the particular orientation of the collaborating laboratory. The important issue is the definition of those clinical circumstances in which monitoring of circulating haloperidol levels will be useful. Shader and Greenblatt (1979) have enumerated several circumstances concerning neuroleptic treatment in clinical practice where quantitation of drug levels might be indicated. Further research utilizing these assay techniques should clarify the utility of monitoring serum haloperidol levels as an aid to treatment of schizophrenic patients with this neuroleptic drug.

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Haloperidol plasma levels in relation to antimanic effect

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INTRODUCTION

In the treatment of mania, lithium (Li) has been found in several controlled studies as clearly superior to placebo, but haloperidol, when compared to Li, seems to be faster acting, at least with regard to motor activity (Shopsin *et al.*, 1975). Hence, haloperidol has increasingly been recommended as the antimanic drug of choice (Shaw, 1979). However, the recommended dose of haloperidol for mania varies extremely, from 4 mg to 100 mg daily (Hollister, 1978). The haloperidol dose required obviously depends on the severity of the manic state, but might also depend on the metabolism of the drug, which varies in different patients (Forsman, 1977). To our knowledge the relationship between plasma levels of haloperidol and antimanic effect has not been evaluated. In the present study we have measured plasma levels of haloperidol in manic patients treated with a fixed haloperidol dose and examined the relationship between plasma levels, antimanic effect and side effects.

MATERIAL AND METHODS

The study was carried out in the period between April 1979 and May 1980 in the Department of Psychiatry, Rigshospitalet, Copenhagen. Patients with severe mania obtaining a score of 11 or more on the Bech–Rafaelsen Mania Scale (Bech *et al.*, 1979) were included in the study. Exclusion criteria were organic brain disease, schizophrenia, alcoholism, drug addiction and somatic disorders requiring medical treatment. Patients receiving ECT or Li treatment were also excluded.

A total of 16 patients (14 women and two men) entered the study. However, five patients were excluded due to failure in drug administration, erroneous collection of blood samples or need for parenteral treatment. Ten women and one man with a median age of 35 years (range 23–70 years) completed the study.

The diagnostic classification of the patients was based on the MULTI-CLAD system (Kramp *et al.*, 1979). This classification was performed by a psychiatrist, who otherwise did not take part in the study. By this system the patients were grouped in two categories: definite mania and possible mania. The evaluation of the clinical effect of haloperidol was performed by rating scales. The antimanic effect was measured by the Bech–Rafaelsen Mania Scale (Bech *et al.*, 1979), and the haloperidol induced side effects were measured by a modified version of the Simpson Scale (Simpson and Angus, 1970). Patients with a rating score of 10 or less at day 7 were defined as responders. The raters were blinded to the plasma levels. On the three rating days during the treatment, namely after 2 days (day 3), 4 days (day 5) and 6 days (day 7) of treatment, blood samples were drawn for measuring plasma haloperidol. The tablets or mixture of haloperidol were given in a fixed dosage of 5 mg at 08.00 and 20.00, that is 10 mg haloperidol daily. The patients were observed during the intake of the medication.

Diazepam (5–40 mg per day) was allowed if sedation was needed. Neuroleptic side effects were treated with biperiden (2–10 mg per day). Blood samples were taken from fasting patients at 08.00 on day 3, day 5 and day 7 before the morning dose of haloperidol was given, that is 12 h after the preceding drug intake. Plasma samples were stored at −80 °C until radioimmunoassay (RIA) was performed.

Statistical analyses were performed using non-parametric test methods (Siegel, 1956). As the level of statistical significance, $P<0.05$ was used (one-tailed). To compare the sex ratio in the present study with other studies, the Fisher test was used. The relationship between plasma levels of haloperidol, age of the patients and rating scores was analyzed by the Spearman test. Similar analyses were completed for testing the predictive value of plasma level of haloperidol on day 3 when correlated to day 5 and day 7. To demonstrate the individual dispersion of plasma haloperidol between patients, Friedman's test was used. The two groups of responders and of non-responders were compared by a Mann–Whitney test. The RIA method was used for measuring haloperidol levels in the plasma. The assay was performed by means of a commercially available haloperidol RIA kit (Institut National des Radioelements, Belgium) (J. Heykants, personal communication).

The data on each patient are illustrated in table 1.

RESULTS

The patients were divided into two groups according to the MULTI-CLAD score: definite ($n=7$) and possible mania ($n=4$). Due to the small number of

Table 1 Plasma levels of haloperidol (in nanograms per milliliter) and mania score during treatment

No	Classification*	Sex	Age	Day 1	Day 3		Day 5		Day 7	
				Manic score	Manic score	Plasma level	Manic score	Plasma level	Manic score	Plasma level
1	D	f	25	30	22	4.5	15	3.8	7	3.5
2	D	f	24	27	26	1.8	22	2.2	11	2.7
3	D	f	58	31	11	3.2	8	3.5	8	3.6
4	D	f	44	32	24	3.2	17	3.2	15	4.0
5	D	f	36	19	8	4.2	17	4.6	8	4.8
6	D	f	35	20	14	2.6	17	2.8	16	3.2
7	D	f	32	30	23	3.7	12	3.4	12	4.1
8	P	f	70	34	19	3.0	14	3.4	9	3.0
9	P	m	23	32	26	1.9	8	1.3	9	1.7
10	P	f	33	26	7	4.3	4	5.0	2	4.0
11	P	f	40	27	18	2.9	19	1.7	17	0.8
Median (range)			35 (23–70)	30 (19–34)	19 (7–26)	3.2 (1.8–4.5)	15 (4–22)	3.4 (1.3–5.0)	9 (2–17)	3.5 (0.8–4.8)

*D, Definite mania; P, possible mania.

patients, we have united the groups, and in the following the total number of patients is therefore 11. As the final sex ratio was 10:1, this study gives information on these relationships on females only.

Plasma levels on day 3 were shown to be of predictive value concerning the plasma levels on day 5 and day 7 (r_S=0.87, P<0.01 and r_S=0.74, P<0.01). Furthermore, the plasma levels obtained on day 3, day 5 and day 7 were found stable within the group of 11 patients as indicated by the Friedman test (χ^2=0.95, k=3, n=11, P<0.05). Using the Friedman test, we found that the dispersion in plasma haloperidol levels between the patients was significantly larger than within the patients from day 3 to day 7 (χ_r^2=25.78, k=11, n=3, P<0.01). No significant correlation between haloperidol plasma levels and total scores on the mania scale was demonstrated on day 3 (r_S=−0.38), day 5 (r_S=−0.39) or day 7 (r_S=−0.37). Likewise, when the patients were grouped according to the mania rating score on day 7 in responders (n=6) and non-responders (n=5), no differences was seen in the haloperidol levels (median 3.5 ng ml^{-1}, range 1.7–4.8 ng ml^{-1} and median 3.2 ng ml^{-1}, range 0.8–4.1 ng ml^{-1}, respectively). Decrease in total mania score from day 1 to day 3 indicating clinical improvement was significantly correlated to plasma level on day 3 (r_S=0.57, P<0.05). Furthermore, a significant negative correlation was demonstrated between plasma level of haloperidol on day 3 and total mania score on day 7 (r_S=−0.61, P<0.05). An item analysis of the rating scale for mania was carried out and divided into four subgroups, i.e. the sum of scores on items no. 1 and 2, concerning motor and verbal activity, the sum of scores of the remaining items, i.e. items no. 3–11, item no. 6 concerning mood and item no. 7 concerning self-estimation. The sum of scores in each group was correlated to plasma levels and no significant correlation was found on day 3 and day 5 for any of the subscales: day 3 (items no. 1+2, r_S=−0.12; items no. 3–11, r_S=−0.38; item no. 6, r_S=−0.44; item no. 7, r_S=−0.21); day 5 (items no. 1+2, r_S=−0.20; items no. 3–11, r_S=−0.38; item no. 6, r_S=−0.36; item no. 7, r_S=−0.19).

On day 7 a significant correlation to plasma levels of haloperidol and items no. 1+2 (r_S=−0.58, P<0.05) was found, but not to the remaining items (items no. 3–11, r_S=−0.49; item no. 6, r_S=−0.22; item no. 7, r_S=−0.09). A significant positive correlation between plasma levels of haloperidol and total score on the side-effect rating scale emerged on day 3 and day 7 (r_S=0.79, P<0.01, and r_S=0.54, P<0.05, respectively), but not on day 5 (r_S=0.32).

DISCUSSION

In the present study, the clinical improvement seen on day 3 was slightly but significantly correlated to plasma levels on day 3. Furthermore, the plasma levels on day 3 seemed to be of predictive value for the clinical outcome on day 7. However, we were unable to demonstrate a lower therapeutic limit

for plasma haloperidol levels after 6 days' treatment of manic patients, which is in contrast to plasma level studies on the outcome of haloperidol treatment in schizophrenia (Tune *et al.*, 1980). However, the correlation between verbal motor activity and plasma levels of haloperidol is in agreement with the findings of Shopsin *et al.* (1975), who indicated that haloperidol especially exerts its effect on the psychomotor activity component of mania. On the other hand, the lack of correlation between subscales and plasma levels is in agreement with the construction of the mania rating scale, as its items have been selected due to their high intercorrelation coefficients (Bech *et al.*, 1979).

We found that steady state of plasma haloperidol levels seems to be reached within 2 days of treatment, which is earlier than reported in a study by Forsman (1977). Concerning haloperidol-induced side effects, a significant correlation was seen between total rating score and plasma levels on day 3 and to a lesser extent on day 7, whereas no correlation could be demonstrated on day 5. This dispersion in the correlation might be due to the treatment with biperiden.

We found no correlation between haloperidol plasma level and the age of the patients. The sex ratio in our material was 10 : 1 (female versus male).

It has for many years been generally agreed that the sex ratio in mania is 1 : 1, but in recent studies on mania the ratios seem to be much more skewed, for instance, 14 : 3 (McNamee *et al.*, 1972) and 14 : 6 (Petterson *et al.*, 1973). Although these ratios did not differ from the sex ratios among our patients, our results are representative only for females. The number of patients in the present study is small, but our results might indicate an adjustment of plasma haloperidol level after 2 days' treatment to the highest level in this study, i.e. 5 ng ml^{-1}.

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Haloperidol plasma levels and clinical response in schizophrenia

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INTRODUCTION

The recent availability of chemical techniques makes it possible to measure plasma levels of psychotropic drugs in relation to clinical response in mental disorders. Although most of the work in this area deals with tricyclic antidepressants, there is a growing interest in pharmacokinetic parameters of antipsychotic medication in schizophrenic illness.

Haloperidol, one of the most frequently used antipsychotic drugs, is of great interest for clinical studies in light of the fact that its pharmacokinetic parameters have been extensively studied (Forsman and Öhman, 1977). Recent investigations have shown that there is wide interindividual variation in plasma levels of haloperidol, an observation consistent with reports indicating that high dosage of haloperidol may increase the response to this drug in chronic resistant schizophrenic patients (McCreadie and McDonald, 1977).

The present study was undertaken to collect preliminary data on the pharmacokinetics of haloperidol in schizophrenic patients and to assess the reliability of plasma level evaluation in relation to clinical response.

PATIENTS AND METHODS

Twenty patients (10 male and 10 female), aged 21 to 66 (mean, 37), suffering from schizophrenia according to the diagnostic criteria of Feighner *et al.* (1972) were included in the present investigation. They were hospitalized for an acute psychotic relapse and were treated with oral haloperidol after a wash-out period of 7 days. All patients were in good physical health

with no evidence of any organic disease. None of them was on a long-acting neuroleptic treatment at the moment of relapse. Haloperidol was given orally three times a day starting at doses of 6 mg, allowing for a weekly increase of 6 mg according to the clinical state of the patients. An antiparkinsonian drug (procyclidine) was used only if severe extrapyramidal symptoms appeared during the study. Nitrazepam was given in the evening if necessary. The active study period lasted for 4 weeks. Blood samples were drawn once a week in the morning, 10 h after the last oral dose for the radioimmunological assay of haloperidol (IRE, Belgium).

The Brief Psychiatric Rating Scale (BPRS) evaluation was done once a week on the same day, the rater being unaware of the dosage and the plasma levels of haloperidol.

RESULTS

Table 1 summarizes the clinical characteristics of the patients' samples. Weight, steady state levels at the end of the study and the amelioration score (difference between the BPRS score at the end of the wash-out period and the score at the end of the study) are also illustrated in this table.

Table 1 Clinical and pharmacokinetic data

Case	Sex	Age	Weight (kg)	Steady state (ng ml^{-1})	Amelioration score, (BPRS)
1	F	26	45	1.4	3
2	M	21	70	9.2	39
3	M	63	85	5.5	21
4	M	21	73	15.7	76
5	M	46	92	9.4	38
6	F	39	69	3.5	4
7	F	66	60	5.5	19
8	M	22	83	1.2	0
9	M	23	55	3.2	13
10	F	32	70	3.2	8
11	F	23	51	5.6	6
12	F	29	50	10.6	24
13	F	28	48	1.9	5
14	M	30	70	4.1	22
15	M	51	64	12.5	37
16	F	37	56	3.6	5
17	M	40	68	10.1	24
18	F	31	66	0.2	2
19	M	22	60	2.7	2
20	F	30	65	8.5	7

Figure 1 shows the distribution of steady state plasma levels of haloperidol in patients taking 6 mg of the drug orally. There is a large interindividual variation of haloperidol plasma levels with values ranging from a low of 1.3 ng ml^{-1} to a high of 11.3 ng ml^{-1}, thus showing a ninefold variation between the patients studied. Those data are consistent with those previously published (Morselli *et al.*, 1979; Forsman and Öhman, 1976).

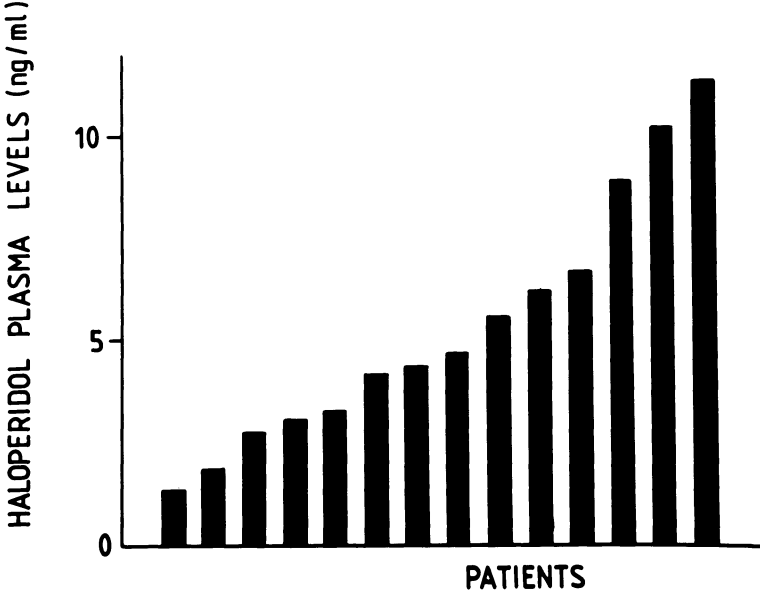

Figure 1 Distribution of steady state plasma levels in schizophrenic patients receiving 6 mg of haloperidol orally.

Figure 2 is an attempt to correlate steady state plasma levels of haloperidol at the end of the study with clinical improvement as measured by changes in BPRS scores. The amelioration scores plotted in this figure are differences between baseline scores at the end of the wash-out period and the scores registered at the end of the study. It is quite clear that amelioration scores are highly correlated with haloperidol plasma levels, the correlation coefficient being $r = 0.87$ at the level of significance of 0.001. Patients with a plasma level below 4 ng ml^{-1} tend to show poor clinical response or no response at all to the medication.

Patients above the level of 9 ng ml^{-1} show clear-cut therapeutic response to haloperidol. Patients with drug levels between 4 and 6 ng ml^{-1} show intermediate responses to the antipsychotic medication. Since none of the patients in this study had a serum level exceeding 20 ng ml^{-1}, we could not assess if there is a relationship between clinical deterioration and very high levels of haloperidol.

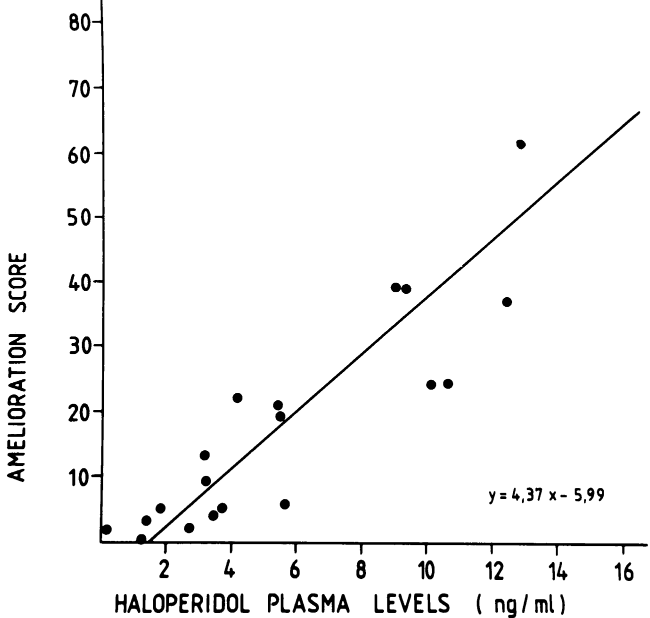

Figure 2 Correlation between haloperidol plasma levels and clinical response ($r = 0.87$, $p < 0.001$).

CONCLUSION

In this study, clinical response to haloperidol correlates very well with the serum concentration of this antipsychotic drug – that is non-responders show low serum drug levels. These results are in good agreement with a recent report of Tune *et al.* (1980) using a receptor assay and showing that schizophrenic patients with haloperidol plasma levels below 50 ng of chlorpromazine equivalents show a poor response to haloperidol.

We would like to emphasize the preliminary nature of the study. Some aspects of the investigation are open to criticism; in particular, the variable dosage regimen and the small number of patients. However, our data indicate that the determination of plasma levels of haloperidol could be of clinical value in the treatment of schizophrenic patients.

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Section Five

Pharmacokinetics and Plasma Level/Effect Relationships

(B) Antidepressants

Pharmacokinetics and plasma level/effect relationships of tricyclic antidepressants: an update

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INTRODUCTION

The research on pharmacokinetics of tricyclic antidepressants (TCA) has developed in two phases. Through the 1960s the bulk of research was concentrated on qualitative aspects of distribution, metabolism and excretion. By the introduction of methods for drug concentration assay in human blood in the early and mid-1970s, the research has concentrated on quantitative aspects of TCA kinetics and the difficult field of relating clinical pharmacokinetics and pharmacodynamic and therapeutic measurements. In the 1980s a major challenge will be to combine the kinetic/therapeutic relationships to a multi-axial diagnostic system that takes both clinical and biological classification procedures into account. This seems to be the most precise way to improve our diagnostic procedures toward a better identification of those patients that are genuinely responsive to treatment with antidepressants. Further research on factors influencing the kinetics of antidepressants, and kinetic research on the newer antidepressants, is also needed.

PLASMA LEVEL/EFFECT RELATIONSHIPS

In contrast to the development in the field of neuroleptics (Morselli *et al.*, this volume) the last two years' research on plasma level/effect relationship of antidepressants has not added much to the general conclusions that could be made in earlier reviews (Gram, 1977*b*, 1978).

In 1971 Åsberg *et al.* suggested – on the basis of a 2 week study on 29

patients – a so called 'curvilinear' relationship between plasma level and therapeutic effect of nortriptyline (NT). Table 1 summarizes the studies that have confirmed this finding. Three of these studies (Kragh-Sørensen *et al.*, 1973, 1976; Montgomery *et al.*, 1978) seem to meet reasonable research criteria in terms of diagnostic classification, compliance-control, blindness, etc. These studies, involving 72 patients, all point out that the antidepressive effect is poor or lacking when the NT levels are above 175–200 $\mu g\ l^{-1}$. With a dose interval-sampling interval of 24–12 h (Montgomery *et al.*, 1978), the critical plasma level limit can be expected to be slightly higher than with the 8–7 h schedule used in earlier studies (Kragh-Sørensen *et al.*, 1973, 1976), provided that the effect is related to the *average* steady state level (Gram *et al.*, 1980). Whereas the concept of an *upper* effective plasma level limit thus is reasonably well established in these studies, the *lower* effective plasma level of NT is still only tentatively defined to about 50–60 $\mu g\ l^{-1}$. Indeed, several studies have failed to find such a relationship (Burrows *et al.*, this volume), but it has not been ruled out that this is not related to methodological factors (Gram, 1977*b*; Gram *et al.*, 1980), and these results underline that plasma level monitoring should be used only in the context of proper clinical diagnostic practice.

Table 1 Nortriptyline: plasma level/effect studies confirming studies by Åsberg *et al.* (1971)

	N	Patient status	Diagnosis	Duration (weeks)
Kragh-Sørensen *et al.*, 1973	30	In-patients	Endogenous	4
Kragh-Sørensen *et al.*, 1976	24	In-patients	Endogenous	4+2
Ziegler *et al.*, 1976*b*, 1977	18+1	Out-patients	Mixed?	6
Montgomery *et al.*, 1977*	36	In-patients	Mixed?	3–6
Montgomery *et al.*, 1978	18	In-patients	Endogenous	4

*Open, non-blind study with variable dosage.

Table 2 summarizes the results of the three studies on imipramine (IMI) that fulfil reasonable criteria for design, etc., to be conclusive (Olivier-Martin *et al.*, 1975; Glassman *et al.*, 1977; Reisby *et al.*, 1977). The studies are generally in good agreement, showing that in endogenously depressed patients there is a direct relationship between the plasma levels (imipramine+desipramine (DMI)) and the antidepressive effect, such that optimal effect is obtained when the levels are above 250 $\mu g\ l^{-1}$. None of these studies have given any indications of a poor therapeutic response in patients developing high or very high levels, and the relationship to toxicity at high levels is not clear. As discussed by Kragh-Sørensen *et al.* (this volume), the most disturbing side effect of IMI is orthostatic hypotension occurring at low sub-therapeutic plasma levels (Glassman *et al.*, 1979; Thayssen *et al.*, 1981).

The relative contribution of the two compounds, IMI and DMI, to the therapeutic effect is not clear. On the basis of our data analyses (Gram *et al.*,

Table 2 Imipramine: plasma level/effect studies

	Olivier-Martin *et al.*, 1975		Glassman *et al.*, 1977		Reisby *et al.*, 1977	
	N=18	*N*=6	*N*=42	*N*=18	*N*=51*	*N*=15
Diagnosis	Endogenous	Non-endogenous	Endogenous	Delusional	Endogenous	Non-endogenous
Duration (weeks)	3		4		4	
Relationship (IP+DMI)		—		—		—

*Defined as endogenous score on Newcastle Scale I and/or II.

1978; Reisby *et al.*, 1977) we have suggested that perhaps the antidepressive effect requires plasma levels of both IMI and DMI above a certain level, indicating that simultaneous effect on two different receptor systems might be needed. Other independent analyses by our group and others, assuming the same effect of both compounds, indicate that IMI might be more potent than DMI (Gram *et al.*, 1978; Perel *et al.*, 1978).

Table 3 summarizes the studies carried out on amitriptyline (AMI). AMI is now the most studied TCA in this field, with a total of 320 patients. However, the quantity has not contributed to the clarity, and – as indicated in table 3 – a general conclusion is not possible at the present time. In some of these studies, the lack of appropriate diagnostic classification and lack of drug-compliance control probably explain the negative findings. Several of the studies (Braithwaite *et al.*, 1972; Montgomery and Braithwaite, 1975; Ziegler *et al.*, 1976*a*; Montgomery *et al.*, 1979) indicate a lower effective level of AMI+NT of about 100 $\mu g\,l^{-1}$, whereas the demonstration of an upper limit (Montgomery *et al.*, 1979; Vandel *et al.*, 1978) is much less consistent. AMI may be difficult to study, if the parent compound, AMI, and the metabolite, NT, have inherently different plasma level/effect relationship profiles.

Table 4 summarizes recent studies on clomipramine published within the last few years (Della Corte *et al.*, 1979; Träskman *et al.*, 1979; Reisby *et al.*, 1979). These studies differ in several respects of methodology and design, and this may explain why the results are so different.

DIAGNOSTIC CLASSIFICATION

As indicated in tables 2 and 4, the existence of a plasma level/effect relationship seems to be closely related to the diagnosis of endogenous depression. The data of Glassman *et al.* (1977) indicated that in patients exhibiting pronounced non-depressive delusional symptoms, the effect of IMI was generally poor, and no relationship to the plasma levels could be established. These patients did *not* respond to higher IMI levels (A. H. Glassman, personal communication).

For both IMI and clomipramine, Reisby *et al.* (1977, 1979) found no relationship between plasma levels and therapeutic effect in non-endogenous, reactive-neurotic depressions, although the recovery rate was the same as in the endogenous group (Gram *et al.*, 1980). In the IMI study (Reisby *et al.*, 1977), very few of the non-endogenous patients had levels above the critical level (IMI+DMI $>200-250\,\mu g\,l^{-1}$) found in the endogenous group, and the data thus do not exclude that higher drug levels would have been effective in some of the non-responding, non-endogenous patients. Several of the non-endogenous patients were characterized by responding in spite of having very low plasma levels, indicating either spontaneous or placebo response or much lower effective plasma level limits than in the endogenously depressed patients.

Table 3 Amitriptyline: plasma level/effect studies

	N	Patient status	Diagnosis	Relationship
Braithwaite *et al.*, 1972	15	In- and out-patients	Mixed	
Montgomery and Braithwaite, 1975	28	In-patients	Mixed	
Ziegler *et al.*, 1976*a*, 1977	18+4	Out-patients	Mixed	
Kupfer *et al.*, 1977, 1978	16+9	In-patients	Endogenous	
Coppen *et al.*, 1978	54	In-patients	Mixed	—
Vandel *et al.*, 1978	62	?	Mixed	
Montgomery *et al.*, 1979	65	?	Endogenous	(NT)
Robinson *et al.*, 1978	49	Out-patients	Mixed	—

Table 4 Clomipramine: plasma level/effect studies

	Della Corte *et al.*, 1979	Träskman *et al.*, 1979		Reisby *et al.*, 1979	
	N=50	*N*=17	*N*=10	*N*=17	*N*=15
Diagnosis	Mixed	Endogenous, high HIAA	Endogenous, low HIAA	Endogenous	Non-endogenous
Duration (weeks)	3	3		5	
Relationship	CDMI	CDMI CIP	—	CIP	—

The significance of differentiating between endogenous and non-endogenous, reactive-neurotic depressions has increasingly gained acceptance in recent years in relation to drug therapy as well as in biological research (Bielski and Friedel, 1976; Carroll, 1980). However, the criteria for making this distinction seem to vary between centers. The Newcastle diagnostic inventories are increasingly used, but the relative merit of the different Newcastle scales (Bech *et al.*, 1980) or other operationalized systems for depression diagnosis has not been examined systematically. In the studies on IMI and clomipramine (Reisby *et al.*, 1977, 1979) a combined score obtained from two of the Newcastle scales (No. I: Carney *et al.*, 1965; No. II: Gurney *et al.*, 1972) was used such that patients that scored non-endogenous on one or both of these scales were classified as non-endogenous. In the majority of patients the classification agreed on the two scales, but about 20 per cent of the patients scored endogenous on No. II and non-endogenous on No. I. Separate analyses of these patients from the IMI study ($n=14$) showed a significant relationship ($r_S=0.51$, $p<0.05$) between plasma levels and antidepressive effect that was essentially identical to that found in the 37 patients primarily classified as endogenously depressed (Bech *et al.*, 1980). The number of patients in the clomipramine study was too small to permit similar analyses. Plasma level/effect relationships in patients thus may be used to establish which diagnostic scales most precisely differentiate between patients with or without a true drug-related response. These results underline the necessity of internationally established scoring systems for diagnostic classification of depressed patients. Both in plasma level/effects studies and in studies on biological diagnostic markers (Carroll, 1980) the use of different or poorly defined diagnostic procedures may become a major source of apparent disagreement between different research centers. As suggested in recent studies on clomipramine by Träskman *et al.* (1979), classification of patients by biochemical tests may be useful to identify patients showing a plasma level related response. Indeed the possibility of different effective plasma levels in different types of depressions should always be considered.

PHARMACOKINETICS

The various aspects of pharmacokinetics relevant to plasma level/effect studies as well as to the clinical application of plasma level monitoring of antidepressants have been discussed in detail in previous reviews (Gram, 1977*b*, 1980; Gram *et al.*, 1980). Once it has been decided to adjust drug dose on the basis of plasma concentration measurements, a rapid and reliable method for dose adjustment will be needed. For NT a schedule using samples taken after 7–10 days of treatment has been shown to be feasible and reliable (Kragh-Sørensen *et al.*, 1980; Kragh-Sørensen and Larsen, 1980). A scheme for earlier dose adjustment has been suggested by Cooper and Simpson (1978) and Braithwaite *et al.* (1978), using a single-

dose–single-sample prediction, and Kragh-Sørensen *et al.* (1980) predicting from blood samples drawn early in the course of treatment. The latter procedure has obvious practical advantages and has shown a moderate to good reliability, but the clinical applicability of this procedure has to be finally established in larger patient materials. Preliminary data on lithium (Li) indicate that this principle for prediction may also be applicable for this drug.

We have previously developed the theoretical basis for using only within-patient plasma level measurements for steady state level prediction on the basis of the following equation (Gram *et al.*, 1980):

$$C_\infty = \frac{C_1^2}{2C_1 - C_2} = \frac{C_2^2}{2C_2 - C_4}, \qquad (1)$$

where C_1, C_2 and C_4 denote the concentration measurements after the first, second and fourth doses, and C_∞ the concentration at steady state. However, for both NT and Li the equation generally gives too high predicted values of C_∞, and in some cases the denominator $(2C_1 - C_2)$ becomes close to zero. This is explained by the course of plasma concentration build-up shown for NT (Kragh-Sørensen *et al.*, 1980). In the majority of patients the rise in plasma concentration during the first 3–4 days is almost linear and not curved as it theoretically should be. Obviously, changes in drug kinetics take place during the early phase of administration, but the nature of these changes can not as yet be settled. The error made by disregarding terms for absorption and distribution (two-compartment model) in deriving equation (1) can at most account for an overestimate of C_∞ of 2–3 per cent. The plasma concentration reached after 3–4 days is very often close to the final steady state level, and it may be sufficiently accurate to regulate the dose on this basis. However, in some patients the NT concentration may continue to rise over several weeks (Kragh-Sørensen *et al.*, 1976), and this may indicate that there are non-linear kinetics in occasional patients, although proportionality between NT dose and steady plasma levels has been shown in large patient materials of all ages (Kragh-Sørensen and Larsen, 1980).

For IMI, recent results from our research unit indicate that non-linear kinetics probably is a more general problem (Bjerre *et al.*, 1981). In a study on elderly depressed patients (age 63–78) it was found that in all patients (n=6) that had the IMI dose increased, the plasma concentration of the metabolite DMI rose disproportionally (figure 1), whereas the IMI concentration generally rose proportionally to the increase in dose. Reviewing the records from earlier studies on IMI (Gram *et al.*, 1977; Reisby *et al.*, 1977), one case (female, age 63) was disclosed in which similar disproportional changes of the DMI level followed reduction of the IMI dose. These data indicate that disproportionality is an almost universal phenomenon in elderly patients, whereas available data from younger IMI-treated patients do not permit conclusions concerning this group. Single dose studies on younger healthy volunteers have indicated first-order kinetics (Gram *et al.*, 1976), but this may not be representative for multiple dosing in patients.

The disproportionality most likely is related to a limited capacity of hydroxylation metabolism. The interaction between neuroleptics and IMI

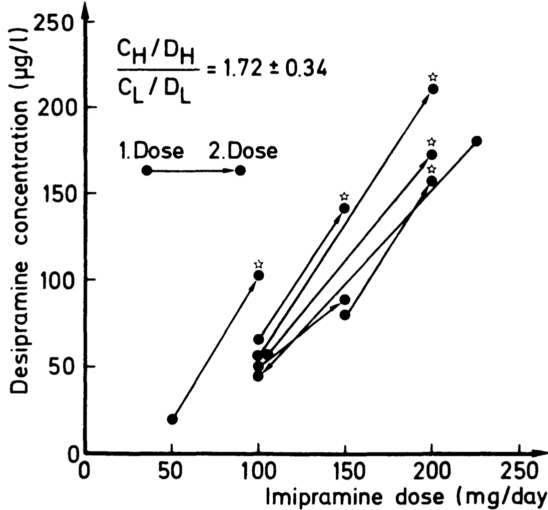

Figure 1 Corresponding *dose of imipramine* and *plasma concentration* of the active metabolite *desipramine* in seven elderly patients (age 63–78). *C*, concentration; *D*, dose; H, high dose; L, low dose. Starred points indicate that the new steady state level has not been reached.

also affects mainly the hydroxylation of DMI (Gram *et al.*, 1974; Gram, 1975, 1977*a*); elderly patients seem to be more susceptible to this effect. In two patients, a daily dose of only 8 mg perphenazine thus resulted in a clear rise of the DMI levels (Bjerre *et al.*, 1981).

A decreased capacity for hydroxylation will affect mainly DMI because this compound has lower affinity to the microsomal enzymes than has the parent compound, IMI (von Bahr, 1972). The presence of IMI thus may be important, and the DMI plasma concentration levels may not necessarily show the same pattern of non-linearity if DMI is given as the parent drug (see the discussion of linearity of NT kinetics above). The age variations in steady state levels in IMI-treated patients appear to affect the IMI levels as much as the DMI levels (Nies *et al.*, 1977; Gram *et al.*, 1977). Nies *et al.* (1977) also found an effect of age in AMI-treated patients on AMI levels, but *not* on the levels of the demethylated metabolite (NT). This may indicate that the demethylation also changes by age, or that the first-pass elimination of IMI and AMI decreases (systemic availability increases) as a consequence of changes in hepatic blood flow in the elderly.

The effect of age on the kinetics of TCA thus may be quite complex, and different for different compounds, and at least partly related to the dose levels studied.

As will be discussed elsewhere (Kragh-Sørensen *et al.*, this volume) dose- and age-dependent kinetics is only one of several problems associated with the use of IMI in the elderly, and the interplay between pharmacokinetics and dynamics seems to be particularly complex in this group of patients.

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Pharmacokinetics of high clearance drugs and their metabolites

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INTRODUCTION

The overall objective in describing mathematically the time course of drug and metabolite concentrations in the body is twofold: (1) to give a reasonable summary and explanation of the characteristics of the data, and (2) to make predictions and design future studies. Forecasting the results from multiple dosing, effects of disease states, and pharmacologic/toxicologic activity from limited drug concentration data are major goals of predictive pharmacokinetics.

