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# Regulation of Blood Pressure by Central Neurotransmitters and Neuropeptides*

ATHINEOS PHILIPPU

**Contents**



Institut für Pharmakodynamik und Toxikologie der Universität Innsbruck, Peter-Mayr-Str. 1, A-6020 Innsbruck, Austria
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## 1 Introduction

The central regulation of arterial blood pressure has been extensively investigated in recent decades. The techniques which have been used include the following:

1. Investigations on the effects of centrally applied agonists and antagonists on blood pressure, as well as on blood pressure changes elicited by stimulation of distinct brain areas
2. The identification of neurons by fluorescence microscopy and immunochemistry in discrete brain areas involved in blood pressure regulation
3. Determination of the levels of neurotransmitters and neuropeptides and of the turnover of neurotransmitters in brain areas of normal and hypertensive animals
4. Investigations on the effects of selective lesioning or ablation of brain structures on blood pressure
5. Determination of the release of neurotransmitters in distinct brain areas of normal and hypertensive animals

A few years ago, the main bulk of scientific work on central blood pressure regulation concerned mainly neurotransmitters such as catecholamines, acetylcholine, histamine, serotonin and GABA ($\gamma$-aminobutyric acid). The discovery of neuropeptides greatly increased the number of potential endogenous substances which may be of importance for cardiovascular control. Expansion of our knowledge, however, has not yet led to a thorough understanding of the central regulatory mechanisms. Despite an impressive number of separate pieces of information, the mosaic is far from complete. The coexistence of neurotransmitters and neuropeptides in several areas involved in cardiovascular control, or even their localization in one and the same neuron, as well as the possible interactions between neurotransmitter and neuropeptide systems, have blurred rather than clarified the image. For these reasons alone, it is essential to consider both the neurotransmitters and the neuropeptides when the mechanisms involved in central blood pressure regulation are surveyed. I hope that juggling with the many neurotransmitters and neuropeptides will not make it impossible to see the wood for the trees.

A brief outline of the mapping of neurotransmitters and neuropeptides will be presented in each section. Most of the studies concerning the mapping of these substances have been carried out in the rat and, to a lesser extent, in the mouse, the cat and other animal species. Unless otherwise stated, mapping is based on the results obtained in the rat. The distribution and mapping of those neurotransmitters and neuropeptides will be described mainly in the areas which seem to be involved in central blood pressure regulation. This outline, which is by no means complete, may help towards an understanding of the mutual influences of the neurons involved in central blood pressure regulation. Further details can be found from the literature quoted in this review.

## 2 Functional Significance of Neuronal Pathways in Blood Pressure Regulation

The most logical and simplest experimental approach for the identification of brain areas involved in the central regulation of blood pressure is the study of blood pressure changes elicited by electrical stimulation or selective destruction of distinct brain structures. Such experimental procedures have led to the identification of brain areas which, when stimulated or lesioned, alter the arterial blood pressure. The areas which lead to a rise in blood pressure when stimulated include the posterior hypothalamus (Karplus and Kreidl 1918, 1927), the locus coeruleus (Fallert and Polc 1970; Przuntek and Philippu 1973), the area postrema (Ferrario et al. 1979) and the fastigial nucleus of the cerebellum (Miura and Reis 1970). A pressor response is also elicited by electrical stimulation of the *rostral* part of the ventrolateral medulla (Loeschcke et al. 1970; Trouth et al. 1973; Neumayr et al. 1974), the lateral and medial amygdaloid nuclei (Torii and Kawamura 1960; Mogenson and Calaresu 1973), the raphe nuclei (Fallert and Polc 1970; Smits et al. 1978; Kuhn et al. 1980) and the parabrachial nucleus. The latter nucleus has connections with the amygdaloid complex, the hypothalamus, the nucleus of the solitary tract, the medullary reticular formation and the nucleus ambiguus (Mraovitch et al. 1982). On the other hand, a fall in blood pressure follows electrical stimulation of the anterior hypothalamus (Folkow et al. 1959), the nucleus of the solitary tract (Seller and Illert 1969), the central amygdaloid nucleus (Morin et al. 1951; Torii and Kawamura 1960; Mogenson and Calaresu 1973) and the *caudal* ventrolateral medulla (Blessing and Reis 1982). A pressor response accompanied by bradycardia is also induced by stimulation of the trigeminal complex (Kumada et al. 1975).

Using this information and that from electrophysiological studies a simplified scheme can be drawn which indicates some of the areas and the relationship between the various structures involved in blood pressure regulation (Fig. 1). The nucleus of the solitary tract is the primary site of termination of the buffer nerve fibres (carotid sinus nerve and aortic depressor nerve) which arise in the carotid sinus and the aortic arch. In some animal species the nucleus of the solitary tract projects directly to the central amygdaloid nucleus, which in turn projects to the nucleus of the solitary tract and to the dorsal motor nucleus of the vagus (for review see Spyer 1981; Calaresu et al. 1984). The central amygdaloid nucleus receives a projection from the anterior hypothalamus (Conrad and Pfaff 1976). A bidirectional cardiovascular pathway exists between the ventrolateral medulla and the nucleus of the solitary tract (Ciriello and Caverson 1986). The medullary neurons of the *rostral* ventrolateral medulla are under the inhibitory influence of the nucleus of the solitary tract and of the *caudal* ventrolateral medulla. This focal pressor area of the rostral ventrolateral medulla may be the "vasomotor

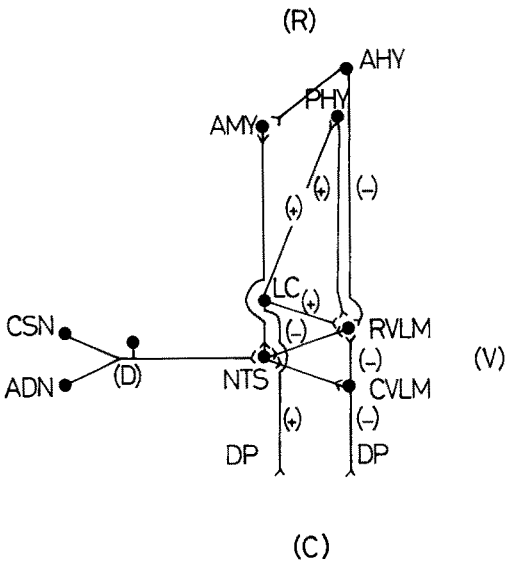


**Fig. 1.** A schematic representation of the interconnections between the main brain areas involved in blood pressure regulation. *CSN*, carotid sinus nerve; *ADN*, aortic depressor nerve; *NTS*, nucleus of the solitary tract; *AHY*, anterior hypothalamus; *PHY*, posterior hypothalamus; *AMY*, amygdaloid complex; *LC*, locus coeruleus; *DP*, descending pathways; *RVLM*, rostral ventrolateral medulla; *CVLM*, caudal ventrolateral medulla. The rostral ventrolateral medulla corresponds to the lateral reticular nucleus (see Sect. 2.2). (+), Excitatory influence; (−), inhibitory influence; (*R*), rostral; (*C*), caudal; (*D*), dorsal; (*V*), ventral

centre" (Dittmar 1870; Alexander 1946; Dampney 1981; Ross et al. 1984) whose existence has been often questioned (for review see Hilton and Spyer 1980). The anterior hypothalamus inhibits, while the posterior hypothalamus excites cardiovascular neurons of the rostral ventrolateral medulla (Ciriello and Calaresu 1977). The neurotransmitters involved in these neurophysiological events will be discussed in the following chapters.

## 3 Catecholamines

### 3.1 Mapping of Catecholamine-Containing Neurons

Fluorescence microscopy and immunochemistry have been widely used for identifying neurotransmitters and neuropeptides in the various brain areas. In order to map the pathways, neurotransmitters and neuropeptides have been determined in intact animals, as well as in animals after selective lesions.

For detailed mapping of the monoaminergic pathways various experimental approaches have been used, such as the selective destruction of nerve terminals by neurotoxins and the depletion of the monoamine stores by drugs. The immunohistochemical identification of catecholamine-containing (dopamine, noradrenaline, adrenaline) neurons has been made by demonstrating the presence of the enzymes tyrosine hydroxylase (TH), dopamine-$\beta$-hydroxylase (DBH) and phenylethanolamine-*N*-methyltransferase (PNMT). The presence of PNMT indicates that the cells are able to synthesize adrenaline.

The presence of TH suggests that the neurons possess dopamine as a neurotransmitter, while the presence of TH *and* DBH suggests that the monoamine is noradrenaline.

In this review "noradrenergic" and "adrenergic" refer to noradrenaline-containing and adrenaline-containing neurons, respectively.

### *3.1.1 Brainstem*

Noradrenaline-containing cell bodies are present in the cell groups A1–A7 and the adrenergic cell bodies in the cell groups C1 and C2 (Dahlström and Fuxe 1964; Ungerstedt 1971; Hökfelt et al. 1974; Swanson and Hartman 1975; Poitras and Parent 1978) (Fig. 2).

#### *Ventrolateral Medulla*

Noradrenergic and adrenergic cell bodies are found in the A1 and C1 cell groups, respectively. The cell bodies form a column in which the ratio of noradrenaline/adrenaline-containing cell bodies varies; the noradrenaline-containing cell bodies are mainly located in the caudal, the adrenaline-containing cell bodies in the rostral part of the column (for review see Hökfelt et al. 1984a). The noradrenergic neurons of the A1 cell group project to structures of the forebrain such as the paraventricular nucleus and the supraoptic nucleus (Swanson and Hartman 1975; Palkovits et al. 1980; Sawchenko and Swanson 1982; Saper et al. 1983). The cell bodies of the A1 cell group are

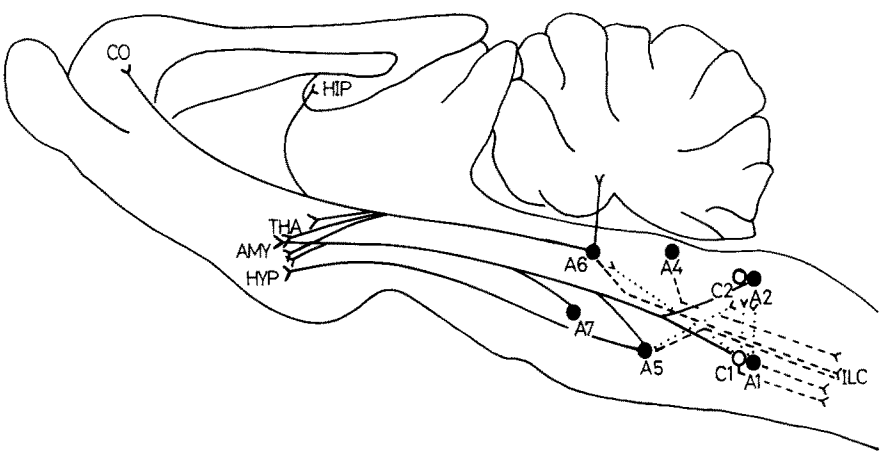


**Fig. 2.** Schematic representation of noradrenergic and adrenergic pathways which may play a role in blood pressure regulation. *Filled circles*, noradrenaline-containing cell bodies; *open circles*, adrenaline-containing cell bodies; *solid lines*, ascending pathways; *broken lines*, descending pathways; *dotted lines*, connections between noradrenergic (A1–A7) and adrenergic (C1 and C2) cell groups; *HIP*, hippocampus; *CO*, cortex; *THA*, thalamus; *HYP*, hypothalamus; *ILC*, intermediolateral column of the spinal cord

connected with the noradrenergic cell bodies of the A2 cell group of the dorsomedial medulla (Dahlström and Fuxe 1964; Palkovits and Jacobowitz 1974), the adrenergic cell bodies of the C1 cell group (Granata et al. 1985a) (Fig. 2) and the noradrenergic cell bodies of the A6 cell group (Sawchenko and Swanson 1982). In rats (Dahlström and Fuxe 1965), cats (Fleetwood-Walker and Coote 1981) and chickens (Smolen et al. 1979), neurons of the A1 cell group project to the intermediolateral column of the spinal cord. However, in the rat an abundant innervation of the spinal cord by neurons of the A1 cell group has been questioned (Ross et al. 1981a; Westlund et al. 1981) (Fig. 2). The adrenergic cell bodies of the C1 cell group project to the hypothalamic median preoptic nucleus (Saper et al. 1983), the dorsal motor nucleus of the vagus, the nucleus of the solitary tract, the paraventricular nucleus and the arcuate nucleus (Fuxe et al. 1975). Moreover, cell bodies of the C1 cell group project to the spinal cord (intermediolateral column) (Ross et al. 1981a, 1983; Saper et al. 1983; Goodchild et al. 1984).