The purpose of this paper is to give an overview of approaches for analyzing pharmacokinetic data pertaining to high clearance drugs and their metabolites. Data for the tricyclic antidepressants (TCA) will be used for illustration and the concept of transit time analysis for describing metabolite disposition will be introduced.

SHAM CHARACTERISTICS

An appropriate starting point in dealing with raw data collected in a pharmacokinetic study, such as serum or plasma concentration–time data, is to subject them to graphical or computer analysis to determine the SHAM characteristics for each data set (figure 1). This acronym refers to the slopes of the curves (S), their heights or intercepts (H), the total area under the concentration–time curve (A), and the first moment of the curve (M) (Caprani *et al.*, 1975; Lassen and Perl, 1979). The iterative computer program NONLIN (Metzler, 1969) and the LaGrange polynomial algorithm for determining area (Yeh and Kwan, 1978) are useful for curve-

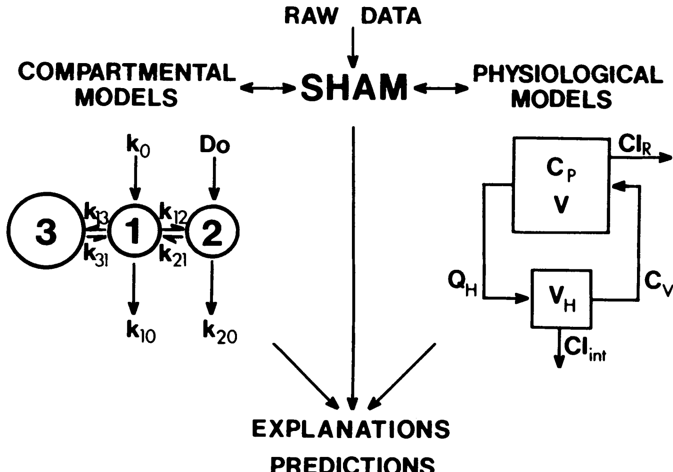

Figure 1 Pharmacokinetic data are analyzed by determining the SHAM characteristics (see text), assigning models, or using model-independent calculations to explain the data and make predictions.

fitting and area analysis. Computer analysis using regression programs is preferred to hand calculations because of the rapidity, flexibility, and less biased nature of this approach.

At this point four basic parameters can describe most types of pharmacokinetic data. Often this may be enough. For example, from the terminal plasma concentration decline following single or multiple drug doses, the half-life can be computed to predict the dosing time required in that individual to attain steady state conditions (Gibaldi and Weintraub, 1971). The SHAM analysis serves to summarize the fundamental characteristics of any curve without the need for any model assumptions. The SHAM properties are generally exact to the degree of intensity of blood sampling and the accuracy of the terminal slope value. The latter is crucial for determining A and M by extrapolation of the last data point to time infinity.

COMPARTMENTAL MODELS

Concentration–time data for the TCA are frequently analyzed with the use of compartmental models (Gram *et al.*, 1976; Jorgensen and Hansen, 1976; Ziegler *et al.*, 1978). A one- or two-compartment open model is common. The one-compartment model assumes the drug to be homogenously distributed throughout the body. The two-compartment model assumes a central compartment containing the blood volume and the highly perfused tissues

such as liver, kidneys, and lungs. The peripheral compartment consists of the poorly perfused tissues such as muscle and fat (Riegelman *et al.*, 1968).

The choice of models will partly depend on the purpose of the analysis. For example, figure 2 shows desipramine (DMI) concentration–time data which were computer-fitted to both a one- and a two-compartment open model. If the major purpose of the analysis is to describe the terminal log–linear disposition phase of drug concentration, then both models describe the data equally well. However, if the purpose is to characterize the input of the drug to the body, as would be desirable in a bioavailability study, then the one-compartment model is less suitable as the distributive and absorptive phases are merged in one term reflecting the early time course of the data. Realistically, neither a one- nor a two-compartment open model is likely to reflect the true disposition of desipramine. As the tricyclics undergo an extensive pre-systemic elimination (Gram, 1977), a more appropriate analysis may be to consider the major eliminating organ, the liver, as a compartment separate from the central compartment (Gibaldi and Feldman, 1969). This type of model is depicted in figure 1, where compartment 2 represents the liver when drug input occurs by a first-pass route of administration. To predict accurately tissue concentrations (for example, in the central nervous system), at least a two- or a three-compartment model will be required (Westlake, 1971; Van Rossum, 1973).

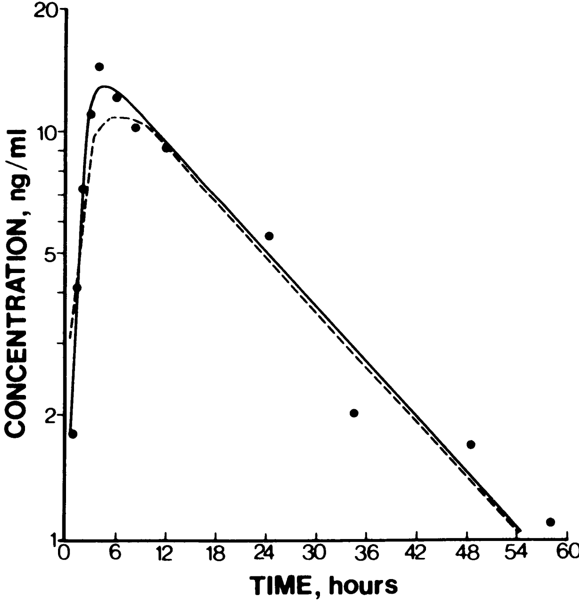

Figure 2 Plasma concentrations of desipramine (●) as a function of time after a 50 mg dose in a volunteer. Data are computer fitted to a one-compartment open model (dashed line) or a two-compartment open model (solid line).

An inherent problem in compartmental analysis is determining the adequacy of model assignment (Wagner, 1975). Fortunately, some measures exist to help determine the goodness of data fit. Subjectively, the computer-predicted concentrations can be inspected to insure that no systematic deviations occur from the observed data. Objectively, correlation coefficients, the sum of the unweighted and weighted squared deviations, and the Akake Information Criterion (Yamaoka, 1978*a*) may be used.

PHYSIOLOGICAL MODELS

Physiological models, concepts, and constructions have become the preferred vehicles for examining pharmacokinetic data. Volume and clearance parameters for describing drug disposition are more realistic than the use of rate constants and indefinite compartmental models. Elaborate physiological models incorporate real physicochemical and physiological characteristics such as blood flows, diffusion processes across membranes, metabolic activity of eliminating organs, and thermodynamic properties such as binding and partitioning to describe drug disposition (Dedrick, 1973; Chen and Andrade, 1976).

A simple hepatic perfusion model which is applicable to TCA can be employed to assess the expected disposition characteristics of high clearance drugs and their metabolites (figure 3). The drug dose (Do) can be input either intravenously ($Do_{(IV)}$) into a distribution space (V) resulting in a plasma concentration (C_p), or orally ($Do_{(oral)}$) to stimulate a first-pass route of administration. The oral dose enters the liver by the hepatic blood flow (Q_H) where it distributes within the volume of the liver (V_H) and emerges

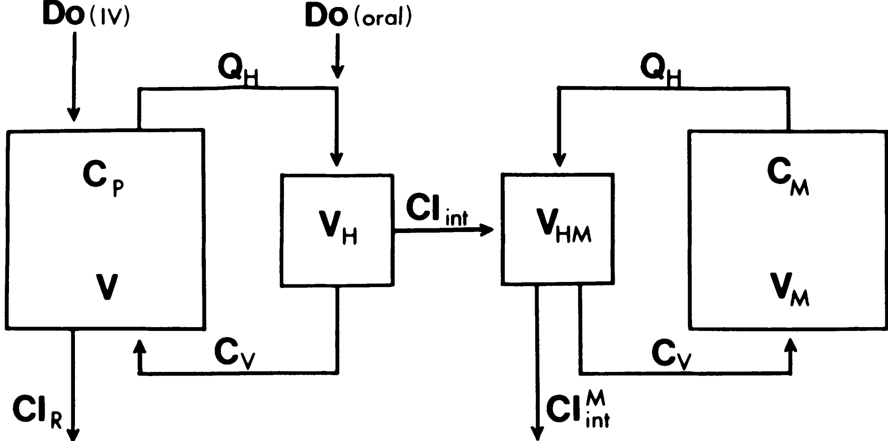

Figure 3 Physiological perfusion model used to assess disposition characteristics of high clearance drug and metabolites (abbreviations explained in the text).

with a concentration in the venous blood (C_V). The parent drug can be eliminated either through the liver by intrinsic metabolic clearance (Cl_{int}) to form a metabolite or by a secondary systemic pathway such as renal clearance (Cl_R). The metabolite, in turn, occupies a characteristic hepatic volume (V_{HM}) and distribution space (V_M). The plasma concentration of the metabolite (C_M) declines according to V_M and its intrinsic metabolic clearance (Cl^M_{int}). Equations have been derived (Pang and Gillette, 1978) which describe the area under the concentration–time curve for parent drug and metabolite after parent drug administration intravenously (AUC_{IV}, AUC^M_{IV}) and orally (AUC_{po}, AUC^M_{po}):

$$AUC_{IV} = \frac{Do(Q_H + Cl_{int})}{Q_H Cl_{int} + Cl_R(Q_H + Cl_{int})}, \tag{1}$$

$$AUC^M_{IV} = \frac{Do \cdot Q_H \cdot Cl_{int}}{Cl^M_{int}[Q_H \cdot Cl_{int} + Cl_R(Q_H + Cl_{int})]}, \tag{2}$$

$$AUC_{po} = \frac{Do \cdot Q_H}{Q_H \cdot Cl_{int} + Cl_R(Q_H + Cl_{int})}, \tag{3}$$

$$AUC^M_{po} = \frac{Do \cdot Cl_{int}(Q_H + Cl_R)}{Cl^M_{int}[Q_H \cdot Cl_{int} + Cl_R(Q_H + Cl_{int})]}. \tag{4}$$

The major conceptual differences among these equations is the fact that Do/AUC_{IV} yields the systemic clearance of drug (Cl_p) while Do/AUC_{po} is a function primarily of the intrinsic clearance (Wilkinson and Shand, 1975). They permit evaluation of the role of route of administration on disposition of the parent drug, including formation of metabolites. Inspection of equations (2) and (4) reveals that AUC^M_{IV} and AUC^M_{po} are equivalent only when secondary clearance of the parent drug is zero. Simulations show that increasing secondary clearance causes a reduction in AUC^M_{IV} in direct proportion to the reduction in AUC_{IV} (figure 4). However, the reduction in AUC_{po} is not accompanied by a proportionate reduction in AUC^M_{po}.

A rigorous assessment of TCA disposition data in humans with physiological models is hindered by the difficulty in obtaining quantitative information on individual blood flows, organ partition coefficients, and organ sizes. Nevertheless, the physiological approach with clearance terms and constructions to describe drug disposition is preferred over vague compartmental models and rate constants.

TRANSIT TIMES

A model-independent approach can characterize TCA disposition utilizing transit times. The mean residence time, or transit time, $\bar{t}$, which is calculated

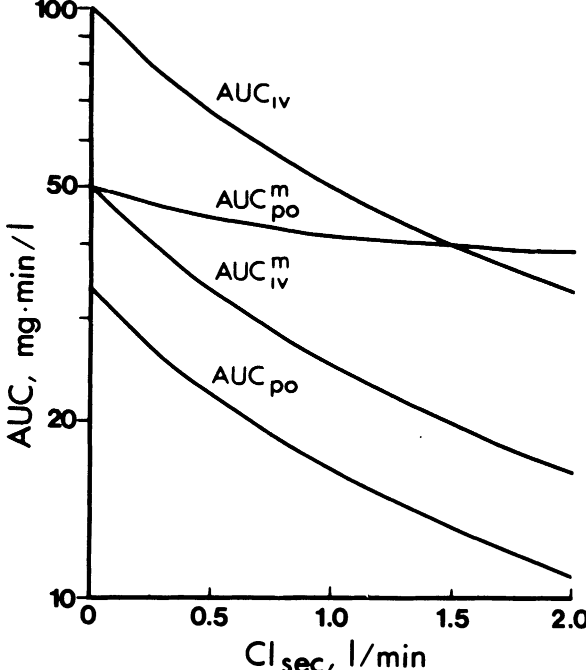

Figure 4 The effects of altering the secondary clearance (Cl_{sec}) on the area under the curve relationships for the hepatic perfusion model. Parameters used were: $Do = 100$ mg, $Q_H = 1.5$ l min^{-1}, $Cl_{int} = 3.0$ l min^{-1}, $Cl^{M}_{int} = 2.0$ l min^{-1}, and Cl_R or Cl_{sec} varies from 0 to 2.0 l min^{-1}.

as the quotient of the first moment and the area under the curve, is a useful characteristic finding increasing utility in pharmacokinetic studies. As a time-average value, $\bar{t}$ is the average length of time spent by a drug molecule from its entry into the body until its exit (Oppenheimer *et al.*, 1975; Lassen and Perl, 1979). For intravenous doses, it is determined fundamentally by the volume of distribution at steady state (V_{Dss}) and systemic clearance:

$$\bar{t} = V_{Dss}/Cl_p \tag{5}$$

if it can be assumed that $V \gg V_H$ (figure 3). Rearrangement allows a model-independent method for calculation of V_{Dss} (Benet and Galeazzi, 1979) from $\bar{t}$ and Cl_p. Numerical integration of the function $t \cdot C_p$ versus t provides an easy method of generating $\bar{t}$ from any type of experimental data:

$$t = \int_0^{\infty} C_p \cdot dt/A = M/A. \tag{6}$$

In addition, $\bar{t}$, because of its model-independent nature, provides a much needed alternative to calculation of absorption rate constants in bioavaila-

bility studies (Yamaoka, 1978*b*). For an intramuscular dose of drug the transit time, $\bar{t}_{im}$, is

$$\bar{t}_{im} = \frac{V_{Dss}}{Cl_p} + \bar{t}_{abs}, \tag{7}$$

where $\bar{t}_{abs}$ is the transit time for the input process and is equal to the reciprocal of the absorption rate constant for a simple first-order absorption process. Similarly, the transit time for an oral dose of drug, $\bar{t}_{po}$, is

$$\bar{t}_{po} = \frac{V_{Dss}}{Cl_p} + \bar{t}_{abs}. \tag{8}$$

Note that both equations employ $Cl_p = Do/\mathrm{AUC}_{iv}$ for the case of high clearance drugs.

It is evident that the transit time for a metabolite given directly, $\bar{t}_m$, is related to the fundamental characteristics of the metabolite as

$$\bar{t}_m = \frac{V^M_{Dss}}{Cl^M_{int}}, \tag{9}$$

where V^M_{Dss} is the volume of distribution at steady state of the metabolite. While it may be impossible to determine either V^M_{Dss} or Cl^M_{int} without direct administration, the transit time of metabolite formed from parent drug, $\bar{t}_{p \to m}$, can be determined as

$$\bar{t}_{p \to m} = \bar{t} + \bar{t}_m, \tag{10}$$

where $\bar{t}$ is $\bar{t}_{im}$. Substituting equation (7) into equation (10),

$$\bar{t}_{p \to m} = \frac{V_{Dss}}{Cl_p} + \bar{t}_{abs} + \bar{t}_m \tag{11}$$

gives $\bar{t}_{p \to m}$ for systemic administration of parent drug. The relationship for oral administration of the parent drug must account for first-pass availability of metabolite (F^*):

$$\bar{t}_{p \to m} = \frac{V_{Dss}}{Cl_p} + \bar{t}_{abs} + \bar{t}_m F^*. \tag{12}$$

If it is assumed that $\bar{t}_{abs}$ is similar for oral and intramuscular doses, $\bar{t}_{p \to m}$ following first-pass metabolism of parent drug should be shorter than when metabolite is formed by introduction of parent directly into the systemic circulation because of the effect of F^*. This can be visualized in figure 3 by considering the steps involved in formation and elimination of metabolite when parent drug is introduced into either V or V_H.

This theory can be extended further to sequentially formed, multiple

metabolites. Consider as an example 2-hydroxydesipramine (2-OH-DMI), the first-pass metabolite of desipramine (DMI) (DeVane *et al.*, 1980). When DMI is administered orally, 2-OH-DMI should have a shorter $\bar{t}$ than if DMI must first be formed by administration of a precursor, imipramine (IMI). Furthermore, the transit time of 2-OH-DMI should be even longer if IMI is administered intramuscularly rather than orally, thus avoiding the first-pass formation of DMI. To test this theory, transit times for 2-OH-DMI were calculated from data obtained in a three-way cross-over study where four normal volunteers were given single doses of oral DMI, oral IMI, and intramuscular IMI (figure 5). For each of the volunteers, the transit time of 2-OH-DMI was longer when IMI was administered intramuscularily than when DMI was given orally. In volunteers 2, 3, and 4 intramuscular IMI produced longer transit times of 2-OH-DMI than did oral IMI, consistent with theory. Only in volunteer 1 did the observed results deviate substantially from theory, as the transit time was longer from oral IMI than from intramuscular. This result may be due to differences in absorption characteristics between the two doses. Recalling that $\bar{t}$ is determined from the time that drug enters the body, any increased lag time occurring before the beginning of absorption of the oral dose that was not present with the intramuscular dose would create an artifactually long transit time for resulting metabolites. Thus, the close agreement between the nature of the observed transit times indicates that this characteristic is a useful addition to pharmacokinetic analysis of high clearance drugs and their metabolites. The

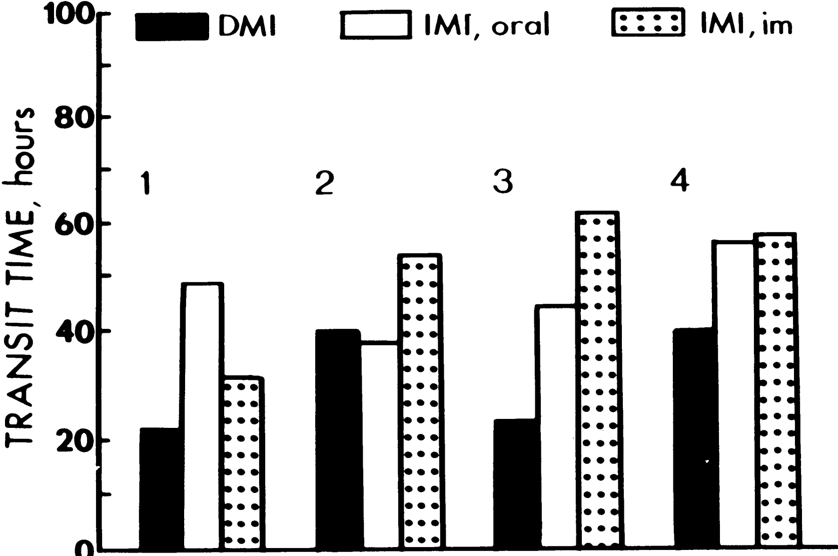

Figure 5 Mean transit time of 2-hydroxydesipramine in four volunteers who participated in a cross-over study of single dose desipramine, imipramine, and intramuscular imipramine.

transit time concept also has direct clinical relevance as it reflects the duration of time that each compound resides in the body and can serve to compare a series of drug analogs or metabolites.

SUMMARY

Pharmacokinetic data may be analyzed by several possible approaches for the purpose of interpreting data and making predictions. The SHAM characteristics are the starting point from which compartmental or physiological models may be developed. The latter are preferred but require more extensive data for use than is often available. The inherent physiological volume and clearance parameters may be calculated by model-independent methods to allow comparisons of different sets of data.

Transit-time analysis is proposed as a useful, model-independent approach to accompany the area value in the fundamental pharmacokinetic characterization of metabolites of high clearance drugs.

ACKNOWLEDGEMENT

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Prediction of plasma levels and clinical response in depression

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Those of us who treat patients daily continue to be disappointed with the relatively low response rate seen with the tricyclic antidepressants (TCA) – in the region of 30–50 percent of the patients do not show a response within a trial period in patients who have been selected for the response to antidepressants.

The response is slow and uneven and if these drugs were being used to treat a fulminating illness like pneumonia they would have been abandoned long ago. Compassionate clinicians continue to pray for the development of an antidepressant which will act rapidly in a higher proportion of cases in order to reduce the amount of suffering and morbidity caused by inadequately treated depression.

One approach to improving response with conventional TCA has been to pay attention to plasma levels.

There are more studies on the relationship of plasma concentrations and clinical response on nortriptyline (NT) than any other TCA. Åsberg *et al.* (1971), Kragh-Sørensen *et al.* (1973, 1976), Ziegler *et al.* (1976, 1977) and Montgomery *et al.* (1977, 1978*a*) (figure 1) have all reported a significant relationship between plasma NT concentrations and clinical response. Studies by Lyle *et al.* (1974) and Burrows *et al.* (1972, 1974) did not demonstrate a significant relationship. There appears to be a consensus that in treating endogenous depression with NT the best clinical outcome is associated with plasma concentrations approximately between 50 and 175 or 200 $\mu g\, l^{-1}$ and that a poorer response is seen in patients with concentrations outside this range.

In a rather elegant double-blind study, Kragh-Sørensen *et al.* (1976) demonstrated the significant clinical advantage of adjusting the dosage of those patients with high plasma NT concentrations to bring them within the optimum therapeutic range for 2 weeks at the fourth week of treatment.

There have also been a large number of studies investigating the relationship between plasma concentrations of amitriptyline (AMI) and clinical response. This is hardly surprising, since AMI is the most widely prescribed

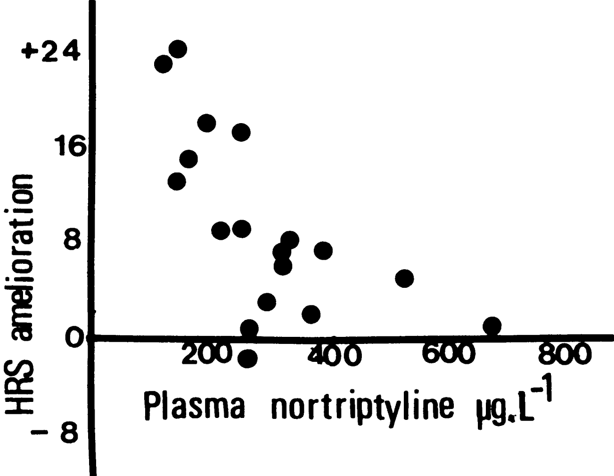

Figure 1 Relationship between amelioration of depression scores (difference in Hamilton scores between weeks 0 and 4) and mean plasma nortriptyline levels.

antidepressant in the world. A relationship has been reported by Braithwaite *et al.* (1972), Montgomery and Braithwaite (1975), Ziegler *et al.* (1977), Kupfer *et al.* (1977), Vandel *et al.* (1978), Montgomery *et al.* (1979*a*, *b*) and Moyes and Moyes (1978). All of these studies, with the

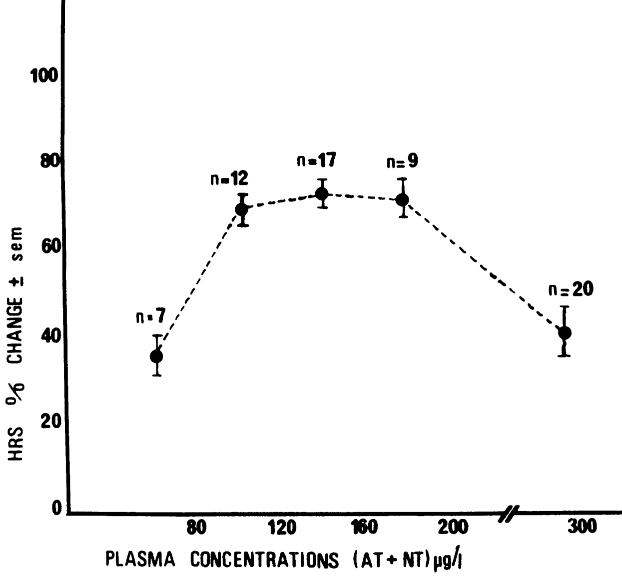

Figure 2 Plasma levels of amitriptyline and clinical response as HRS percentage change.

exception of Kupfer *et al.* (1977), either reported a curvilinear relationship (Montgomery and Braithwaite, 1975; Vandel *et al.*, 1975; Montgomery *et al.*, 1979*a*, *b*; Moyes and Moyes, 1978) between drug plasma concentration and response or had findings which were consistent with the curvilinear hypothesis that the optimum response is associated with intermediate concentrations of AMI plus NT of between 80 and 200 $\mu g\, l^{-1}$. Coppen *et al.* (1978), Liisberg *et al.* (1978) and Robinson *et al.* (1979) did not report the existence of a relationship. However, in the study of Liisberg *et al.* (1978), of the three patients developing high plasma concentrations (>200 AMI+NT) two were non-responders, which is certainly consistent with the curvilinear hypothesis. Likewise, the study by Coppen *et al.* (1978) reported that high plasma concentrations of NT were associated with a significantly poorer outcome measured as percentage change of Hamilton Rating Scale (HRS) (Hamilton, 1967). This very interesting observation, which was confirmed in the study of Montgomery *et al.* (1979*a*), suggests that high concentrations of NT may be responsible for inhibiting the antidepressant effect of AMI (figures 2 and 3). It is these same high concentrations of NT

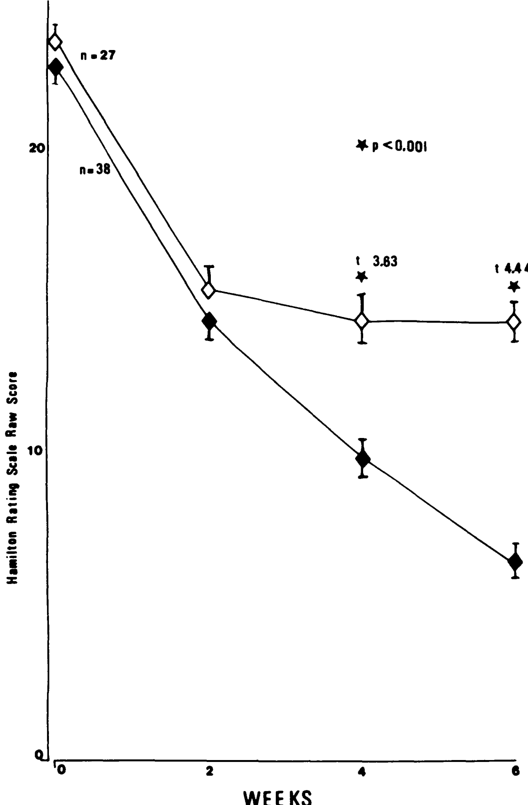

Figure 3 Response of patients developing amitriptyline plus nortriptyline levels inside and outside the therapeutic range 80–200 $\mu g\, l^{-1}$.

which have been implicated as a cause of cardiotoxicity (Burrows *et al.*, 1976).

There have been rather fewer studies investigating the possible relationship between kinetics and response with mianserin. Montgomery *et al.* (1978*b*), in a large study, proposed the existence of a curvilinear relationship for mianserin with a significantly poorer outcome associated with very high plasma concentrations and the suggestion that very low levels were also associated with a poorer response (figure 4). Perry *et al.* (1978) and Russell (1978) reported a poorer response associated with low plasma mianserin concentrations. Very high levels were not observed in these two studies or indeed in the study by Coppen *et al.* (1976), where no relationship was observed. It would appear from these studies that high concentrations of mianserin do not occur sufficiently often in routine dosage to create much of a problem.

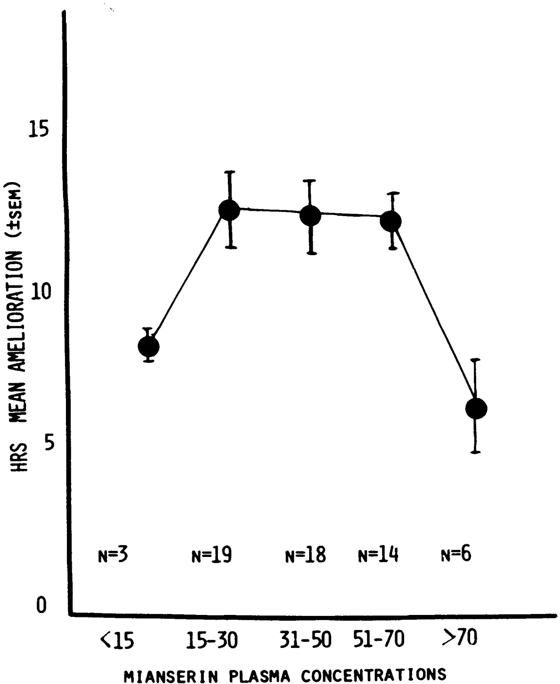

Figure 4 Plasma levels of mianserin and clinical response as amelioration of HRS.

No relationship has been reported between plasma concentrations of maprotiline and antidepressant response in studies by Angst and Rothweiler (1974), Mathur *et al.* (1976), Fensbo (1976), Norman *et al.* (1978) or Montgomery *et al.* (1980*a*). Angst and Rothweiler (1974) suggested that the poor response seen in the two patients who developed very high concentrations of maprotiline may be evidence of the upper limit of an

optimum therapeutic range. However, since no other study has found patients developing these very high concentrations, they are unlikely to present the same sort of problems seen with NT or AMI. Indeed in the double-blind group comparison study of maprotiline and AMI by Montgomery *et al.* (1980*a*), high plasma drug concentrations were observed in 70 percent of the AMI-treated group and were associated with a poorer response compared with those developing moderate concentrations. In the maprotiline-treated group (who had a better response), there was no relationship between drug plasma concentrations and response. Likewise, high levels of norzimelidine, the active metabolite of zimelidine, have been reported to be associated with a poor response in endogenous depression by Montgomery *et al.* (1980*b*) (figure 5). No relationship was reported by Coppen *et al.* (1979), who were investigating a mixed diagnostic group.

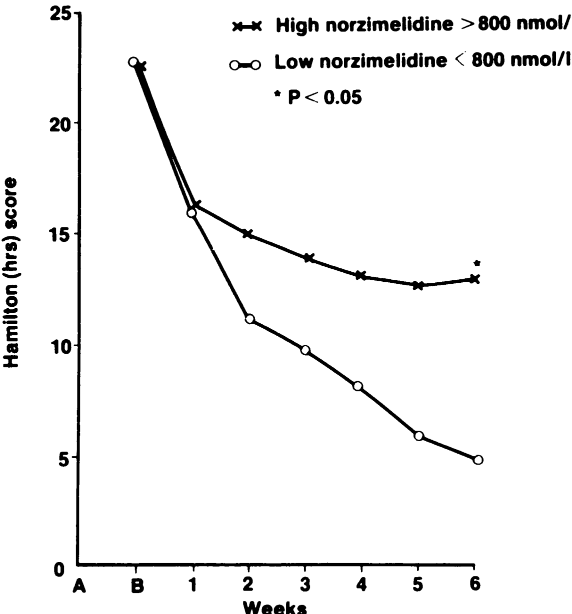

Figure 5 Response of patients developing norzimelidine levels above and below 800 nmol l^{-1}.

The majority of investigators who found a relationship between plasma levels and response have studied patients with defined endogenous depression. Indeed, in the studies of Montgomery *et al.* (1978*c*) on mianserin the relationship was found in the group with endogenous depression defined by the Newcastle scales and not in the reactive depression group. It is important to avoid generalizing from the findings with endogenous depression to other groups of depressed patients. It may well be that the studies of Burrows *et al.* (1972, 1974), Robinson *et al.* (1979) and Coppen *et al.* (1978), in failing to use recognized diagnostic criteria, included too high a proportion of patients

without endogenous depression to demonstrate a plasma level response relationship.

A 10-fold interindividual variation in plasma levels has been reported with a constant dose. In any arbitrary dosage regime chosen, one patient may develop levels 10 times those of another. It would be more logical to move from an arbitrary dosage regime toward using plasma levels to determine dosage and improve response.

Waiting for the achievement of steady state plasma levels and their subsequent measurement may involve unnecessary delay during which time some patients would have developed inappropriate levels. Prediction of plasma levels using complex pharmacokinetic measures is cumbersome, expensive and difficult to use.

In my group, we have been investigating prospectively in a variety of antidepressants the use of simple 24 h or 48 h spot levels after a single oral dose as predictors of steady state plasma concentrations and clinical response. These simple tests are convenient to use in an in-patient or clinic setting. The patient is given a timed single oral dose under supervision and a blood sample is taken 24 and 48 h later. For NT (Montgomery *et al.*, 1979*c*) the response of patients developing levels above the recommended range (200 $\mu g\ l^{-1}$) was 20 percent improvement of HRS score, whereas the response of patients inside the range was 81 percent. In this study on NT a close relationship ($r=0.97$) was observed between the 48 h spot plasma level and steady state levels. This allowed us to examine the use of the 48 h level as a predictor of responders and non-responders (HRS<>8). The point biserial correlations ($0.61, p<0.05$) showed the 48 h level to be a significant predictor of response (figure 6).

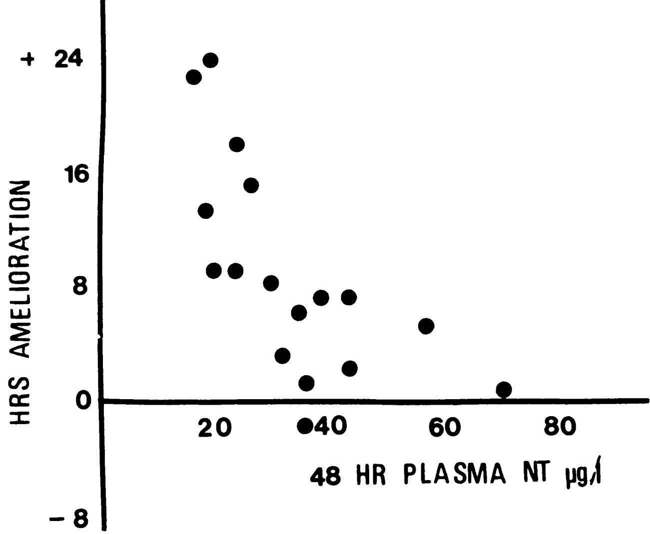

Figure 6 Comparison between the amelioration of the Hamilton scores after 4 weeks treatment with nortriptyline compared with the 48 h spot level after a single oral dose of 100 mg.

For AMI the response in the 38 patients who developed plasma levels of AMI+NT within the recommended range (80–200 μg l^{-1}) was 72 percent and outside the range 43 percent. Of those outside the recommended range, seven patients were below the range and 20 above. It appears from our investigations that the high levels of NT, the metabolite of AMI, determine non-response, although a combination of the two drugs may be responsible for some of the effect.

The prediction of steady state levels of AMI+NT is complicated by the metabolism of AMI to NT. However, the correlation, $r=0.7$, of the 48 h AMI+NT level with steady state plasma levels appears adequate for prediction. The point biserial correlation is not the most appropriate statistic for a curvilinear relationship. However, in this study, where a substantial number of patients with a poor response developed high levels of NT when treated with AMI, there was a significant point biserial correlation (0.44, $p<0.05$) with the 48 h NT level.

The disadvantage of inappropriate high levels of NT is probably more serious than inappropriate low levels. Failure to respond with high levels may be complicated by cardiotoxicity, which has been demonstrated by Fensbo (1976) to be significantly more common in patients developing levels above 200 μg l^{-1}. This is in agreement with the findings of Burrows *et al.* (1976). Taylor and Braithwaite (1978) have demonstrated, using non-invasive techniques, that there is a linear relationship between changes in systolic time intervals and plasma levels. The predictor tests may, therefore, be used both to improve response and to avoid unwanted and possibly dangerous side effects.

We have examined this phenomenon with zimelidine and found that high levels greater than 800 nmol l^{-1} were associated with a significantly poorer response than lower levels. Both zimelidine and norzimelidine appear to predict non-response, point biserial correlation on the 48 h predictor test 0.67 and 0.66 respectively, although the plasma level relationship was only evident for norzimelidine.

For clomipramine and maprotiline, we could detect no plasma level/clinical response relationship and the 48 h point biserial correlations are appropriately low. If a significant plasma level/response relationship has not been established, it is obviously unlikely that the prediction of plasma levels will help judge response. The same is likely to be true for non-endogenous depression where most studies have failed to determine a plasma level/response relationship. Prediction of levels for non-endogenous depression are, therefore, unlikely to be useful in the prediction of response, although they may help in avoiding toxic plasma levels.

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Kinetic aspects of nortriptyline dose regimen

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INTRODUCTION

The kinetics of nortriptyline (NT) have been extremely well documented by a series of investigations during the past 10 years. Such studies on NT, as well as other tricyclic antidepressants (TCA), have been of increasing interest ever since the discovery of markedly different plasma levels in patients given the same dosage (Hammer and Sjöqvist, 1967) – a phenomenon which is one of several explanations for the variability in the therapeutic response to these compounds. Detailed investigations of factors determining the variability naturally became highly important, as did studies on the predictability and maintenance of suitable drug levels in the individual patient – especially since the existence of an optimal therapeutic plasma range was suggested by several investigators (Åsberg *et al.*, 1971; Kragh-Sørensen *et al.*, 1973, 1976; Ziegler *et al.*, 1976; Montgomery *et al.* 1977).

The kinetic information that has accumulated through several investigations (Alexanderson 1972*a,b*; Gram and Fredricson Overø, 1972, 1975; Gram *et al.*, 1974; Fredricson Overø *et al.*, 1975) clearly indicate that the administration of NT as one daily dose would result in roughly the same plasma concentrations as the traditional administration of three daily doses. The recommended dosage regimen of two to four daily doses has generally remained unchanged, however, although some use of a once-daily scheme has been noted (Ayd, 1974; Ziegler *et al.*, 1976, 1977; Maguire *et al.*, 1976). The advantages of a once-daily dose regimen as compared to repeated intake during the day have been much debated (Ayd, 1972, 1974; Schubert and Miller, 1978) and it has been maintained that the once-daily scheme greatly improves patient compliance.

With this background, it appears rational to utilize existing kinetic data for its fundamental purpose, namely prediction, and to calculate concentration patterns attained with different dose regimens. Since kinetic models and predictions thereof are true only as long as they are not demonstrated to be

insufficient or false, experimental confirmation and clinical evaluation appears to be the next logical step before an altered dose regimen can be recommended.

PREDICTION OF NORTRIPTYLINE CONCENTRATION PATTERNS

Simulations using previous data

It has been suggested (Fredricson Overø *et al.*, 1975) that the kinetics of NT may be described by a two-compartment open model and first-order rate constants for its distribution and elimination have been presented (Gram and Fredricson Overø, 1975; Fredricson Overø *et al.*, 1975, 1977). The rate constant for absorption has not been and is not easily evaluated, however, and for the present purpose different first-order rate constants were assigned to the absorption process. Plasma concentration curves were then simulated according to the suggested model by means of a suitable computer program established by Dr V. Hansen at H. Lundeck & Co. The relevant equations for single dose and steady state curves in the central (C_1, 'plasma') as well as peripheral (C_2, 'tissue') compartments are shown in figure 1. Predictions were made with data from 12 subjects participating in previous studies (patients A, B, and C from Fredricson Overø *et al.* (1977) and subjects 1–5 and 7–10 from Gram and Fredricson Overø (1975); subject 6 and patient D were excluded because of poor parameter estimations). In a recently published study (Fredricson Overø, 1980) predictions were based upon data from subjects 5, 8, and 10 and absorption rate constants of 0.2, 0.35 and 0.5 h^{-1}. For the present calculations rate constants of 0.35, 0.50

Single dose Two compartment model simulation

$$C_1 = \frac{k \cdot D}{V_1}\left[\frac{K_{-1}-\alpha}{(k-\alpha)(\beta-\alpha)} \cdot e^{-\alpha t} + \frac{K_{-1}-\beta}{(k-\beta)(\alpha-\beta)} \cdot e^{-\beta t} + \frac{K_{-1}-k}{(\alpha-k)(\beta-k)} \cdot e^{-kt}\right]$$

$$C_2 = \frac{k \cdot K_{-1} \cdot D}{V_1}\left[\frac{1}{(k-\alpha)(\beta-\alpha)} \cdot e^{-\alpha t} + \frac{1}{(k-\beta)(\alpha-\beta)} \cdot e^{-\beta t} + \frac{1}{(\alpha-k)(\beta-k)} \cdot e^{-kt}\right]$$

Steady state Two compartment model simulation

$$C_1 = \frac{k \cdot D}{V_1}\left[\frac{K_{-1}-\alpha}{(k-\alpha)(\beta-\alpha)} \cdot \frac{e^{-\alpha t}}{(1-e^{-\alpha \tau})} + \frac{K_{-1}-\beta}{(k-\beta)(\alpha-\beta)} \cdot \frac{e^{-\beta t}}{(1-e^{-\beta \tau})} + \frac{K_{-1}-k}{(\alpha-k)(\beta-k)} \cdot \frac{e^{-kt}}{(1-e^{-k\tau})}\right]$$

$$C_2 = \frac{k \cdot K_{-1} \cdot D}{V_1}\left[\frac{1}{(k-\alpha)(\beta-\alpha)} \cdot \frac{e^{-\alpha t}}{(1-e^{-\alpha \tau})} + \frac{1}{(k-\beta)(\alpha-\beta)} \cdot \frac{e^{-\beta t}}{(1-e^{-\beta \tau})} + \frac{1}{(\alpha-k)(\beta-k)} \cdot \frac{e^{-kt}}{(1-e^{-k\tau})}\right]$$

Figure 1 Equations for single dose and steady state concentration curves in the central and peripheral compartments of a two compartment model.

and 0.65 h^{-1} were chosen and a total daily dose of 150 mg NT base with a first-pass reduction of 50 percent (Gram and Fredricson Overø, 1975). Curves were calculated for once-daily and t.i.d. dose regimens and compared to existing experimental data.

Rate of absorption

The predicted single dose plasma concentrations are summarized in figure 2 for the slower (k_a=0.35) and intermediate (k_a=0.50) absorption rates. The shapes of the curves are reminiscent of previously published patterns (Alexanderson, 1972*b*; Gram and Fredricson Overø, 1972; Gram *et al.*,

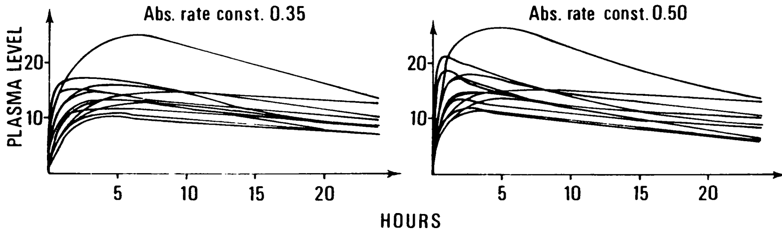

Figure 2 Single dose plasma concentration curves predicted for 12 individuals according to the two compartment model with absorption rate constants of 0.35 h^{-1} (left) and 0.50 h^{-1} (right).

1974), particularly those combined with the slower absorption rate constant. The k_a=0.50 curves – and to even greater extent the k_a=0.65 ones – display early peak concentrations, which are unusual in the data material referred to above. Neither was it a common finding in the very same individuals (1–5, 7–10) for whom experimental oral data exist (Gram and Fredricson Overø, 1975). A comparison between predicted and observed data in terms of the time at which maximal plasma concentration is attained (table 1) clearly indicates an absorption rate constant of 0.35 to be more realistic than 0.50. With k_a=0.65 absorption obviously gets too rapid, while k_a=0.20 in a recent study (Fredricson Overø, 1980) proved to give too slow a process. For the present purposes it seems reasonable to conclude, therefore, that the absorption of NT is reasonably well described by a first-order rate constant of about 0.35 h^{-1}.

Plasma level fluctuation

The degree of fluctuation within a given dose interval is determined by the relationship between the rates of absorption, distribution and elimination. Invasion rates which exceed the distribution rates inevitably cause transient accumulation in the plasma compartment and thus more or less pronounced fluctuation. The relatively slow absorption of NT – a rate constant of

Table 1 Predicted and observed times of peak level after nortriptyline administration

Subject	Single dose t_{max} (h)			
	Predicted times based on absorption rate constants (h^{-1}) of 0.35	0.50	0.65	Observed
1	3.5	2	1	4
2	5	3	2	6
3	5	2.5	2	9
4	5.5	2	2	7
5	6	4	3	7
7	5	2	2	7
8	7	5	4	4
9	1	1	1	6.5
10	9	6.5	4.5	7
A	5	2	1	—
B	3	1	1	—
C	5	4	3	—
Mean	5.0	2.9	2.2	6.4
S.D.	2.0	1.7	1.2	1.6

0.35 h^{-1} corresponds to a 2 h absorption half-life – is therefore of decisive importance, since it is lower than the indicated distribution rate constant (K_1) of 0.4–5.4 h^{-1} (mean 2.1) and thus makes it relatively easy for the distribution process to cope with the invasion of drug. Pronounced peak levels are consequently not observed. The fluctuation as expressed by the ratio between maximal and minimal levels is likewise limited as summarized in table 2.

With a t.i.d. regimen and an absorption rate constant of 0.35 h^{-1} the predicted max/min ratio under steady state conditions is seen to be in the 1.04–1.28 range with a mean of 1.14. It is only slightly higher (mean 1.17) with the 0.5 h^{-1} absorption constant.

Fluctuation inevitably gets more pronounced when the dose interval is prolonged. Once-daily administration thus predictably gives rise to the slightly higher max/min ratios of about 1.7 (range 1.21–2.45), 1.8 (range 1.25–2.94) and 2.0 (range 1.27–3.34) with absorption rate constants of 0.35, 0.50 and 0.65, respectively (table 2).

The simulated curves indicate fluctuation to be twice as pronounced in subject B as in subject 10 under a once-a-day regime. This variability is well explained by differences in elimination half-life, as illustrated in figure 3. Pronounced correlation is seen between higher ratios and shorter half-lives. Linear correlation coefficients of −0.84 and −0.80 for absorption rates governed by 0.35 and 0.50 constants were calculated. The data are suggestive of a curved relationship, however.

The data of the 12 subjects, which were used for the present simulations,

Table 2 Predicted fluctuation of nortriptyline steady state concentrations upon administration t.i.d. or once daily

Subject	Max/min ratio				
	t.i.d. based on absorption rate constants (h^{-1}) of		Once daily based on absorption rate constants (h^{-1}) of		
	0.35	0.50	0.35	0.50	0.65
1	1.18	1.25	1.93	2.17	2.38
2	1.18	1.24	2.03	2.25	2.45
3	1.12	1.16	1.60	1.74	1.84
4	1.12	1.16	1.57	1.71	1.80
5	1.14	1.19	1.90	2.06	2.17
7	1.13	1.17	1.64	1.80	1.91
8	1.09	1.11	1.50	1.59	1.65
9	1.14	1.19	1.56	1.73	1.87
10	1.04	1.05	1.21	1.25	1.27
A	1.10	1.14	1.41	1.52	1.63
B	1.28	1.39	2.45	2.94	3.34
C	1.10	1.14	1.54	1.65	1.73
Mean	1.14	1.17	1.70	1.84	2.00
S.D.	0.06	0.07	0.33	0.44	0.53

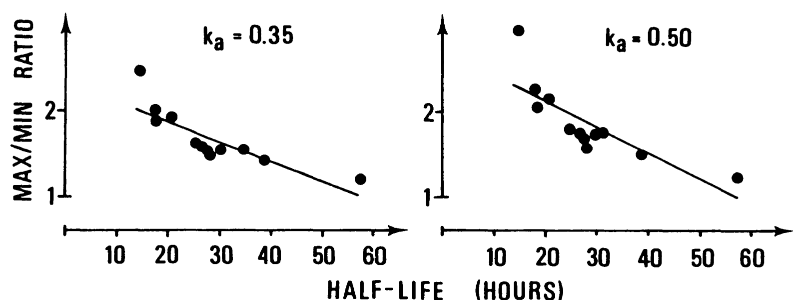

Figure 3 Relationship between predicted steady state max/min concentration ratio at once-daily administration and half-life in 12 individuals.

cover a threefold range of half-lives. It seems reasonable to assume, therefore, that the predicted range of fluctuation of 1.2–2.5 for the most likely absorption rate would be applicable to the majority of patients.

The plasma concentration patterns predicted to be attained under steady state conditions at administration once or three times daily are illustrated in figure 4. A comparison with the steady state data recorded during an 8 h dose interval by Alexanderson (1972*b*) in one subject and by Kragh-Sørensen *et al.* (1974) in five, reveals that the predicted flat curve corresponds well to reality.

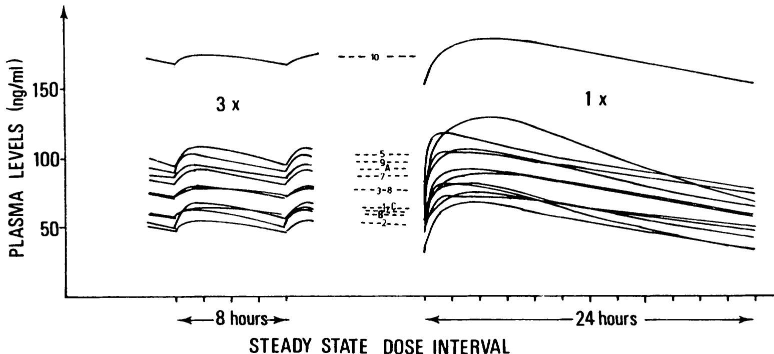

Figure 4 Predicted steady state plasma levels in 12 individuals given the same daily dose t.i.d. (left) or once daily (right). The dotted lines with numbers denote mean concentration and identification of subject.

The difference between the t.i.d. and once-daily curves is seen to be limited (figure 4). In spite of more pronounced fluctuation – a mean max/min ratio of 1.70 as compared to 1.14 – the once-a-day curve patterns still appear rather flat. The mean concentration is – self-evidently – the same regardless of dose regimen. It is attained between 12 and 13 h after a daily dose (absorption rate constant 0.35 h^{-1}) and is indicated on figure 4 for the 12 individuals.

The similarities thus established between predicted and experimental plasma levels after a single dose as well as under steady state conditions strongly favor the applicability of the two-compartment model as an approximation of NT kinetics. Consequently, there seems to be little if any reason to doubt the limited fluctuation predicted for once-daily administration.

Concentration pattern in 'tissue'-compartment

The discussion above has been restricted to plasma concentrations – the only feasible sampling point. The concentration pattern for the peripheral, 'tissue', compartment is easily obtained by data simulation, however, and might be worth a moment's consideration.

As illustrated by table 3, maximal drug levels are attained later in the peripheral than in the central compartment – as one would expect. At steady state conditions with once-daily administration, maximal 'tissue' levels are reached after about 6 h as compared to about 3 h in the plasma compartment. (The latter figure is different from that related to a single dose, as is inherent in the mathematics.) The concentration pattern of the 'tissues' is thus even flatter than that of plasma, as also indicated by the somewhat lower max/min ratios (table 3), which range from 1.2–2.0 with a mean of 1.5 in tissues and 1.2–2.5, mean 1.7, in plasma.

Since it is not known which of the two compartments is of greatest significance for the clinical effect, it appears relevant to know that the non-observable compartment is also the more constant one.

Table 3 Predicted steady state conditions at once-a-day administration of nortriptyline (absorption rate constant=0.35 h^{-1})

Subject	t_{max}			Max/min ratio	
	Central compartment	Peripheral compartment		Central compartment	Peripheral compartment
1	2	6		1.9	1.7
2	3<	6		2.0	1.8
3	3	6		1.6	1.5
4	3	6		1.6	1.5
5	4	6		1.9	1.8
7	3	6		1.6	1.5
8	5	6		1.5	1.4
9	1	6		1.6	1.4
10	5	7		1.2	1.2
A	2	6		1.4	1.3
B	2	6		2.5	2.1
C	2	4		1.5	1.5
			Mean	1.7	1.5
			S.D.	0.3	0.3
			Range	1.2–2.5	1.2–2.0

PLASMA LEVELS AND CLINICAL OBSERVATIONS IN PATIENTS TREATED BY A ONCE-DAILY NORTRIPTYLINE DOSE

Having accepted the roughly twofold fluctuation of plasma levels as being within reasonable limits, the crucial question arises as to whether the prediction can be confirmed by experimental data, that is by measurements of maximal and minimal levels in patients given a daily dose. Such data have been collected in a recent study at the Risskov psychiatric hospital (Pedersen and Lund Sørensen, 1980). In contrast to a few earlier studies in which the daily dose was given at night (Ziegler *et al.*, 1976, 1977; Maguire *et al.*, 1976), the 21 endogenously depressed patients of the Risskov study received a daily morning dose, since it was considered essential to obtain full information on any effects that might appear in relation to peak concentrations.

Plasma levels

Blood samples for indication of the fluctuation of drug levels were obtained during the third week of dosage 5½ and 23½ h after the morning dose. The latter time should give a rather accurate estimate of the minimal drug level; the former a reasonable estimate of the maximum. The finding from the present predictions that several subjects may attain peak values somewhat earlier suggest that the 5½ h value may provide an underestimate. On the

other hand, the flatness of the curve makes the actual choice of time less critical.

The max/min ratio calculated from these two points in 21 patients was in the 1.10–2.16 range with a mean value of 1.54 (Fredricson Overø, 1980). The indicated fluctuation is thus well compatible with the predicted range of 1.21–2.45 with a mean of 1.70 (table 2) for once-daily administration with the most probable absorption rate constant.

Further blood samples were taken 29 and 48 h after administration of drug – a placebo dose was given one day and NT dosage was then resumed. In the four plasma samples, monoexponentially declining NT levels were recorded in the majority of subjects, thus enabling rather accurate estimation of the biological half-life. In the remaining patients the data were somewhat more scattered, but without particular tendencies – half-life estimation was therefore based upon all data points even in these cases. The half-lives were found to vary from 24 to 86 h (Fredricson Overø, 1980), as further illustrated in figure 5, where the estimated half-lives are plotted against the estimated max/min ratios. In spite of some scattering, a tendency toward lower ratios at longer half-lives is observed. Included in the figure are the linear regression lines from the predicted relationship illustrated in figure 3. Several data are seen to gather themselves close to these lines, while others are rather distant. Considering the errors involved in drug assay, as well as half-life estimation, this is perhaps not surprising. However, lower than expected ratios could be explained by underestimation of the maximum level – higher ratios might be taken as an indication of more complex absorption processes.

On the whole, however, predicted and experimental data agree fairly well and it appears justifiable to conclude that this kind of prediction is sensible

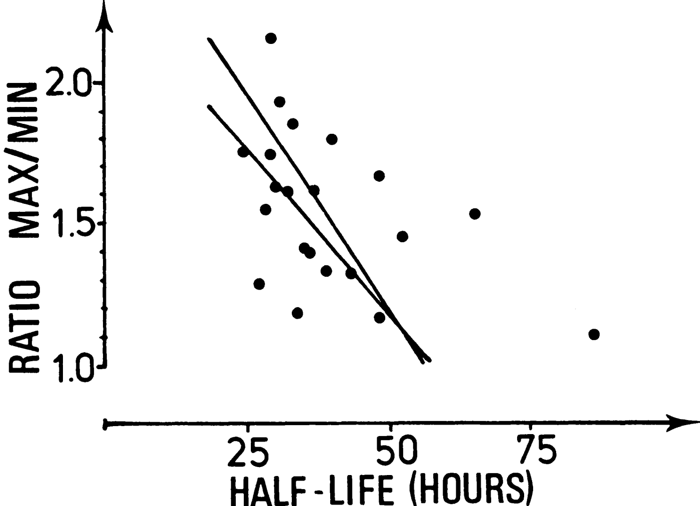

Figure 5 Relationship between estimated half-life and observed max/min concentration ratio in 21 patients, treated with 150 mg NT once daily. The lines are the linear regression lines of figure 3.

and that the fluctuation in NT plasma levels remains limited even at once-daily administration.

It might be added that the compatibility of predicted and observed data indicates that the rapidly disintegrating tablet used in patient studies is absorbed like the drug solution of volunteer studies and predictions.

Therapeutic effect

The therapeutic effect of NT, given as a daily 150 mg morning dose, was concluded to be quite as favorable as when given by the traditional t.i.d. scheme (Pedersen and Lund Sørensen, 1980). In 19 out of 21 patients steady state plasma levels were recorded to be within the 45–150 ng ml^{-1} range (minimum in dose interval) and 18 of these displayed a good therapeutic effect, as did one of the two patients with higher levels (250 ng ml^{-1}). The data are thus generally supportive of the proposed optimal concentration range hypothesis.

Side effects

Side effects were recorded to be very moderate and not more pronounced with the once-daily regimen than with t.i.d. administration (Pedersen and Lund Sørensen, 1980). Sedation was not observed – interestingly enough, since sedation has been brought forward as an argument for bedtime administration (Ziegler *et al.*, 1976; Maguire *et al.*, 1976). ECG recordings were made before NT administration and after 2 weeks' treatment, just before dosage as well as $5\frac{1}{2}$ h later. No serious cardiac influence was reported (Lanng Nielsen, 1980) and the increased pulse rate corresponded to what is observed in patients receiving the traditional t.i.d. dose.

CONCLUSION

The predictions based upon existing kinetic information and the data obtained from patients given a once-daily dose regimen are thus in complete agreement as regards the similarity of plasma levels at once-daily and t.i.d. administration. Furthermore no clinical differences have been observed. There seems to be every reason, therefore, to prefer the more practical once-a-day dose regimen for NT.

It should be realized that this line of argument is not necessarily true for TCA in general. Thus a tertiary amine like amitriptyline is more rapidly absorbed (as one would anticipate because of the higher lipophilicity and lower pK_a). Transient accumulation in the plasma compartment and more pronounced fluctuation is thus to be expected and has also been indicated experimentally (Jørgensen, 1977). Whether this degree of fluctuation is

acceptable or not is a question still open for discussion. For the secondary amine drug NT, however, there seems to be little reason to doubt the acceptability of the fluctuation – even at once-daily administration.

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The pharmacokinetics and metabolism of tricyclic antidepressant drugs in patients with chronic renal failure

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INTRODUCTION

The evaluation and treatment of depression in patients with chronic renal failure (CRF) sometimes presents a difficult clinical problem. Although the appearance of symptoms of depression in such patients is a common occurrence, there is disagreement over the actual incidence of formal primary depressive illness. However, many studies have reported a high incidence of depression and suicide in patients on hospital dialysis treatment (Shea *et al.*, 1965; Retan and Lewis, 1966; Reichsman and Levy, 1972; Cadnapaphornchai *et al.*, 1974). Patients with CRF who are psychiatrically ill have a diminished chance of survival (Farmer *et al.*, 1979); thus the need for effective treatment is particularly pressing.

In those cases where psychotropic medication is indicated, the choice of drug is limited by complications associated with their side effects and by the wish to avoid further dietary restrictions. Use of tricyclic antidepressants (TCA) has been advocated in some cases (Caramand *et al.*, 1968; Levy, 1976) but the response to treatment has not been investigated systematically (Czaczkes and de Nour, 1978). Our own clinical impression, like that of others (Buchanan *et al.*, 1977), is that the response to treatment is often disappointing. Possible reasons for this include the nature of the depressive

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illness and diagnostic considerations, the effect on mood of the metabolic disturbances associated with renal failure, impaired drug elimination or plasma protein binding, and the interaction between antidepressants and other prescribed medication.

Deterioration in renal function can result in the accumulation of drugs and their metabolites that are normally excreted by glomerular filtration. Quantitative, and possible qualitative, changes in serum proteins may alter the protein binding of drugs. Uremia may also influence hepatic function with resultant effects on drug metabolism. All these changes in drug handling may necessitate dosage modifications if optimal drug plasma levels and avoidance of toxic side effects are to be achieved. Dialysis treatment can further complicate dosage regimens by clearing significant amounts of drug and metabolites.

For these reasons, therefore, we embarked on a series of studies designed to investigate the pharmacokinetics and plasma protein binding of TCA in patients with CRF and the relevance to the treatment and outcome of depressive illness (Rosser *et al.*, 1979). The results of some of these investigations are described in this presentation.

EXPERIMENTAL METHODS

Plasma protein binding studies with maprotiline

Patients and volunteers

The serum protein binding of maprotiline was investigated in a group of 22 patients (15 male and seven female) on chronic hemodialysis. They underwent dialysis for 10 h, twice weekly, on a Meltec Multipoint artificial kidney (1.0 m^2). Ten milliliters of clotted blood samples were obtained from each patient immediately before dialysis to provide 3–4 ml of serum. The serum samples were stored in glass tubes at 4 °C for between 2 and 4 days prior to determination of protein binding. A group of 14 healthy volunteers (seven male and seven female) were used as control subjects for the study. Ten mililiters of clotted blood samples were obtained from each subject and treated in the same way as the patient samples.

Determination of serum proteins

Serum concentrations of albumin and α_1-acid glycoprotein (AAG) were measured using a radial immunodiffusion technique (M-Partigen, Boehring-Hoechst, UK). Each sample was measured in duplicate against control serum samples of known protein concentration.

Determination of maprotiline binding

Maprotiline binding studies were carried out using ^{14}C-labeled drug (S.A.=4.5 μCi mg^{-1}) obtained from Ciba-Geigy, Basle, Switzerland, at a concentration of 600 ng ml^{-1} serum. The measurement of serum protein binding of maprotiline was carried out using equilibrium dialysis at 37 °C according to the original technique described by Ernebo *et al.* (1971). Serum (0.5 ml) was equilibrated with 0.5 ml of pH 7.4 phosphate buffer containing labeled drug for 17 h. Following equilibration, 250 μl aliquots of the protein and dialysate solution were mixed with 10 ml of scintillation fluid (NE 260, Nuclear Enterprises, Edinburgh, Scotland) and radioactivity determined by liquid scintillation counting. Quench corrections were made using an internal standardization method. Serum drug binding was expressed as the percentage of unbound drug calculated from the ratio of the activity found in buffer and serum samples. Each binding measurement was carried out in duplicate.

Pharmacokinetic studies with nortriptyline

Patients

Twenty patients with chronic renal failure consented to participate in the study, for which approval was obtained from the Charing Cross Hospital Ethical Committee. The patients formed two groups: 12 who were being managed conservatively, and eight who were training for home dialysis and dialyzed for 10 h twice weekly on Meltex Multipoint (1.0 m^2) artificial kidneys.

Drug dosage and sampling schedule

A single oral dose of 75 mg nortriptyline (NT) was administered to each group of patients according to the following protocol:

(1) *Patients not being dialyzed.* The dose of NT was administered at 09.00 on the first day of study. Heparinized venous blood samples (10 ml) were collected beforehand, and at 24, 30, 48, 54 and 72 h afterwards.

(2) *Patients on dialysis.* These patients received a single dose of NT at 09.00 on the day after dialysis. The blood sampling regime was followed as detailed above, the last sample being drawn prior to the next dialysis.

Measurement of plasma nortriptyline concentrations

The plasma was separated and stored at −20 °C until analysis. Plasma NT concentrations were determined by a specific gas–liquid chromatographic

(GLC) procedure with nitrogen-selective detection (Dawling and Braithwaite, 1978; Braithwaite, 1979).

Pharmacokinetic analysis

From these measurements a log plasma NT concentration versus time profile was constructed for each patient, and a linear least-squares regression analysis fitted to the terminal elimination phase (β-phase). From this line, plasma NT half-life ($t_{1/2}$) and total hepatic intrinsic clearance (Cl_i) were calculated using the formulae

$$t_{1/2}=0.693/\beta \text{ (h)},$$

$$Cl_i=D/(AUC)_\beta \text{ (l h}^{-1}\text{)}$$

(Wilkinson and Shand, 1975), where, assuming complete absorption and essentially hepatic metabolism, β is the gradient of the β-elimination phase, D is the dose administered, and $(AUC)_\beta$ is the area under the extrapolated β-slope, which has been shown to be a reliable approximation for the total area under the plasma concentration–time curve (Alexanderson, 1972).

Investigation of hydroxylated metabolites of nortriptyline

Patients

(1) Thirteen of the patients (four male, nine female) who formed part of the previous pharmacokinetic study of NT were investigated in more detail by measurement of conjugated and unconjugated plasma 10-OH-NT concentrations at 24, 48 and 72 h following administration of the single oral dose of NT. The age of the patients ranged between 18 and 73 years (mean age 53 years; S.D. 16 years).

The results obtained in this group of patients were compared with those obtained in a group of eight normal healthy volunteers (four male, four female) aged between 20 and 37 years (mean age 24 years) who were given a single oral dose of 75 mg NT.

(2) A further four patients on chronic hemodialysis who were receiving treatment with NT for depression were each investigated over a 1–2 month period. NT was prescribed as a single night-time dose of 50–75 mg. The patients were dialyzed for 10 h twice weekly on a Meltec Multipoint (1.0 m^2) artificial kidney, and on each occasion 10 ml plasma samples were taken pre- and post-dialysis for measurement of NT and conjugated and unconjugated 10-OH-NT.

Measurement of conjugated and unconjugated 10-hydroxynortriptyline

Conjugated and unconjugated 10-OH-NT were measured using a modification of the method used for the determination of NT (Dawling and Braith-

waite, 1978; Braithwaite, 1979). Both compounds were separated by differential extraction and converted to 10, 11-dehydronortriptyline by acid hydrolysis at 90 °C prior to GLC (Dawling *et al.*, 1980).

RESULTS

The age (mean and S.D.) of the patients and volunteers and results obtained for serum protein concentrations and percentage unbound maprotiline are shown in table 1. No significant difference in binding between the two groups was found (Mann-Whitney U test, $U=110$, $p>0.05$) although the uremic patients displayed a significantly ($F=6.8$, $p<0.01$) larger interindividual variation in binding. There was no significant correlation ($r=+0.16$) between serum AAG concentrations and the unbound fraction of maprotiline, but a significant negative correlation ($r=0.50$, $p<0.05$) was found with serum albumin concentrations. The serum concentrations of AAG found in the uremic patients were slightly, but significantly, higher ($p<0.01$) than those obtained in volunteer subjects.

The results obtained in the single dose pharmacokinetic study of NT are shown in table 2. The patients were 10 male and 10 female, with a mean age of 49 years (range 18–73 years). the median NT half-life was 25.2 h (range 14.5–140.0 h) and the median NT clearance was 32.3 l h^{-1} (range 8.1–122.0 l h^{-1}). Plasma NT half-life and clearance in the dialyzed and non-dialyzed patients did not differ ($U=47$ for $t_{1/2}$; $U=29$ for clearance). The relationship between age and plasma NT half-life or clearance showed no significant linear correlation ($r=0.27$ for $t_{1/2}$; $r=0.38$ for clearance). There was no significant correlation between plasma NT clearance and glomerular filtration rate (^{51}Cr-EDTA clearance) in the 12 patients not being treated with hemodialysis.

Plasma NT half-life and clearance in these patients were compared with those in two groups of healthy subjects, the details of which are shown in table 2. The elder group of volunteers were taken from the work of Alexanderson (1973). The clearance values reported by Alexanderson were calculated from the dose taken as hydrochloride salt and $(AUC)_\beta$ as free base, so they were corrected by multiplying by 0.878. Because of the narrow age span (47–53 years) and homogeneity of the subjects (five pairs of monozygotic and six pairs of dizygotic twins), the patients were also compared to a group of 30 younger, non-related volunteers studied in this laboratory. No significant differences could be detected in NT kinetics between Alexanderson's volunteers and this group of patients. The patients with CRF also had similar NT half-lives to the younger volunteer group, but their NT clearances differed significantly when examined with the Mann-Whitney U-test ($U=179$, $Z=-2.4$, $p<0.02$).

The mean plasma concentrations (±S.E.M.) of NT, conjugated 10-OH-NT and unconjugated 10-OH-NT 24, 48 and 72 h after a single 75 mg dose of NT in 13 CRF patients compared with eight healthy volunteers are shown in

Table 1 Serum protein binding of maprotiline in CRF patients and healthy volunteers

Group	Age (years)		Albumin ($g\,l^{-1}$)		α_1-Acid glycoprotein ($g\,l^{-1}$)		Percentage of free maprotiline		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Range
Young healthy volunteers (N=14)	22.6	3.3	Not determined		0.73	0.15	10.5	1.0	9.1–12.4
CRF patients (N=22)	45.7	13.2	39	6	0.90	0.21	10.0	2.5	6.1–16.5

Table 2 Comparison of nortriptyline kinetics in patients with chronic renal failure and healthy volunteers

Group	Number		Age (years) (mean±S.D.)	Nortriptyline half-life (h), median (range)	Nortriptyline clearance ($l\,h^{-1}$), median (range)
	M	F			
Dialyzed patients	5	3	45±14	28 (15–35)	40 (11–96)
Non-dialyzed patients	5	7	52±14	23 (15–140)	30 (8–122)
Combined patients	10	10	49±15	25 (15–140)	32* (8–122)
Young healthy volunteers	19	11	26± 5	23 (15–51)	51* (16–115)
Older healthy volunteers (Alexanderson, 1973)	12	10	50± 2	31 (18–93)	44 (14–100)

* U=179, p<0.02.

figure 1. The ratio of conjugated and unconjugated 10-OH-NT to that of NT at the various time points in both patients and volunteers is shown in table 3. As can be seen, both conjugated and unconjugated 10-OH-NT concentrations appeared to be higher in CRF patients, particularly those of the conjugated metabolite.