*Dorsomedial Medulla and Pons*

*Noradrenergic and Adrenergic Neurons.* Noradrenaline-containing cell bodies form the A2–A7 cell groups, adrenaline-containing cell bodies the C2 cell group. The A2 and C2 cell groups lie within the nucleus of the solitary tract and the dorsal motor nucleus of the vagus. The noradrenergic cell bodies are mainly found in the caudal part of the dorsal vagal complex, while the adrenergic cell bodies are mainly located in the rostral part (Koda and Bloom 1983; for review see Hökfelt et al. 1984a). The medial rostral part of the adrenergic cell group C2 has been named C3 (Howe et al. 1980). The cell bodies of the nucleus of the solitary tract (but not those of the A2 cell group) project to the noradrenergic A1 cell group of the ventrolateral medulla (Sawchenko and Swanson 1982).

In the area postrema small cell bodies are present which contain noradrenaline or adrenaline (Armstrong et al. 1982a). Interestingly, adrenergic cell bodies were not found in the guinea-pig brain (Cumming et al. 1986).

The noradrenaline-containing A5 cell group is located among the fibres of the rubrospinal tract mainly at the level of the superior olive of the pons (Dahlström and Fuxe 1964; Blessing et al. 1978).

*Dopamine-Containing Neurons.* Dopamine-containing cell bodies seem to form a separate cell group in the medial part of the dorsal motor nucleus of the vagus, as well as in the area postrema (Armstrong et al. 1982a). Dopaminergic cell bodies and nerve terminals are also present in the locus coeruleus (McRae-Degueurce and Milon 1983; Westerink and De Vries 1985). The dopaminergic nerve terminals probably originate from cell bodies located in the ventral mesencephalic tegmental regions (McRae-Degueurce and Milon 1983).

*Noradrenergic Pathways*

From the noradrenaline-containing cell bodies of the ventrolateral and dorsomedial medulla two noradrenergic pathways emerge, namely the dorsal and the ventral bundles.

The dorsal noradrenergic pathway arises from the A6 cell group which is identical with the locus coeruleus (Dahlström and Fuxe 1964; Ungerstedt 1971) and the locus subcoeruleus which lies ventral to the locus coeruleus (Maeda and Shimizu 1972; Olson and Fuxe 1972; Chu and Bloom 1974). The noradrenergic neurons of this complex (Fig. 2) mainly project to the frontal cortex, the hippocampus (Andén et al. 1966; Fuxe et al. 1968; Ungerstedt 1971; Jones and Moore 1977; Ader et al. 1980; Nagai et al. 1981), the amygdaloid complex (Jones and Moore 1977; Fallon et al. 1978), the thalamus (Maeda and Shimizu 1972; Kobayashi et al. 1974; Jones and Moore 1977), the cerebellum (Bloom et al. 1971; Bloom and Battenberg 1976; Nagai et al. 1981) and several hypothalamic nuclei and areas, such as the lateral hypothalamic area and the periventricular, supraoptic and paraventricular nuclei (Fuxe 1965; Ungerstedt 1971; Lindvall and Björklund 1974; Jones and Moore 1977; Sawchenko and Swanson 1982). Within the supraoptic nucleus, the noradrenergic nerve endings terminate preferentially in those regions which contain vasopressin (McNeill and Sladek 1980). Some of the ascending axons cross over to terminate in the contralateral hypothalamus (Philippu et al. 1979a). Noradrenergic nerve terminals that originate from the locus coeruleus are also found in the dorsal raphe nucleus (Fuxe 1965; Loizou 1969; Sakai et al. 1977a, b).

The ventral pathway arises mainly from the cell bodies of the A1, A2, A5 and A7 cell groups. The ventral noradrenergic pathway innervates the preoptic area, various hypothalamic nuclei, structures of the limbic system and the nucleus of the solitary tract (Ungerstedt 1971). In particular, the cell bodies of the areas A1 and A2 project to the paraventricular nucleus, the cell bodies of the area A1 to the supraoptic nucleus (Sawchenko and Swanson 1982), and those of the A5 cell group to the nucleus of the solitary tract, the dorsal motor nucleus of the vagus and the spinal cord (Satoh et al. 1977; Loewy et al. 1979a; Blessing et al. 1981a; Westlund et al. 1981). In the cat, the ventral bundle innervates the hypothalamus and the cerebral cortex (Maeda and Shimizu 1972; Maeda et al. 1973).

The external layer of the ventral medulla oblongata is densely innervated with catecholaminergic terminals (Smialowska et al. 1985). Descending noradrenergic pathways to the rat spinal cord originate from the A4–A6 cell groups (Ader et al. 1979; Loewy et al. 1979a; Loewy and Neil 1981; Blessing et al. 1981a; Nagai et al. 1981; Westlund et al. 1983). In the cat, neurons from the A2 and A6, but not the A5 cell group, innervate the spinal cord (Fleetwood-Walker and Coote 1981).

*Adrenergic Pathways*

Adrenergic nerve terminals originating from the adrenergic cell bodies are present in the dorsal motor nucleus of the vagus, the nucleus of the solitary tract, the locus coeruleus and the raphe nuclei, as well as in the hypothalamus (arcuate nucleus, dorsomedial hypothalamus, paraventricular hypothalamus) (Hökfelt et al. 1974; Fuxe et al. 1975; Van der Gugten et al. 1976).

### *3.1.2 Hypothalamus*

Dopamine-containing cell groups exist in the dorsal and posterior hypothalamus. These cell bodies seem to project into the limbic system and the cortex. It seems likely that nerve terminals of the dopaminergic perikarya also lie within the hypothalamus (Fuxe et al. 1974).

## 3.2 Cardiovascular Effects of Catecholamines and Related Drugs

### *3.2.1 Cerebroventricular System*

The intracerebroventricular administration of noradrenaline leads to a fall in blood pressure and bradycardia. However, the opposite cardiovascular effects have also been reported. Central administration of a plethora of drugs that either stimulate or block $\alpha$- or $\beta$-adrenoreceptors also led to conflicting results (for review see Philippu 1980). For example, it has been reported that the central administration of the $\alpha$-adrenoreceptor blocking agent phentolamine either does not affect blood pressure (Heise and Kroneberg 1973), leads to a fall in blood pressure and bradycardia (Vollmer and Buckley 1977), or leads to a pressor response and tachycardia (Day and Roach 1974). Probably, the cardiovascular response may be influenced by several factors, such as anaesthesia, species differences and the site of injection and thus the site of drug action.

Since the site of drug injection may qualitatively influence the cardiovascular response, blood pressure and heart rate changes elicited by a drug injected into the ventricular system of the brain is the sum of possibly opposite effects of the drug on different brain structures. Hence, investigation of these overall changes in blood pressure and heart rate is of limited importance. To get an idea of the importance of various brain structures in blood pressure regulation, the effects of drugs applied to distinct brain areas should be investigated.

Anaesthetics may also interfere with the cardiovascular effects of centrally applied drugs (for review see Philippu 1980). Toda et al. (1969) demonstrated that in anaesthetized rabbits the intracerebroventricular injection of

adrenaline lowers blood pressure and heart rate, while in conscious rabbits the amine leads to a rise in blood pressure and bradycardia. More recently, it has been shown that the intracerebroventricular administration of noradrenaline lowers blood pressure in anaesthetized rats, but increases it in unanaesthetized animals (Corrêa et al. 1985). Central administration of noradrenaline also increases the release of vasopressin (Bhargava et al. 1972; Kuhn 1974; Milton and Paterson 1974). The pressor response to noradrenaline is inhibited by $H_1$- and $H_2$-receptor antagonists (Corrêa et al. 1985). On the other hand, noradrenaline and other $\alpha$-receptor agonists are ineffective in hypophysectomized and in Brattleboro rats which are deprived of vasopressin (Corrêa et al. 1985; Hiwatari and Johnston 1985). Since the central administration of histamine also elevates plasma vasopressin (Blackmore and Cherry 1955; Bhargava et al. 1973; Dogterom et al. 1976; Tuomisto et al. 1980), it seems probable that noradrenaline releases histamine, which in turn releases vasopressin thus leading to the rise in blood pressure (Corrêa et al. 1985). On the other hand, in anaesthetized dogs the fall in blood pressure elicited by noradrenaline is associated with a decrease in the release of vasopressin. Central administration of the $\alpha$-adrenoreceptor blocking drug phenoxybenzamine abolishes the fall in blood pressure elicited by noradrenaline and attenuates the inhibition of the vasopressin release (Kimura et al. 1981). These findings indicate that the central cardiovascular effects of noradrenaline are partly mediated by hypophyseal vasopressin.

If catecholaminergic neurons were indeed involved in cardiovascular regulation, then chemical sympathectomy with 6-hydroxydopamine (6-OHDA) would be expected to affect blood pressure. 6-OHDA causes a short-term release of catecholamines which is followed by a long-term depletion. As early as 1972 it was shown that the central administration of this neurotoxin to rats (Haeusler et al. 1972a) and conscious rabbits (Chalmers and Reid 1972) elicits an immediate fall in blood pressure and bradycardia. These cardiovascular effects have been attributed to the destruction of catecholaminergic nerve terminals and release of catecholamines. However, Korner et al. (1978) reported that in both conscious and anaesthetized rabbits, the intracisternal injection of 6-OHDA leads to hypertension and bradycardia which are inhibited by centrally administered phentolamine. The pressor response to the intracisternal injection of 6-OHDA resembles that observed on electrical stimulation of the hypothalamus (Feigl 1964; Forsyth 1970) and which has been attributed to release of catecholamines from hypothalamic nerve terminals (Philippu et al. 1973a) (see Sect. 3.2.3). A pressor response to 6-OHDA immediately after its central administration to conscious animals has also been reported (Lewis et al. 1974; Elliot et al. 1985a). Once more it seems likely that the conflicting results might be due partly to differences in the distribution of the neurotoxin when injected into the intracerebroventricular system. This view is supported by the finding that intracisternal ad-

ministration of 6-OHDA to pontine decerebrate preparations elicits an acute fall in blood pressure (Korner et al. 1978).

In spinal rats, the intracerebroventricular injection of 6-OHDA inhibits the pressor response to carotid occlusion, thus indicating the involvement of central catecholaminergic mechanisms (Kubo et al. 1985a). Since the central administration of noradrenaline enhances the release of vasopressin (see above), the increased release of this peptide might be the reason for the pressor response (see Sect. 8.2).

The reason why the pattern of the cardiovascular response depends on the site of drug administration is difficult to understand. Recently, it was reported that the fall in blood pressure elicited by the intracisternal administration of noradrenaline is reversed to a pressor response by the $\alpha_2$-adrenoreceptor blocking drug yohimbine. This rise in blood pressure is prevented by an intracisternal injection of the $\alpha_1$-receptor antagonist prazosin. These findings have been interpreted as indicating that $\alpha_2$-receptors mediate a depressor response, while stimulation of the $\alpha_1$-receptors leads to a rise in blood pressure (Bousquet and Schwartz 1983). Different densities of $\alpha_1$- and $\alpha_2$-receptors in various brain structures may explain the significance of the site of drug administration for the cardiovascular response. However, the inhibition of the central hypotensive effect of clonidine by the $\alpha_1$-receptor antagonist prazosin (see Sect. 3.4) does not support this idea.

### *3.2.2 Brainstem*

#### *Ventrolateral Medulla*

The first suggestion concerning the involvement of the ventrolateral medulla in cardiovascular control was made by Loeschcke and Koepchen (1958), who observed that procaine applied to the cat medulla leads to a fall in blood pressure. Feldberg and Guertzenstein (1972) found that pentobarbital locally applied to the ventral surface of the cat medulla causes a fall in blood pressure, thus confirming the observation of Loeschcke and Koepchen (1958). In rabbits (Fallert and Bucher 1966) and rats (Granata et al. 1983) electrolytic lesions of this area also result in irreversible hypotension, while electrical stimulation of the ventrolateral medulla of the cat elicits a pressor response. However, in the dog, electrolytic lesions change neither the blood pressure nor the sympathetic discharges (Laubie and Schmitt 1983).