Table 4 shows the mean 'steady state' plasma concentrations of NT, and conjugated and unconjugated 10-OH-NT pre- and post-dialysis in four patients receiving antidepressant treatment with NT. The mean pre-dialysis ratio of unconjugated and conjugated 10-OH-NT to that of NT in the four patients was 1.4 and 41.3 respectively.

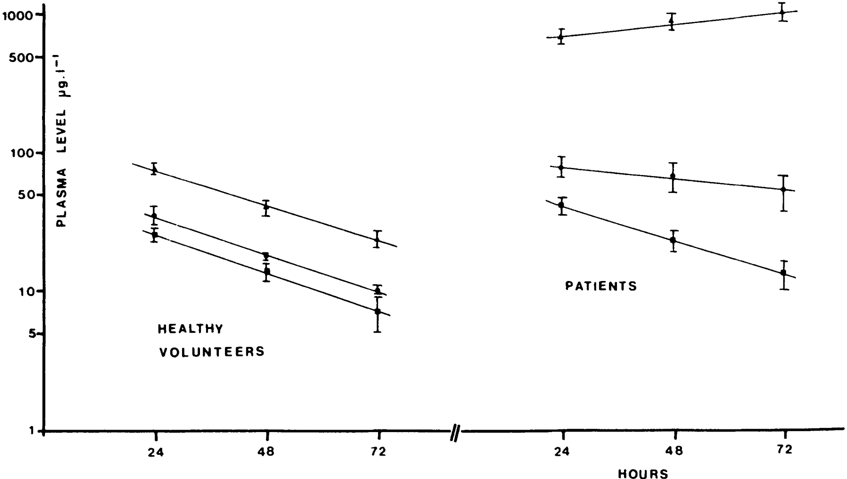

Figure 1 Mean (±S.E.M.) plasma concentrations of NT (■), conjugated 10-OH-NT ((▲) and unconjugated 10-OH-NT (●) in CRF patients and volunteers 24, 48 and 72 h after a single oral dose of NT.

DISCUSSION

The finding of an average unbound fraction of maprotiline of 10.5 percent in the sera of normal subjects agrees with the results of Reiss *et al.* (1975) who reported a bound fraction of 91 percent in healthy subjects when using similar techniques and conditions. The binding characteristics of this drug in the dialysis patients were similar to those in the control group. Reidenberg *et al.* (1971), in their study of desmethylimipramine binding in uremic subjects, were also unable to demonstrate a difference in binding compared to healthy volunteers. However, in this study, the dialysis patients displayed significantly ($p<0.01$) greater interindividual differences in the unbound maprotiline fraction (6.1–16.5 percent) than did the normal subjects

Table 3 Ratio of conjugated (10-OH-NT-conj) and unconjugated (10-OH-NT) 10-hydroxynortriptyline to that of nortriptyline (NT) in CRF patients and healthy volunteers

Group	24 h		48 h		72 h	
	10-OH-NT / NT	10-OH-NT-conj / NT	10-OH-NT / NT	10-OH-NT-conj / NT	10-OH-NT / NT	10-OH-NT-conj / NT
CRF patients (*N*=13)	1.4	3.0	1.3	2.9	1.4	2.4
Healthy volunteers (*N*=8)	1.9	16.3	2.9	37.7	3.9	74.9

Table 4 Mean (±S.E.M.) steady state plasma drug concentrations (in micrograms per liter) during nortriptyline treatment

	Patient			
	1 60/M	2 45/F	3 63/F	4 60/M
NT				
Pre-dialysis	145 (9)	97 (5)	192 (23)	151 (4)
Post-dialysis	164 (8)	49 (2)	163 (26)	105 (11)
10-OH-NT, free				
Pre-dialysis	117 (10)	235 (8)	249 (20)	136 (5)
Post-dialysis	130 (9)	141 (6)	249 (28)	112 (6)
10-OH-NT, conjugated				
Pre-dialysis	5607 (516)	5407 (1326)	5760 (288)	6130 (281)
Post-dialysis	2356 (238)	2651 (291)	2742 (112)	2096 (104)

(9.1 – 12.4 percent). In an attempt to explain the increased interindividual differences in protein binding of maprotiline, the relationship of the serum concentrations of albumin and AAG to the degree of binding was examined. A number of basic drugs, including imipramine (IMI), have been shown to have a strong affinity for AAG (Piafsky and Borgå, 1977; Piafsky *et al.*, 1978). This protein is an 'acute phase' reactant (Schmid, 1975) and may be elevated in a number of disease states with a resultant enhanced binding capacity for certain basic drugs. The results of the present study failed to show any correlation between the concentrations of AAG and the binding of maprotiline, which is in agreement with a previous investigation in geriatric patients where much higher AAG concentrations were observed (Braithwaite *et al.*, 1978). In fact, there was a weak, but significant, negative correlation between maprotiline binding and serum albumin concentrations. This indicates that albumin may be more important for determining the binding of maprotiline than AAG.

The finding of extreme interindividual variability in both NT half-life and clearance in the pharmacokinetic study is similar to observations made by other workers in physically healthy subjects (Alexanderson, 1972, 1973; Gram and Fredricson Overø, 1975). The 20 patients with CRF showed a 10-fold variation in NT half-life (14.5 – 140 h), while plasma clearance varied 15-fold (8.1 – 122 l h^{-1}). The use of additional medication and the coexistence of other disease states in these patients may have enhanced the naturally occurring variability. However, there was no significant difference in NT pharmacokinetics between the patients treated with hemodialysis and those who were being managed conservatively. To estimate the effect which chronic renal failure may have on NT kinetics, the NT half-life and clearance values obtained in this study were compared with previously published data where these had been calculated similarly. No differences were detected in the comparison in the similarly aged volunteers, although a significant difference in NT clearance was observed between these patients and the group of younger volunteers (table 2; $U = 179$, $p < 0.02$). This study demonstrated no predictable alteration in NT kinetics in CRF as measured by the drug's half-life and total plasma clearance.

NT is metabolized by hepatic microsomal demethylation and hydroxylation (von Bahr, 1972). In CRF, hepatic drug oxidation mechanisms are usually unaltered (Levy, 1977; Reidenberg, 1977), and this is suggested in the present study by the lack of a relationship between NT kinetics and glomerular filtration rate. NT is finally excreted in the urine following glucuronide conjugation of its hydroxylated metabolite, 10-OH-NT (Alexanderson and Borgå, 1973), and it is possible that these compounds may accumulate in renal insufficiency. The hydroxy-metabolites of both NT and IMI have recently been shown to be active with respect to synaptosomal uptake of neurotransmitter amines, and it is thought likely that their presence in significant amounts in plasma influences the therapeutic and toxic effects of these drugs (Bertilsson *et al.*, 1979; Potter *et al.*, 1979). The present studies have shown that in patients with CRF receiving chronic treatment with NT plasma concentrations of free (unconjugated) 10-OH-NT may not be unusually high despite the presence of abnormally high

concentrations of conjugated metabolite. Thus, in the small group of patients investigated, the ratio of unconjugated 10-OH-NT to NT concentrations would appear to be similar to those found in healthy volunteers in the present study (table 3) and those reported by Bertilsson *et al.* (1979). On the other hand, the ratio of pre-dialysis concentrations of conjugated 10-OH-NT to NT concentrations was much higher than in healthy volunteers and similar to those found in other CRF patients following single oral dosage (table 3). A comparison of the plasma drug concentrations pre- and post-dialysis indicated that generally very little NT or free 10-OH-NT was removed whereas levels of conjugated 10-OH-NT were consistently halved. This finding was confirmed by the negligible amounts of NT and unconjugated NT relative to that of conjugated 10-OH-NT found in the dialysate (Dawling *et al.*, 1980).

In conclusion CRF does not appear to be associated with any predictable alteration in TCA pharmacokinetics apart from an accumulation in conjugated hydroxylated metabolites. These studies have, however, indicated the possibility of increased variability in drug handling which may make antidepressant drugs more difficult to use in such patients. The potential danger of side effects and the difficulty in their evaluation in patients with CRF may indicate the need to monitor plasma drug concentrations in certain clinical situations.

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Factors affecting the biphasic concentration : effect relationships of tricyclic antidepressants

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Tricyclic antidepressants (TCA) have a perplexing proclivity to antagonize their own effects. This proclivity can result in apparently erratic results and paradoxical dose: response relationships. Besides being of academic interest, this phenomenon can have important clinical implications. While many patients with an affective syndrome respond favorably to TCA, some do not and others even worsen. This variability in efficacy is due, in part, to marked interindividual differences in steady state concentrations achieved on a given dose (Alexanderson, 1973) and the fact that these drugs exert different actions at different concentrations.

TCA are not unusual in this respect. Many psychotropic agents are pharmacologically complex and have varying effects depending upon their concentration at a target tissue. Conceptualization of drug effects thus is better understood from the vantage of concentration: response relationships rather than dose: response relationships. This shift has occurred as a result of improvement in analytical techniques, permitting accurate determination of drug concentrations in both body fluids and tissues. Although this article will center on the pharmacology of TCA, especially in regard to their cardiac actions, the discussion will develop a basic premise – better understanding and prediction of drug effects through quantification of drug concentrations in the body.

Although TCA are most commonly considered to be catecholaminergic agonists, they have several other effects (table 1). TCA act as

Table 1 Pharmacological effects of TCA

I. Neurotransmitter re-uptake blockade: catecholamines, serotonin
II. Bind to muscarinic cholinergic receptors: atropine-like effects
III. Direct membrane activity: (a) membrane stabilization; (b) inhibition of $Na^{+}:K^{+}$ ATPase activity; (c) local anesthesia

catecholaminergic agonists by blocking the re-uptake of biogenic amines into the presynaptic neurons from which these neurotransmitters are liberated in response to a depolarizing stimulus (Alpers and Himwich, 1969). By blocking the re-uptake mechanism, TCA increase the amount and duration of neurotransmitter exposure at the postsynaptic receptors. They are indirect acting agents since the presynaptic neuron must fire before the TCA can exert their agonistic effect of blocking re-uptake. Although best known for these effects, TCA also have potent anticholinergic (Snyder and Yamamura, 1977) and direct membrane stabilizing properties (Elonen, 1974; Elonen *et al.*, 1974). With regard to the latter, TCA exceed both cocaine and mepivacaine in terms of potency as local anesthetics.

There are three major phenomena where one or more TCA have been shown to have biphasic effects: (1) antidepressant action of nortriptyline (NT), (2) alteration in cerebral microcirculation induced by amitriptyline (AMI) and (3) cardiac effects of all TCA. In these biphasic phenomena, the drug exerts an initial effect at low concentrations but then blocks it as the dose or concentration increases. Such a concentration : response relationship suggests that different mechanisms are responsible for the various phases and that these mechanisms predominate at different tissue concentrations. Each of the three examples will be briefly reviewed. The apparent mechanisms underlying the cardiac effects will then be discussed as a model for explaining complex concentration : response relationships.

Several investigators have reported an inverted U-shaped relationship when the antidepressant action of NT is plotted against its steady state plasma concentration. This relationship means that there is: (1) a minimal concentration theshold (50 ng ml^{-1}) below which NT does not exert antidepressant actions, and (2) a maximum concentration (150 ng ml^{-1}) above which NT apparently blocks its own antidepressant effectiveness (Åsberg, 1974). This critical range (approximately 50–150 ng ml^{-1}), wherein NT ameliorates affective symptoms, has been termed NT's 'therapeutic window'.

Such a range has not been as clearly defined for any other TCA. However, this finding is of clinical importance because of the large interindividual variability in elimination rates for these drugs. This variability can cause up to 30–40-fold differences in steady state drug concentrations between patients receiving the same dose of a TCA (Hullin, 1977). Without measuring drug concentration, a clinician has no reliable means of knowing whether a patient is above or below such a range. Given this inverted U-shaped relationship, he can not simply assume that an inadequate response means that the dose administered is too small. Poor response may be due to excessive drug concentrations as well as to insufficient concentrations. If this erroneous assumption is made, the dose may be increased until toxic side effects occur. Conversely, the clinician may decide the patient has a syndrome which is not responsive to this form of therapy (figure 1).

All TCA have potent effects on cerebral fluid dynamics (Preskorn and Hartman, 1979; Preskorn *et al.*, 1980*c*). They increase the movement of diffusion-limited substances from the vascular compartment into the brain by increasing the permeability of the blood : brain barrier and by altering

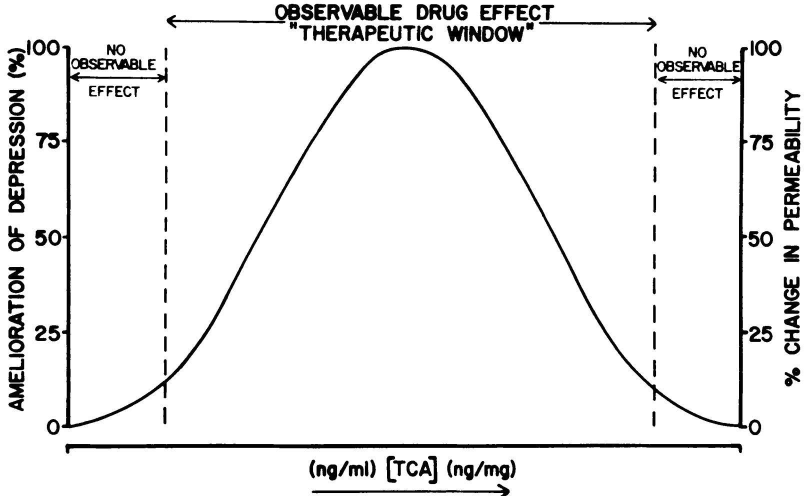

Figure 1 The curvilinear relationship between TCA concentration and effect. An inverted U-shaped curve results: (a) when the antidepressant effect of nortriptyline is plotted against its plasma concentration (Åsberg, 1974) and (b) when the cerebrovascular effects of amitriptyline is plotted against its brain concentration (Preskorn *et al.*, 1980*c*).

cerebral blood flow. AMI has been the most extensively studied TCA with regard to this effect on the cerebral microcirculation. As with the antidepressant effect of NT, there initially appears to be a paradoxical dose : response relationship for AMI's vascular effects. As the dose administered is increased, the response occurs earlier but is also shorter lived (Preskorn and Hartman, 1979).

This apparent paradox has been clarified by plotting effect against brain concentration of AMI rather than against dose administered. Such a plot reveals another inverted U-shaped relationship, demonstrating that the drug-induced alteration in cerebral fluid dynamics occurs within a critical concentration range (Preskorn *et al.*, 1980*c*). Thus, a minimum concentration is required before AMI exerts this effect. Moreover, above a critical concentration AMI aborts its own vascular action (figure 1).

AMI is demethylated *in vivo* to form NT (Preskorn *et al.*, 1980*e*). A subject treated with amitriptyline is thus exposed to both agents. Although AMI probably does not require conversion to NT to be active (Preskorn and Hartman, 1979), it is noteworthy that the same type of curvilinear relationship holds when the magnitude of blood : brain barrier alteration is plotted against brain concentrations of NT in subjects treated with AMI (Preskorn *et al.*, 1980*c*). A similar concentration : effect relationship thus is found for this cerebrovascular action of AMI and NT as for the antidepressant action of NT (figure 1).

Before pursuing this last point, TCA exhibit another curvilinear concentration : effect relationship for which the underlying mechanisms are reasonably well established. TCA are potent cardioactive drugs. Although

most physicians are aware of the serious cardiotoxicity that can occur in overdose patients (Jefferson, 1975), many do not realize that these drugs exert positive cardiac effects at lower concentrations (figure 2). Unlike the earlier examples of NT and AMI, the biphasic relationship between drug concentration and cardiac effects has been documented with all TCA. The discussion will now focus on this phenomenon as a model of the complex actions of these drugs and the usefulness of quantitating drug levels to predict drug effect.

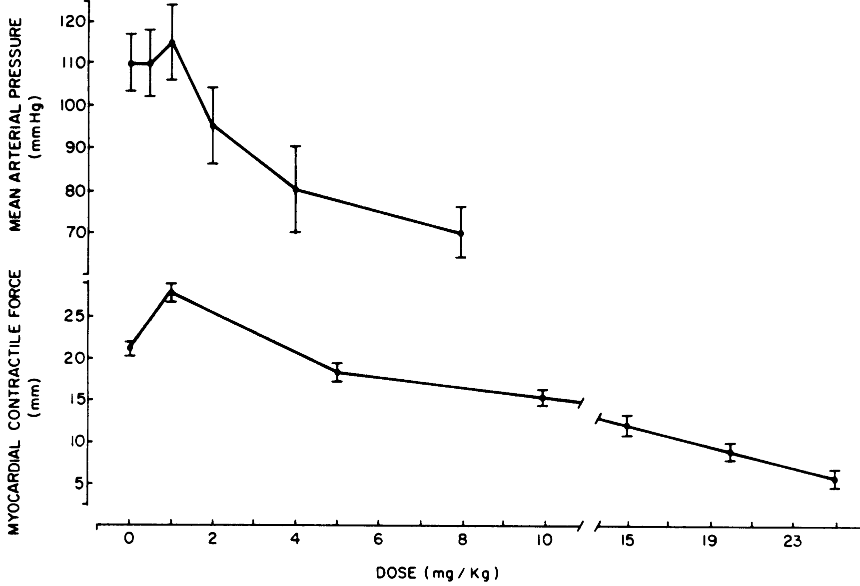

Figure 2 The curvilinear relationship between i.v. dose of desipramine and its *in vivo* cardiac effects (Laddu and Somani, 1969). The increase in myocardial contractility is adrenergically mediated while the later impairment of myocardiality contractility is due to the membrane stabilizing properties of the drug.

The positive chronotropic and inotropic phase caused by low dose TCA is not only concentration-dependent but also is adrenergically mediated. Desipramine (DMI) (10^{-9}–10^{-7} M) potentiates the positive chronotropic response to norepinephrine (NE) in isolated rat atria (Babulova *et al.*, 1973). Similarly, perfusion of a cat heart preparation with imipramine (IMI) (10^{-6} M) enhances and markedly prolongs the positive inotropic effect of NE (Sigg *et al.*, 1963). In other studies, the tachycardia induced by the addition of protriptyline or DMI (10^{-7}–10^{-6} M) to a rat atria preparation is inhibited by propranolol or atrial adrenergic denervation by 6-hydroxydopamine (6-OH-DA) pretreatment (Babulova *et al.*, 1973; Franco *et al.*, 1976). Guinea-pig atria exposed to similar concentrations of DMI show a rate increase that can be completely suppressed by reserpine pretreatment (Brunner *et al.*, 1971). The positive cardiac effects of low

concentrations of TCA then are prevented by propranolol, suppressed by reserpine pretreatment, and potentiated by NE. All this evidence supports an adrenergic mechanism underlying the positive phase of this phenomenon (figure 3).

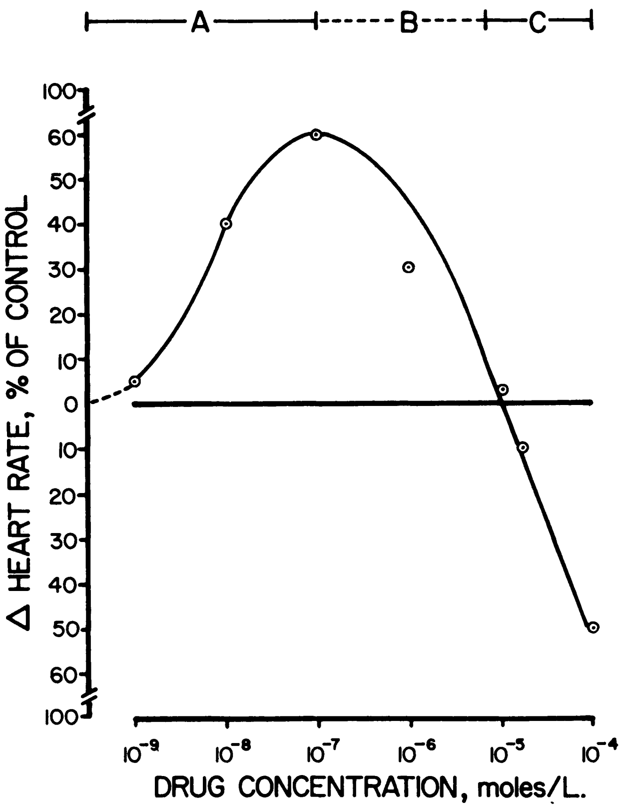

Figure 3 The curvilinear relationship between TCA concentration in the perfusate and the chronotropic effects on isolated heart preparations. This graph is a composite of results from several studies (Babulova *et al.*, 1973; Brunner *et al.*, 1971; Elonen *et al.*, 1974; Franco *et al.*, 1976; Langslet *et al.*, 1971). At low concentrations (10^{-9}–10^{-7} M), TCA potentiate the positive chronotropic (↑ heart rate) effects of NE (phase A). This phase can be blocked by pretreatment with reserpine, 6-OH-DA, and adrenergic antagonists. At high concentrations ($>10^{-5}$ M), TCA impair tissue excitability and thus cause a decrease in the spontaneous rate (phase C). This phase is unaffected by adrenergic agonists or antagonists. Phase C occurs at concentrations where TCA exert local anesthetic actions. Furthermore, the TCA produce the same effect as lidocaine at similar concentrations and when given together the effects of TCA and lidocaine are additive. Phase B represents a transition phase between the specific neurohormonally mediated effects of TCA and their direct membrane effects.

These effects seen at low doses of TCA are overcome at high doses by cardiotoxic effects. In *in vitro* experiments, the TCA tissue concentration has been demonstrated to be a direct function of the concentration of TCA in the fluid bathing the tissue and the length of exposure (Babulova *et al.*, 1973). The myocardial tissue uptake of TCA is not saturated in such a bath even after several hours of incubation. Binding first appears to occur at specific sites on the myocardial cell membrane. With continued exposure, non-specific binding predominates.

Cardiotoxic effects therefore can be produced by either increasing the length of incubation or by increasing the drug concentration (figure 3). A shift from NE potentiation to inhibition occurs in rat atria exposed to a bath containing a constant concentration of DMI. In isolated guinea-pig atria, concentrations of IMI under 10^{-6} M have a positive inotropic effect, whereas concentrations of 10^{-5} M diminish myocardial contractile force by one-third. Higher concentrations ($>10^{-4}$ M) cause a 75 percent incidence of atrial pump failure and arrest (Brunner *et al.*, 1971).

These *in vitro* findings are further supported by *in vivo* studies. DMI 1 mg kg^{-1} intravenously (i.v.), produces an increase in heart rate, myocardial contractile force, and blood pressure in vagotomized dogs (Kaumann *et al.*, 1965; Laddu and Somani, 1969). These cardiovascular effects are completely abolished by the β-adrenergic blocker, dicholoroisoproterenol, and attenuated by guanethedine or reserpine pretreatment. Intravenous administration of IMI, AMI, and NT (0.32–1 mg kg^{-1}) to dogs causes an increase in heart rate, and myocardial contractility (Baum *et al.*, 1976; Sigg *et al.*, 1963). Plasma concentrations ranging from 35 to 100 ng ml^{-1} of DMI and from 40 to 120 ng ml^{-1} of protriptyline are associated with positive chronotropic effects in rats (Bianchetti *et al.*, 1977). These experimental results parallel similar positive chronotropic effects observed in patients having plasma levels of TCA within this range (Burgess *et al.*, 1978). Thus, these concentration-dependent positive effects occur at plasma levels substantially below the levels at which serious cardiotoxicity occurs.

Negative cardiac effects occur when the TCA tissue concentrations – plasma levels in the clinical situation – exceed the range where the initial phase is observed. In dogs, there is a decrease in heart rate and cardiac output due to myocardial depression at i.v. doses of IMI greater than 2 mg kg^{-1} (Sigg *et al.*, 1963). In cats, a 3 mg kg^{-1} i.v. dose of IMI causes an 80 percent decrease in the amplitude of cardiac contractions, a reduction in heart rate by 30–40 beats min^{-1}, and widening of the QRS complex. The majority of cats die from this drug-induced cardiac failure (Brunner *et al.*, 1971). Injection of AMI (2 mg kg^{-1} i.v.) in rats causes a 19 percent decrease in heart rate, a 38 percent decrease in blood pressure, a 16 percent increase in the duration of the PQ interval, and a 31 percent increase in the duration of the QRS interval within 5 min after drug administration (Thorstrand *et al.*, 1976).

Direct measurement of the negative inotropic effects of TCA is not ethically permissible in man. However, hemodynamic studies in patients on TCA have reported drug-induced impairment of myocardial contractility. In one study, 18 patients with a history of angina or previous myocardial infarction were given i.v. IMI. At routine doses, IMI administration resulted

in an increase in left ventricular end-diastolic pressure (LVEP) reaching a maximum 30–45 min after injection (Bianmino *et al.*, 1975). In man, NT can also cause an increase in systolic time interval measurements due to drug-induced impairment of left ventricular myocardial contractility (Taylor and Braithwaite, 1978).

Based upon animal research, the cardiotoxic effects of TCA are most likely the result of direct membrane action of the drugs and not their catecholaminergic effects (Bianchetti *et al.*, 1977; Bonaccorsi *et al.*, 1977; Elonen, 1974; Elonen *et al.*, 1974). The rank order potency of the various TCA for NE potentiation in the rabbit heart is: protriptyline>NT>AMI>doxepin (Elonen, 1974). In contrast, the rank order of potency in terms of inducing cardiotoxicity is: doxepin, AMI>NT>protriptyline (Langslet *et al.*, 1971).

Although not widely recognized for these membrane actions, TCA are potent local anesthetics, being approximately three times as potent as lidocaine, four times as potent as tetracaine, and seven times as potent as mepivacaine. IMI, for example, can inhibit excitability and block impulse conduction in desheathed nerve preparations (Guerrero and Molgo, 1974). Furthermore, several TCA can equal or surpass the ability of quinidine, procainamide, propranolol, or lidocaine to suppress or prevent experimentally induced ventricular arrhythmias (Baum *et al.*, 1976; Fekete and Borsky, 1964, Marmo *et al.*, 1972; Schmitt *et al.*, 1970). TCA pretreatment increases the resistance of the myocardium to oubain-induced ventricular arrhythmias and cardiac arrest (Baum *et al.*, 1971; Wilkerson and Sanders, 1978). In dogs, AMI is more effective than lidocaine in converting ventricular arrhythmias – resulting from acute myocardial infarction – to sinus rhythm (Wilkerson and Sanders, 1978). These antiarrhythmic effects of IMI have also been demonstrated clinically in patients with both atrial and ventricular premature depolarization (Bigger *et al.*, 1977; Kantor *et al.*, 1978).

To summarize the cardiac findings, TCA at low tissue concentrations act to potentiate the positive effects of NE on myocardial performance. At higher tissue concentrations, the drugs bind avidly but non-selectively to myocardial cells and diminish myocardial performance by interfering with membrane excitability. These two mechanisms underlie the different phases of TCA cardiac effects. Thus the NE potentiation predominates at low concentrations, but is blocked at higher concentrations due to drug-induced membrane stabilization (figure 3).

Conceivably, similar mechanisms may be responsible for the other biphasic concentration : effect relationships seen with TCA. The cardiac effects can serve as a useful model since objective quantification and elaborate *in vitro* and *in vivo* manipulations are possible in this system, thus permitting elucidation of various mechanisms. The brain, conversely, is not as accessible, nor is quantification as direct nor as accurate. These experimental limitations are even more problematic for studying clinical phenomena such as the curvilinear relationship existing between the antidepressant action and plasma concentration of NT.

However, the cerebrovascular effects of TCA can serve as an experimental intra-axial model of a similar concentration : effect relationship. This

latter model does permit direct quantification in terms of changes in the brain uptake of diffusion-limited substances and also permits experimental manipulations. While the relationship, if any, between the drug-induced blood:brain barrier effect and its clinical usefulness is unknown; several observations – both kinetic and dynamic – suggest that it may exert this cerebrovascular effect in man. First, all TCA share this action. Further there is a strong correlation ($r = 0.96$) between their sedative effects in man and their cerebrovascular potency (Preskorn and Hartman, 1980). Second, AMT has been shown to enhance the cerebral extraction of water in two widely different mammalian species: rats and monkeys (Preskorn *et al.*, 1980*c,d*). Moreover, the effect occurs acutely in both species at plasma concentrations seen in patients receiving routine antidepressant chemotherapy. Third, the drug-induced barrier alteration is potentiated by chronic TCA administration designed to mimic clinical treatment. Under these conditions, the effect is observable continuously thoughout the dosage interval and occurs at steady state concentrations as low as 70 ± 13 ng ml^{-1}, well below the upper limits of the therapeutic range for AMI (Preskorn *et al.*, 1980*a*). Taken together, then, these data suggest that AMI could exert this effect in man irrespective of whether this cerebrovascular effect contributes to its clinical actions. Based on these considerations, elucidation of the pharmacological and neural mechanisms underlying this action of antidepressants may serve as a useful central model in comprehending some of their clinical effects.

The initial phase (figure 1), where AMI increases the diffusibility of water across the blood:brain barrier, appears to be adrenergic mediated. First, this increase is blocked by prior 6-OH-DA ablation of central adrenergic neurons (Preskorn *et al.*, 1980*b*) and is attenuated by phenoxybenzamine pretreatment. Second, neither serotoninergic or cholinergic mechanisms appear to play a role in producing the effect. Thus, the initial increase in cerebral extraction – like the initial positive chronotropic effects of TCA – appears to be adrenergically mediated. As the brain concentration of AMI rises, this initial increase is blocked. In an analogous way to the cardiac effects, this blockade may be due to drug-induced membrane stabilization. Studies are ongoing to test this possibility.

Results from these latter studies will increase our knowledge of the effects of TCA on a system of vital importance to normal cerebral function, the cerebral microcirculation. While awaiting these developments, the major conclusion from this discussion can be drawn from the cardiac studies. When confronted with a curvilinear dose:response relationship, the possibility that the drug exerts opposing effects at different concentrations must be considered.

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Biochemical indices of the effects of the selective MAO inhibitors clorgyline, pargyline and deprenyl in man

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Drugs which inhibit monoamine oxidase (MAO) affect the metabolism of many biogenic amines and their metabolites. All of the MAO-inhibiting antidepressants available in the USA as prescription drugs are non-selective, and retard the oxidative deamination of the entire list of substrates for the enzyme presented in table 1. These non-selective inhibitors include phenelzine, tranylcypromine and isocarboxazid, as well as such previously widely used drugs as nialamide and iproniazid.

A few MAO inhibitors exist which appear to inhibit preferentially the deamination of some biogenic amines, with lesser effects on other amines. Clorgyline, for example, inhibits 50 percent of the deamination of serotonin (5-HT) by human brain cortex homogenates *in vitro* at concentrations of

Table 1 A partial listing of biogenic amine substrates for monoamine oxidase

Indoleamines:	5-Hydroxytryptamine
	5-Methoxytryptamine
	Tryptamine
Catecholamines:	Norepinephrine
	Epinephrine
	Dopamine
Non-catechol phenylethylamines:	Tyramine
	3-Methoxytyramine
	Phenylethylamine
	Phenylethanolamine
	Octopamine
Metabolites and others:	Tele-methylhistamine
	Kynuramine
	Normetanephrine
	Metanephrine

2×10^{-10} M (table 2). Phenylethylamine metabolism remains unaffected by clorgyline until high concentrations (10^{-6} M) are added. Other propargylamine derivitives such as deprenyl and pargyline exhibit reverse selectivity, inhibiting phenylethylamine deamination in human and rat brain *in vitro* at lower concentrations than those required to inhibit 5-HT deamination (table 2). Substrate selectivity with these drugs is not absolute, and at high inhibitor concentrations selectivity is lost. Other MAO inhibitors (MAOIs) which have been demonstrated to exhibit substrate selectivity include harmine and harmaline, Lilly 51641 and some structurally related cyclopropylamines, 5-phenyl-3-(*N*-cyclopropyl)ethylamine-1,2,4-oxadiazole (PCO), and a number of recently described agents including FLA336, MD780515 and R011-1163. Interestingly, some other drugs such as the tricyclic antidepressants and amphetamines, whose primary mechanism of action has generally been thought to be not related to MAO inhibition, also demonstrate some selective inhibitory effects on MAO.

Table 2 Inhibition of MAO activity in human cortex by clorgyline, deprenyl and pargyline, *in vitro*

Substrate	IC_{50} (nmol)		
	Clorgyline	Deprenyl	Pargyline
Serotonin (1 mM)	0.2	500	160
Phenylethylamine (0.2 μM)	1410	30	30

Data from Murphy *et al.* (1979*b*).

The basis for the preferential inhibition of the deamination of some substrates by these selective inhibitors is thought to reside in the existence of two closely related forms of the enzyme, MAO-A and MAO-B, which differ in some properties, including their intracellular accessibility to the inhibitors, their sensitivity to these drugs, and their capacity to deaminate certain monoamine substrates. Among the substrates examined, 5-HT and, to a lesser extent, norepinephrine (NE), are preferentially deaminated by MAO-A, while phenylethylamine, phenylethanolamine, *o*-tyramine, tele-methylhistamine and benzylamine are preferential substrates for MAO-B. Several recent reviews of the characteristics of the MAO and of selective MAOIs have been published (Fowler *et al.*, 1978; Wolstenholme and Knight, 1976; Murphy, 1978; Singer *et al.*, 1979).

Most studies defining the substrate-selective characteristics of these inhibitors have examined changes in MAO-A versus MAO-B activity *in vitro* or following acute drug administration *in vivo* in animals. A smaller number of studies have measured changes in tissue concentrations of monoamines following drug treatment in animals, principally rodents. Very few investigations of the chronic administration of these inhibitors have been accomplished, and some of these have suggested a loss in selectivity with continued drug administration.

Only meager information is available concerning the changes in monoamine metabolism produced by selective MAOIs in man. Nonetheless, selective MAOIs including deprenyl, clorgyline, pargyline and Lilly 51641, have begun to be evaluated in patients with Parkinson's disease and depression, in anticipation of possible therapeutic advantages from their more specific actions, with the possibility of greater safety and fewer adverse effects. The absence of information regarding amine metabolic changes in man is particularly of concern because the data from rodents may not be readily extendable to man, due to substantial rodent–primate differences in the proportion of MAO-A versus MAO-B activity, especially in brain (Garrick *et al.*, 1979).

This paper reviews some recent approaches our group has taken to evaluate to what extent the selectivity of several MAOIs is maintained *in vivo* in man during longer term drug administration. The biological data presented in this review were obtained during the course of two clinical studies conducted at the NIH Clinical Center: (1) a comparative evaluation of the clinical and biochemical effects of clorgyline and pargyline in depressed patients, described in two preliminary reports (Lipper *et al.*, 1979; Murphy *et al.*, 1979*b*); and (2) an evaluation of deprenyl as an adjunct to L-Dopa treatment in Parkinson's disease patients, also described in detail elsewhere (Eisler *et al.*, 1979).