The pressor area of the ventrolateral medulla was found to correspond to the reticular nuclei (Loeschcke et al. 1970; Trouth et al. 1973). Because of its localization, the area has been called nucleus reticularis lateralis (Meessen and Olszewski 1949; Palkovits and Zaborszky 1977; Bousquet et al. 1980), but other terms have also been used, such as the ventrolateral reticular nucleus, the rostral ventrolateral medulla, the pressor area of the lateral reticular for-

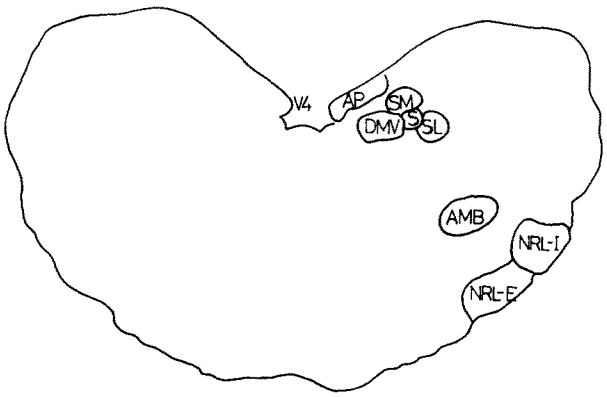


**Fig. 3.** Frontal section of the cat brain; *P* 13.5 mm posterior to the zero point which corresponds to the imaginary interaural line. *V4*, fourth ventricle; *AP*, area postrema; *SM*, medial nucleus of the solitary tract; *S*, solitary tract; *SL*, lateral nucleus of the solitary tract; *DMV*, dorsal motor nucleus of the vagus; *AMB*, nucleus ambiguus; *NRL-I*, lateral reticular nucleus (internal division); *NRL-E*, lateral reticular nucleus (external division)

mation and the lateral medullary pressor area. Structures adjacent to the lateral reticular nucleus may also participate in the stimulation-induced pressor response (Willette et al. 1983). The localization of the lateral reticular nucleus is shown in Fig. 3.

The *rostral* ventrolateral medulla is included in the baroreflex pathway (Ciriello and Calaresu 1977; Bousquet et al. 1980; Dampney 1981; McAllen et al. 1982; Yamada et al. 1984). Within the region of the pressor area are located neurons of the adrenergic C1 cell group, which project to the spinal cord (Ross et al. 1981a, 1983; Goodchild et al. 1984). The C1 cell group appears to be included in the baroreceptor pathway. Hence, it has been postulated that the adrenergic C1 cell group is responsible for tonic vasomotor control (Dampney 1981; Ross et al. 1983, 1984; Reis et al. 1984).

The idea that adrenaline neurons of the C1 cell group belong to the vasomotor neurons of the *rostral* ventrolateral medulla has been recently questioned, because pretreatment of rats with the PNMT inhibitor LY 134046 (8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine) does not influence either the pressor response or the tachycardia to electrical stimulation of the *rostral* ventrolateral medulla. Moreover, intrathecal injections of phentolamine or propranolol in doses which do not block peripheral $\alpha$- or $\beta$-receptors do not affect the stimulation-induced cardiovascular effects (Connor and Drew 1987).

The adrenergic sympathoexcitatory neurons from the *rostral* ventrolateral medulla are under the inhibitory influence of the nucleus of the solitary tract (Granata et al. 1983, 1985a).

This area of the ventrolateral medulla seems to mediate vasodepressor responses elicited in the nucleus of the solitary tract, because bilateral electro-

lytic lesions of the *rostral* ventrolateral area abolish the fall in blood pressure and the bradycardia caused by electrical stimulation of the vagus, or by distension of the carotid sinus. The *rostral* ventrolateral medulla also mediates the depressor response to stimulation of the caudal ventrolateral medulla, since tetrodotoxin or 6-OHDA injected into the C1 cell group of the *rostral* ventrolateral medulla abolish the pressor response to kainic acid injected into the *caudal* ventrolateral medulla (Granata et al. 1985b, 1986).

In cats, clonidine applied to the "chemosensitive area S" of the ventral surface of the brainstem (Schläfke and Loeschcke 1967) through Perspex rings leads to a fall in blood pressure (Bousquet and Guertzenstein 1973; Dhawan et al. 1975). The area S is situated at the *rostral* ventrolateral medulla. A fall in blood pressure is also observed when the *rostral* ventrolateral medulla is superfused with clonidine through a push-pull cannula (Sinha et al. 1975). When injected into this area, clonidine elicits a hypotensive effect at lower doses (50–100 ng) than those required when the drug is injected into other brain areas, such as the nucleus of the solitary tract (see Sect. 3.2.2.3) (Bousquet et al. 1981a; Bousquet and Schwartz 1983; Sinha et al. 1985). Moreover, bilateral lesions of the "chemosensitive area S" abolishes the fall in blood pressure elicited by intravenous injection of clonidine (Bousquet et al. 1975). Laubie and Schmitt (1977) were, however, unable to confirm this finding. This discrepancy might be due to the positioning of the electrolytic lesions. The high sensitivity of the "chemosensitive area S" to clonidine might indicate that this structure of the ventrolateral medulla is the main site of the drug action. This is supported by the recent finding that the hypotensive effect of intravenously administered clonidine is inhibited when the $\alpha_2$-receptor antagonist idasoxan is microinjected into the *rostral* part of the lateral ventricular nucleus (Gatti et al. 1988) (see Sect. 3.4).

The affinity of noradrenaline for $\alpha_1$-adrenoreceptors is approximately equal to that for $\alpha_2$-receptors (Starke et al. 1974). Microinjections of noradrenaline into the *rostral* ventrolateral medulla are also effective, although the lowest dose necessary to decrease blood pressure is approximately 200 times higher than that of clonidine. On the other hand, the $\alpha_1$-adrenoreceptor agonist phenylephrine was found to be ineffective. As might be expected, the depressor response to clonidine is inhibited by the selective $\alpha_2$-adrenoreceptor antagonists idazoxan and piperoxan, but not by prazosin which blocks $\alpha_1$-adrenoreceptors (Sinha et al. 1985). Thus, in this area $\alpha_1$-adrenoreceptors do not seem to be involved in the hypotensive action of clonidine, although prazosin inhibits the fall in blood pressure when clonidine is intracerebroventricularly administered (see Sect. 3.4).

As already mentioned (see Chap. 2), electrical stimulation of the *caudal* ventrolateral medulla lowers blood pressure. The depressor response is frequency-dependent and whereas electrical stimulation at a low frequency (20 Hz) leads to a fall in blood pressure, stimulation at a high frequency

(100 Hz) elicits a pressor response (Blessing and Reis 1982). In the rabbit, the depressor area lies 1 mm posterior to the rostral border of the area postrema. The area possesses two vasodepressor regions; one is located in the nucleus of the trigeminal nerve while the other seems to coincide with the noradrenergic A1 cell group of the ventrolateral medulla (Blessing and Reis 1982).

Day et al. (1983) stimulated electrically the *caudal* ventrolateral medulla in the rat by using extremely thin electrodes. They also found that stimulation of this area at various frequencies either decreases (low frequency) or increases (high frequency) blood pressure. However, the area which lowered blood pressure when stimulated did not coincide with the noradrenergic A1 cell group, but with a segment of the nucleus ambiguus lying adjacent to the rostral third of the A1 cell group. Electrical stimulation of the A1 cell group never decreased blood pressure. Thus, direct involvement of noradrenergic neurons of the A1 cell group in the blood pressure changes evoked by stimulation or lesion of the *caudal* ventrolateral medulla seems to be doubtful.

From the A1 cell group of the *caudal* ventrolateral medulla originate ascending catecholaminergic pathways (see Sect. 3.1.1.1) which terminate in the paraventricular nucleus. Interruption of the primary afferents to the nucleus of the solitary tract by bilateral lesions increases blood pressure and catecholamine levels in the paraventricular nucleus. The latter finding has been interpreted as indicating decreased neuronal activity in this area due to a reduced catecholamine release. In turn, the reduced release of catecholamines enhances the release of vasopressin, thus leading to the rise in blood pressure (Zukowska-Grojec et al. 1983, 1985). The involvement of vasopressin is supported by the finding that the pressor response to bilateral lesions is abolished (Barnes et al. 1984; Kubo and Amano 1986) by d($CH_2$)$_5$Tyr(Me)arginine vasopressin (TMAV; Kruszynski et al. 1980), which blocks the vascular vasopressin $V_1$-receptors. It seems that impulses from the nucleus of the solitary tract and the *caudal* ventrolateral medulla inhibit the release of vasopressin in the hypothalamus, thus decreasing blood pressure. Consistent with this view is the observation that electrolytic destruction of A1 cell group neurons increases blood pressure and plasma vasopressin (Blessing et al. 1982).

However, different results exist concerning the role of noradrenaline in the release of vasopressin. Electrophysiological studies have shown that increases in blood pressure elicited by electrical stimulation of the *caudal* ventrolateral medulla are accompanied by enhanced activity of the vasopressin-secreting supraoptic neurons. Since the injection of the neurotoxin 6-OHDA into the supraoptic nucleus abolishes the facilitatory effect of electrical stimulation without changing the basal activity pattern, it has been concluded that noradrenergic afferents facilitate the activity of vasopressin neurons (Day and Renaud 1984). Furthermore, injection of noradrenaline into the third ventricle, into the supraoptic nucleus or into the paraventricular hypothalamic

nucleus increases circulating vasopressin (Bhargava et al. 1972; Kuhn 1974; Milton and Paterson 1974; Bridges et al. 1976; Benetos et al. 1986) (see Sect. 3.2.1).

The noradrenaline-containing A5 cell group of the pons has also been implicated in the central regulation of blood pressure. In rats (Loewy et al. 1979b) and rabbits (Woodruff et al. 1986), electrical stimulation of this cell group increases blood pressure and decreases heart rate. The pressor response to electrical stimulation is eliminated by microinjections of 6-OHDA into this area (Loewy et al. 1979b; Woodruff et al. 1986). The decrease in heart rate is abolished by bilateral vagotomy (Loewy et al. 1979b; Woodruff et al. 1986) or by destruction of the nucleus of the solitary tract (Woodruff et al. 1986), thereby indicating that bradycardia is due to activation of the baroreceptor reflex.

The importance of this area for cardiovascular control has been confirmed by the observations that increases in blood pressure elicited by peripheral administration of noradrenaline (Andrade and Aghajanian 1982; Guyenet 1984), angiotensin II or vasopressin (Guyenet 1984) reduce the firing rate of A5 neurons, while the fall in blood pressure caused by nitroprusside increases the rate of firing in this area (Andrade and Aghajanian 1982). Since transections of pathways to the hypothalamus and to the nucleus of the solitary tract, as well as bilateral vagotomy, do not affect the pressor response elicited by electrical stimulation of the A5 cell group, it seems that projections of the area to the intermediolateral cell column excite preganglionic sympathetic neurons to elicit the rise in blood pressure (Loewy et al. 1979b; Woodruff et al. 1986).

### *Nucleus of the Solitary Tract*

As mentioned in Chap. 2, the afferent neurons arising from the carotid sinus and aortic arch terminate in the nucleus of the solitary tract. Electrical stimulation of the nucleus lowers the arterial blood pressure (Seller and Illert 1969), while bilateral electrolytic lesions abolish the baroreceptor reflex and lead to an acute, fulminating neurogenic hypertension. This hypertension is mediated by $\alpha$-receptors, because it is inhibited by the intravenous injection of the $\alpha$-adrenoreceptor blocking drug phentolamine (Doba and Reis 1973). Interruption of the primary afferents by bilateral transections lateral to the nucleus of the solitary tract also leads to hypertension which is associated with tachycardia (De Jong and Palkovits 1976; Zukowska-Grojec et al. 1983, 1985).

The occurrence of catecholaminergic neurons in the nucleus of the solitary tract (see Sect. 3.1.1.2) led to a thorough investigation of the importance of catecholaminergic systems of this nucleus for the baroreflex. It was found that bilateral injections of the neurotoxin 6-OHDA into the nucleus of the

solitary tract increase the arterial blood pressure for approximately 48 h. Moreover, 6-OHDA leads to a long-lasting (2 weeks) lability of the blood pressure (Snyder et al. 1978). Similar effects are elicited by selective electrolytic lesion of the noradrenergic A2 cell group (Talman et al. 1980a).