In these studies, patients received the MAOIs for 4 weeks or more, in doses of 10–40 mg per day for clorgyline (average dose, 28 mg per day), 30–130 mg per day for pargyline (average dose, 90 mg per day) and 5–10 mg per day for deprenyl. Plasma, urine and cerebrospinal fluid (CSF) samples were collected prior to MAOI administration and again after 4 weeks of treatment. Preliminary clinical data from these studies indicated that clorgyline had significant antidepressant and antianxiety effects; pargyline had minimal therapeutic effects but, like clorgyline, did produce quite marked orthostatic hypotension; and deprenyl provided only minimal additional benefit to the Parkinson's disease patients receiving L-Dopa (Lipper *et al.*, 1979; Eisler *et al.*, 1979).

Platelet MAO activity was measured using ^{14}C-benzylamine as the substrate (Murphy *et al.*, 1976). As indicated in figure 1, pargyline and deprenyl treatment for 4 weeks was associated with essentially complete inhibition of the MAO-B enzyme in platelets. Clorgyline administration, in contrast, did not lead to a significant change in platelet MAO activity (Murphy *et al.*, 1979*b*). These data suggest that clorgyline, even when given for 4 weeks, maintains its selectivity as an MAO-A inhibitor in not appreciably inhibiting the platelet MAO-B enzyme and, of course, that the two MAO-B inhibitors were given in sufficient dosage to reduce markedly MAO-B activity in platelets.

Plasma amine oxidase activity was also measured in these studies using a previously described assay (Murphy *et al.*, 1976). This enzyme, unlike the mitochondrial MAO found in tissues, utilizes a different cofactor (pyridoxal instead of FAD) and has a different range of substrates and inhibitors. It does not have the properties of either MAO-A or MAO-B, and is insensitive to clorgyline, pargyline and deprenyl *in vitro*, with no inhibition observed

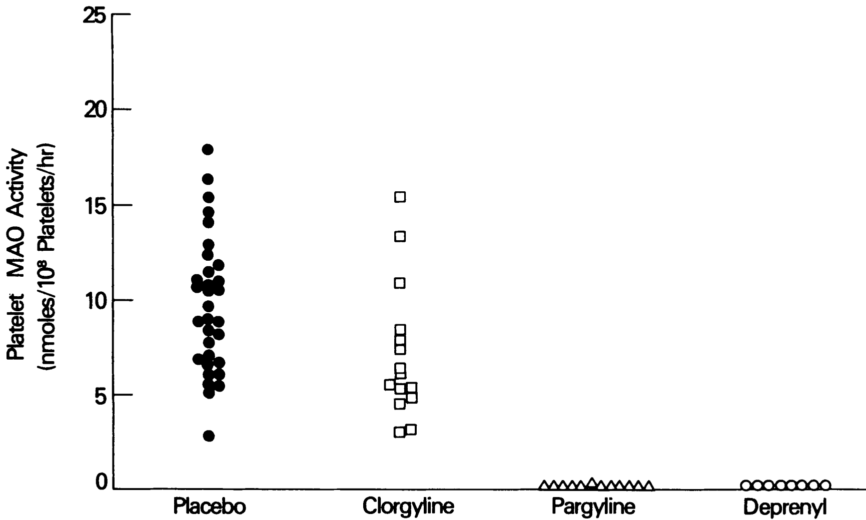

Figure 1 Platelet monoamine oxidase activity during pretreatment placebo period and during the fourth week of treatment with clorgyline, pargyline or deprenyl.

until over 1000-fold higher concentrations ($>10^{-3}$ M) of these drugs are used. Nonetheless, plasma amine oxidase activity was reduced somewhat during the 4 weeks of pargyline treatment, and to a lesser extent during clorgyline treatment (figure 2; Murphy *et al.*, 1979*c*). Deprenyl, however,

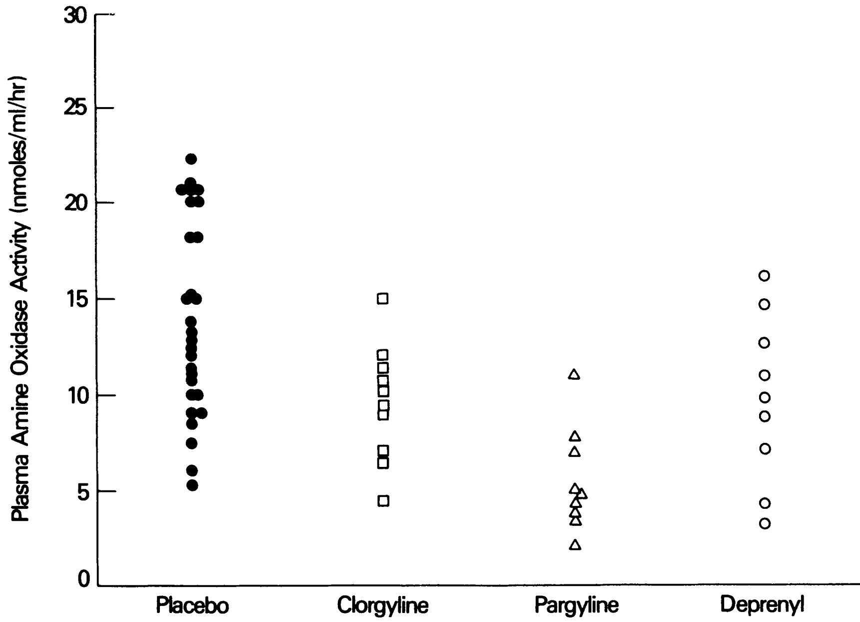

Figure 2 Plasma amine oxidase activity during the pretreatment placebo period and during the fourth week of treatment with clorgyline, pargyline or deprenyl.

did not affect its activity (in marked contrast to deprenyl's effects on platelet MAO-B), suggesting further that changes in this enzyme do not follow a pattern consonant with the MAO-A/MAO-B typology (Eisler *et al.*, 1979).

Among the other blood constituents measured in this study, changes in plasma concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) indicated a greater effect of clorgyline on this metabolite of NE, an MAO-A substrate (Pickar *et al.*, 1981). As indicated in figure 3, clorgyline treatment was associated with a marked reduction in plasma MHPG. Somewhat lesser reductions were observed with pargyline, and negligible reductions with deprenyl. These differences thus support the hypothesis that clorgyline would have the greatest effect on the metabolism of NE. These observations also suggest that deprenyl treatment remained selective, but that pargyline was probably inhibiting MAO-A as well as MAO-B. Since deprenyl and pargyline are nearly equipotent inhibitors of MAO *in vitro*, it may be that the higher pargyline doses used in this study exceeded those at which selectivity for MAO-B could be maintained.

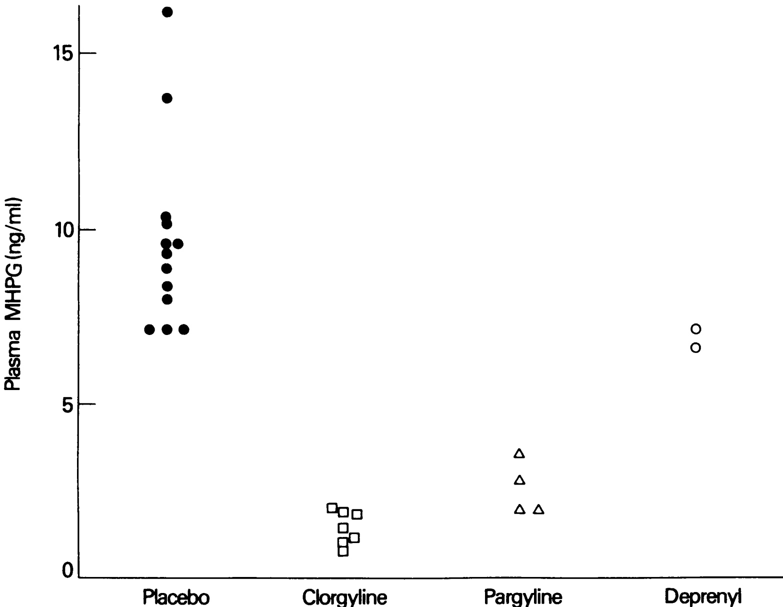

Figure 3 Plasma MHPG concentrations during the pretreatment placebo period and during the fourth week of treatment with clorgyline, pargyline or deprenyl.

Like the plasma MHPG results, plasma NE concentrations were reduced by clorgyline (figure 4). Pargyline also led to a similar reduction in plasma NE (Murphy *et al.*, 1978). The basis for this effect is not known, but it may be related to the clinically evident orthostatic hypotension produced by these drugs, and it may reflect MAOI-related changes in central noradrenergic mechanisms regulating peripheral sympathetic activity. In the parkinsonian patients, no change in plasma NE accompanied treatment with

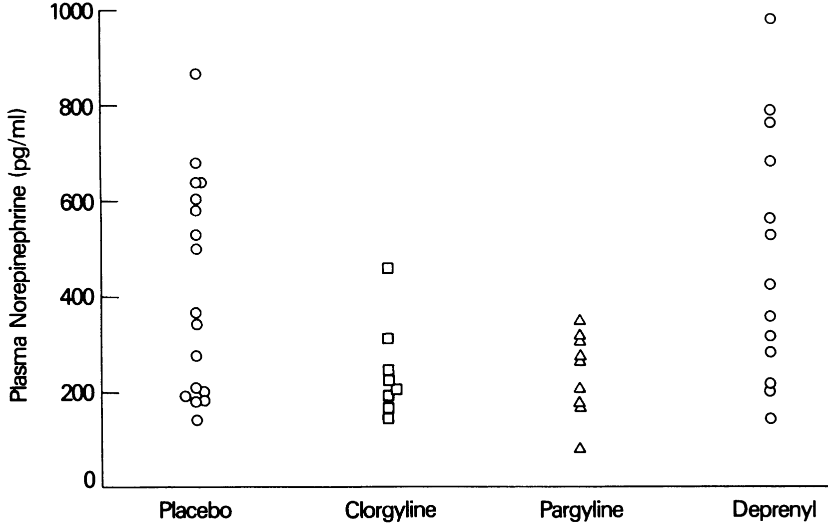

Figure 4 Plasma norepinephrine concentrations during the pretreatment placebo period and during the fourth week of treatment with clorgyline, pargyline or deprenyl.

deprenyl (Eisler *et al.*, 1979). These changes are consistent with the rank order of reductions in plasma MHPG produced by the three drugs.

Mean CSF NE concentrations, unlike those in plasma, were not altered by treatment with either clorgyline or pargyline (figure 5; Major *et al.*, 1979*b*). Considerable individual differences in both baseline values and those obtained during drug treatment were present, however, and a significant correlation between the magnitude of change in CSF NE and clinical

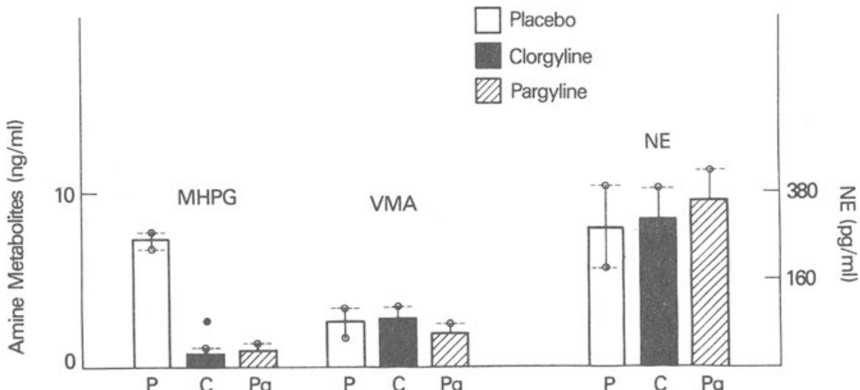

Figure 5 Cerebrospinal fluid concentrations of NE, MHPG and VMA during the pretreatment placebo period and during the fourth week of treatment with clorgyline or pargyline.

antidepressant response was observed (Major *et al.*, 1979*a*). Marked reductions in CSF MHPG (but not in VMA) occurred with clorgyline and pargyline. Like the changes in NE and MHPG in the periphery noted above, these data are consistent with a large inhibiting effect on MAO-A by clorgyline, as well as by pargyline. In other studies not depicted in figure 5, CSF NE and MHPG were not found to be altered by deprenyl administration to parkinsonian patients (Eisler *et al.*, 1979).

The dopamine (DA) metabolites homovanillac acid (HVA) and, to a lesser extent, 3,4-dihydroxyphenylacetic acid (DOPAC) in CSF were reduced more by pargyline than by clorgyline (figure 6; Major *et al.*, 1979*b*). This is consistent with data indicating that DA is predominantly deaminated by MAO-B in man (Glover *et al.*, 1977; Garrick and Murphy, 1980). It is also consistent with the platelet MAO reductions in indicating that both in the CNS and in periphery MAO-B is altered to greater extent by pargyline than by clorgyline. No significant reductions in CSF HVA were observed in the parkinsonian patients treated with deprenyl, although CSF DA concentrations were elevated and urinary HVA concentrations were reduced in these patients (Eisler *et al.*, 1979). This difference from the pargyline treatment results may represent the pargyline versus deprenyl dosage difference or may have resulted from an interaction of the drug effect with the altered central DA system in the parkinsonian patients.

The major 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), was reduced approximately 50 percent in CSF by both clorgyline and pargyline.

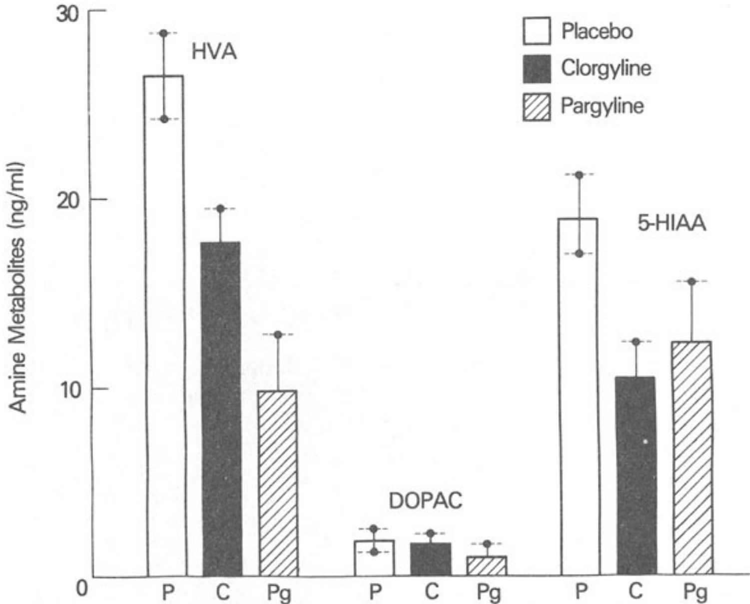

Figure 6 Cerebrospinal fluid concentrations of HVA, DOPAC and 5-HIAA during the pretreatment placebo period and during the fourth week of treatment with clorgyline or pargyline.

This reduction in 5-HIAA is substantially less than that observed for CSF MHPG, although both NE and 5-HT are preferred substrates for the MAO-A enzyme. The equivalent reduction produced by pargyline also suggests that pargyline may be acting, in part, to inhibit MAO-A, although there is evidence that in primates, including man, 5-HT is also deaminated to some extent by MAO-B (Garrick *et al.*, 1979).

Urinary phenylethylamine excretion was increased approximately 100-fold by pargyline administration, but was essentially unaffected by clorgyline treatment (figure 7; Karoum *et al.*, 1980). These data, like the platelet MAO results, confirm the marked MAO-B inhibition produced by pargyline, and also confirm that clorgyline had minimal effects on MAO-B activity. Urinary phenylethylamine excretion has also been reported to be markedly increased following deprenyl administration (Elsworth *et al.*, 1978).

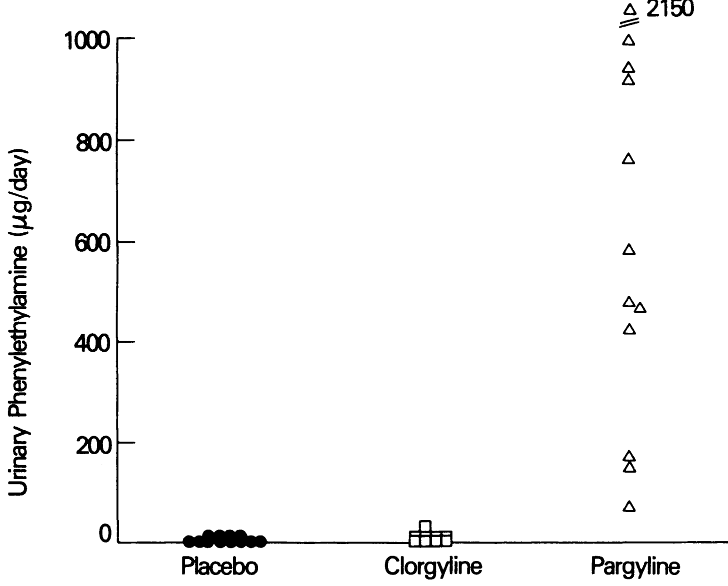

Figure 7 Urinary phenylethylamine excretion during the pretreatment placebo period and during the fourth week of treatment with clorgyline or pargyline.

SUMMARY

Consistent reductions in platelet MAO-B activity and marked, non-overlapping elevations in urinary phenylethylamine excretion in subjects treated with pargyline and deprenyl provided clear evidence that MAO-B activity was markedly inhibited during treatment with these two MAO-B

inhibitors in all patients. These two measurements would seem to provide the most useful indices of MAO-B inhibition during chronic treatment with MAOIs in man. Reductions in plasma and CSF MHPG concentrations were maximal with clorgyline treatment, and may provide the most useful index of MAO-A inhibition, in the absence of a readily available human tissue source of the enzyme, as is available for MAO-B activity assay in human platelets. The combined data indicate that in the doses used, clorgyline maintained high selectivity as an MAO-A inhibitor, and deprenyl as an MAO-B inhibitor, while pargyline more markedly inhibited MAO-B, but also inhibited MAO-A. Selectivity was maintained with clorgyline and deprenyl for at least a 4 week period of drug administration in man.

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Section Six

Toxicity and Plasma Drug Levels

Cardiovascular effects of antidepressants*

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INTRODUCTION

A great variety of symptoms have been reported after the ingestion of an overdose of a tricyclic antidepressant (TCA). These include disorientation, ataxia, vomiting, coma, convulsions, ECG changes and dysrhythmias (Hamilton and Mahapatre, 1972). As some of the TCA appear to show an affinity for myocardial tissue (Jefferson, 1975; Elonen *et al.*, 1975; Bianchetti *et al.*, 1977), it is not surprising that a large proportion of individuals experiencing overdose of these drugs show signs of cardiotoxicity. This is compelling evidence for a serious cardiotoxicity in overdose; even at therapeutic dose levels there is strong evidence to show that changes in cardiac parameters may occur with nortriptyline (NT) (Taylor and Braithwaite, 1978; Burrows *et al.*, 1976), imipramine (IMI) (Kristiansen, 1961; Bigger *et al.*, 1977), clomipramine (CI) (Singh, 1972) and amitriptyline (AMI) (Smith and Rusbatch, 1967; Scollins *et al.*, 1972), although these changes are not apparent in all patients (Jefferson, 1975; Vohra *et al.*, 1975*a,b,c*). Using surface ECG recording and in other studies using His bundle electrocardiography (HBE), Burrows *et al.* (1976) found that intracardiac conduction was prolonged in patients taking therapeutic doses of TCA, and Taylor and Braithwaite (1978), using systolic time interval measurements, found changes indicating that a deterioration in cardiac function occurred with NT which was correlated with the plasma level. In TCA overdose the effects produced by the cardiotoxicity of these drugs can be most dangerous and may be difficult to correct (Manoguerra and Weaver, 1977).

*This paper has been modified from the chapter entitled 'Cardiotoxicity of antidepressants: experimental background' in *Stress and the Heart*, 2nd edn (ed. D. Wheatley), Raven Press, New York.

For these reasons, the relative cardiotoxicity of currently available antidepressants in overdose and at therapeutic dose levels is of particular interest and has been investigated by many workers using a variety of methods.

ANIMAL STUDIES

With regard to tests for cardiotoxicity, it is difficult to make a critical assessment of the various animal models which have been used, mainly because it is hard to obtain a single quantitative expression of the 'true' cardiotoxicity of these compounds in man.

The effects of several TCA have been compared in a number of relatively uncomplicated isolated preparations such as the guinea-pig (Greeff and Wagner, 1971; Kato *et al.*, 1974), rat (Mundo *et al.*, 1974) and rabbit (Hughes and Coret, 1974) atria, cat papillary (Biamino, 1976; Biamino *et al.*, 1975) and rat ventricle (Prudhommeaux *et al.*, 1968). There appears to be general agreement among workers that at high concentrations ($>300\ \mu g\ l^{-1}$; $>1\times10^{-6}$ M) the TCA produce a negative inotropic response and, in preparations showing spontaneous activity, a negative chronotropic effect as well. Although some differences have been observed in the effects of TCA when applied to isolated tissues in low concentrations (Mundo *et al.*, 1974), there is general agreement that at high concentrations any differences between the compounds are small (Dumovic *et al.*, 1976; Brunner *et al.*, 1971; Greeff and Wagner, 1969).

In isolated guinea-pig atria, Dumovic *et al.* (1976) found that at equal concentrations (1×10^{-5} M; 3 mg l^{-1}), there were some statistically significant differences between the abilities of NT, protriptyline, desmethylimipramine (DMI), AMI, IMI and doxepin to reduce rate and force of contraction, although the maximum mean effect on rate (doxepin and IMI) was a reduction of about 5 percent and the maximum mean effect on force (doxepin) a reduction of about 13 percent. Differences of this size are unlikely to be clinically significant.

The experiments on isolated tissues indicate that many of the older TCA have a similar degree of cardiotoxicity, while some of the newer agents, nomifensine and mianserin for example, would seem to have less harmful effects on the heart at similar concentrations. One feature of all the work done in isolated tissues with regard to cardiotoxicity at therapeutic plasma levels is the concentration used in the experiments. In poisoning, plasma levels of AMI may be very high (Petit *et al.*, 1977), but in therapeutic use only rarely exceed 300 $\mu g\ l^{-1}$ (1×10^{-6} M) and are usually very much lower (Braithwaite and Widdop, 1971; Jørgensen, 1975). One other feature of these experiments, particularly in relation to cardiotoxicity at therapeutic dose levels, is that all these isolated tissue experiments involve acute administration of the antidepressants, whereas chronic exposure is the rule in the human therapeutic situation.

As with isolated tissues, no single *in vivo* method has become accepted as the best method to assess cardiotoxicity; a great variety of methods have been used and experiments have been carried out in mice (Elonen, 1974), rats (Nemec, 1974; Bonaccorsi *et al.*, 1977), guinea-pigs (Burrows *et al.*, 1976), rabbits (Hughes and Radwan, 1979), cats (Forika *et al.*, 1971) and dogs (Dhumma-Upakorn and Cobbin, 1977). At very low doses, the TCA produce an increase in heart rate, contractility, blood pressure, coronary and aortic flow and automaticity (Elonen, 1974; Baum *et al.*, 1976) and both the atropinic and the norepinephrine (NE) uptake blocking properties of these drugs may be involved in these effects (Bonaccorsi and Garattini, 1978). At higher doses there is general agreement that heart rate is reduced, blood pressure falls, automaticity is decreased, the PQ and PR intervals lengthen, the width of the QRS complex is increased and changes in the T wave occur (Nemec, 1974; Baum *et al.*, 1976; Thorstrand *et al.*, 1976; Kurioka and Taniwa, 1974; Desager *et al.*, 1979). Ventricular extrasystoles, bigeminy, trigeminy, A-V block and ventricular fibrillation may all be seen (Nemec, 1974; Kurioka and Taniwa, 1974). The ECG changes and dysrhythmias which are found in animals are very similar to those seen in man and occur at plasma levels which are within the range found in overdose patients (Bianchetti *et al.*, 1977; Bonaccorsi *et al.*, 1977). Quantitatively, IMI, AMI, NT and doxepin appear to be only marginally different in their cardiotoxicity (Burrows *et al.*, 1976; Nemec, 1974; Baum *et al.*, 1976).

Of the newer antidepressants, iprindole had less effect on ventricular and atrial conduction velocity or ventricular excitability than did IMI, but iprindole is not without cardiotoxicity since a significant prolongation of the A-V conduction time was seen (Baum *et al.*, 1971). Nomifensine was required in higher dose and produced smaller changes in the PQ interval in the guinea-pig ECG than did either AMI or IMI (Hoffmann, 1977).

The animal studies confirm the considerable cardiotoxic potential of the TCA. The studies also indicate that not all antidepressants are likely to show an equivalent cardiotoxicity in man. Although many of the older TCA show approximately equal cardiotoxicity this is not true of the newer antidepressants. The experiments with mianserin indicate a substantially reduced cardiotoxicity on a weight-for-weight basis compared with many other antidepressants. If there is a risk of overdose, then it would appear that mianserin would provide a safer alternative than the more traditional TCA.

HUMAN STUDIES

Cardiovascular effects of therapeutic doses of tricyclics

The cardiovascular effects of TCA are well known and constitute the major impetus in the search for safer and more effective agents. Epidemiological studies have documented the cardiovascular side effects of the tricyclics and this has been the subject of several reviews (Jefferson, 1975; Thorstrand,

1976; Freeman *et al.*, 1969; Bonaccorsi and Garattini, 1978; Boston Collaborative Drug Surveillance Program, 1972; Coull *et al.*, 1970; Moir, 1973).

The Boston Collaborative Drug Surveillance Program (1972) reported on 237 patients who received AMI, IMI, DMI, protriptyline or NT. The incidence of sudden death and death rate was not different in these patients and a similar group of hospitalized patients from the same wards who did not receive antidepressants. Similarly, the survey found no difference in cardiotoxicity between 80 patients with cardiovascular disease receiving tricyclics and 3994 patients with cardiovascular disease not receiving tricyclics.

This apparent lack of cardiotoxic effects of the antidepressants is in contrast to the Aberdeen General Hospitals Group studies (Coull *et al.*, 1970; Moir, 1973). A group of 864 patients who entered hospital in a 40 month period and who received AMI were studied. Of these, 119 had a diagnosis of cardiac disease and were matched by age, sex, cardiac diagnosis and length of hospitalization with a group of patients not taking antidepressants. Among the AMI group there were 23 deaths (19 percent of patients) compared with 15 deaths (12 percent) in the control group. This difference was not statistically significant. When compared for 'sudden unexpected cardiac death', 13 of 23 AMI deaths met the criteria compared with three of 15 controls, a significant difference. A further investigation of 87 cardiac patients receiving IMI compared with 87 control cardiac patients, revealed four unexpected deaths in the IMI group compared with two in the control group. The authors advocated caution in the prescribing of AMI and IMI to patients with cardiac disease. Both studies serve to point out the dangers associated with chronic tricyclic administration.

Electrocardiographic changes and therapeutic drug plasma levels

There are studies of electrocardiographic changes during 'pure' tricyclic therapy. Such studies are summarized in Table 1. The general pattern of ECG changes which emerges from these studies is ST-T changes, increases in PR and QRS width, bundle branch block and sinus tachycardia. These changes are reversible, in patients without pre-existing heart disease, when the drugs are withdrawn. Clinically the cardiovascular side effects of the tricyclics are potentially dangerous and warrant close scrutiny in patients taking these drugs.

In addition to monitoring ECG changes, some studies have monitored drug plasma levels. Freyschuss *et al.* (1970) studied 40 depressed patients treated with NT. Pulse rate, blood pressure and ECG were recorded before treatment and during treatment, when steady state plasma levels of NT were achieved. Mean steady state NT levels were 47 ng ml^{-1} for the 75 mg group and 93 ng ml^{-1} for the 150 mg group. Heart rate and blood pressure increased during drug treatment. In one patient (a 51 year old male) a right bundle branch block appeared. When drug therapy was withdrawn his ECG returned to normal. In the remaining patients the ECG records showed no signs of adverse effects of NT. Plasma levels did not correlate with the increase in pulse rate observed.

Electrocardiographic changes were studied in a group of 32 patients receiving various doses of AMI, NT, IMI and doxepin (Vohra *et al.*, 1975*b*). Plasma levels were determined in the 20 patients who received NT. The mean plasma concentration was 182 ng ml^{-1} (range 60–392 ng ml^{-1}). Heart rate increased in 26 of the 32 patients and this increase tended to be greater when the pretreatment heart rate was low. The PR interval increased in all subjects following drug administration; QT interval showed no significant changes. The ST-T wave changes found in these patients were considered unremarkable by the authors. Three patients on NT showed prolongation of the QRS, which did not occur with the patients receiving other drugs. There was no correlation between plasma NT levels and increased heart rate or PR interval. It was tentatively suggested that doxepin had less effect on the cardiovascular system than nortriptyline.

Intracardiac conduction studies using HBE also provided some support for the hypothesis that doxepin is less cardiotoxic than NT, and suggested that plasma levels of NT in excess of 200 ng ml^{-1} are more often associated with cardiac abnormalities (Vohra *et al.*, 1975*a*). The relationship between the His bundle electrogram and the standard ECG is shown in figure 1. HBE studies were carried out in 12 patients before and at least 2 weeks after they had been on therapeutic doses of NT. Plasma for NT determination was collected at the same time as the HBE recordings were made. Mean NT level was 209 ng ml^{-1} (range 75–490 ng ml^{-1}). Five patients showed significant prolongation of the H–V interval and of these four had plasma levels in excess of 200 ng ml^{-1}. The effect on the A–V conduction was variable. These authors suggest that prolongation of the H–V interval may account for the increased incidence of sudden deaths in cardiac patients taking tricyclics (Coull *et al.*, 1970; Moir, 1973).

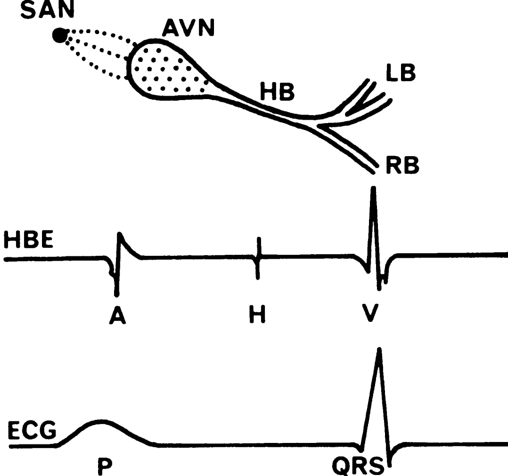

Figure 1 His bundle electrogram (HBE) with the standard ECG. An anatomical model of the conduction system at the top of the figure is orientated to show the sites of origin of the electrical waves. The A-H and H-V intervals represent proximal and distal intracardiac conduction respectively.

Table 1 Electrocardiographic effects of therapeutic doses of tricyclic antidepressant drugs

Patient population	Drug* and dose	Electrocardiographic findings	References†
23 patients	IMI, up to 200 mg per day	In 15 patients there were alterations in the ECG which were often first manifest or were aggravated by physical exercise; 'stress or cardiovascular disease may predispose to IMI-induced ECG changes'.	8
82 patients	IMI, 75–200 mg per day	Serial ECGs over a treatment period of 30 days revealed no direct drug effect. The 'changes that did occur could be explained by the presence of complicating factors'.	10
36 adults 15 adults	AMI DMI } 'ordinary therapeutic doses'	T-wave changes (flattening, isoelectricity, inversion) in two patients on AMI. These changes were fully reversible and 'did not produce any cardiac symptoms'. No changes with DMI. Ten patients with an abnormal control ECG had no changes during treatment.	12
65 patients ($\bar{x}$=60 years age)	AMI, 75–225 mg per day	Treatment of three or more weeks during which one or more ECGs were taken revealed ECG changes in 18 percent of patients. These included isoelectric T waves, ST segment depression and frequent extrasystoles. 'Possible changes in 32%, no changes in 50%'.	18
40 patients (20–72 years)	NT, 75–150 mg per day	After 3 weeks of drug therapy the ECG at rest and during exercise revealed no direct effects except one patient who developed a right bundle branch block during the work test (ECG response to exercise testing after drug withdrawal was normal).	6
40 patients ($\bar{x}$=50 years age)	DOX, range of daily doses ($\bar{x}$=150 mg per day)	One or more ECGs over 18–41 months revealed some transient tachycardia in five patients. Otherwise 'no adverse cardial effects were detected clinically or electrographically'.	1

14 children with enuresis (5–10 years)	IMI, with 25/75 mg per day	No significant changes in serial ECGs.	9
30 patients	'Recommended' doses of tricyclics for > 3 weeks	Non-specific flattening of T waves. Two patients developed primary heart block (reversible in one case).	11
27 children with enuresis (5–12 years)	IMI, 25–75 mg per day	'No substantial ECG changes'.	9
32 patients (19–57 years)	NT, IMI, AMI, DOX, 75–400 mg per day ($\bar{x}$= 175 mg per day)	Moderate increase in heart rate; mild increase in PR interval and no significant effect on the QTc. Repolarization changes in four patients.	15
12 patients	NT, 150 and 200 mg per day	Distal atrioventricular conduction (H-V interval) increased in five patients after 2 weeks' therapy).	14
7 children with hyperkinetic/ aggressive behavior disorders (7–10 years)	IMI, 5 mg kg^{-1} per day	In all cases the repolarization phase was affected (decreased T-wave amplitude and increased T-wave width). Three children evidenced primary A-V block.	16

Table 1 (continued)

Patient population	Drug* and dose	Electrocardiographic findings	References†
9 patients (>65 years)	IMI, 75–100 mg per day	An increased heart rate 'clinical meaningful changes were observed in four patients'. Two developed sinus tachycardia, one developed non-specific ST-T-wave abnormalities and one developed left axis deviation and complete bundle branch block.	13
47 patients ($\bar{x}$=43 years age)	AMI, IMI, MAP, MIA, TRI, 40–300 mg per day	No serious disturbances of cardiac rhythm after 3 weeks therapy. Increases in PR and QRS proved reversible after drug withdrawal.	4
15 patients (18–50 years)	AMI, 75–200 mg per day ($\bar{x}$=132 mg per day)	Significant increase in heart rate (mean increase of 16 min^{-1}). Those patients having rate change > 16 min^{-1} had significantly higher AMI and AMI+NT plasma levels.	18
17 patients (20–65 years)	NT, 50–150 mg per day	Significant increases in heart rate and non-specific ST-T wave changes during treatment. Weak positive correlation between the change in heart rate and the plasma NT level ($r=0.46$; $p<0.05$; one-tailed test).	17
27 female patients ($\bar{x}$=32 years age)	AMI (100–200 mg per day; $n=14$); MIA (80–100 mg per day; $n=13$)	ECGs recorded before and after 3 weeks of drug treatment revealed an increase in heart rate and PR interval in 12 and 11 of the 14 AMI patients respectively.	2

8 healthy male volunteers (20–40 years)	IMI, 0.5 mg kg^{-1} intravenously infused over 30 min	Slight increase in heart rate. H-V interval increased to pathologic values in two volunteers. Prolongation of repolarization in the ventricle.	3
66 patients (20 men, 46 women)	AMI, IMI, MAP, MIA, TRI, 40–300 mg per day	After 3 weeks of therapy heart rate and PR interval were increased ($P<0.02$; $P<0.05$). Significant flattening of the T-wave was also noted. When therapy was maintained for 13 months only heart rate continued to be increased; all other values had returned to normal.	5
7 patients (6 had prior ECG abnormalities)	IMI, 175–400 mg per day ($\bar{x}$=271 mg per day)	Slight increases in heart rate, PR, QRS and QTc intervals (see text).	7

* *Abbreviations:* AMI, amitriptyline; DMI, desipramine; DOX, doxepin; IMI, imipramine; MAP, maprotiline; MIA, mianserin; NT, nortriptyline; TRI, trimipramine.