These findings are difficult to interpret, because more than one catecholamine is present as a neurotransmitter in the nucleus of the solitary tract (see Sect. 3.1.1). The acute effects of 6-OHDA might be due to the release of noradrenaline or adrenaline from damaged nerve terminals, since adrenaline nerve endings do not seem to be resistant to 6-OHDA as postulated earlier (Jonsson et al. 1976). This view was based on the observation that centrally applied 6-OHDA did not affect PNMT activity. However, determination of hypothalamic adrenaline levels revealed that 6-OHDA depletes adrenaline nerve terminals (Tessel et al. 1978). Fety and Renaud (1983) and Fety et al. (1984) also came to the conclusion that adrenaline-containing neurons might be sensitive to 6-OHDA, because central administration of the neurotoxin decreased DBH activity in the C2 adrenergic region, indicating that unchanged PNMT activity does not necessarily prove the functional integrity of adrenaline neurons.

Nevertheless, the lability of blood pressure after catecholamine depletion by 6-OHDA or electrolytic lesion suggests that catecholaminergic neurons of the nucleus of the solitary tract group modulate the baroreceptor reflex. Local administration of various sympathomimetics and sympatholytics also provided evidence for the involvement of catecholamines and the baroreflex control.

In anaesthetized animals, injections of noradrenaline into the nucleus of the solitary tract decrease blood pressure and heart rate (De Jong 1974; Struyker-Boudier et al. 1975; Sinha et al. 1975; Kubo and Misu 1981a). The cardiovascular response to noradrenaline seems to be dose dependent, because low doses of the amine lower heart rate without influencing blood pressure (Gurtu et al. 1982). Several sympathomimetics have been *monolaterally* injected into the nucleus of the solitary tract to characterize the type of $\alpha$-adrenoreceptors involved in the cardiovascular effects of noradrenaline. The most potent agonist was found to be adrenaline, followed by noradrenaline, $\alpha$-methylnoradrenaline, clonidine and tyramine (Zandberg et al. 1979; Kubo and Misu 1981a). The antagonism by yohimbine of the cardiovascular effects of these sympathomimetics (Zandberg et al. 1979; Rockhold and Caldwell 1980; Kubo and Misu 1981a; Kubo et al. 1987) might suggest the involvement of $\alpha_2$-receptors in the depressor response. Since central administration of 6-OHDA blocked the cardiovascular effects of tyramine without influencing those of noradrenaline or clonidine, the $\alpha_2$-adrenoreceptors in this area seem to be postsynaptically located (Kubo and Misu 1981a). Decreases in blood pressure elicited by noradrenaline (Kubo and Misu 1981a) or $\alpha$-methylnoradrenaline (De Jong and Petty 1982) are also inhibited by the $\alpha_1$-receptor blocking agent prazosin, but the *monolateral* injection of the

$\alpha_1$-receptor agonist phenylephrine into the nucleus of the solitary tract was found to be ineffective (Kubo and Misu 1981a). Very recently, it was reported that *bilateral* injections of the $\alpha_1$-receptor agonists methoxamine, phenylephrine or St 587 (2-(chloro-5-trifluoromethylphenylimino)imidazolidine) increase blood pressure and heart rate. These effects are inhibited by prazosin (Kubo et al. 1987). In the nucleus of the solitary tract, it seems that stimulation of postsynaptically located $\alpha_2$-receptors decreases blood pressure, while stimulation of $\alpha_1$-adrenoreceptors increases blood pressure and heart rate. However, the inhibition by prazosin of the depressor responses to noradrenaline and $\alpha$-methylnoradrenaline injected monolaterally (see above) is still puzzling.

In cats, superfusion of the nucleus of the solitary tract with clonidine through a push-pull cannula did not affect the arterial blood pressure (Philippu et al. 1973a). Schoener and Pitts (1985) found that in rats superfusion of the nucleus of the solitary tract with low concentrations of clonidine decreases blood pressure and heart rate. The negative results obtained in cats might be due to the unfavourable relationship between the size of the nucleus of the solitary tract on the one hand and the size of the push-pull cannula on the other. Nonetheless, superfusion of the nucleus of the solitary tract with clonidine attenuates, while superfusion with the $\alpha$-adrenoreceptor blocking agent tolazoline increases the pressor response to electrical stimulation of the posterior hypothalamus (Philippu et al. 1973a, 1974). The findings indicate that the hypothalamic influence of the baroreceptor reflex is mediated through $\alpha$-adrenoreceptors of the nucleus of the solitary tract.

Clonidine treatment and withdrawal from clonidine treatment affect DBH and PNMT activities in the A1/C1 cell groups of the ventrolateral medulla (see Sect. 3.4). In the A2/C2 cell groups which correspond to the nucleus of the solitary tract, treatment with clonidine for 7 days does not influence DBH and PNMT activities, but DBH activity is reduced during clonidine withdrawal (Atkinson et al. 1986). Hence, the cardiovascular effects of clonidine are not associated with turnover changes of noradrenaline and adrenaline in the nucleus of the solitary tract. Different results have been reported by Fuxe et al. (1979b), who investigated the effects of clonidine on the turnover of noradrenaline and adrenaline in the dorsal midline area of the caudal medulla oblongata. This area is not homogeneous; among other structures, the area contains the nucleus of the solitary tract, the dorsal motor nucleus of the vagus, the commissural nucleus and the nucleus of the hypoglossal nerve (Fuxe et al. 1979a, b). Injection of clonidine into the dorsal midline area of the caudal medulla oblongata decreases the adrenaline turnover, while the noradrenaline turnover is not influenced. However, the change in the adrenaline turnover does not seem to be causally related to the clonidine-induced fall in blood pressure, because intraperitoneal administration of the drug also decreases the adrenaline turnover but blood pressure is not influenced (Fuxe et al. 1980a).

The results obtained with the various sympathomimetics and sympatholytics suggest that stimulation of $\alpha_2$-receptors of the nucleus of the solitary tract decreases blood pressure and heart rate. However, Vlahakos et al. (1985) reported that the pattern of cardiovascular response to locally applied noradrenaline greatly depends on anaesthesia. In conscious rats, administration of noradrenaline into the nucleus of the solitary tract (the drug was given either by microinjection, or the nucleus superfused with noradrenaline through a push-pull cannula) leads to a rise in blood pressure and bradycardia. Microinjection of clonidine into the nucleus of the solitary tract also increases blood pressure (see Sect. 3.4). Ether, pentobarbital or urethane abolish or reverse the pressor response to noradrenaline.

Based on the foregoing findings, it may be suggested that stimulation of $\alpha$-receptors located in the nucleus of the solitary tract modulates the baroreflex. In this nucleus, serial synapses have been described and experiments with 6-OHDA revealed that some of these synapses are catecholaminergic (Chiba and Kato 1978). Thus, a non-catecholaminergic baroreflex (see later) might be influenced by catecholaminergic neurons, the postsynaptic $\alpha$-receptors being located on the non-catecholaminergic neurons of the baroreflex arc. In this connection, it is of interest to note that subnuclear regions of the nucleus of the solitary tract associated with the inputs from the carotid sinus baroreceptors show a high density of $\alpha_2$-adrenoreceptors (Unnerstall et al. 1984; Robertson and Leslie 1985). A high density of these receptors is also present in the dorsal motor nucleus of the vagus (Robertson and Leslie 1985).

Acute sinoaortic denervation elicits a rise in blood pressure, which is associated with a decreased noradrenaline level and an increased noradrenaline and adrenaline turnover on the dorsal midline area of the caudal medulla. All these changes disappear 4 weeks after denervation. The adrenaline turnover was also found to be reduced in the caudal medulla of spontaneously hypertensive rats (SHR). The results have been interpreted as indicating that the hypertension may be due to the increased release of noradrenaline, while the adrenaline release is enhanced so as to counteract the rise in blood pressure (Fuxe et al. 1979c, 1983a; Yukimura et al. 1981).

Very recently, the effects of experimentally induced blood pressure changes on the release of catecholamines of the nucleus of the solitary tract have been investigated. Determination of the release of endogenous catecholamines revealed that moderate increases in blood pressure reduce the rate of release of adrenaline, while pronounced pressor responses additionally diminish the rate of release of noradrenaline in superfusates of the nucleus of the solitary tract (Fig. 4). Supposing that the release of noradrenaline and adrenaline is inhibited so as to countertact the rise in blood pressure, it would seem that noradrenergic and adrenergic neurons possess a hypertensive function in the nucleus of the solitary tract of the cat. There are no indications that endo-

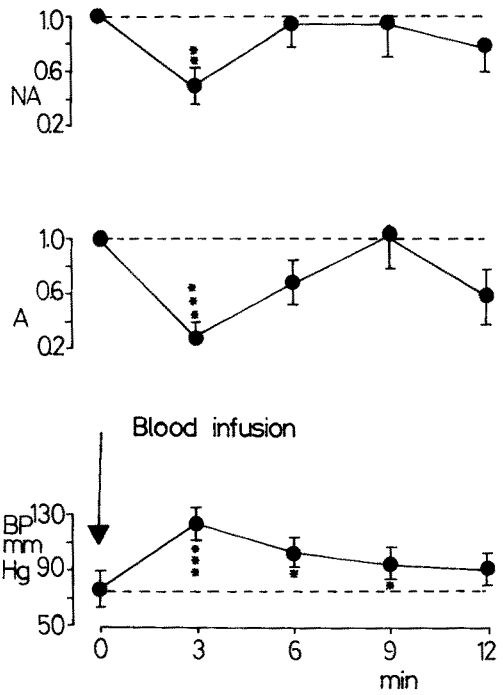


**Fig. 4.** Effects of a rise in blood pressure on the release rates of noradrenaline and adrenaline in the nucleus of the solitary tract of the cat. The nucleus was superfused with artificial CSF through a push-pull cannula at a rate of 150 µl/min and the catecholamines were radioenzymatically determined in the superfusate. To elicit a pressor response, blood (7 ml/kg) was intravenously infused. The rates of release of catecholamines in the sample before blood infusion were taken as 1. *NA*, noradrenaline; *A*, adrenaline; *BP*, mean arterial blood pressure. Means of 7–9 experiments ±SEM. $*P<0.05$, $**P<0.01$, $***P<0.001$ (Kobilansky et al. 1988)

genous adrenaline or noradrenaline exert a hypotensive action on this area (Kobilansky et al. 1988). A qualitative change in the pattern of catecholamine response to the rise in blood pressure as a result of anaesthesia is improbable, because anaesthetics only quantitatively influence the release of endogenous catecholamines (see Sect. 3.2.3). Since noradrenaline applied to the nucleus of the solitary tract of conscious rats increases blood pressure (Vlahakos et al. 1985), it is intriguing to speculate that in the conscious cat exogenous catecholamines would also lead to a pressor response when administered to this nucleus.

There are conflicting results concerning the cardiovascular effects of dopamine. Microinjection of this amine into the nucleus of the solitary tract of anaesthetized rats was found either to lower (Zandberg et al. 1979) or to increase blood pressure (Granata and Woodruff 1982). There is no plausible explanation for this discrepancy. In the cat, experimentally induced decreases in blood pressure reduce the rate of dopamine release in the nucleus of the solitary tract, indicating that dopamine may possess a hypotensive action in this area (Kobilansky et al. 1988).

### *Locus Coeruleus*

Fluorescence microscopy and immunochemistry have shown that the noradrenergic nerve terminals of the hypothalamus originate in cell bodies

located in the loci coeruleus and subcoeruleus (see Sect. 3.1.1.3). The course of the ascending catecholaminergic pathways has been confirmed by stimulation experiments. Monolateral electrical stimulation of the locus coeruleus increases the release of endogenous noradrenaline and adrenaline in the ipsilateral posterior hypothalamus. Catecholamine release is also enhanced in the contralateral hypothalamus, although to only one-third of the extent found in the ipsilateral hypothalamus. Probably, one-third of the ascending axons cross over to terminate at the contralateral hypothalamus (Philippu et al. 1979a).