† *References*: 1, Ayd (1971); 2, Brogden *et al.* (1978); 3, Brorson and Wennerblom (1978); 4, Burckhardt *et al.* (1976); 5, Burckhardt *et al.* (1978); 6, Freyschuss *et al.* (1970); 7, Kantor *et al.* (1978); 8, Kristiansen (1961); 9, Martin and Zaug (1975); 10, Muller *et al.* (1961); 11, Muller and Burckhardt (1974); 12, Schou (1962); 13, Shader (1975); 14, Vohra *et al.* (1975*a*); 15, Vohra *et al.* (1975*b*); 16, Winsberg *et al.* (1975); 17, Ziegler *et al.* (1977*a*); 18, Ziegler *et al.* (1977*b*).

In a subsequent study, Burrows *et al.* (1977) compared the effects of therapeutic doses of doxepin and NT on intracardiac conduction. A total of 17 patients, all diagnosed as suffering from a primary depressive illness, with no history of cardiovascular disease, were admitted to the study. After a 1 week placebo period, patients were randomly allocated to treatment with 150 mg per day of NT or 150 mg per day of doxepin. After 3 weeks' treatment, patients were allowed a 10 day 'washout' period and then crossed over to the other drug. Standard ECG and high speed, high fidelity ECG were recorded in the placebo period and after 3 weeks' treatment on each drug. Plasma samples were collected at the same time as ECG recordings were made. The mean steady state NT plasma level was 196 ng ml^{-1} (range 29–416 ng ml^{-1}) and mean doxepin plasma level was 52 ng ml^{-1} (range 30–118 ng ml^{-1}). Pretreatment ECGs showed no significant abnormalities. Six patients showed a significant prolongation of the QRS complex while receiving NT, but only one patient showed prolongation on doxepin. There was no correlation between plasma NT or plasma doxepin levels and prolongation of the QRS width. Five of the six patients on NT, who showed significant changes of QRS width, had plasma levels in excess of 170 ng ml^{-1}. Six other NT patients also had plasma levels greater than 170 ng ml^{-1} but did not show any QRS prolongation. This study does not support the contention that conduction disturbances are seen more frequently at plasma levels in excess of 200 ng ml^{-1}. The number of patients studied here is small and does not eliminate such a possibility entirely. The study does support the authors' original proposal, that doxepin has less effect on intracardiac conduction. Doxepin plasma levels were, on the whole, much lower than those of NT. Possibly a lower bioavailability of doxepin accounts for its apparent lack of cardiotoxicity.

Cardiographic abnormalities were noted in a study of 17 depressed patients treated with NT (50–150 mg per day) for 3 weeks (Ziegler *et al.*, 1977*b*). significant increases in heart rate were observed and a number of patients exhibited ST segment and T wave abnormalities. Most of these changes were regarded as clinically insignificant. In two patients clinically significant ECG abnormalities developed during treatment. A 65 year old male developed a first degree heart block (plasma NT 129 ng ml^{-1}). Premature ventricular contractions were observed in a 33 year old female during treatment (plasma NT 176 ng ml^{-1}). This abnormality disappeared when her dose was halved and her plasma level fell to 80 ng ml^{-1}. There was a significant correlation between plasma NT level and change in heart rate for the group as a whole.

Electrocardiographic function was studied in a group of 15 patients being treated with AMI (75–200 mg per day) for 3 weeks (Ziegler *et al.*, 1977*a*). Heart rate increased significantly (mean increase was 16 beats min^{-1}). Patients with high plasma AMI or high total plasma tricyclic levels (AMI+NT) were those who most often showed the greatest increase in heart rate.

Kantor *et al.* (1975) studied seven depressed patients (six with prior ECG abnormalities) who received 175–400 mg of IMI per day. Mean heart rate determined by computer analysis of the continuous 24 h ECG recordings

increased significantly but slightly in each patient. There were also significant increases in PR, QRS and QT intervals during IMI treatment. Plasma tricyclic levels in these patients remained within the therapeutic bounds for this compound.

As a general conclusion from these studies it could be stated that usual doses of the tricyclics can lead to electrocardiographic changes in some patients and that these changes are most likely to be associated with high plasma levels of the drug. Plasma level monitoring on the other hand is not necessarily a guide to ECG changes.

Cardiovascular effects of tricyclic antidepressant overdosage

The electrocardiographic features of tricyclic overdosage include prolongation of intra- and atrioventricular conduction, sinus abnormalities including ST deviation, which may simulate acute myocardial infarction, ventricular arrhythmias, profound bradycardia, supraventricular tachycardia and asystole (Vohra and Burrows, 1974). Some or all of these complications are observed in tricyclic overdosage. What is apparent from the studies reported is the unpredictability of TCA poisoning. Severe complications are often seen in patients who ingest only small amounts of TCA while mild or no symptoms may occur in some patients who ingest two or more grams of a tricyclic. This uncertainty may be related to pharmacokinetic differences between patients. Some individual cases of TCA overdosage are presented in table 2.

Electrocardiographic changes and plasma levels in overdosage

Vohra *et al.* (1975*a*) studied 14 patients admitted with an overdosage of doxepin (n=6), NT (n=3), IMI (n=2) and AMI (n=3). None of the doxepin patients had prolongation of the H–V interval, but seven of the eight patients with overdoses of the other drugs did. Average overdose in both cases was the same (1.3 g as compared to 1.4 g). Plasma doxepin levels in one patient were $>2\ \mu g\ ml^{-1}$ soon after admission and rapidly fell over the next 24 h (Maguire, 1977). Plasma levels in other patients were not reported.

Electrocardiographic parameters and plasma tricyclic levels were determined in a study of 15 patients with overdoses (Spiker *et al.*, 1975). Ten patients overdosed with AMI, and one each with AMI plus protriptyline, doxepin, IMI, DMI and IMI+DMI. There was no correlation between total plasma tricyclic levels and maximum heart rate, lowest diastolic, or systolic blood pressure, level of consciousness, pupil size or P–R interval. Maximum QRS duration showed a strong positive correlation ($r=0.75$, $P<0.01$) with total tricyclic levels. Excluding one patient with pre-existing bundle branch block, intraventricular conduction delays were often associated with plasma levels in excess of 1000 ng ml^{-1}. As plasma levels decreased, the QRS width returned to normal. This study was confirmed in a group of 36 overdosed

Table 2 Selected cases of tricyclic antidepressant overdosage

Age	Sex	Drug*	Amount ingested	Electrocardiographic findings	References†
19	F	IMI	3.75 g	Intraventricular conduction block; complete right bundle branch block; death 13 h after O/D	10
1.5	M	IMI	1.5 g	Prolonged QRS width, depressed ST, ventricular beats, complete heart block	1
70	F	IMI	2.5 g	Cardiac arrest after 17 h	5
2	M	IMI	600–650 mg	Irregular beat 60/168 min^{-1}, AV dissociation, prolonged QRS, depressed ST segments	13
59	F	DMI	2.5 g	Prolonged QRS (0.14 s as compared to 0.08 s before O/D)	17
2	F	DMI	2.5 g	Progressive slowing of the heart; death after O/D	2
59	M	NT	1.25 g	Lengthening of PR interval, inverted T waves	3
2	F	DBZ	160 mg	Supraventricular and ventricular tachycardia, ST-T-wave changes	14
27	F	IMI	1.6 g	Cardiac arrest after 30 h	22
22	F	AMI	1.5 g	Supraventricular tachycardia, non-specific ST-T-wave changes	15
2.5	F	IMI	760 mg	First degree A-V and intraventricular block	4
1.8	M	IMI	500 mg	Supraventricular tachycardia	4
1.5	M	DBZ	1.5 g	Prolonged QRS width; supraventricular tachycardia; first degree A-V block	4
30	F	AMI	2.5 g	Prolonged QRS width	18
29	M	AMI	2.5 g		
27	F	AMI	2.5 g		
35	M	AMI	600 mg		
1.5	?	AMI	400 mg	Ventricular premature beats	7
2.3	?	IMI	500 mg	Right bundle branch block; complete heart block	7

27	F	NT	2.0 g	Intraventricular conduction defects; prolonged QRS width and prolonged H-V conduction time	21
20	F	IMI	2.0 g		
35	F	NT	1.5 g		
17	M	NT	625 mg		
22	F	DMI	2.0 g	Right ventricular conduction defect; first degree A-V block	
16	F	AMI	1.5 g	Sinus tachycardia; ST-T-wave abnormalities	16
36	F	AMI	2.5–3.0 g	Intraventricular conduction defect	
26	F	AMI	750 mg	No abnormal findings	
22	F	AMI	750–1000 mg	Prolonged QRS width	19
22	M	AMI	1.0 g	Sinus tachycardia	12
45	F	AMI	500 mg	Sinus tachycardia	12
49	F	AMI	500 mg	ST-T-wave changes	12
59	F	AMI	750 mg	No abnormal findings	12
39	F	AMI	500 mg	No abnormal findings	12
28	F	NOM	1.5 g	Sinus tachycardia	11
43	F	NOM	3.5 g	Sinus rhythm; minor T-wave flattening	20
54	M	AMI	?	QRS widening; ST-T-wave abnormalities	6
39	F	MIAN	580 mg	First degree heart block	8
53	F	MIAN	600 mg	Left axis deviation	9

* *Abbreviations:* AMI, amitriptyline; IMI, imipramine; DBZ, dibenzepine; NT, nortriptyline; DMI, desipramine; NOM, nomifensine; MIAN, mianserin.

† *References:* 1, Arneson (1961); 2, Bickel *et al.* (1967); 3, Brackenridge *et al.* (1968); 4, Brown *et al.* (1973); 5, Davies and Allaye (1963); 6, Giller *et al.* (1979); 7, Goel and Shanks (1974); 8, Green and Kendall-Taylor (1977); 9, Jansen *et al.* (1977); 10, Michon *et al.* (1959); 11, Montgomery *et al.* (1978); 12, O'Brien (1967); 13, Prout *et al.* (1965); 14, Rosenberg *et al.* (1969); 15, Slovis *et al.* (1971); 16, Spiker *et al.* (1975); 17, Tchen *et al.* (1966); 18, Thorstrand (1974); 19, Tobias and Das (1976); 20, Vohra *et al.* (1978); 21, Vohra *et al.* (1975*c*); 22, Williams and Sherker (1971).

patients all of whom had a QRS width >100 ms and a total plasma tricyclic level in excess of 1000 ng ml^{-1} (Petit *et al.*, 1976).

Spiker and Biggs (1976) found that tricyclic plasma levels could remain above 1000 ng ml^{-1} up to 96 h after overdose. They suggest that sustained plasma levels may play a role in sudden death 3–6 days after overdose. Clinically such patients should be monitored cardiographically up to 6 days, while antidepressant therapy, if commenced immediately after the overdose, may aggravate a delicate cardiological situation.

Forty patients ingesting TCA overdoses were studied as a pharmacological model to determine whether plasma tricyclic levels correlated with major adverse effects or electrocardiographic findings (Petit *et al.*, 1977). Patients were grouped into those with total plasma tricyclic levels above ($n=13$) and below ($n=27$) 1000 ng ml^{-1}. It was found that significant differences between the two groups did exist. The mean plasma level of all those patients who died, had cardiac arrest, required respiratory support, developed unconsciousness or grand mal seizures was ⩾1000 ng ml^{-1}. Similarly, the mean plasma level of all patients who experienced electrocardiographic abnormalities (ventricular rate ⩾120 min^{-1}; QRS width >100 ms; cardiac arrhythmias; bundle branch block) was > 1000 ng ml^{-1}. When each of these groups was analyzed individually, significantly more patients, with the specific major adverse effect, had plasma levels above than below 1000 ng ml^{-1}. These authors again demonstrated the trend for cardiotoxic symptoms of the tricyclics to be associated with elevated plasma levels.

Electrocardiographic changes were studied following AMI and IMI overdosage in 30 patients (Bailey *et al.*, 1978). There were 19 cases of AMI overdose and 11 of IMI. Plasma tricyclic levels were measured within 2–4 h following admission and ECGs were recorded 4–6 h after admission. On admission, plasma tricyclic levels ranged from 29 to 1260 ng ml^{-1} for AMI (total drug plasma levels) and from 123 to 1732 ng ml^{-1} for IMI (total drug levels). Prolonged QT interval, widened QRS complex, abnormal ST segments and flattened T waves were observed on admission ECGs. There was a significant difference between total tricyclic levels for patients with widened QRS complex and those without widening (821 ng ml^{-1} and 396 ng ml^{-1}, respectively). There was no correlation between plasma level and the extent of QRS broadening. Further, the range of plasma levels observed in this study overlap with those observed in patients receiving chronic oral doses, thus making the use of plasma levels alone an unreliable index of tricyclic overdosage. A parent drug-to-metabolite (P/M) ratio may be a better predictor of overdosage. A P/M ratio less than 2 is more usual in steady state levels while P/M greater than 2 strongly suggests an overdose, but its absence does not exclude it. These authors concluded that QRS broadening, arrhythmia and increased total plasma tricyclic levels represent serious cardiotoxicity.

On the basis of the studies described, the most reliable and readily available clinical index of TCA overdosage is prolongation of the QRS width by 100 ms or more. This will almost certainly be associated with elevated plasma levels of the drug. Plasma levels are not indicative of cardiotoxicity *per se*, but the finding of a P/M ratio of greater than 2 may be of some value in

diagnosing toxicity. Treatment of tricyclic overdose has been described by others (Vohra and Burrows, 1974).

Studies with new antidepressants

Nomifensine

Nomifensine is a new antidepressant of unique chemical structure. In controlled clinical studies the drug has been shown to be an effective antidepressant with few side effects, particularly those of a cardiovascular nature (Acebal *et al.*, 1976; Angst *et al.*, 1974; Madalena *et al.*, 1973; Moizeszowicz and Subira, 1977).

The effects of nomifensine on the cardiovascular system were investigated in 10 in-patients suffering from depressive illness (Burrows *et al.*, 1978*b*). Each patient was studied before treatment and at the end of a 3 week period of nomifensine. Standard 12-lead ECG and HBE recordings were made at these times. Plasma levels of nomifensine were measured during the treatment period. The results of the study are shown in table 3. Nomifensine was associated with an increase in heart rate in seven of the 10 patients, which was not statistically significant. Nomifensine had no effect on H–V interval, QRS width and other electrocardiographic parameters (A–H, PR and QT intervals and ST or T wave changes were not apparent), nor did the drug affect blood pressure significantly. There was no significant correlation between free or total plasma levels of nomifensine and any of the parameters measured in the study. The mean level of free nomifensine was 45 ng ml^{-1} and of total (free+conjugated) was 185 ng ml^{-1} for the group of patients who received 100 mg per day. For the patients who received 200 mg per day, mean free nomifensine was 75 ng ml^{-1} and mean total was 370 ng ml^{-1}.

Table 3 The effects of nomifensine on distal intracardiac conduction, heart rate and QRS width in 10 patients receiving up to 200 mg of the drug per day

Case no.	Age (years)	Sex	Dose (mg per day)	H-V interval (ms)		Heart rate (beats min^{-1})		QRS width (s)	
				Pre	Post	Pre	Post	Pre	Post
1	34	F	100	48	50	81	76	0.08	0.08
2	62	M	100	60	60	92	100	0.08	0.08
3	18	F	100	50	55	84	95	0.07	0.07
4	54	M	100	40	40	63	60	0.10	0.10
5	40	M	100	45	45	86	92	0.06	0.06
6	31	F	200	45	45	67	57	0.09	0.09
7	33	F	200	40	40	67	77	0.08	0.08
8	38	F	200	45	50	89	99	0.06	0.06
9	59	F	200	45	45	65	92	0.08	0.08
10	63	F	200	60	60	82	86	0.08	0.08

Wide interindividual variations in plasma levels were demonstrated in this study. On the basis of this investigation, nomifensine appears to be without serious effect on the cardiovascular system of man at these doses.

Reports of nomifensine overdosage in man also suggest that the drug is without serious cardiotoxic properties (Montgomery *et al.*, 1978; Vohra *et al.*, 1978). Nomifensine is apparently without serious effect on the cardiovascular system in both therapeutic doses and overdosage.

Mianserin

Mianserin is a tetracyclic piperazino-azepine compound, effective in the treatment of depressive illness (Brogden *et al.*, 1978). No consistent effect on heart rate, PR interval, QRS width or T wave amplitude was observed when mianserin was administered to 13 depressed patients in doses of 80–120 mg for 3 weeks (Peet *et al.*, 1977). In two depressed patients, mianserin prolonged the QT interval after 1 week of treatment, but by week 2 this abnormality had returned to normal. No other effects on the ECG were noted (Burgess *et al.*, 1978*a*, *b*). In a comparative study of the long term effects of antidepressant treatment, four patients treated with 40 mg of mianserin per day showed increased PR interval, QT and QRS width and T wave flattening after 3 weeks' treatment (Burckhardt *et al.*, 1978). These electrocardiographic changes returned to normal after 13 months of treatment. Only heart rate continued to be increased. In a study of 60 patients with pre-existing heart disease, 35 received 30 or 60 mg of mianserin per day for 3 weeks in a double blind trial (15 patients received a placebo) (Kopera and Schenk, 1977). Heart rate, blood pressure and ECG were monitored at various intervals throughout the trial. There were no sudden unexpected deaths and no differences between groups on the cardiovascular parameters monitored.

Intracardiac conduction was measured in 10 depressed in-patients by HBE before and after 3 weeks' treatment with 60 mg of mianserin per day (Burrows *et al.*, 1979). Blood pressure, heart rate and ECGs were recorded at the same time. Heart rate increased in six of the 10 patients, but this was not considered to be clinically significant by the investigators. No other significant changes in cardiovascular parameters were observed during the study. This investigation supports the other studies described and suggests that mianserin is without significant effects on the cardiovascular system.

On overdosage mianserin does not appear to cause the severe complications of most other antidepressants (Crome *et al.*, 1978; Jansen *et al.*, 1977). One death has been reported in a woman who ingested 600 mg of mianserin and a large dose of lorazepam (Crome and Newman, 1977); plasma mianserin was within normal limits (110 ng ml^{-1}) while the lorazepam level was 500 ng ml^{-1}, about two to three times levels observed with chronic dosing. The time elapsed between blood sampling and overdosage was not known.

The cardiovascular safety of mianserin in both therapeutic and overdoses is established by these studies. Mianserin may be of particular value in the

treatment of the depressed patient with heart disease and represents a significant advance in the treatment of depressive illness.

Zimelidine

Zimelidine (Astra H102/09) is a selective serotonin uptake inhibitor with a bicyclic structure. Limited clinical studies have shown that it possesses antidepressant effects (Benkert *et al.*, 1977; Coppen *et al.*, 1979). Burgess *et al.* (1978*b*) compared the cardiovascular effects of AMI, mianserin and zimelidine by using high speed surface ECG and systolic time intervals. Patients were studied before and after 3 weeks' drug therapy. Neither mianserin or zimelidine affected systolic time intervals. Zimelidine tended to increase QT interval and slow heart rate. None of the drugs affected blood pressure. On the basis of the eight mianserin and seven zimelidine patients studied it was concluded that both drugs are safer than AMI. In two cases of overdosage with zimelidine, both patients experienced few adverse reactions (Astra Pty Ltd., unpublished data). The safety of zimelidine awaits further studies.

Cardiovascular effects of lithium

The use of lithium salts (Li) for the treatment of manic-depressive disorders is well established, although its mechanism of action is not known (Hollister, 1976). The effects of Li on the heart are generally regarded as being infrequent, innocuous and reversible (Tilkian *et al.*, 1976). Four effects of Li on the heart have been noted: in order of decreasing frequency of occurrence they are T-wave flattening (Schou, 1962; Hansen and Amdisen, 1978); sinus node abnormalities (Wilson *et al.*, 1976); ventricular arrhythmias (Tseng, 1971; Jaffe, 1977) and myocarditis (Tseng, 1971; Swedberg and Winblad, 1974).

Most reports describing the cardiovascular effects of Li have been anecdotal or retrospective and controlled investigations are few. The effects of therapeutic levels of Li were investigated in six healthy male volunteers aged 18–23 years (Dumovic *et al.*, 1980). Subjects were studied before Li and after 2 weeks' treatment, when steady state plasma levels were obtained. A 12-lead ECG and a high speed, high fidelity ECG were used to record electrical activity of the heart. Systolic time intervals at rest and during periods of exercise were also recorded. Some minor T-wave flattening occurred in three subjects but there was no effect on any other ECG parameters. There was no correlation between plasma Li levels and the magnitude of T-wave depression. Serum potassium levels remained within normal limits for all volunteers throughout the study. It has been proposed that slow, partial intramyocardial depletion of potassium and its replacement by Li may explain the T-wave flattening seen on the ECG (Tilkian *et al.*, 1976). The observations in this study do not exclude this possibility. Systolic time intervals were not affected by Li treatment.

This study supports the contention that Li is without serious cardiotoxicity, at least in the short term. Clearly more systematic studies of the effects of long term administration of Li on the heart are required. In view of the question of the nephrotoxicity of chronic Li treatment which has recently been raised (Burrows *et al.*, 1978*a*; Hullin *et al.*, 1979), serious consideration should be given to the use of Li in patients with pre-existent heart disease.

CONCLUSION

In both animal and human studies the TCA exert significant effects on the cardiovascular system, as demonstrated by the characteristic changes observed in the ECG or by HBE. Studies of the electrocardiographic effects in relationship to plasma levels of the tricyclics, after either therapeutic doses or overdosage, so far have failed to demonstrate toxic levels of these drugs, ECG changes occurring both with therapeutic and overdoses. At present the best indication of tricyclic overdose is a significant prolongation (>100 ms) of the QRS complex on the ECG. Significant cardiotoxicity is associated with elevated plasma levels of the tricyclics. Studies with newer antidepressants such as nomifensine, mianserin and zimelidine have shown that these compounds lack the cardiotoxicity of the older drugs. Further studies to confirm these preliminary findings are required, but if they are less cardiotoxic, then they represent a significant advance in the treatment of depression, especially in patients with pre-existent heart disease. The risk of suicide, a significant component of the depressive syndrome, by overdose with these agents is markedly reduced. Regular monitoring of cardiovascular status in patients receiving prophylactic treatment with antidepressants is to be advised.

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Cardiovascular effects of the tricyclic antidepressants: implications for new research

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In the preceding chapter, Dr Burrows and his colleagues have reviewed the usual cardiovascular effects of tricyclic antidepressants (TCA). We would like now to focus in greater detail on several cardiovascular effects which are clinically important but have, until recently, been poorly understood. The specific effects we will review are the antiarrhythmic properties of tricyclic compounds, their effect on left ventricular performance, and their propensity to produce orthostatic hypotension. We will discuss why these effects have been poorly understood for so long and will review the implications of these effects on the clinical use of TCA and on our understanding of the basic pharmacology of these drugs.

Five years ago Vohra and Burrows, on the basis of a series of His-bundle studies, suggested that the TCA had many quinidine-like effects on cardiac conduction (Vohra *et al.*, 1975*a*, *b*; Burrows *et al.*, 1976). Our own studies of a patient with an imipramine (IMI)-induced heart block (Kantor *et al.*, 1975) supported their observation and prompted us to examine the possibility that, in addition to its quinidine-like effect on conduction, IMI might also exhibit quinidine-like antiarrhythmic effects. We have reported on two depressed patients both of whom had a significant number of ventricular premature depolarizations (VPDs) which declined dramatically during IMI treatment (Bigger *et al.*, 1977). To demonstrate that this antiarrhythmic effect was a primary result of the drug and not secondary to recovery from depression, we restudied one patient 10 months after he had discontinued the IMI and was free of depression. In the non-depressed drug-free state, this patient's frequency of VPDs was virtually identical to his frequency of VPDs before IMI treatment. Thus, the reduction of his VPD frequency appeared related to his taking IMI, not to his improved mood.

We recently completed a study of 44 seriously depressed patients who were treated with IMI (Giardina *et al.*, 1979). Of these 44 patients, 11 had

more than 10 VPDs per hour, a rate at which many cardiologists would institute treatment. In general, a drug that produces more than 50 percent reduction in the frequency of VPDs is considered a good antiarrhythmic compound and a drug that reduces VPDs by more than 75 percent is considered an excellent compound. In 10 of our 11 IMI-treated patients, there was more than a 90 percent reduction in the frequency of VPDs. While extensive information on this antiarrhythmic effect of tricyclic drugs is available only for IMI, animal studies suggest that this characteristic is common to all the usually used tricyclic compounds (Wilkerson, 1978).

In retrospect, it is fairly easy to see why for almost two decades this powerful antiarrhythmic activity of IMI remained obscure. In overdose, especially severe overdose, IMI is likely to cause arrhythmias. It was then assumed that the arrhythmias observed during overdose would be likely to occur at normal therapeutic doses in vulnerable individuals, especially those patients with pre-existing VPDs. Obviously, this is not true. The assumption that a drug will at a lower dose do slightly less of what it does at a higher dose can be a misleading extrapolation. For example, quinidine itself causes arrhythmias in overdose; unfortunately, for many years this fact was overlooked by psychiatrists.

The clinical implications of the antiarrhythmic effect of the tricyclic drugs are fairly obvious. Certainly these drugs are not a threat to a patient with pre-existing arrhythmias. As a matter of fact, it is clear that ventricular arrhythmias are likely to benefit from IMI treatment at the same time that the depression improves. If a problem is likely to exist in the treatment of a depressed patient with VPDs, it will be in the area of drug–drug interactions. Although it has not yet been scientifically demonstrated, one would strongly suspect that the quinidine-like effects of tricyclic compounds would be additive to other class I antiarrhythmic drugs. In other words, since the tricyclics are class I antiarrhythmic drugs one would expect that someone already on quinidine who is treated with IMI or an IMI-like compound could actually receive an excess of antiarrhythmic drug. This could result in excessive prolongation of cardiac conduction.

Perhaps more important than the safety of using these drugs in patients with pre-existing VPDs is the implication this observation has for patients who take an overdose of TCA. In view of the fact that tricyclic drugs have impressive antiarrhythmic properties of their own, it seems futile, if not dangerous, to use similar antiarrhythmic drugs to treat the ventricular arrhythmias caused by tricyclic overdose. In all likelihood, a number of patients have been killed because, after taking an overdose of a tricyclic compound, they were treated with drugs of the same antiarrhythmic family. Serious tricyclic overdoses are probably best treated in the same way as overdoses of quinidine (Hoffman and Bigger, 1971).

The antiarrhythmic properties of the TCA may tell us something about the effect of these drugs on the brain. It is far easier to examine isolated cardiac tissue than it is to examine isolated brain tissue. Two pairs of authors have now studied the effects of TCA on isolated Purkinje tissues as well as on ventricular muscle (Weld and Bigger, 1980; Rawling and Fozzard, 1979). Both have found imipramine to have striking effects on the fast

sodium channels (see figure 1). This startling observation on isolated cardiovascular tissue raises the question of whether IMI also affects sodium channels in nerve fibers. Cases of death from IMI overdose have long been known to show IMI tightly bound to the heart (Moccetti *et al.*, 1971). IMI is also known to bind tightly to isolated Purkinje fibers, though it is not clear whether sodium channels are involved in this phenomenon. One might even wonder if there is any connection between this binding and the recently described high affinity site for IMI on human platelets (Briley *et al.*, 1980). The relevance of these cardiovascular effects to the mode of action of IMI on the brain remain to be seen; however, it is tempting to speculate that IMI's effect on the fast sodium channel in some way relates to what we refer to as 're-uptake blockade' in neuronal tissue. It is difficult to imagine that a pharmacological effect so dramatic as that seen in cardiac tissue does not exist in some form in neuronal tissue as well.

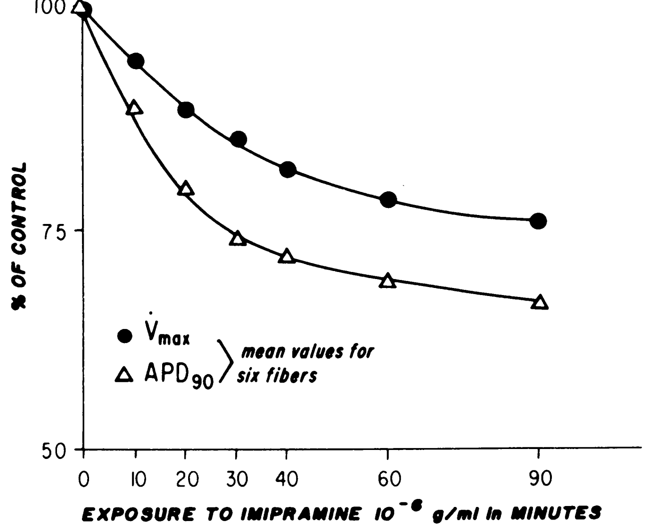

Figure 1 Time course of action potential changes during exposure to IMI. V_{max} (●) is the maximal upstroke velocity; APD_{90} (△) is the action potential duration at 90 percent repolarization. (Reproduced with permission from Weld and Bigger, 1980.)

Another important but largely unexplored topic is the effect of TCA on left ventricular performance. For many years, left ventricular performance could be measured accurately only via cardiac catheterization, a procedure which cannot be justified for most psychiatric patients. Therefore, studies of the effects of TCA on cardiovascular function have generally relied on indirect measures of ventricular performance. Both Müller and Burckhardt in Germany (Müller and Burckhardt, 1974; Burckhardt *et al.*, 1978) and Taylor and Braithwaite (1978) in England reported that ventricular performance as measured by systolic time intervals appeared to decline in the presence of TCA. However, we have reservations about the accuracy of

systolic time intervals as a measure of left ventricular performance in patients taking TCA. Systolic time intervals have been used to assess the effects of a number of drugs on the mechanical activity of the heart. An integral part of the method is the measurement of the pre-injection period – the interval between the beginning of ventricular depolarization, as documented on the ECG, and the rise in pressure in the carotid artery. The TCA may distort or even invalidate this method of assessing left ventricular performance because they alter cardiac conduction (Giardina *et al.*, 1979). In order to evaluate the accuracy of systolic intervals as a measure of left ventricular performance in patients on TCA, we used both systolic time intervals and echocardiography to assess cardiovascular function in a group of patients without overt cardiac disease. Like Müller and Burckhardt (1974), Burckhardt *et al.* (1978) and Taylor and Braithwaite (1978), we found that the systolic time intervals were abnormal during TCA treatment. In contrast, however, there was no change in cardiac performance as measured by echocardiography (Giardina *et al.*, 1981).

Recently, non-invasive techniques utilizing short-lived radionuclides have been developed to measure left ventricular performance (Marshall *et al.*, 1977). In one method, a bolus of technetium-99 is injected into an antecubital vein and a series of cardiac images are obtained on a scintillation camera and stored on a computer. In this technique the radioactivity present in the heart during diastole and systole is measured during a series of normal ventricular contractions. The fraction of radioactivity ejected from the heart during an average contraction can be accurately determined, yielding a relatively precise index of ventricular performance. We are presently involved in a series of studies using this methodology to determine whether the cardiac function of patients with left ventricular impairment is further compromised by treatment with antidepressant drugs.

In the course of our studies of the cardiovascular effects of tricyclic drugs, we have become increasingly impressed by the ability of these compounds to produce orthostatic hypotension (Glassman *et al.*, 1979; Roose *et al.*, 1980). In our experience, orthostatic hypotension is the most common serious cardiovascular complication of therapeutic concentrations of these drugs. The magnitude of the fall in blood pressure (as measured in millimeters of mercury) does not seem to increase with age. However, it appears obvious that the risk inherent in any given drop of blood pressure increases greatly with age (that is, an orthostatic blood pressure fall of 40 mm Hg is likely to be far better tolerated by someone 35 years old than by someone 65 years old). Furthermore, we have recently had reason to wonder whether patients with congestive heart failure are at higher risk for developing orthostatic hypotension than those patients without pre-existing heart failure. In spite of the frequency and severity of this symptom, little is understood of the mechanism involved. U'Prichard *et al.* (1978) suggested that the degree of orthostatic hypotension correlates well with the α-adrenergic blocking activity of the tricyclic drugs. However, our own studies of orthostatic hypotension raise questions about this supposition. We have found little difference in the ability of IMI and desmethylimipramine to cause orthostatic hypotension in spite of the fact that they show significant differences in

α-adrenergic binding activity (Roose *et al.*, 1980; U'Pritchard *et al.*, 1978). On the other hand, nortriptyline (NT), whose α-adrenergic binding activity is very similar to that of IMI, seems to cause far less postural hypotension. U'Prichard's binding studies were conducted with WB401, a compound now thought to bind primarily to α_1-noradrenergic sites. Recently the French group of Lecrubier *et al.* (1980) has shown that yohimbine, an α_2-blocking agent, is capable of diminishing the orthostatic effect of IMI. This raises the question of whether there are differences in α_2-adrenergic blocking activities between various tricyclic drugs. This would be of interest on theoretical grounds because IMI and NT, although they vary in their potential to induce orthostatic hypotension, are equally effective antidepressant compounds. The α_2-blocking action of these drugs could be excluded as the mechanism of their antidepressant effect if binding studies would show that these equally effective tricyclic compounds differed significantly in their α_2-blocking ability. At any rate, the combination of such basic pharmacology with carefully documented clinical differences between the drugs offers a unique tool for exploring the way in which these drugs work. Such observations also have practical significance; clearly, in the treatment of older patients particularly vulnerable to the consequences of orthostatic hypotension, antidepressant drugs without significant orthostatic effects that still retain potent antidepressant activity are quite valuable.