In addition to the release of catecholamines in the hypothalamus, electrical stimulation of the locus coeruleus leads to a rise in blood pressure (Przuntek and Philippu 1973), which is inhibited by central administration of 6-OHDA (Ogawa 1978). The pressor response appears to be due partly to stimulation of the hypothalamus by ascending catecholaminergic pathways, because electrolytic or chemical lesions of the hypothalamus attenuate the rise in blood pressure elicited by stimulation of the locus coeruleus (Przuntek and Philippu 1973; Maruyama 1981). Moreover, the pressor response to stimulation of the locus coeruleus is inhibited when $\alpha_2$-adrenoreceptor stimulating agents are applied to the hypothalamus (Maruyama 1981). These findings were confirmed by Gurtu et al. (1984), who also observed that, in cats, electrical stimulation of the locus subcoeruleus leads to a rise in blood pressure and heart rate. Microinjections of guanethidine into the posterior hypothalamus abolish the cardiovascular response to stimulation of the locus coeruleus, while the response elicited by stimulation of the locus subcoeruleus is not affected by this drug. The cardiovascular response to electrical stimulation of the locus coeruleus seems to be due to activation of the descending hypothalamoadrenal pathway, because such stimulation is ineffective in adrenalectomized animals (Gurtu et al. 1984). In rats, stimulation of the locus coeruleus elicits a biphasic pressor response; the first phase is prevented by peripheral administration of 6-OHDA but not by adrenalectomy, while the second phase is abolished by adrenalectomy, as well as by central or peripheral administration of the neurotoxin (Gauthier 1981; Drolet and Gauthier 1985).

These findings suggest the involvement of noradrenaline neurons of the locus coeruleus in blood pressure regulation (Przuntek and Philippu 1973). Additional evidence for this view is given by the observations that experimentally induced blood pressure changes alter the activity of noradrenaline neurons in the locus coeruleus; increases in blood pressure depress, while decreases in blood pressure enhance the activity of the noradrenaline neurons (Svensson and Thorén 1979; Ward et al. 1980; Elam et al. 1985; Olpe et al. 1985). Increases in blood pressure in the carotid sinus also inhibit the activity of the vasopressin neurons of the supraoptic nucleus, an effect which is abolished by injection of 6-OHDA into the locus coeruleus (Banks and Harris 1984).

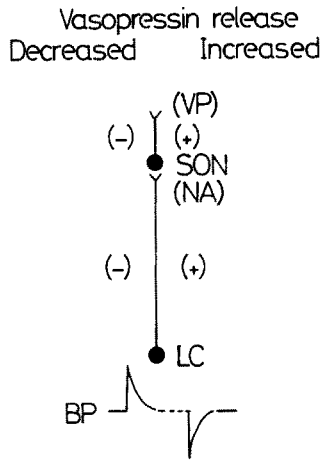


**Fig. 5.** Effects of blood pressure changes on the activities of noradrenaline-containing and vasopressin-containing neurons of the locus coeruleus and the supraoptic nucleus, respectively. *BP*, blood pressure; *LC*, locus coeruleus; *SON*, supraoptic nucleus; +, increased neuronal activity; –, decreased neuronal activity; *NA*, noradrenaline; *VP*, vasopressin

The activation of the noradrenergic pathway from the locus coeruleus seems to increase blood pressure by stimulating the release of vasopressin in the supraoptic nucleus (see Sect. 8.2) (Fig. 5). These findings are in agreement with the observation that the pressor response to stimulation of the locus coeruleus is greater in deoxycorticosterone acetate (DOCA)-salt hypertensive rats than in normotensive rats. Since the enhanced pressor response is also found in prehypertensive DOCA-salt-treated rats, it seems that the locus coeruleus is involved in the development, rather than in the maintenance, of hypertension (Chida et al. 1983). Moreover, the activity of the noradrenaline neurons is found to be reduced in DOCA-salt hypertensive rats and SHR (Olpe et al. 1985). Finally, injection of the $\alpha_1$-adrenoreceptor agonist phenylephrine into the locus coeruleus leads to a fall in blood pressure, which has been attributed to activation of somatic and/or dentritic receptors leading to feedback inhibition of the noradrenaline release (Sinha et al. 1984). It should be kept in mind that the last-mentioned results were obtained in anaesthetized animals; experiments with conscious animals might help to clarify the pattern of the cardiovascular response to catecholamines.

Results different from those obtained in DOCA-salt hypertensive rats were obtained in SHR. The concentrations of dopamine and its metabolite DOPAC (3,4-dihydroxyphenylacetic acid), as well as the rate of DOPA accumulation after DOPA-decarboxylase inhibition, were found to be increased in 4-week old SHR, thus indicating an increased activity of noradrenergic neurons of the locus coeruleus in SHR (Koulu et al. 1986a). Indeed, it has been shown that the rate of DOPAC formation in the locus coeruleus correlates well with the noradrenergic activity in this brain region (Buda et al. 1983; Gonon et al. 1983). These findings were interpreted as indicating an enhanced catecholaminergic activity in the locus coeruleus of SHR during the early stage of hypertension so as to counteract the increasing blood pressure.

Since the activity of the noradrenergic neurons has been found to be decreased in experimentally induced hypertension (Svensson and Thorén 1979; Ward et al. 1980; Elam et al. 1984a; Olpe et al. 1985), it cannot be excluded that the enhanced catecholaminergic activity observed by Koulu et al. (1986a) mainly reflects activities of dopaminergic or adrenergic cell bodies and nerve terminals (see Sects. 3.1.1.2 and 3.1.1.4) of the locus coeruleus. Another possible explanation is the involvement of the ascending noradrenergic pathway in the genesis of the hypertension rather than in counteracting the rise in blood pressure. Nonetheless, the locus coeruleus seems to play a key position in regulation of blood pressure and also in experimentally induced hypertension (see also Sect. 3.4). This idea is supported by alterations in the dentritic architecture in SHR. In these animals the locus coeruleus possesses increased number and length of dendritic branches, as well as an increase in the number of secondary branch points (Felten et al. 1984).

Other changes of the catecholamine metabolism in SHR will be presented in Section 3.3.

### *3.2.3 Hypothalamus*

Electrical stimulation of the hypothalamus either increases or decreases the arterial blood pressure, the pattern of response depending on the area stimulated: thus, stimulation of the *posterior* part of the hypothalamus leads to a pressor response (Karplus and Kreidl 1918, 1927), while stimulation of the preoptic region lowers blood pressure (Kabat et al. 1935). The sympathoinhibitory depressor hypothalamic area has been precisely characterized by Folkow et al. (1959). Stimulation of this area leads to reproducible and constant decreases in blood pressure in both rats (Folkow et al. 1959, 1964) and cats (Phillippu and Schartner 1976) (Fig. 6).

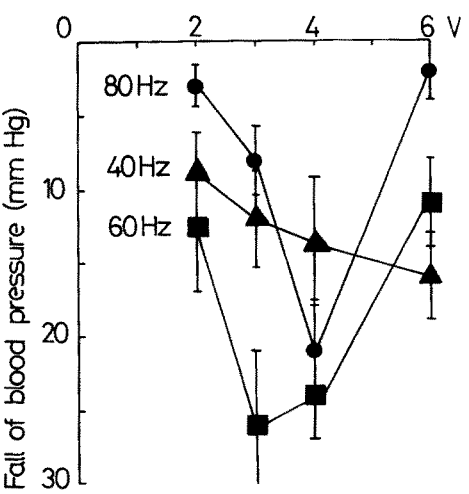


**Fig. 6.** Effects of voltage and frequency on the depressor response to electrical stimulation of the anterior hypothalamic area. The cat hypothalamus was stimulated for 60 s with 40, 60 or 80 Hz. Mean values of 5–20 experiments ±SEM (modified from Philippu and Schartner 1976)

Hypothalamic stimulation has been widely used for studying effects of drugs on blood pressure changes. Superfusion of the cat hypothalamus through the third ventricle with desipramine, which inhibits the neuronal reuptake of catecholamines, enhances the pressor response to stimulation of the *posterior* hypothalamus (Przuntek et al. 1971). On the other hand, intracerebroventricular injection of 6-OHDA leads to pronounced depletion of the noradrenaline stores in the hypothalamus and decreases the rise in blood pressure elicited by electrical stimulation of the *posterior* hypothalamus (Przuntek et al. 1971; Gupta et al. 1972; Haeusler 1975). The findings indicate that the pressor response to electrical stimulation of the *posterior* hypothalamus might be due to release of catecholamines from the hypothalamic catecholaminergic nerve terminals (Przuntek et al. 1971). This view has been supported by experiments in which the *posterior* hypothalamus was superfused with drugs through a push-pull cannula and electrically stimulated with the non-insulated tip of a cannula. It has been shown that hypothalamic superfusion with $\alpha$- or $\beta$-adrenoreceptor agonists enhances (Philippu and Kittel 1977; Philippu and Stroehl 1978; Philippu et al. 1979b; Philippu 1984), while superfusion with $\alpha$- or $\beta$-adrenoreceptor antagonists inhibits (Philippu et al. 1973a, 1974; Philippu and Kittel 1977; Philippu and Stroehl 1978) the pressor response to hypothalamic stimulation (Table 1). Involvement of hypothalamic adrenoreceptors in the pressor response is also supported by the observation that microinjection of adrenaline or noradrenaline in the *posterior* hypothalamus increases blood pressure and heart rate (Struyker-Boudier et al. 1974, 1975; Borkowski and Finch 1978; Zawoiski 1980).

The question whether $\alpha_1$- or $\alpha_2$-receptors of the *posterior* hypothalamus are involved in the pressor response cannot be answered with certainty, because this response is enhanced by hypothalamic superfusion with tramazoline ($\alpha_2$-receptor agonist) and phenylephrine ($\alpha_1$-receptor agonist). Moreover, the pressor response is inhibited by both yohimbine ($\alpha_2$-receptor antagonist) and prazosin ($\alpha_1$-receptor antagonist) (K. Wiedemann and A. Philippu, unpublished observations). On the other hand, $\beta_1$-receptors seem to be predominantly involved in the pressor response. Although the pressor response is inhibited by $\beta_1$- and $\beta_2$-adrenoreceptor blocking agents (Table 1), enhancement of the pressor responses by the $\beta_1$- and $\beta_2$-receptor agonist isoprenaline is abolished by the selective $\beta_1$-antagonist atenolol, but it is only slightly inhibited by the $\beta_2$-receptor antagonist butoxamine. Moreover, the $\beta_2$-adrenoreceptor stimulating drugs terbutaline and salbutamol are ineffective, while the $\beta_1$-receptor agonist tazolol enhances the pressor response to hypothalamic stimulation (Philippu and Stroehl 1978).

In addition to adrenoreceptors, dopamine receptors also seem to be involved in the pressor response, because hypothalamic superfusion with dopamine, apomorphine or bromocriptine greatly enhances the rise in blood pressure elicited by hypothalamic stimulation (Philippu 1984). Furthermore,

**Table 1.** Effects of hypothalamic superfusion with drugs affecting $\alpha$-, $\beta$-adrenoreceptors or dopamine receptors on blood pressure changes elicited by electrical stimulation of the hypothalamus; the involved receptors are stated in parentheses

| | Hypothalamus Posterior | Anterior |
|---|---|---|
| Response to electrical stimulation | Pressor | Depressor |
| *Agonists* | | |
| Noradrenaline ($\alpha$) | I | |
| Adrenaline ($\alpha$, $\beta$) | I | |
| Isoprenaline ($\beta_1$, $\beta_2$) | I | Ne |
| Orciprenaline ($\beta_1$, $\beta_2$) | I | |
| Tazolol ($\beta_1$) | I | |
| Terbutaline ($\beta_2$) | Ne | |
| Salbutamol ($\beta_2$) | Ne | |
| Dopamine | I | |
| Bromocriptine | I | |
| *Antagonists* | | |
| Phentolamine ($\alpha_1$, $\alpha_2$) | D | D |
| Tolazoline ($\alpha_1$, $\alpha_2$) | D | D |
| Prazosin ($\alpha_1$) | D | |
| Yohimbine ($\alpha_2$) | D | D |
| Piperoxan ($\alpha_2$) | D | D |
| Propranolol ($\beta_1$, $\beta_2$) | D | Ne |
| Sotalol ($\beta_1$, $\beta_2$) | D | |
| Practolol ($\beta_1$) | D | |
| Metoprolol ($\beta_1$) | D | |
| Atenolol ($\beta_1$) | D | Ne |
| Butoxamine ($\beta_2$) | D | Ne |

Effects on blood pressure: *I*, increase; *D*, decrease; *Ne*, no effect (for references see text)

the enhancing effect of dopamine is inhibited by the dopamine receptor antagonist haloperidol (Fig. 7).

The importance of the *posterior* hypothalamus as a pressor area is underlined by the results obtained in SHR where electrical stimulation of the *posterior* hypothalamus leads to a pressor response which is greater than that in normotensive rats (Juskevich et al. 1978; Buñag and Takeda 1979). The increased pressor response in SHR might be due to changes in the hypothalamic adrenoreceptors, because binding studies revealed an increased density of $\alpha_1$-adrenoreceptors in the hypothalamus of the hypertensive rats (Yamada et al. 1985). Moreover, the noradrenaline release from slices of the posterior hypothalamus by yohimbine is decreased in SHR, which suggests a diminished $\alpha_2$-mediated auto-inhibition of the noradrenergic neurotransmission (Kubo et al. 1986a).