Again, in a theoretical way, it is interesting to compare those tricylic effects that develop immediately to those that develop later, as well as effects to which tolerance develops to those where it does not. In many instances, precise data are simply not available. However, it is clear that in spite of clinical impressions to the contrary, orthostatic hypotension develops at very low plasma levels of IMI and tolerance does not develop over many weeks of repeated measurements. On the other hand, heart rate tends to increase on IMI initially and then to return toward baseline, even in the face of rising plasma levels of the drug. Similarly, the sedative effects of doxepin and amitriptyline often occur after a single dose while the antidepressant effect of these drugs occurs much later. It has been suggested that the delay in the antidepressant effect is related to the time required to reach steady state, but this, at best, is only part of the answer. On the other hand, the development of IMI's antiarrhythmic activity is very much related to the time required to reach adequate plasma level.

Thus, we hope we have illustrated that the careful study of the side effects of antidepressant drugs serves not only to provide a sounder basis for therapeutics and, therefore, promotes the more rational use of these compounds, but also supplies us with tools to pursue a more fundamental understanding of the basic pharmacology of the drugs. It allows us to separate the activities of the drugs that are fundamental to their antidepressant action from those that are not. We can also explore the relationship between the drugs' effects on sodium channels in cardiac tissue and the so-called 'blockade of re-uptake' of neurotransmitter substances in the brain. Finally, it may even be possible to investigate the complex consequences of stimulating or blocking various receptors in the central nervous system.

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Antidepressant treatment with imipramine and nortriptyline in elderly patients

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INTRODUCTION

While there is still disagreement on the importance of monitoring the plasma level concentration of tricyclic antidepressants (TCA) to achieve better therapeutic effect (Gram and Kragh-Sørensen, this volume), there is general agreement on the significance of plasma concentration monitoring in achieving greater safety in the treatment of elderly patients. This agreement should be seen in relation to the expected increase in undesired effects, in particular cardiovascular reactions in the elderly who may display higher plasma levels on conventional doses than younger patients do. However, at the present time our knowledge of cardiovascular effects of TCA in the elderly is rather limited, and the practical value of TCA plasma concentration monitoring has not been studied systematically.

At the Clinical Psychopharmacology Research Unit at Odense University, therefore, we have established a standardized investigation program for elderly depressed patients treated with various TCA. Patients above 60 years of age with endogenous depression are examined in the research program that includes the following: (1) an open, controlled TCA treatment schedule with plasma level monitoring with 1 week on placebo and then 6 weeks of active treatment; (2) registration of diagnoses, degree of depression and effect of treatment by use of diagnostic and quantitative rating scales; (3) weekly registration of orthostatic blood pressure (BP) reaction (6 min standing); (4) systolic time interval and 24 h monitoring of electrocardiogram in the placebo week, and in the second and third week of active therapy.

So far we have used this study design in patients treated with imipramine (IMI) and nortriptyline (NT). In this paper, comparable results for the two

drugs concerning orthostatic hypotensive effect and changes in systolic time intervals (STI) in relation to drug plasma levels will be discussed. For further details, see Thayssen *et al.* (1980).

THE HYPOTENSIVE EFFECT OF IMIPRAMINE AND NORTRIPTYLINE

Recently Glassman *et al.* (1979) concluded from a study on the hypotensive effect of IMI in depressed patients that (1) there was no tendency to diminished orthostatic reaction or decreased frequency of subjective hypotensive symptoms over time (4 weeks' treatment); (2) the orthostatic BP drop was independent of age, sex, plasma levels (IMI + desipramine (DMI)) and pre-existing heart disease; (3) the best predictor of orthostatic hypotension during treatment was the degree of orthostatic BP drop before treatment.

In our study on IMI, dose-dependent kinetics (Bjerre *et al.*, 1980) and hypotensive reactions resulted in variable and low or subtherapeutic plasma concentrations (2nd week: IMI + DMI 54–319 ng ml^{-1}, median 74 ng ml^{-1}).

In spite of this we found a statistically significant increase in orthostatic BP drop from the placebo week to active treatment (figure 1). In two female patients this increased drop in orthostatic systolic blood pressure caused a fall and fracture of the collum femoris. The supine BP was not significantly influenced by IMI. Our results suggest a more pronounced effect on the

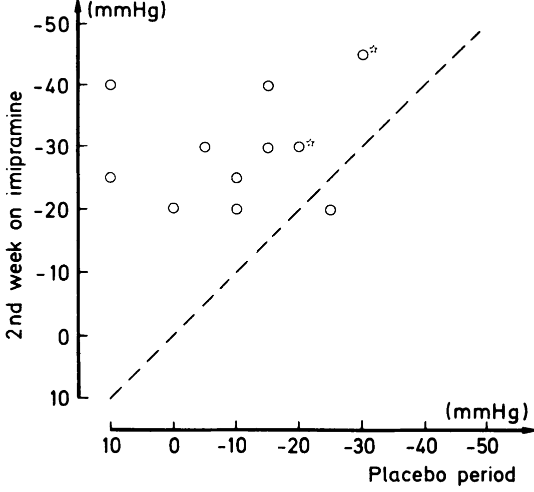

Figure 1 Systolic orthostatic blood pressure drop before and during imipramine treatment in 11 elderly patients (age 62–84 years). Starred points indicate fall with fracture.

orthostatic BP with increasing drug levels affecting as much the duration of the BP drop as the maximal magnitude of the BP drop. This pattern was very clear in a 79 year old woman that was followed during the days immediately before and after discontinuation of the IMI treatment (figure 2). It can be seen that the systolic BP slowly normalized after discontinuation of the IMI treatment. Even within 24 h, a quicker return to normal systolic BP can be seen when the patient stands up, but the immediate BP drop is unchanged.

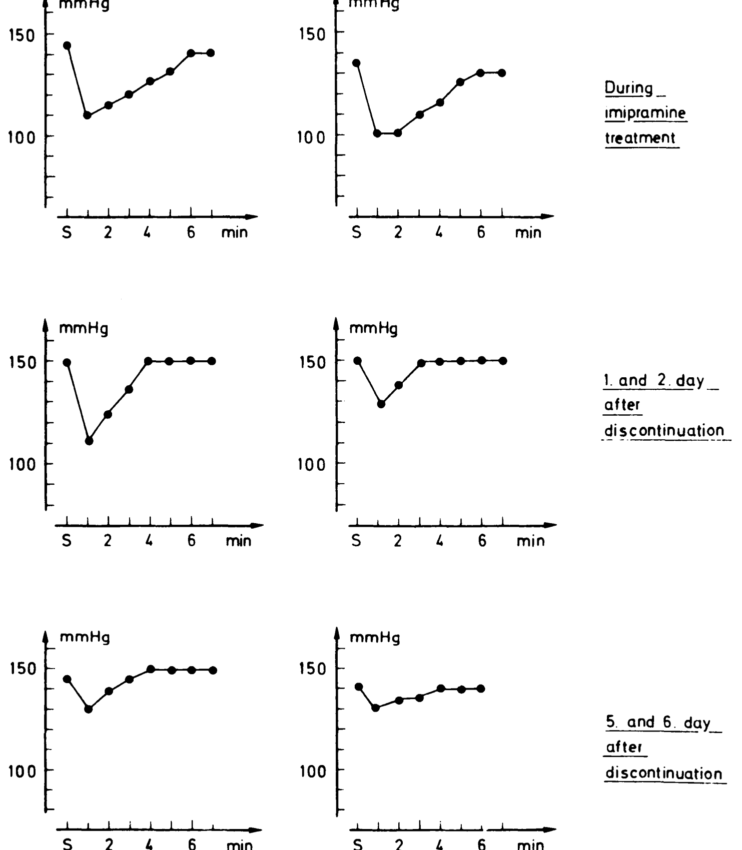

Figure 2 Systolic blood pressure in supine position (S) and after 1–7 min standing in an imipramine-treated 79 year old female patient.

In contrast to Glassman *et al.* (1979), we found a moderate positive correlation between orthostatic BP drop and the IMI levels ($r_s = 0.38$), whereas there was no correlation to the DMI levels. The data from the 79 year old woman followed after discontinuation also illustrates this (figure 3). The reduction in average systolic BP drop closely followed the decline in IMI plasma concentration, both being about 50 percent after the first 24 h, whereas the DMI level had only dropped 15–20 percent at this time.

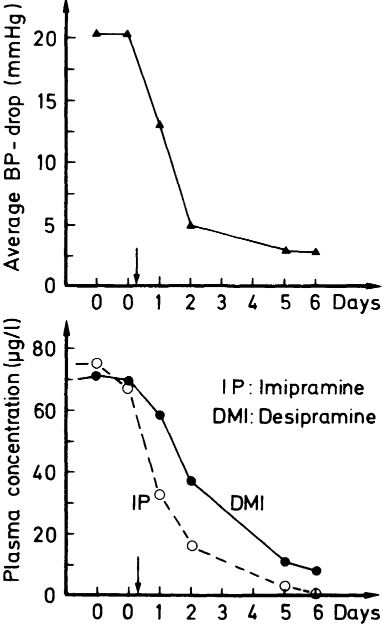

Figure 3 Systolic orthostatic blood pressure drop before (0) and after (1–6) discontinuation (↓) of imipramine treatment in a 79 year old female patient. Average of systolic pressure during BP drop the first 6 min standing.

As stated by Glassman *et al.* (1979), the best predictor of orthostatic hypotension during IMI treatment was the degree of orthostatic reaction before treatment. As shown in figure 1, our limited material did not indicate a similar relationship, but the two patients with falls and fractures were among those patients with the largest orthostatic BP drop in the placebo period.

In contrast to IMI, NT only caused a moderate, and clinically not significant, drop in orthostatic BP. Ten elderly endogenously depressed patients of age 61–84 (mean 70 years) all with therapeutic NT levels (2nd week: 45–185 ng ml^{-1}) were studied. In none of these patients were troublesome hypotensive effects seen, and the orthostatic BP reaction was significantly lower than after IMI treatment (Thayssen *et al.*, 1980). The difference between IMI and NT is illustrated in figures 2, 3, 4 and 5, which show the hypotensive effect of the two drugs in the same patient. This 79 year old female patient was treated on two occasions with IMI and later with NT. Although the effect of NT was less pronounced than for IMI, the effect of increasing plasma concentration was the same (figure 5), influencing more the duration of BP drop while standing than the magnitude of the orthostatic BP drop (figures 2 and 4). These results with NT accord with those of Freyschuss *et al.* (1970) who studied 40 younger patients treated with NT (mean age 44 years) and found no change in postural BP. It seems, therefore, justified to conclude that NT treatment does not give rise to clinically significant hypotensive effects in any age group.

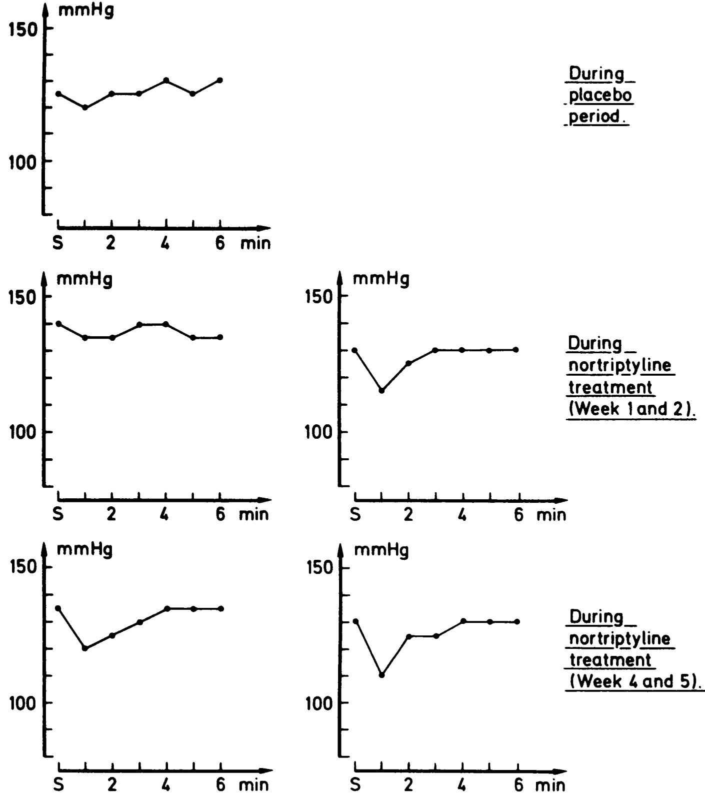

Figure 4 Systolic blood pressure in supine position (S) and after 1–6 min standing in a nortriptyline-treated 79 year old female patient.

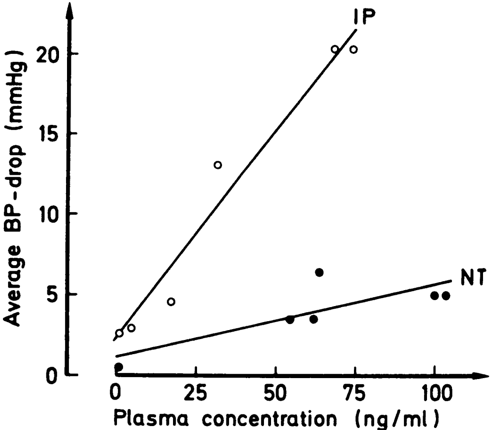

Figure 5 Correlation between imipramine (○) or nortriptyline (●) plasma concentration and average drop in systolic blood pressure (6 min) in a 79 year old female patient.

SYSTOLIC TIME INTERVAL MEASUREMENTS DURING IMI AND NT TREATMENT

During IMI treatment we found no changes in the ratio pre-ejection period (PEP) or the PEP/LVET ratio (LVET: left ventricular ejection time). These results contrast with earlier studies (Burgess *et al.*, 1979; Raeder *et al.*, 1978; Taylor and Braithwaite, 1978). The relatively low plasma levels (IMI + DMI) in our patients may possibly explain these differences. Our results for NT indicate that this drug significantly influences PEP and the PEP/LVET ratio (Thayssen *et al.*, 1980). These results agree with earlier studies (Taylor and Braithwaite, 1978) in which a significant correlation between PEP and the ratio PEP/LVET and NT plasma concentrations was found.

Within the plasma NT concentration range, in our patients the changes in STI were the same as those found by Taylor and Braithwaite (1978) in their investigation involving mostly younger patients (age 22–71, mean 46 years, $n = 8$). These results suggest that impairment of ventricular contractility in elderly is of the same magnitude as in younger patients. This is in accordance with an earlier investigation in which no cardiotoxicity was observed in elderly depressed patients during NT treatment controlled by plasma concentration measurements (Sørensen *et al.*, 1978).

Due to the low plasma concentrations in the IMI-treated patients (low or subtherapeutic levels) compared to the NT-treated patients (therapeutic levels) the significance of the weaker effect of IMI on the STI measurement is difficult to assess.

CONCLUSION

The data from our research program have pointed out some clinically significant differences between IMI and NT in the treatment of elderly depressed patients.

The disproportionality between dose and steady state levels of the metabolite DMI (Bjerre *et al.*, 1980), and the sometimes severe orthostatic hypotension occurring at subtherapeutic drug levels, seriously limits the use of IMI in the elderly. Possibly the orthostatic BP reaction can be predicted from pretreatment values, but the present data are not sufficient to establish the absolute safety of this procedure. For NT this study, in line with earlier observations in the elderly (Kragh-Sørensen and Larsen, 1980), showed direct proportionality between dose and steady state levels. The hypotensive effect of therapeutic levels of NT was clearly less pronounced than that of IMI and did not require dose reduction in any of the patients. A possible risk associated with the use of NT in the elderly might be indicated by the changes in STI, but the clinical significance of these moderate changes is not clear. Altogether NT treatment guided by drug level monitoring and

maintenance of therapeutic plasma levels seems to be reasonably safe in the elderly.

Systematic studies on the use of other antidepressants in elderly patients are needed, however.

ACKNOWLEDGEMENTS

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Neurological side effects and plasma and CSF levels

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INTRODUCTION

The occurrence of neurological symptoms of extrapyramidal type represents a major problem in the treatment of schizophrenic patients with antipsychotic drugs. Some of these symptoms were described a few years after the introduction of chlorpromazine (CPZ) in psychiatry. Delay and Deniker (1961) included them in their *'syndrome neuroleptique'*, a symptom complex they considered to be typical for the new antipsychotic drugs that were introduced during the 1950s. It has been estimated that between 40 and 50 percent of the population of schizophrenic patients develop these symptoms during treatment with the different types of neuroleptics that are currently available on the market.

Two types of extrapyramidal manifestations with different clinical pictures and probably also different pathophysiological backgrounds have been shown to occur (table 1):

Table 1 Neurological manifestations during neuroleptic treatment

Acute:	Pseudoparkinsonism
	Acute dystonia
Late:	Tardive dyskinesia

(1) *Pseudoparkinsonism* usually appears early during the treatment. It is often dose-dependent and is conventionally treated by dose reduction or the administration of anticholinergic drugs. Typically it involves all the major symptoms of the Parkinson syndrome, that is, akinesia, rigidity, tremor and salivation. *Acute dystonia* also occurs early during treatment. It has an irregular appearance and is responsive promptly to the administration of

anticholinergic drugs. This side effect is manifested by tonic contractions of the tongue, mouth and masticatory muscles.

Both pseudoparkinsonism and acute dystonia are considered to be related to the blockade of dopamine (DA) receptors in the basal ganglia. These *acute* extrapyramidal symptoms can be produced by all the neuroleptic compounds available on the market. The highest frequencies of these manifestations are obtained, however, by phenothiazine, thioxanthene and butyrophenone compounds with piperazine groups in the side chains. These compounds are also known to have the strongest potency in blocking receptors for DA in the basal ganglia.

(2) *Tardive dyskinesia* typically appears after several years of treatment with neuroleptic drugs, but it can also manifest itself during the first month of treatment. The symptoms consist of rapid movements of the tongue, lips and jaws. These symptoms are not amendable to treatment by antiparkinsonian drugs. Moreover, they have a tendency to increase during withdrawal of the neuroleptic compounds that produce them. In many patients tardive dyskinesias have been shown to be irreversible. They seem to occur in a high frequency in older patients but they can also be seen in young individuals in early phases of treatment. The pathophysiological mechanism involved in the syndrome is still unclear. However, development of supersensitive DA receptors and irreversible toxic effects involving cell loss in the basal ganglia have been suggested.

The acute as well as the late appearing neurological symptoms have been shown to occur with phenothiazine, thioxanthene and butyrophenone neuroleptics. These types of neurological signs do not appear during treatment with other types of psychoactive compounds such as tricyclic antidepressants, monoamine oxidase inhibitors or sedatives. Therefore, it has been generally recognized that these symptoms are part of the pharmacodynamic effect spectrum of neuroleptics and are not related to the production of specific toxic drug metabolites or other pharmacokinetic variables. This is one reason why the neurological symptoms of antipsychotic drug treatment have been studied predominantly by groups interested in pharmacodynamics whereas groups interested in pharmacokinetics have paid relatively little attention to them. The rather irregular appearance of the extrapyramidal symptoms in a fraction of the patients treated with neuroleptic drugs represents another problem for the quantitative analysis of this type of side effect in relation to pharmacokinetic variables.

PHARMACOKINETICS AND ACUTE EXTRAPYRAMIDAL SYMPTOMS

A number of studies have indicated that the appearance of Parkinsonism during antipsychotic drug treatment is related to the dosage administered. It seems logical to assume that this relationship is due to the accumulation of high tissue concentrations in high dose treatment with neuroleptics. These

high concentrations should then directly cause the appearance of the side effect. However, only few studies have been designed to give an answer to this question.

A few years ago we were interested in analyzing the possible occurrence of relationships between concentrations of CPZ in plasma and cerebrospinal fluid (CSF) and various clinical effect profiles in schizophrenic patients (Wode-Helgodt *et al.*, 1978). For this purpose we designed a study where schizophrenic patients were given CPZ for 4 weeks in fixed doses. The general protocol of the study is depicated in figure 1. On a blind basis

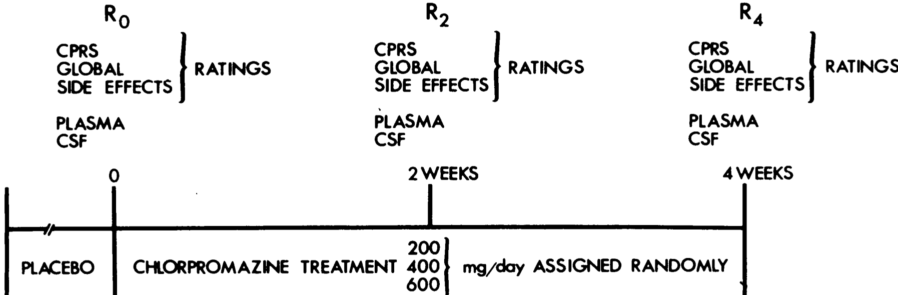

Figure 1 Protocol for chlorpromazine study.

patients were given 200, 400 or 600 mg of CPZ per day. Ratings of psychopathology and side effects were performed after 2 and 4 weeks' treatment. At the same time intervals, concentrations of CPZ in serum and CSF were determined by mass fragmentography (Alfredsson *et al.*, 1976). The occurrence of extrapyramidal symptoms of acute type were rated according to a modified version of the Simpson and Angus rating scale (Simpson and Angus, 1970; table 2). The items that were rated on the basis

Table 2 The modified Simpson and Angus rating scale

Observed items	*Reported items*
1. Gait	7. Somnolence
2. Elbow rigidity	8. Muscular tension
3. Tremor	9. Tremor
4. Salivation	10. Dry mouth
5. Akathisia	11. Increased salivation
6. Dystonia	12. Constipation
	13. Trouble starting urine
	14. Ejaculation disturbance
	15. Potency disturbance
	16. Amenorrhea
	17. Lactation
	18. Blurred vision
	19. The head feels heavy
	20. Headache
	21. Vertigo

of observation were gait, elbow rigidity, tremor, salivation and dystonia. Rating scores obtained in these items were added and were assigned to represent a global measurement of observed extrapyramidal symptoms ($\Sigma 6_{EP}$).

Table 3 Observed side effects in CPZ-treated psychotic patients

Item		Men			Women		
	Dose CPZ (mg)	200	400	600	200	400	600
	n	5	5	5	8	8	7
		Freq. %	Freq. %	Freq. %	Freq. %	Freq. %	Freq. %
1. Gait							
2 weeks		20	0	20	0	0	14
4 weeks		20	40	40	0	12	43
2. Elbow rigidity							
2 weeks		20	20	0	0	25	29
4 weeks		40	40	40	0	37	57
3. Tremor							
2 weeks		20	60	60	12	50	86
4 weeks		40	20	40	0	37	57
4. Salivation							
2 weeks		0	0	0	0	12	14
4 weeks		0	0	0	0	0	14
5. Akathisia							
2 weeks		0	0	0	0	12	0
4 weeks		0	0	0	0	25	14
6. Dystonia							
2 weeks		0	0	0	0	0	0
4 weeks		0	0	20	0	0	14

Table 3 presents the frequency of patients that displayed different types of side effects. The occurrence of gait disturbance, elbow rigidity and tremor were the most predominant side effects observed. Approximately 50 percent of the patients exhibited these side effects after 4 weeks treatment with the highest CPZ dose. Salivation and dystonia appeared to occur in a lower frequency. The rating of the global score of acute neurological signs and its relationship to the dose of CPZ administered in men and women is shown in figure 2. In both sexes there appears to be a relationship between the total amount of neurological symptoms and the dose of CPZ administered. The results obtained gave no indication for a sex difference in the occurrence of these side effects.

Concentrations of CPZ and two of its active metabolites, desmethylchlorpromazine and 7-hydroxychlorpromazine (7-OH-CPZ) were determined in the plasma of these patients on the same occasion as the rating of extrapyramidal symptoms was performed. CSF concentrations of CPZ were also determined. This design allowed an analysis of the possible relation-

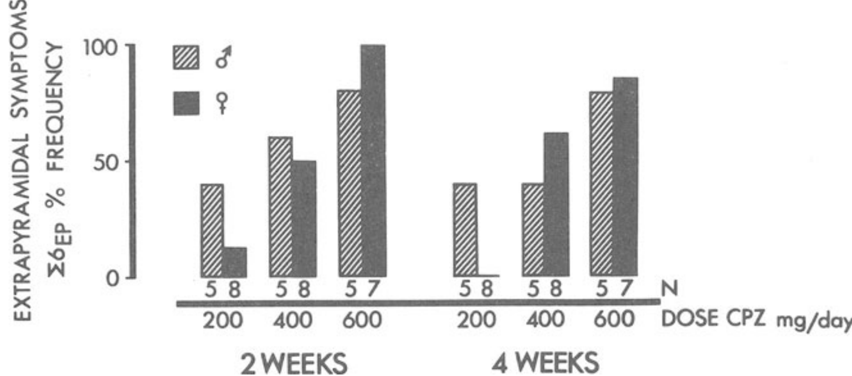

Figure 2 Extrapyramidal side effects during CPZ treatment.

ships between the neurological side effects and the drug concentrations in these body fluids. The drug concentrations obtained in plasma are presented in figure 3. CPZ occurs in the highest amount in plasma. The two metabolites occur in concentrations which are less than 20 percent of the concentration of the parent compound. There is a clear-cut relationship between the dosage of CPZ administered and the mean concentrations of the metabolites in plasma. Also, similar relationships were obtained for the CPZ concentration in CSF. The analysis of the quantitative relationships between different pharmacokinetic variables as the dose and the concentration of CPZ in the body fluids is presented in table 4. There are highly significant correlations between the CPZ concentration in the CSF and the extrapyramidal global score after 4 weeks of treatment. There was also a significant correlation with the dose of CPZ, but the highest correlation coefficients for the side effects were with the CPZ concentration in CSF.

Table 4 Relationships (r) between pharmacokinetic data and acute extrapyramidal side effects $\Sigma6_{EP}$ in CPZ-treated psychotic patients

	Duration of treatment					
	2 weeks			4 weeks		
Sex	M+F	M	F	M+F	M	F
n=	31	12	19	28	12	16
CPZ dose (mg)	0.51‡	0.39	0.56‡	0.57§	0.59*	0.54*
CPZ dose (mg kg^{-1})	0.36*	0.31	0.39	0.44†	0.49	0.38
CPZ conc. in plasma	0.50‡	0.12	0.71§	0.68§	0.53	0.73‡
CPZ conc. in CSF	0.53‡	0.33	0.62‡	0.71§	0.75†	0.73‡

r=Spearman's rank correlation.
*P<0.001. ‡P<0.02.
†P<0.01. §P<0.05.

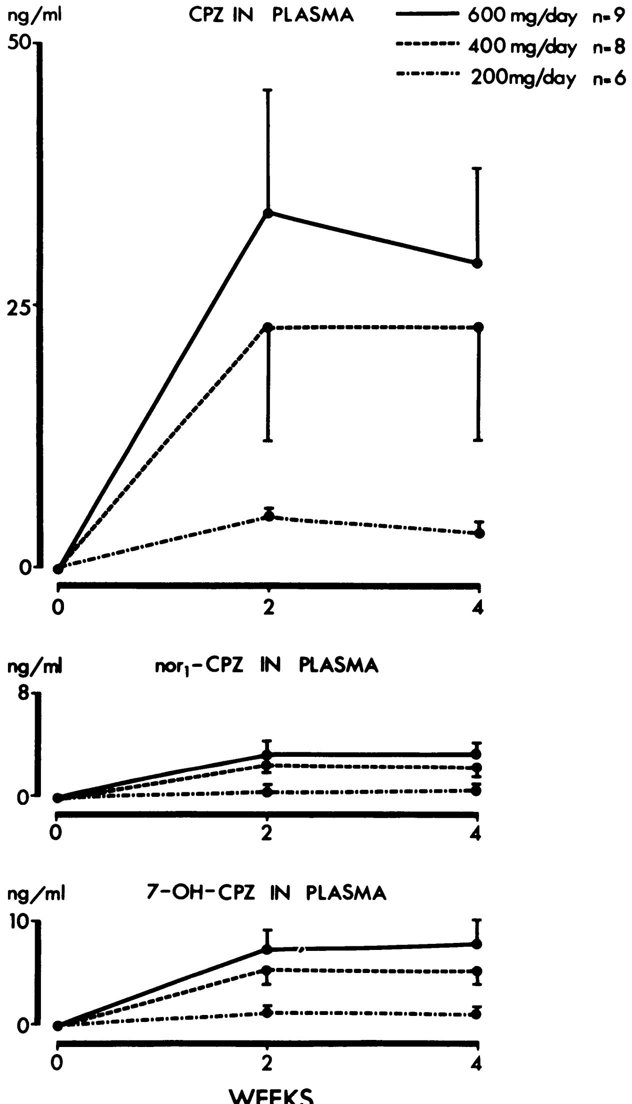

Figure 3 Chlorpromazine and metabolites in plasma from patients treated with three different doses.

These observations, obtained in a double-blind study, clearly indicate the occurrence of quantitative relationships between the frequency and intensity of acute neurological symptoms and drug concentrations in the body fluids of CPZ-treated patients. This kind of result is generally encouraging to the clinical psychiatrist and indicates that behavioral neurological symptoms can be reliably and validly measured by a conventional rating scale.

The results are also compatible with the view that the concentration of unchanged CPZ in the central nervous system (CNS) is directly responsible for the manifestation of the neurological side effects in CPZ-treated patients.

In our earlier studies, we found that the sum of the CPZ and active metabolite concentrations of the plasma did not result in any higher correlation with the occurrence of extrapyramidal symptoms than the CPZ concentration did. On the basis of this, we made the tentative suggestion that the active metabolites of CPZ (which, in plasma, constitute only a minor fraction of the concentration of the parent compound) did not markedly participate in the production of extrapyramidal symptoms. At that time, our mass fragmentographic methodology did not allow the reproducible quantification of the active metabolites in the CSF. Later, however, an improvement of the sensitivity for estimation of 7-OH-CPZ in CSF has allowed us to do quantitative estimates of the concentration of this metabolite also in drug-treated patients. The results obtained indicate that the proportion of 7-OH-CPZ in relation to the parent compound is higher in CSF than in plasma. Thus, whereas in the plasma the concentration of 7-OH-CPZ is much lower than that of CPZ, they are of the same magnitude in the CSF

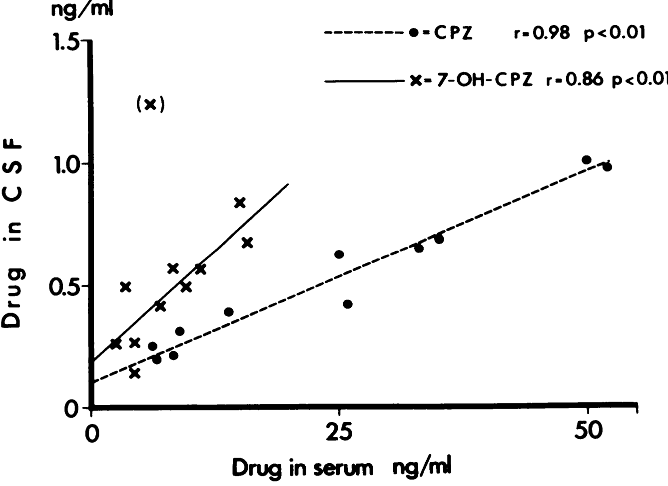

Figure 4 Correlation between serum and CSF levels of CPZ and 7-OH-CPZ.

(figure 4). So far we have not been able to do any quantitative blind study on the relationship between the concentration of 7-OH-CPZ in CSF and the occurrence of different clinical effect variables in drug-treated patients. Therefore we can not as yet exclude the possibility that 7-OH-CPZ also plays a major role in some of the pharmacodynamic effects of CPZ treatment (figure 4).

PROTEIN BINDING OF CHLOPROMAZINE IN CSF

In our earlier studies on CPZ concentrations in CSF, we found that the concentration in CSF was, on the average, 2–3 percent of the plasma concentration (Alfredsson *et al.*, 1976). Subsequent studies demonstrated that the degree of protein binding of CPZ in plasma is about 99 percent, which signifies a free fraction of only 1 percent. This finding necessitated a modification of our initial hypothesis that the CPZ concentration in the CSF reflects the free fraction in plasma. One reason for the unexpectedly high CPZ concentration in CSF could be a significant degree of protein binding of CPZ in this body fluid. With the use of equilibrium dialysis and CPZ-saturated chambers we have now initiated a systematic study on the protein binding of CPZ in CSF (Alfredsson and Sedvall, 1981). We found that there is a significant degree of protein binding of CPZ in CSF. There is also a marked individual variation (figure 5). The degree of binding of CPZ added

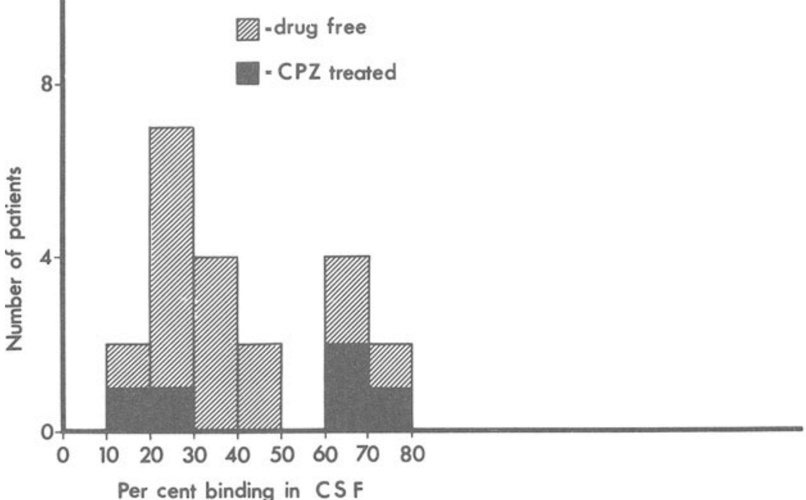

Figure 5 Protein binding of chlorpromazine in CSF.

in vitro is similar to the degree found during the *in vivo* treatment of patients. Serum diluted to the same protein concentration as in the CSF gives about the same protein binding of the drug as found in native CSF (figure 6).

The marked interindividual variation in the degree of protein binding of CPZ in CSF is an important factor which further complicates the possibility of finding high correlations between drug concentrations in this body fluid and clinical and biochemical effect variables. However, the possibility of compensating for the individual variation by measurement of protein binding in equilibrium dialysis should theoretically make it possible to reduce this variation.