Injection of noradrenaline into the paraventricular nucleus of the hypothalamus also leads to a pressor response associated with an increased plasma

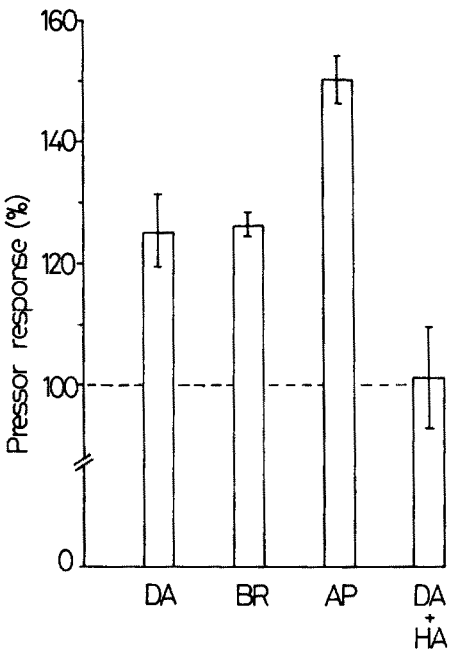


**Fig. 7.** Effects of dopamine agonists and haloperidol on the pressor response to electrical stimulation of the posterior hypothalamus of the cat. *DA*, dopamine ($10^{-3}$ mol/l); *BR*, bromocriptine ($10^{-5}$ mol/l); *AP*, apomorphine ($10^{-5}$ mol/l); *HA*, haloperidol ($10^{-5}$ mol/l). The hypothalamus was superfused with drugs through a push-pull cannula and electrically stimulated with the non-insulated tip of the cannula. The pressor response in control animals (hypothalamic superfusion with artificial CSF) was taken as 100%. Mean values of 6 experiments ±SEM (K. Wiedemann and A. Philippu, unpublished results)

level of arginine-vasopressin. The rise in blood pressure is prevented by systemic administration of the $V_1$-receptor antagonist TMAV. It seems that noradrenaline injected into the paraventricular nucleus enhances the release of vasopressin which in turn induces the pressor response (Benetos et al. 1986).

The fall in blood pressure caused by stimulation of the *anterior* hypothalamic area is strongly dependent on frequency and voltage (Fig. 6). Increases in frequency and/or voltage often result in a rise rather than in a fall in blood pressure. When appropriate stimulation parameters are used, it is possible to obtain reproducible and constant depressor responses. By using this experimental set-up it was shown that superfusion of the *anterior* hypothalamic area with $\alpha$-receptor antagonists through a push-pull cannula leads to a concentration-dependent inhibition of the depressor response (Philippu and Schartner 1976), while superfusion with $\beta$-agonists or $\beta$-antagonists is ineffective (Iijima and Philippu 1980). Thus, in the *anterior* hypothalamus, in contrast to the *posterior* hypothalamus, $\alpha$- but not $\beta$-receptors seem to be involved in the blood pressure change elicited by electrical stimulation.

The following observations underline the specificity of the effects obtained by superfusing the hypothalamus with $\alpha$- or $\beta$-adrenoreceptor agonists and antagonists:

1. Superfusion with agonists or antagonists enhances the response to hypothalamic stimulation without influencing the "resting" blood pressure, thus excluding leakage into the circulation.

2. The effects of the agonists are inhibited by superfusion with the corresponding antagonists and vice versa. Furthermore, the inhibitory effect of β-adrenoreceptor antagonists cannot be attributed to their local anaesthetic property, because (a) equianaesthetic concentrations of local anaesthetics are ineffective, and (b) the pressor response to hypothalamic stimulation is inhibited by (−)-propranolol but not by (+)-propranolol, which is equipotent as a local anaesthetic but does not virtually block β-receptors.
3. The pressor response is also inhibited by β-adrenoreceptor blocking agents deprived of local anaesthetic activity. Finally, β-adrenoreceptor blocking agents inhibit the pressor response to stimulation of the posterior hypothalamus but they do not influence the depressor response elicited by electrical stimulation of the anterior hypothalamus (for references see above).

Clonidine applied to the *anterior* hypothalamic/preoptic area through a push-pull cannula decreases blood pressure and heart rate, as does electrical stimulation. The fall in blood pressure is antagonized by yohimbine but not by prazosin, indicating the involvement of $\alpha_2$-receptors. In contrast, the decrease in heart rate is inhibited by prazosin but not by yohimbine, suggesting involvement of $\alpha_1$-receptors in the bradycardic effect of clonidine (Pitts et al. 1986). Injections of noradrenaline or adrenaline into the *anterior* hypothalamus also lower blood pressure and heart rate (Struyker-Boudier et al. 1974; Borkowski and Finch 1978; Zawoiski 1980), thus underlining the vasodepressor property of the catecholaminergic systems in this area.

Taken together, the findings indicate that catecholamine systems in the two hypothalamic regions exert opposite effects on the cardiovascular system: release of catecholamines in the *posterior* hypothalamus increases, while release in the *anterior* hypothalamus decreases blood pressure, thus contributing to the homoeostasis of the arterial blood pressure. If this is indeed so, a change in the arterial blood pressure should alter the release of catecholamines in the two hypothalamic areas, so as to counteract the blood pressure change.

To prove the involvement of the hypothalamus in the homoeostasis of blood pressure, the *posterior* and *anterior* hypothalamic areas have been superfused with artificial CSF through push-pull cannulae and the release of endogenous catecholamines determined in the superfusates. Several procedures may be used to induce blood pressure changes experimentally, such as (a) intravenous injection of drugs which either increase or decrease blood pressure, (b) controlled bleeding, (c) electrical stimulation of the splanchnic nerve, and (d) transection of the spinal cord. Continuous collection of the superfusates at short time intervals (90, 60 or even 10 s) made possible the close correlation of blood pressure changes with alterations in the rates of release of catecholamines in the hypothalamus.

**Table 2.** Effects of blood pressure changes on the release of catecholamines in the hypothalamus of anaesthetized cats and conscious rabbits

| | Change in BP | Species | Post hypoth. release of | | | Ant. hypoth. release of | | | References |
|---|---|---|---|---|---|---|---|---|---|
| | | | DA | NA | A | DA | NA | A | |
| Bleeding | Fall | Cat | I | I | I | Ne | Ne | Ne | [1] |
| Nitroprusside | Fall | Cat | I | I | I | Ne | Ne | Ne | [1] |
| Nitroprusside | Fall | Rabbit | I | I | I | D | D | D | [2, 3] |
| Chlorisondamine | Fall | Cat | I | I | I | D | D | D | [4] |
| Chlorisondamine | Fall | Rabbit | | | | D | D | D | [3] |
| Noradrenaline | Rise | Rabbit | Ne | Ne | Ne | I | I | I | [2, 3] |
| Tramazoline | Rise | Rabbit | | | | I | I | I | [3] |
| Tramazoline | Rise | Cat | D | D | D | I | I | I | [4] |
| Splanchnic nerve stimulation | Rise | Cat | Ne | Ne | Ne | I | I | I | [5] |
| Spinal transection | Rise | Cat | D | D | D | I | I | I | [4] |
| Spinal transection | Fall | Cat | I | I | I | D | D | D | [4] |

Catecholamines were determined in the superfusate.
Rate of release: *I*, increase; *D*, decrease; *Ne*, no effect; *Post. hypoth.*, posterior hypothalamus; *Ant. hypoth.*, anterior hypothalamus; *DA*, dopamine; *NA*, noradrenaline; *A*, adrenaline.
References: [1] Sinha et al. (1980); [2] Philippu et al. (1981); [3] Robinson et al. (1983); [4] Dietl et al. (1981); [5] Philippu et al. (1980)

In anaesthetized cats, a fall in blood pressure elicited by intravenous injection of nitroprusside, or by controlled bleeding, enhances the release of dopamine, noradrenaline and adrenaline in the *posterior* hypothalamus (Table 2). Transection of the brain caudal to the hypothalamus strongly reduces the resting release of catecholamines in this hypothalamic area and abolishes the increased catecholamine release due to the fall in blood pressure (Sinha et al. 1980). A pronounced and sustained hypotension elicited by spinal transection at C1/C2 additionally increases the release of the three catecholamines in the *anterior* hypothalamus. A similar effect is elicited by intravenous injection of the ganglionic blocking agent chlorisondamine, which also leads to pronounced and long-lasting hypotension (Dietl et al. 1981). On the other hand, a rise in blood pressure elicited by electrical stimulation of the peripheral trunk of the dissected splanchnic nerve increases the release of the catecholamines in the *anterior* hypothalamic area (Philippu et al. 1980). A pronounced long-lasting rise in blood pressure caused by tramazoline also enhances the release of catecholamines in the *anterior* hypothalamus and decreases the rates of release of the catecholamines in the *posterior* hypothalamus. The pronounced pressor response observed immediately after spinal transection affects the release of catecholamines in a similar way (Dietl et al. 1981). It is probable that even moderate increases and decreases in blood pressure enhance the rates of release of the catecholamines in the *anterior* and

**Table 3.** Alterations in the release of catecholamines in the hypothalamus as a consequence of experimentally induced blood pressure changes

| Blood pressure | Release of catecholamines (DA, NA, A) in the hypothalamus | |
|---|---|---|
| | Anterior | Posterior |
| Moderate fall | No effect | Increase |
| Pronounced fall | Decrease | Increase |
| Moderate rise | Increase | No effect |
| Pronounced rise | Increase | Decrease |

*DA*, Dopamine; *NA*, noradrenaline; *A*, adrenaline (for references see legend to Table 2)

*posterior* hypothalamic areas, respectively. Moreover, a pronounced rise in blood pressure additionally reduces the rates of the catecholamine release in the *posterior* hypothalamus, while a pronounced fall in blood pressure also decreases the release of the catecholamines in the *anterior* hypothalamus (Table 2). The results are summarized in Table 3. It is interesting to note that the beginning and duration of the blood pressure changes coincide with the start and duration of the altered catecholamine release. Moreover, chlorisondamine and spinal transection elicit relatively long-lasting decreases in blood pressure which are associated with long-lasting changes in the rates of catecholamine release in the two hypothalamic areas.

Since anaesthetics might interfere with the release of catecholamines in the hypothalamus, the experiments have been repeated in conscious, unrestrained rabbits (Table 2). Although the pattern of catecholamine release was found to be the same as that in anaesthetized cats, alterations in the release of catecholamines by experimentally induced blood pressure changes were much more pronounced in conscious than in anaesthetized animals (Philippu et al. 1981; Robinson et al. 1983). It seems that pentobarbital anaesthesia reduces the responsiveness of hypothalamic neurons to blood pressure changes. This is in agreement with the observation that lower doses of drugs are needed to affect blood pressure in anaesthetized than in conscious animals. However, it cannot be excluded that species differences are involved here.

Very recently, the vasopressor effect of noradrenaline in the *posterior* hypothalamus was confirmed by Kubo et al. (1988) who found that the hydralazine induced fall in blood pressure increases the MOPEG (3-methoxy-4-hydroxyphenylethylene glycol) level in this area. The vasopressor effect of adrenaline is in agreement with results obtained by intracerebral dialysis of the posterior hypothalamus. Electrical stimulation of the $C_1$ area of the *rostral* ventrolateral medulla elicits a pressor response associated with an increased release of adrenaline in the posterior hypothalamus, while the release rate of noradrenaline is not influenced. It seems that stimulation of an adrenergic pathway originating from the *rostral* ventrolateral medulla increases the

adrenaline release in the posterior hypothalamus thus leading to the rise in blood pressure (Routledge and Marsden 1988).

The vasodepressor function of catecholamines in the *anterior* hypothalamus has also been demonstrated in rats with sinoaortic denervation. The arterial blood pressure had returned to normal 4 weeks after denervation but the adrenaline turnover was found to be increased in the *anterior* hypothalamus. It appears that the hypertension due to sinoaortic denervation activates a compensatory, adrenergic mechanism in the *anterior* hypothalamus thus contributing to the normalization of blood pressure (Fuxe et al. 1983a). However, no adrenaline changes were found in hypothalamic nuclei by Saavedra (1979a, b) (see Sect. 3.3).

Although the results suggest that catecholamines released from their nerve terminals in the two hypothalamic areas exert opposite effects on blood pressure, it is not clear how this occurs. It has been proposed that noradrenaline acts on $\alpha$-adrenoreceptors thus leading to a rise in blood pressure, while adrenaline lowers blood pressure by stimulating separate "adrenaline"-receptors (Bolme et al. 1974). The existence of "adrenaline"-receptors has not been confirmed (Wilkening et al. 1980). A probable explanation is the location of postsynaptic adrenoreceptors at ascending non-catecholaminergic neurons which mediate either a rise (*posterior* hypothalamus) or a fall (*anterior* hypothalamus) in blood pressure.

## 3.3 Catecholamines in Experimental and Genetic Hypertension

Long-term changes in blood pressure are of particular interest in studying central cardiovascular mechanisms involved in the homoeostasis of blood pressure. For this purpose several models of hypertension have been developed. There is no doubt that the introduction of genetically hypertensive rat strains together with the genetically similar but normotensive Wistar-Kyoto rats (WKY) (Okamoto 1969) greatly contributed to clarifying the central mechanisms involved in cardiovascular control. In the meantime, at least six types of hypertensive rat strains have been developed (for review see Festing 1984) and used together with other models of experimentally induced hypertension (see below).

Central administration of 6-OHDA to prehypertensive young SHR attenuates the development of hypertension (Haeusler et al. 1972b). Intracerebroventricular injection of the neurotoxin also prevents the development of DOCA-salt hypertension (Okuno et al. 1983). These effects of 6-OHDA suggest the involvement of noradrenaline neurons of the brain in genetic and experimental hypertension. This idea has been supported by changes in catecholamine metabolism. Indeed, since 1970 several changes in activities of enzymes involved in the synthesis of catecholamines, as well as

in levels and turnover rates of catecholamines, have been described in brain regions and brain nuclei of animals with various forms of hypertension. It should be remembered, however, that these biochemical changes might be secondary to alterations in other neurotransmitters or neuropeptides primarily related to the hypertension. Moreover, in most cases, biochemical changes might be either the reason for the development of hypertension or its consequence, and this means that diametrically opposite interpretations of the findings are possible. Finally, conflicting results have been reported which render interpretation particularly difficult.

The approaches used for the investigation of possible changes in the biosynthesis of catecholamines should be critically evaluated. The mere determination of catecholamine levels is insufficient, because level changes might reflect alterations in the rate of synthesis, in the rate of degradation and/or in the rate of release. This uncertainty is lessened when the activity of the corresponding synthesizing enzyme is determined. Turnover determinations are of course very useful, but turnover is usually determined by the rate of disappearance of the amine after inhibition of its biosynthetic enzyme(s). Compounds used as enzymatic inhibitors of biosynthesis might exert additional effects on other neurotransmitters or neuropeptides. Thus, it cannot be excluded that these compounds may indirectly influence the level or the disappearance rate of the amine (Philippu 1984). As already mentioned, determination of neurotransmitter levels or turnover rates in the whole hypothalamus or in the whole medulla oblongata might also lead to erroneous conclusions, because separate structures in these anatomical entities might exert opposite effects on blood pressure regulation. The direct determination of the rate of release in distinct brain areas by microdialysis, voltammetry or push-pull cannulae (for reviews see Hamberger et al. 1985; Knott et al. 1985; Philippu 1985) avoids these disadvantages.

Biosynthetic alterations of catecholamines in hypertension are summarized in Tables 4–7. The reason for the extreme variability of the results is not quite obvious. It might be argued that some of the conflicting findings are due to the existence of genetically different SHR and WKY rats at various laboratories (Festing 1984; Kurtz and Morris Jr. 1987), but conflicting results have also been reported concerning biochemical changes in DOCA-salt hypertensive rats. Pronounced differences in catecholamine levels were found between male and female SHR and WKY rats (Howes et al. 1983, 1984). Whatever the reason(s) may be, the following general conclusions can be drawn:

1. Biochemical changes are found in hypothalamic nuclei and in nuclei of the brainstem.
2. Biochemical changes are not limited to the first weeks of life.
3. Biochemical changes are also observed in DOCA-salt and renal hypertension. Hence, changes in genetically hypertensive rats are not necessarily the reason for the development of the hypertension.

**Table 4.** Genetic hypertension; differences between hypertensive and normotensive animals in hypothalamic catecholamines and their biosyntheses

| Type of hypertension | Age (weeks) | Region | Dopamine | | Noradrenaline | | Adrenaline | | DBH | PNMT | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Level | Turn-over | Level | Turn-over | Level | Turn-over | | | |
| SHR | 6 | Hypothalamus | | | Ne | Ne | | | | | Nakamura et al. (1971a) |
| | 12 | Hypothalamus | | | Ne | Ne | | | | | |
| SHR | 6 | Hypothalamus | I | | I | | I | | | | Howes et al. (1984) |
| SHR | 3 | AHN | | | I | | | | | | Wijnen et al. (1978, 1980a) |
| | 4, 7, 10 | PEN | | | | | I | | | | |
| | 10 | PVN | | | | | I | | | | |
| | 10 | AHN | | | I | | | | | | |
| SHR | 4 | AHN, PVN, PEN, VH, DH | | | D | | | | | | Le Quan-Bui et al. (1980) |
| | 12 | AHN, PVN, PEN, VH, DH | | | Ne | | | | | | |
| SHR | 4 | AHN, PEN, PVN, DMHN | | | D | | | | | | Saavedra et al. (1978) |
| | 14 | AHN, PEN, PVN, DMHN | | | D | | | | | | |
| | 4 | PEN | | | | | | | D | | |
| | 14 | AHN, PEN, VMHN | | | | | | | D | | |
| | 4–14 | PVN | | | | | | | | Ne | |
| SHR | 5 | Hypothalamus | | | Ne | | | | | | Patel et al. (1981a) |
| | 9 | Hypothalamus | | | I | | | | | | |
| | 18 | Hypothalamus | | | Ne | | | | | | |
| SHR | 4 | AHN | Ne | | D | D | Ne | | | | Fujino (1984) |
| | 4 | PVN | Ne | | Ne | Ne | Ne | | | | |
| | 12 | AHN, PVN | Ne | | Ne | Ne | Ne | | | | |
| Sp-SHR | 4 | Hypothalamus | D | | I | Ne | Ne | Ne | | | Fuxe et al. (1979c) |
| Sp-SHR | 15–16 | Hypothalamus | D | | D | D | Ne | Ne | | | Fuxe et al. (1979a) |
| LH | 5 | Hypothalamus | Ne | | Ne | | I | | | | Fuxe et al. (1982a) |
| GSHR | | Hypothalamus | | | I | | D | | | | Iwai et al. (1980) |

*I*, Increase; *D*, decrease; *Ne*, no effect; *SHR*, spontaneously hypertensive rats; *Sp-SHR*, stroke-prone SHR; *LH*, Lyon strain of genetically hypertensive rats; *GSHR*, rats genetically sensitive to salt-induced hypertension; *AHN*, anterior hypothalamic nucleus; *PEN*, periventricular nucleus; *PVN*, paraventricular nucleus; *VH*, ventral hypothalamus; *DH*, dorsal hypothalamus; *DMHN*, dorsomedial hypothalamic nucleus; *VMHN*, ventromedial hypothalamic nucleus; *DBH*, dopamine-$\beta$-hydroxylase; *PNMT*, phenylethanolamine-*N*-methyltransferase

**Table 5.** Experimental hypertension; differences between hypertensive and normotensive animals in hypothalamic catecholamines and their biosyntheses

| Type of hypertension, species | Age (weeks) | Region | Dopamine | | Noradrenaline | | Adrenaline | | PNMT | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Level | Turnover | Level | Turnover | Level | Turnover | | |
| DOCA-salt, rats | 2[a] | Hypothalamus | | | Ne | D | | | | Nakamura et al. (1971b) |
| | 4[a] | Hypothalamus | | | Ne | D | | | | |
| DOCA-salt, rats | 3.5[a] | PEN, PVN | Ne | | Ne | | Ne | | | Wijnen et al. (1977) |
| DOCA-salt, rats | 4[a] | Hypothalamus | Ne | | I | | | | | Zamir et al. (1979) |
| DOCA-salt, rats | 2[a], 4[a], 9[a] | PVN | | | | | | | Ne | Saavedra (1979b) |
| DOCA-salt, rats | 4 | AHN, PVN | Ne | | Ne | Ne | Ne | | | Fujino (1984) |
| DOCA-salt, rats | 1[a], 5[a] | Ant. hypothal. | | | | Ne | | | | Chen et al. (1986) |
| | 1, 5 | Post. hypothal. | | | | Ne | | | | |
| | 20 | Ant. hypothal. | | | | Ne | | | | |
| | 20 | Post. hypothal. | | | | D | | | | |
| Salt, rats | 5[a] | Hypothalamus | Ne | | Ne | | | | | Zamir et al. (1979) |
| RH, rats | 4[a] | Hypothalamus | Ne | | I | | | | | Zamir et al. (1979) |
| RH | 0.3[a] | PVN | | | | D | | | | Wijnen et al. (1977, 1980b) |
| | 0.3[a] | PEN, AHN | | | | Ne | | | | |
| | 1[a] | PEN, PVN, AHN | | | | Ne | | | | |
| SAD, rats | 0.4[a] | Hypothalamus | | | | I | | | | Patel et al. (1981b) |
| | 1.9[a] | Hypothalamus | | | | I | | | | |
| SAD, rats | 0.5[a] | AHN, PEN | Ne | | Ne | | Ne | | | Saavedra and Alexander (1983) |
| | 0.5[a] | MPN | I | | Ne | | Ne | | | |
| | 2[a] | MPN, PEN | Ne | | Ne | | Ne | | | |
| | 2[a] | AHN | Ne | | I | | Ne | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SAD, rats | 3[b] | Ant. hypothal. | Ne | Ne | D | Ne | D | Ne | | Yukimura et al. (1981), |
| | 3[b] | Post. hypothal. | Ne | Ne | Ne | Ne | D | Ne | | Fuxe et al. (1983a) |
| | 4[a] | Ant. hypothal. | D | Ne | Ne | Ne | Ne | I | | |
| | 4[a] | Post. hypothal. | Ne | Ne | Ne | Ne | Ne | Ne | | |
| SAD, rats | 1[a] | Ant. hypothal. | | | D | | | | Ne | Chalmers et al. (1979b, |
| | 1[a] | Post. hypothal. | | | D | | | | D | 1984) |
| | 4[a] | Ant. hypothal. | | | Ne | | | | D | |
| | 4[a] | Post. hypothal. | | | Ne | | | | D | |
| | 1.5[b] | V. medulla | | | | | | | Ne | |

*I*, Increase; *D*, decrease; *Ne*, no effect; *RH*, renal hypertension; *SAD*, sinoaortic denervation; *PVN*, paraventricular nucleus; *PEN*, periventricular nucleus; *AHN*, anterior hypothalamic nucleus; *MPN*, medial preoptic nucleus; *V.*, ventral; *PNMT*, phenylethanolamine-*N*-methyltransferase