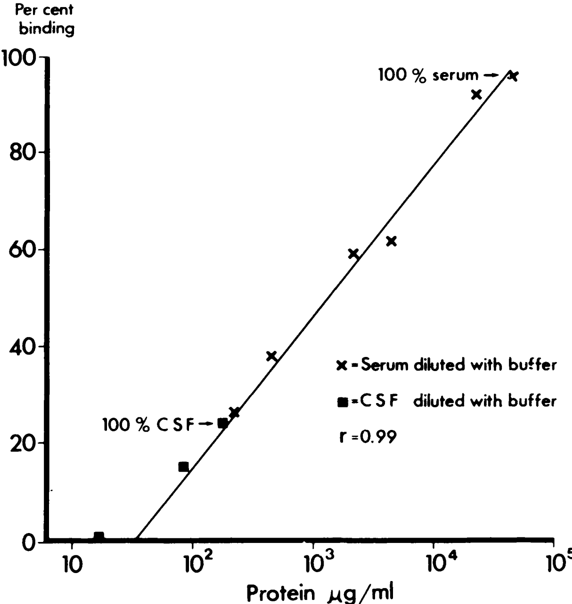

Figure 6 Protein binding of chlorpromazine in solutions with different protein concentrations.

GENERAL CONCLUSIONS

The present study clearly demonstrated the possibility of finding significant correlations between the occurrence of extrapyramidal side effects of the acute type and drug concentrations in the body fluids of CPZ-treated patients. However, the occurrence of active CPZ metabolites in high concentrations in the CNS and the marked degree of protein binding of CPZ and possibly also its metabolites in CSF complicates the possibility of drawing firm conclusions about the role of different pharmacokinetic variables for extrapyramidal manifestations. Further studies on the possible relationship between free CPZ concentrations in the CSF and quantitative aspects of the acute neurological symptoms during neuroleptic treatment may turn out to be useful for further analyzing the pathophysiology of these highly disturbing symptoms.

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Clinical pharmacological approaches to evaluating tardive dyskinesia

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INTRODUCTION

During the last two and one-half decades, neuroleptic medications have provided both a practical efficacious treatment for schizophrenia (Davis *et al.*, 1975), and a theoretical framework for investigating psychoses and movement disorders. Tardive dyskinesia (TD), a potentially irreversible neurological syndrome of involuntary hyperkinetic movements in the oral, facial, limb and truncal regions, is linked with chronic use of neuroleptics and poses a critical problem in the long term management of schizophrenia. The pathophysiological basis underlying the development of TD is yet to be completely clarified, but evidence indicates that it involves at least a primary reduced dopamine (DA) neurotransmission followed by relative DA overactivity that has been characterized as DA receptor hypersensitivity, and perhaps involves specific subtypes of DA receptors. This may lead to an imbalance between the reciprocal DA and acetylcholinergic influences in the nigrostriatal systems. In addition, recent studies in animal models of movement disorders have suggested that neuroanatomical and neurochemical interrelations in the basal ganglia which are mediated by GABA and the endorphins may also play a role in TD (Tarsy and Baldessarini, 1976; Gerlach, 1979*a*; Jeste and Wyatt, 1979; Mackay and Sheppard, 1979).

To increase our understanding of the mechanisms of irreversible TD, we conducted a series of pharmacological trials with agonists and antagonists of the central nervous system (CNS) neurotransmitters DA, acetylcholine, GABA, and peptide fragments of the β-lipotropin hormone_{61-91} ($\beta\text{-LPH}_{61-91}$). With this approach, hypotheses that evolve from animal models can be tested in the clinic, the relative importance of disturbances in neurotransmitters can be investigated, and ultimately, leads for new therapeutic agents can be identified and evaluated.

METHODS

The research methods described below were generally applied in all the investigations. Psychiatric in-patients who had received neuroleptics for 2 years or more, had stable TD for at least 6 months prior to the investigations, and who were physically healthy gave informed consent to participate in these controlled trials. Baseline medications being taken at the time a study was initiated were continued throughout the entire investigation. Since the symptoms of TD and other movement disorders can fluctuate due to non-specific factors, a placebo period at the beginning and end of each long-term drug trial was used to control for non-specific effects. In the design that utilized single dose injections over a wide dosage range, a placebo was used so that drug effects could be compared to placebo as well as to each other.

Tardive dyskinesia and parkinsonian symptoms were recorded at regular intervals on videotapes during a standardized examination that included sitting, standing, walking, distraction by conversation, and performing voluntary movements of non-affected muscle groups, such as writing. Recordings of 5 min duration were made at the same time each day. Scoring of symptoms from the randomly sequenced videotapes was done by raters who were experienced in evaluating movement disorders, and who were blind to the treatment protocol. The rating scale for TD included evaluations of the eight body regions: jaw, tongue, lips, face, head, trunk, and lower and upper extremities. Scoring ranged from 0 to 6 (absent to severe) for each body region assessed during the examination (Gerlach, 1979*a*). Parkinsonian symptoms were rated with a modified Webster's Scale and were scored from 0 to 3 (absent to severe) (Webster, 1968).

Statistical analyses of the drug effects on TD and parkinsonism were done with Wilcoxon's test for paired differences, unless otherwise specified.

DOPAMINE AND TARDIVE DYSKINESIA

Tardive dyskinesia is hypothesized to develop, at least in part, from a primary reduced DA neurotransmission followed by a compensatory relative DA overactivity, perhaps as DA receptor hypersensitivity. From this pathophysiologic point of view, the following four aspects of the relationship between DA and TD will be discussed: (1) the antihyperkinetic effect of different types of antidopaminergic drugs on TD; (2) the potential of antidopaminergic drugs to induce DA hypersensitivity and develop or aggravate TD; (3) the hypothesis of DA receptor modification, suggesting that DA receptor hypersensitivity may be reversed by treatment with DA agonists; (4) the ability of antidopaminergic drugs to induce not only parkinsonism and dystonia but also hyperkinesia in the initial phase of treatment.

Antihyperkinetic effect of antidopaminergic drugs

All CNS antidopaminergic drugs have antihyperkinetic effects. These influences, however, vary from drug to drug, depending on (1) striatal antidopaminergic potency; (2) inherent anticholinergic effect; (3) mechanisms of action (pre- and postsynaptic receptor blockade, presynaptic catecholamine depletion, or synthesis inhibition); (4) dosage; and (5) sedative effect. Therefore, it is not possible to characterize in a specific, simple formula the antihyperkinetic effects of individual drugs.

To illustrate the antihyperkinetic potency of various antidopaminergic drugs, figures 1 and 2 show the TD scores before, during, and after treatment with various receptor-blocking neuroleptics and the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine (AMPT). Haloperidol, a potent DA receptor-blocker, strongly suppressed TD (figure 1). This observation is in accordance with other clinical studies showing that haloperidol (Kazamatsuri *et al.*, 1972) and another specific antidopaminergic drug, pimozide (Claveria *et al.*, 1975), markedly decreased TD. Thioridazine, a weaker DA receptor-blocker, also has antihyperkinetic effects, although less than haloperidol ($p < 0.05$) (figure 1). The antihyperkinetic effect of thioridazine and other 'high dose' neuroleptics might intensify in the course of prolonged treatment, however (Gerlach and Simmelsgaard, 1978). This could also apply to clozapine which has only minimal or no antihyperkinetic effect in short term studies (Gerlach *et al.*, 1975; Gerlach and Simmelsgaard, 1978), but more pronounced suppression of TD during long term treatment (Simpson *et al.*, 1978).

Successful suppression of TD may involve producing or aggravating parkinsonism (Claveria *et al.*, 1975; Gerlach and Simmelsgaard, 1978). This problem can partly be managed by using selective type 2 DA receptor antagonists like sulpiride and oxiperomide (Casey *et al.*, 1979; Casey and Gerlach, 1980). These drugs differ in some respects from the classical neuroleptics (Kebabian and Calne, 1979). They inhibit apomorphine-induced stereotyped behavior but do not induce marked catalepsy, and increase DA turnover and prolactin secretion without inhibiting the DA stimulation of adenylate cyclase (Jenner *et al.*, 1978). In two separate studies, sulpiride and oxiperomide significantly suppressed TD (figure 2). The parkinsonian score did not significantly change during the treatment but minimally increased in a few patients with pre-existing symptoms of hypokinesia and tremor. During the final placebo phase, TD and parkinsonian scores returned to their baseline level, which suggests that separate mechanisms, at least in part, control hypokinesia and hyperkinesia, and should be further investigated.

AMPT decreased TD approximately 50 percent (figure 2). The movements became slower and in seven out of 24 cases they stopped entirely. The effect of AMPT, in general, is weaker than the effect of postsynaptic DA receptor blockers, not only as an antihyperkinetic drug, but also as an antischizophrenic agent (Magelund *et al.*, 1979).

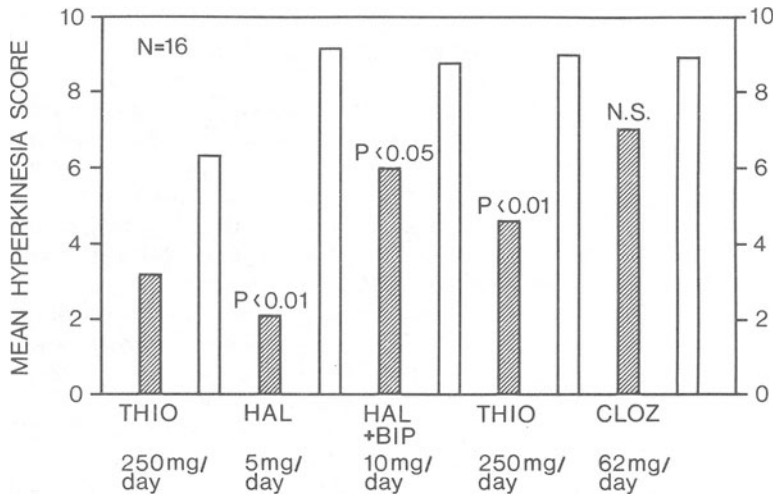

Figure 1 Mean hyperkinesia scores for 16 psychiatric patients with tardive dyskinesia. Each patient was first treated with thioridazine (THIO) for 3 months. After 4 weeks without medicine the patients were treated with four different neuroleptic periods of haloperidol (HAL), haloperidol + biperiden (HAL + BIP), THIO, and clozapine (CLOZ), all of 4 weeks' duration and all interrupted by 4 weeks without drugs. The columns represent mean scores at the end of the treatment periods and the following drug-free periods (Gerlach and Simmelsgaard, 1978).

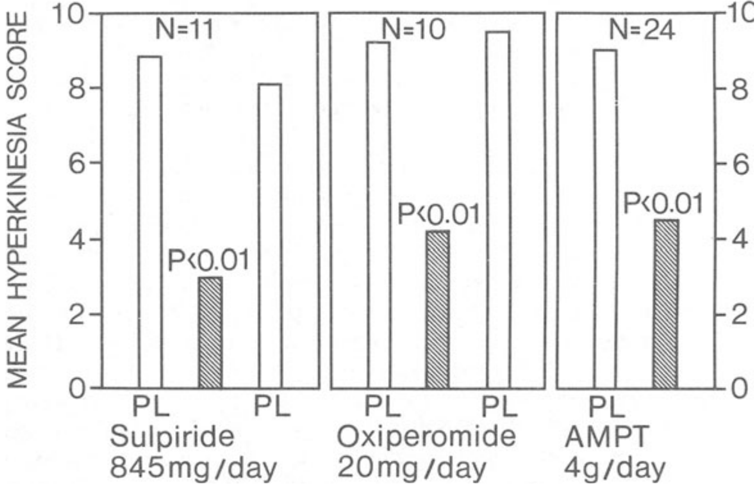

Figure 2 Mean hyperkinesia scores from clinical trials with sulpiride, oxiperomide, and α-methyl-*p*-tyrosine (AMPT). Eleven patients were included in the sulpiride study, 10 in the oxiperomide study, and 24 in the AMPT study. The treatment periods for sulpiride and oxiperomide were 6 weeks; for AMPT, 3 days. The columns represent mean hyperkinesia scores before and at the end of the active treatment periods. A final placebo period of 4 weeks was included in the sulpiride and oxiperomide trials (Gerlach and Thorsen, 1976; Casey *et al.*, 1979; Casey and Gerlach, 1980).

The potential of antidopaminergic drugs to induce TD

There is considerable evidence that neuroleptics can induce reversible or irreversible TD in predisposed subjects. At least three factors in the neuroleptic treatment may contribute to this effect: dosage, duration of treatment, and type of neuroleptic drugs used. Many retrospective epidemiological studies have attempted to clarify the relationship between these factors and the development of TD, but the results have been divergent and inconsistent. This might be expected, however, if the predisposing vulnerability of the individual to TD is of greater importance than the varying doses and duration of treatment. More valid data may come from the development of TD in single vulnerable subjects (monkeys or humans), and such observations suggest that individual predisposition (Gunne and Barany, 1979; Casey *et al.*, 1980*a*) and possibly the dose and duration of treatment are associated with TD onset.

The question whether some neuroleptics are more liable to induce TD than others may be approached in the same way. Although retrospective studies suggested that there is no obvious difference between the ability of particular neuroleptics to induce TD (Crane, 1973; Gardos *et al.*, 1977), the prospective study summarized in figure 1 (Gerlach and Simmelsgaard, 1978) showed that after treatment with haloperidol at 5 mg per day TD was more intensive than after the initial treatment with thioridazine at 250 mg per day ($p<0.01$). These results suggest that haloperidol is more potent than thioridazine in its ability to suppress and produce rebound aggravation of TD. This observation is supported by studies in monkeys in which exacerbation of irreversible TD following a challenge dose of haloperidol (0.05 mg kg^{-1}) was more prolonged than the corresponding reaction to thioridazine (1 mg kg^{-1}) (Gunne and Barany, 1979). In addition, studies in rodents have demonstrated that the degree of DA hypersensitivity is related to the potency and the duration of DA receptor-blocking effect (Christensen *et al.*, 1976; Hyttel, 1977). On the other hand, figure 1 represents treatment periods of only 4 weeks, and a second thioridazine treatment resulted in rebound hyperkinesia of the same intensity as haloperidol treatment.

Whether the proposed D-2 receptor blockers are more or less likely to produce or affect TD than the classical neuroleptics cannot be concluded from the available data. Though the hypotheses about relationships between the different DA receptors, pharmacologic effects, and various dyskinesias are intriguing, these suggestions are unsubstantiated and must be tested in comparative clinical trials and with animal models of TD.

The introduction of clozapine raised expectations that it would be possible to obtain an antipsychotic therapy without neurological side effects. In animal studies, clozapine differed from the classical neuroleptics by inducing catalepsy and antagonizing apomorphine and amphetamine stereotypes only in very high doses (Bartholini *et al.*, 1972) or on intrastriatal application (Fog, 1975). Sayers *et al.* (1975) found that clozapine could antagonize DA receptor hypersensitivity by a mechanism other than blockade of DA receptors. Thus the hypothesis was proposed that clozapine possessed

antihyperkinetic effects without increasing the risk of further aggravating TD, but clinical studies do not suggest any acute marked antihyperkinetic effect. On the other hand, clozapine produced no initial neurological side effects, and in clinical doses did not induce signs of DA hypersensitivity in rodents (Christensen *et al.*, 1976; Sayers *et al.*, 1976), monkeys (Gunne and Barany, 1979), or of TD in humans (Gerlach *et al.*, 1975).

These observations suggest that the TD-inducing effect of neuroleptic treatment is related to a complex interaction between individual vulnerability, the degree and duration of antidopaminergic treatment, and possibly the type of neuroleptic drug.

The effect of DA agonists

Recent animal studies have shown that both the biochemical and behavioral signs of DA hypersensitivity are decreased after prolonged treatment with L-Dopa (Ezrin-Waters and Seeman, 1978; Friedhoff *et al.*, 1980). This suggests that L-Dopa, by counteracting DA hypersensitivity, may have some therapeutic effect in TD. A preliminary study supports this assumption (Alpert *et al.*, 1976). However, in elderly patients with irreversible TD the reduction in hyperkinesia was small after Madopar (L-Dopa+benserazine) 600 mg (corresponding to 3000 mg L-Dopa) per day for 4 weeks; in patients on neuroleptic treatment, Madopar 900 mg (corresponding to 4500 mg L-Dopa) had no effect (Gerlach, 1979*b*).

This result was confirmed in a subsequent trial with Madopar up to 1600 mg (corresponding to 8000 mg L-Dopa) for 8 weeks (Bjørndal *et al.*, 1980*a*). In one patient not receiving neuroleptics, only a slight improvement occurred; otherwise, no clinically significant effect on TD was observed, though four patients experienced psychotropic effects of depression, increased psychosis, or activation. In addition, the DA agonists bromocriptine and CF 25-397 failed to affect TD (Tamminga and Chase, 1980). These results suggest that factors other than reversible DA hypersensitivity may be involved in the pathophysiology of TD. For example, an increased vulnerability of an underlying 'reduced buffer capacity' may be an important variable in determining the development and response to treatment of irreversible TD (Gerlach, 1979*a*).

Another complicating initial syndrome

Finally, it should be noted that not only parkinsonism, but also hyperkinetic movements very much resembling TD occur relatively early in neuroleptic therapy. Delay and Deniker (1969) identified hyperkinesia as well as dystonia in relation to acute and subacute neuroleptic treatment, though in later literature this syndrome has only been recognized in case reports or as part of acute dystonia (Gerlach *et al.*, 1974; Casey and Denney, 1977). Initial hyperkinesia may occur alone or in connection with acute dystonia,

parkinsonism, and/or akathisia, and decreases when the neuroleptic treatment is reduced or when anticholinergic treatment is started or increased.

Slightly simplified, the hypothesis has been stated that the *initial* neurological side effects depend on DA receptor blockade and increased activity in the cholinergic and DA neurons (Gerlach, 1979*a*). Parkinsonism may be related to accentuated cholinergic turnover, whereas hyperkinesia may be related to dopaminergic overactivity. Dyskinesia in the individual subject will then depend on the balance between these two influences.

Later in the course of treatment, when compensatory mechanisms in the cholinergic and DA systems have occurred, a disturbed balance between the DA and cholinergic systems in favor of DA would result in a more simple and relaxed hyperkinetic syndrome, the typical TD (Gerlach, 1979*a*).

CHOLINERGIC MECHANISMS AND TARDIVE DYSKINESIA

Cholinergic hypofunction is a purported pathophysiological factor underlying TD, possibly interacting with DA receptor hypersensitivity. Consequently, anticholinergic drugs can uncover latent TD or aggravate existing TD (Klawans, 1973; Gerlach *et al.*, 1974; Gerlach and Thorsen, 1976; Casey and Denney, 1977). Biperiden, an anticholinergic drug, given together with haloperidol, markedly reduced the antihyperkinetic effect of haloperidol ($p<0.01$) (figure 1). Since anticholinergics aggravate TD, these drugs may also increase the risk of developing TD, though this view has not been proven. This theoretical problem has been investigated clinically in 16 elderly psychiatric patients with TD who were treated with haloperidol and haloperidol+biperiden in randomized sequence (Gerlach and Simmelsgaard, 1978). No significant difference was found between the hyperkinesias following the two treatment periods: eight of the 16 patients developed fewer hyperkinesias after haloperidol+biperiden than after haloperidol, whereas three patients had fewer hyperkinesias after haloperidol alone than after haloperidol+biperiden (figure 1). This result, however, does not exclude the possibility of a differential effect following more prolonged treatment. Though we do not yet know the role of anticholinergics in the pathogenesis of TD, there is ample reason to justify the use of anticholinergic antiparkinsonian drugs when signs of neuroleptic-induced dystonia, parkinsonism, akathisia or initial hyperkinesia are present and a reduction in the antidopaminergic treatment is not feasible due to psychotic symptoms. On the other hand, routine prophylactic administration of anticholinergics should be avoided as this will lead to the prescription of these drugs for many patients who do not need them.

Augmenting CNS cholinergic activity has been a logical experimental and therapeutic approach to the treatment of TD. Physostigmine, an acetylcholinesterase inhibitor, generally decreased TD, but also had variable

effects of either no change, or a temporary aggravation of symptoms (Klawans and Rubovits, 1974; Fann *et al.*, 1974, Gerlach *et al.*, 1974, Tarsy *et al.*, 1974; Davis *et al.*, 1975; Casey and Denney, 1977; Tamminga *et al.*, 1977). The first recent attempts to augment the cholinergic system via precursor loading were with deanol, whose effect on cholinergic mechanisms is unclear (Casey and Denney, 1974; Miller, 1974), but later investigations found this drug to be generally ineffective (Casey, 1977). Choline has moderately reduced symptoms of TD (Davis *et al.*, 1975; Tamminga *et al.*, 1977; Growdon *et al.*, 1977), though side effects limit the widespread use of this agent. Lecithin (phosphatidylcholine) has also been reported to reduce TD (Growdon *et al.*, 1978), but needs to be evaluated further. Though the idea that the cholinergic system could be modified by precursor loading is theoretically attractive, no compounds have yet been developed that are clearly effective and free of side effects.

GABA AND TARDIVE DYSKINESIA

Recent investigation of striato-nigral neuronal mechanisms suggests that hyperkinetic movement disorders may be modified by increasing CNS GABAergic influences (Scheel-Kruger *et al.*, 1979). Gamma-acetylenic GABA (GAG) is an irreversible catalytic inhibitor of GABA transaminase (GABA-T) that raises brain GABA several-fold in rodents and also decreases striatal DA turnover (Jung *et al.*, 1977; Schechter *et al.*, 1977; Palfreyman *et al.*, 1978). Figure 3 shows the effect of increasing doses of GAG at 2 week intervals in nine patients concurrently taking neuroleptics.

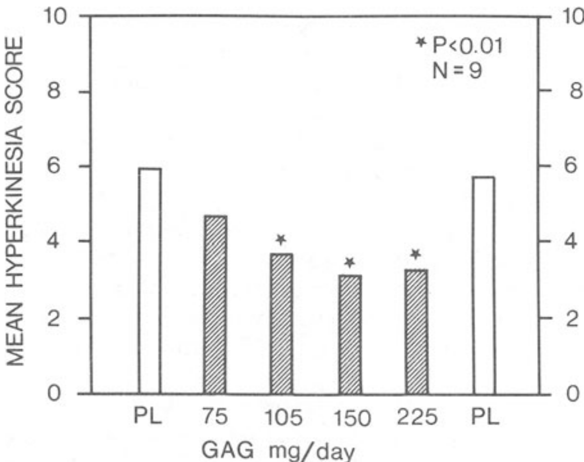

Figure 3 Mean hyperkinesia scores for nine patients with TD during treatment with placebo and increasing doses of gamma-acetylenic GABA (GAG) every 2 weeks (Casey *et al.*, 1980*b*).

Tardive dyskinesia scores were significantly reduced ($p<0.01$) at GAG doses of 105–225 mg per day when compared with pre- and post-treatment placebo scores. There was a significant correlation between the decrease in TD scores during GAG treatment and the strength of neuroleptic dose: those patients who were taking a high amount of neuroleptic medication had substantial improvement with GAG, whereas those patients taking little or no neuroleptic medication had minimal effects during GAG treatment. Parkinsonian scores increased in the four oldest patients, three of whom had mild parkinsonian symptoms prior to GAG. These scores returned to pretreatment levels during the final placebo period (Casey *et al.*, 1980*b*).

The moderate reduction in TD symptoms during GAG treatment is consistent with the proposal that increasing GABA influences is a potentially useful approach for suppressing involuntary hyperkinetic symptoms of extrapyramidal disorders (Chase and Tamminga, 1979). Additional support for this suggestion comes from the observation that the GABA agonist muscimol, or an active metabolite, also decreased TD (Tamminga *et al.*, 1979).

The significantly greater effects of GAG in patients taking higher neuroleptic doses suggest that increased GABA influences may reduce TD by indirect effects on DA mechanisms. Further support of an interaction between GABAergic and DA influences comes from the observation that parkinsonian scores increased in those patients who had pre-existing parkinsonism. If clinical doses of sodium valproate affect brain GABA, additional support for the suggestion that some GABA effects may occur through indirect influences on DA mechanisms comes from a study that found valproate alone was ineffective in TD, but potentiated the ability of haloperidol to reduce dyskinetic symptoms (Nair and Lal, 1979). The negligible to modest effects of valproate, when used alone, in the hyperkinetic syndrome of TD (Linnoila *et al.*, 1976; Chien *et al.*, 1978; Gibson, 1978; Casey and Hammerstad, 1979) and Huntington's disease (Shoulson *et al.*, 1976), illustrate the need for continued drug development. Baclofen, a structural analog of GABA with unclear effects on GABA mechanisms, moderately suppressed TD (Korsgaard, 1976), but potentiated the neuroleptic antihyperkinetic effects (Gerlach *et al.*, 1978; Nair and Lal, 1979).

The currently available GABAergic drugs either have side effects, limited efficacy, or a questionable mode of action. These limitations combine with the seemingly non-specific effect of altered GABA influences in the disparate syndromes of TD, Huntington's disease, parkinsonism, and idiopathic dyskinesias (Chase and Tamminga, 1979) so that no definitive conclusion about the role of GABA in movement disorders can be reached at this time.

NEUROPEPTIDES AND TARDIVE DYSKINESIA

The recent identification of endogenous opiate receptors in the CNS has spurred great interest in the effects of these neurochemical systems in

psychiatric syndromes. As the overlapping hypotheses of altered DA mechanisms in schizophrenia and TD have paralleled each other, so have the research strategies with investigations of the effects of the neuropeptide fragments of β-LPH_{61-91}. Figure 4 shows the effect of the met-enkephalin (β-LPH_{61-65}) synthetic analog (FK 33-824) in TD in comparison with morphine 10 mg and the opiate antagonist naloxone 0.8 mg (Bjørndal *et al.*, 1980*b*). The suppression of hyperkinesia scores with FK 33-824 was statistically significant ($p<0.05$) 1 h after i.m. injection of 3 mg, though the overall clinical impact was only modest. The effects of 1 and 2 mg of FK 33-824 were nearly identical to the 3 mg effects. In two patients who were concurrently receiving a high neuroleptic dose, the hyperkinetic movements disappeared completely, whereas the hyperkinesias were only slightly affected in the other six patients on minimal or no neuroleptic medication. FK 33-824 slightly increased parkinsonism in four patients who had parkinsonian symptoms prior to this drug trial. The altered parkinsonian symptoms were increased bradykinesia, but there was no change in tremor or rigidity. Six hours after the injection of FK33-824, the hyperkinesia and parkinsonian scores had returned to pretreatment levels.

No change in the psychiatric state of the patients was observed. Side effects were dizziness, a feeling of heaviness in the extremities, slurred speech, pursed lips, injection of the conjunctivae, and dryness of the mouth. These effects were present 5–30 min after the injection and did not parallel the time course of the antihyperkinetic actions, though non-specific CNS effects may account for some of the antihyperkinetic effect.

Morphine 10 mg minimally decreased the hyperkinesias for the whole group (figure 4), but substantially decreased symptoms in the two patients

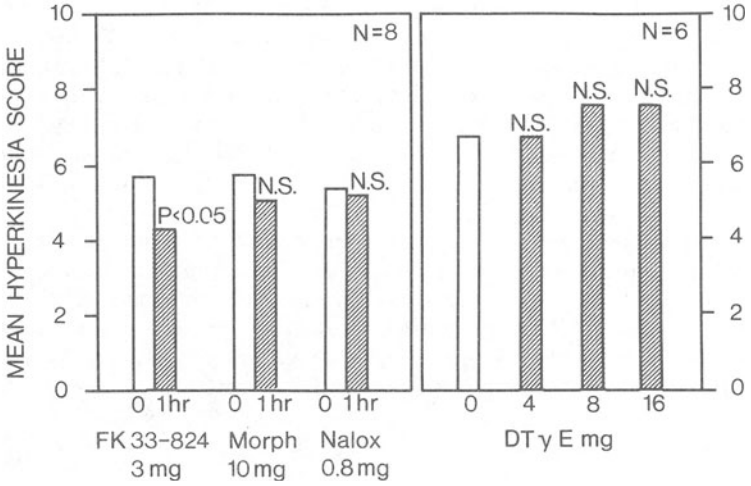

Figure 4 Mean hyperkinesia scores for patients with TD before and 1 h after receiving the met-enkephalin synthetic analogue FK 33-824, morphine, and naloxone (Bjørndal *et al.*, 1980*b*). In a separate study, the effect of single doses of (des-tyr[1])-γ-endorphin (DTγE), 4–16 mg, was compared with placebo over a 2 h period following intramuscular administration.

who were taking a high neuroleptic dose and who also improved during FK 33-824. Three of the patients with reduced hyperkinesia scores also showed increased parkinsonism. The response to morphine was complicated by side effects of drowsiness, dizziness, ataxia, dysarthria, and nausea.

Naloxone 0.8 mg had no consistent effect on the hyperkinesias or the parkinsonian scores (figure 4). No other neurological or mental changes were seen following naloxone.

Recent reports have shown that the β-LPH_{62-77} fragment, des-tyrosine-γ-endorphin (DTγE) produces behavioral effects in animal models which are similar to the effects of neuroleptic drugs, but does not show opiate-like activity (DeWied *et al.*, 1978*a*, *b*). Furthermore, DTγE was reported to have antipsychotic activity in schizophrenic patients (Verhoeven *et al.*, 1978, 1979). In light of these findings in animal and clinical investigations, the role of DTγE was investigated in TD and parkinsonism. Figure 4 shows the effect of placebo and DTγE at single doses of 4, 8, and 16 mg in six patients over 2 h after receiving the drug. There was no significant change in TD or parkinsonian scores during the 2 h following the injection of DTγE. Though the discovery of the endogenous endorphins has led to many enthusiastic investigations of these agents, there has been no consistent effect of either the met-enkephalinergic compound, FK 33-824, or the β-LPH_{62-77}, DTγE, in TD or in parkinsonism. The modest antihyperkinetic and parkinsonian-aggravating effects have been seen only when these drugs were added to concurrent relatively intense antidopaminergic neuroleptic treatment, and correspond to the effect of putative GABA agonists as discussed above. Thus, the early results do not point to a primary role of these specific peptide fragments in the pathophysiology or treatment of TD, and suggest that the effects of these drugs on DA mechanisms are indirect.

CONCLUSION

The pathophysiological mechanisms underlying TD have been partly clarified through the systematic approach of pharmacological manipulations of putative CNS neurotransmitters. Evidence indicates that DA influences are primarily involved in TD, as drugs which decrease the availability of DA consistently suppress hyperkinetic symptoms. The role of DA agonists in TD, however, remains unclear. Future research must clarify and expand the emerging evidence of DA receptor subpopulations and develop compounds that translate the biochemical differences identified in the laboratory into highly specific agents to be used in the clinic. Only through the formulation of new and unique neuroleptics can the hope be realized that drugs will successfully treat schizophrenia without the liability of producing TD. A separate, but related, line of investigation should focus on the clarification of initial hyperkinesia as a clinically similar but pharmacologically distinct entity from TD.

Cholinergic processes play a secondary and incompletely understood role in TD. Additional research is needed to investigate the effects of prolonged

alterations in CNS cholinergic mechanisms produced by continuous antiparkinsonian drugs. Potentially useful areas of inquiry include developing more effective cholinergic agonists and elucidating the effects of precursor loading on CNS cholinergic function. At present, neither the GABA nor endorphin system seems to play a direct role in the pathophysiology or treatment of TD. The indirect effects of these systems on DA mechanisms, however, require substantial additional inquiry. Since this area of drug development is still in the early stages, there will surely be new GABA compounds and molecular subdivisions of β-LPH to be evaluated both in animal models and in the clinic to increase our understanding of TD, other movement disorders, and schizophrenia.

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Neuroleptic-induced 'tardive Tourette's syndrome' and neurotoxicity

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Neurological side effects have been associated with neuroleptic drugs since these drugs were introduced in the treatment of psychiatric disorders. Acute dystonia and parkinsonism may be seen in the initial phases of treatment while a hyperkinetic syndrome may develop later. Fauerby *et al.* (1964) named this late syndrome tardive dyskinesia. The frequent irreversibility of the syndrome may be regarded as a sign of a potential neurotoxic effect of neuroleptic drugs (Gerlach, 1979).

Most investigations of tardive dyskinesia have concentrated on motor dysfunctions in patients; the symptoms have been described as 'extrapyramidal side effects', 'bucco-linguo-masticatory syndrome', 'choreiform syndrome', etc. There are only few observations of 'psychic' side effects and these are, of course, difficult to evaluate since neuroleptic treatment nearly always is used for psychiatric disorders.

In Sct Hans Mental Hospital we have observed three patients with Tourette-like symptoms developed after long term neuroleptic treatment (table 1). We think that these symptoms may be associated with tardive dyskinesia.

The 'spontaneous' Tourette's syndrome is characterized by childhood onset of chronic motor tics and involuntary vocalizations – in many cases of obscene words (coprolalia). Our three patients had not had any such symptoms before neuroleptic treatment (except for some tendency of echopraxia in patient 'E.S.'). After several years of antipsychotic medication they developed a 'tardive Tourette's syndrome' consisting of tics, grunting, shouting, echolalia and echopraxia. In all three patients the symptoms were diminished by antidopaminergic treatment with haloperidol or pimozide (Fog and Pakkenberg, 1980). This treatment may also be used in 'spontaneous' Tourette's syndrome as well as in tardive dyskinesia. Thus, there seems to be a partial overlap between these syndromes.

Table 1 Neuroleptic-induced 'tardive Tourette's syndrome'

Patient	Age (years)	Diagnosis and duration	Main treatment	Tardive dyskinesia	Tics	Gestures	Sounds	Duration
J. L.	17	Schizophrenia, simple; 3 years	Haloperidol (4–10 mg per day), 3 years	0	+	0	+	3 months
A. B.	40	Schizophrenia, paranoid; 16 years	Phenothiazine+ haloperidol (5–15 mg per day), 16 years	0	+	+	+	2 years
E. S.	53	Schizophrenia, paranoid; 12 years	Phenothiazine (for example CPZ, 400–600 mg per day), 11 years	+	0	+	+	3 years

From animal experiments it has been suggested that tardive dyskinesia is associated with dopaminergic supersensitivity (Christensen and Møller-Nielsen, 1979). Long term neuroleptic treatment in rats induces biochemical findings which parallel behavioral findings of enhanced dopamine receptor activity (Clow *et al.*, 1980).

We have earlier reported loss of cells in the rat striatum after long term treatment with a phenothiazine (Pakkenberg *et al.*, 1973); long term treatment with a thioxanthene may also induce morphological changes in certain areas of the rat striatum (Nielsen and Lyon, 1978). These investigations seem to indicate that long duration of treatment and old age of the animals are more important factors than a high dose level (Fog *et al.*, 1980).

Tardive dyskinesia as well as 'tardive Tourette's syndrome' may, therefore, be attributed to a possible neurotoxic effect of neuroleptic drugs.

SUMMARY

Long term neuroleptic treatment may induce tardive dyskinesia; three cases are described in which a 'tardive Tourette's syndrome' consisting of tics, vocalizations, echolalia, echopraxia, etc., occurred after neuroleptic treatment. These syndromes may be associated with neurotoxic effects of neuroleptic drugs since long term treatment with these drugs causes loss of cells in the rat striatum.

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