[a]Weeks after renal operation, beginning of treatment, or denervation

[b]Hours after denervation

**Table 6.** Genetic hypertension; differences between hypertensive and normotensive animals in brainstem catecholamines and their biosyntheses

| Type of hypertension | Age (weeks) | Region | Dopamine | | Noradrenaline | | Adrenaline | | DBH | PNMT | References |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Level | Turn-over | Level | Turn-over | Level | Turn-over | | | |
| SHR | 6 | Medulla | | | Ne | Ne | | | | | Nakamura et al. (1971a) |
| | 12 | Medulla | | | Ne | Ne | | | | | |
| SHR | 6 | Brainstem | I | | I | | Ne | | | | Howes et al. (1984) |
| SHR | 14 | NTS | Ne | | Ne | | Ne | | | | Versteeg et al. (1976) |
| | 14 | A1 | Ne | | I | | | | | | |
| | 14 | A2 | I | | I | | I | | | | |
| SHR | 2, 4, 10 | NTS | | | | | I | | | | Wijnen et al. (1978) |
| | 2, 4 | A1 | I | | I | | I | | | | |
| | 4, 7, 10 | A2 | I | | I | | Ne | | | | |
| SHR | 4 | A1, AP | Ne | | Ne | | D | | | I | Saavedra et al. (1976), |
| | 4 | A2 | Ne | | Ne | | Ne | | | I | Saavedra (1979a), |
| | 4 | NTS | Ne | | Ne | | | | | | Koulu et al. (1986a) |
| | 4 | A1–C1 | Ne | | Ne | | | | | | |
| | 4 | LC | I | | | | | | | | |
| | 4 | A1, A2, LC | | | | | | | Ne | | |
| | 14 | A1, A2, LC | | | | | | | Ne | | |
| | 12 | A1, A2, AP | Ne | | Ne | | Ne | | | Ne | |
| | 14 | LC | Ne | | | | | | | | |
| SHR | 4 | A2, NTS, CN | | | D | | | | | | Le Quan-Bui et al. |
| | 12 | A2, NTS, CN | | | Ne | | | | | | (1980) |
| SHR | 5 | Brainstem | | | | Ne | | | | | Patel et al. (1981a) |
| | 9 | Brainstem | | | | I | | | | | |
| | 18 | Brainstem | | | | Ne | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| SHR | 8, 12 | Medulla | | D | | | | | Nomura et al. (1985), |
| | 4 | A1 | Ne | Ne | Ne | I | | | Fujino (1984) |
| | 4 | NTS | Ne | D | D | Ne | | | |
| | 4 | A2, LC, CN | Ne | Ne | Ne | Ne | | | |
| | 12 | NTS | Ne | D | D | Ne | | | |
| | 12 | A1, A2, LC, CN | Ne | Ne | Ne | Ne | | | |
| SHR, Sp-SHR | 4 | Medulla | | | | | | I | Chalmers et al. (1981) |
| | 17 | Medulla | | | | | | Ne | |
| Sp-SHR | 4 | DCMO | Ne | Ne | Ne | Ne | D | | Fuxe et al. (1979c) |
| Sp-SHR | 15, 16 | DCMO | Ne | D | Ne | Ne | D | | Fuxe et al. (1979a) |
| LH | 5 | DCMO | Ne | Ne | | Ne | D | | Fuxe et al. (1982a) |

*I*, Increase; *D*, decrease; *Ne*, no effect; *SHR*, spontaneously hypertensive rats; *Sp-SHR*, stroke-prone SHR; *LH*, Lyon strain of genetically hypertensive rats; *NTS*, nucleus of the solitary tract; *AP*, area postrema; *LC*, locus coeruleus; *CN*, commissural nucleus; *DCMO*, dorsal part of the caudal medulla oblongata; *PNMT*, phenylethanolamine-*N*-methyltransferase; *DBH*, dopamine-$\beta$-hydroxylase

**Table 7.** Experimental hypertension; differences between hypertensive and normotensive animals in brainstem catecholamines and their biosyntheses

| Type of hypertension, species | Age (weeks) | Region | Dopamine | | Noradrenaline | | Adrenaline | | PNMT | References |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Level | Turn-over | Level | Turn-over | Level | Turn-over | | |
| DOCA-salt, rats | 2[a] | Medulla | | | Ne | D | | | | Nakamura et al. (1971b) |
| | 4[a] | Medulla | | | I | D | | | | |
| DOCA-salt, rats | 4[a] | Pons-medulla | Ne | | I | | | | | Zamir et al. (1979) |
| DOCA-salt, rats | | A1 | | | | | | | I | Saavedra et al. (1976) |
| | | A2 | | | | | | | Ne | |
| | | LC | | | | | | | Ne | |
| DOCA-salt, rats | 3.5[a] | A1, A2, NTS | Ne | | Ne | | Ne | | | Wijnen et al. (1977) |
| DOCA-salt, rats | 2[a], 4[a], 9[a] | A1, LC | Ne | | Ne | | | | I | Saavedra (1979b) |
| | 2[a], 4[a] | A2, AP, CN | Ne | | Ne | | | | Ne | |
| | 9[a] | A2 | Ne | | Ne | | | | I | |
| | 9[a] | AP | Ne | | | | | | Ne | |
| | 9[a] | CN | | | I | | | | | |
| | 2[a], 4[a], 9[a] | NTS, AP | Ne | | Ne | | Ne | | | |
| | 2[a], 4[a] | A1, A2, LC, CN | | | | | Ne | | | |
| | 9[a] | A1, A2, LC, CN | | | | | I | | | |
| DOCA-salt, rats | 4[a] | NTS | Ne | | Ne | D | Ne | | | Fujino (1984) |
| | 4[a] | A1, A2, LC, CN | Ne | | Ne | Ne | Ne | | | |
| Salt, rats | 5[a] | | Ne | | I | | | | | Zamir et al. (1979) |
| RH, rats | 4[a] | | Ne | | I | | | | | Zamir et al. (1979) |
| RH, rats | 0.3[a] | A1 | | | I | I | | | | Wijnen et al. (1977, 1980b) |
| | 0.3[a] | A2, NTS | | | Ne | Ne | | | | |
| | 0.3[a] | CN | | | Ne | I | | | | |
| | 1[a] | A1, A2, NTS, CN | Ne | | Ne | | Ne | | | |
| RH, rats | 2[a] | Brainstem | | | | Ne | | | | Tanaka et al. (1982) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| SAD, rats | 0.5[a] | A1 | | | Ne | | D | | Ne | Saavedra and Alexander (1983) |
| | 0.5[a] | AP | | | Ne | | I | | | |
| | 0.5[a] | NTS, LC | | | Ne | | Ne | | Ne | |
| | 9[a] | A1, AP, LC | Ne | | Ne | | Ne | | Ne | |
| | 9[a] | NTS | Ne | | Ne | | Ne | | I | |
| SAD, rats | 3[b] | DCMO | Ne | Ne | D | I | Ne | I | | Yukimura et al. (1981) |
| | 4[a] | DCMO | Ne | Ne | Ne | Ne | Ne | Ne | | Fuxe et al. (1983a) |
| SAD, rats | 1[a] | LRN | | | I | | | | Ne | Chalmers et al. (1979b) |
| | 1[a] | NTS, LC | | | Ne | | | | Ne | |
| | 4[a] | LRN, NTS, LC | | | Ne | | | | Ne | |
| SAD, rats | 0.4[a] | Hypothalamus | | | | I | | | | Patel et al. (1981b) |

*I*, Increase; *D*, decrease; *Ne*, no effect; *RH*, renal hypertension; *SAD*, sinoaortic denervation; *NTS*, nucleus of the solitary tract; *CN*, commissural nucleus; *LC*, locus coeruleus; *AP*, area postrema; *DCMO*, dorsal midline area of the caudal medulla oblongata; *LRN*, lateral reticular nucleus; *PNMT*, phenylethanolamine-*N*-methyltransferase

[a] Weeks after renal operation, begin of treatment, or denervation; [b] Hours after denervation

The attenuation of the development of hypertension in SHR by intracerebroventricularly applied 6-OHDA has been attributed to the noradrenaline depletion in the brain (Haeusler et al. 1972b). Forebrain noradrenergic innervation does not seem to play a major role in the development of hypertension in SHR, because lesions by 6-OHDA of the ascending noradrenergic bundles do not affect the rise in blood pressure (Van den Buuse et al. 1984a). Moreover, Van den Buuse et al. (1984b, 1986) reported that desipramine, which inhibits neuronal noradrenaline uptake does not influence the effect of 6-OHDA on the development of hypertension. On the other hand, pretreatment with the inhibitor of dopamine uptake GBR-12909 (1(2-(bis(4-fluorophenyl)methoxy)ethyl)-4-(3-phenylpropyl)piperazine) (Heikkila and Manzino 1984) inhibits the effect of 6-OHDA on blood pressure and dopamine depletion. Moreover, electrolytic lesions of the substantia nigra delay the rise in blood pressure in SHR, indicating that dopamine systems of the striatum might be involved in the development of hypertension in SHR (Van den Buuse et al. 1986). However, injection of dopamine into the caudate nucleus does not affect blood pressure, while the drug carbachol either increases or decreases blood pressure according to the region of the nucleus into which it is injected (Pazo and Medina 1983).

Since the dopamine depletion by 6-OHDA attenuates the development of hypertension, and while pretreatment with the inhibitor of dopamine uptake GBR-12909 abolishes the effects of the neurotoxin on blood pressure and dopamine depletion, dopamine seems to possess a hypertensive function. In this connection, it is noteworthy that superfusion of the posterior hypothalamus with dopamine or dopamine receptor agonists greatly enhances the pressor response to hypothalamic stimulation (see Sect. 4.2.2). Furthermore, in SHR the rate of release of dopamine in the posterior hypothalamus is higher, while the release rates of noradrenaline and adrenaline are lower than those in WKY rats. These findings have been interpreted as indicating that the increased rate of release of dopamine in the hypothalamus is the reason, or one of the reasons, for the development of hypertension, while the rates of release of noradrenaline and adrenaline are reduced so as to counteract the rise in blood pressure (Tuomisto et al. 1983). On the other hand, dopamine released in the nucleus of the solitary tract seems to lower blood pressure (see Sect. 3.2.2.2).

## 3.4 Possible Mechanisms of Clonidine Action

The intravenous injection of clonidine (for review of the pharmacological properties of clonidine see Kobinger 1978) elicits an initial rise in blood pressure that is followed by a sustained hypotension and bradycardia, while in anaesthetized animals intracisternal administration immediately lowers blood pressure (Kobinger 1967; Kobinger and Walland 1967; Schmitt et al. 1968; Onesti et al. 1971). Transections of the brain at various levels have

shown that the main site of action of clonidine is the medulla oblongata (Sattler and van Zwieten 1967; Hukuhara et al. 1968; Schmitt and Schmitt 1969). Schmitt et al. (1971) and Haeusler (1973) put forward the view that clonidine may act by stimulating a noradrenergic link in the nucleus of the solitary tract. In the rabbit, clonidine seems indeed to elicit a cardiovascular response by acting on the nucleus of the solitary tract, because bilateral destruction of the nucleus attenuated the fall in blood pressure elicited by the drug (Lipski et al. 1976). However, in anaesthetized dogs and cats (Laubie et al. 1976; Antonaccio and Halley 1977), as well as in conscious rats (Rockhold and Caldwell 1979), bilateral electrolytic lesions of the nucleus of the solitary tract abolish the bradycardia without influencing the hypotensive effect of clonidine. On the other hand, there is good evidence that the main site of the hypotensive action of the drug is situated in the lateral reticular nucleus of the ventrolateral medulla (see Sect. 3.2.2.1).

Binding studies and experiments on isolated organs revealed that the affinity of clonidine for $\alpha_2$-receptors is about ten times higher than for $\alpha_1$-receptors (Starke et al. 1974; U'Prichard et al. 1977; for review see Starke 1981). Clonidine decreases the noradrenaline turnover (Andén et al. 1976), and the release of noradrenaline from brain slices (Farnebo and Hamberger 1971; Starke and Montel 1973). The drug also decelerates the noradrenaline turnover in the locus coeruleus, as well as in the intermediolateral column of the spinal cord and the nucleus of the solitary tract, but the noradrenaline turnover in the cell bodies of the A1 and A2 cell groups is not influenced. This shows that the drug focally inhibits the release of noradrenaline through presynaptic $\alpha_2$-receptors (Lorez et al. 1983). Hence, the hypotensive effect of clonidine may be attributed to an impaired release of endogenous noradrenaline via a presynaptic site of action. Indeed, central administration of yohimbine and piperoxan which preferentially block $\alpha_2$-adrenoreceptors (Starke et al. 1975a, b; Drew 1976) inhibits the central hypotensive action of clonidine (for review see Philippu 1980). However, depletion of catecholamines by pretreatment with reserpine and the tyrosine hydroxylase inhibitor $\alpha$-methyl-*p*-tyrosine does not appreciably affect the cardiovascular effects of clonidine. The latter finding suggests that the hypotension elicited by clonidine may be due to stimulation of postsynaptic $\alpha_1$-adrenoreceptors rather than to stimulation of prejunctional $\alpha_2$-receptors (Haeusler 1974; Kobinger and Pichler 1975, 1976). This idea is supported by the finding that central injection of the neurotoxin 6-OHDA does not influence the hypotensive effect of clonidine (Finch 1975; Warnke and Hoefke 1977; Reynoldson et al. 1979; Kubo and Misu 1981a). However, Dollery and Reid (1973) observed that pretreatment of anaesthetized rabbits with 6-OHDA virtually abolishes the cardiovascular effects of intracisternally applied clonidine. Similar results were recently obtained by Head et al. (1983) who reported that the central cardiovascular effects of clonidine and $\alpha$-methyldopa are markedly reduced two weeks after treatment with 6-OHDA.

The idea that central postsynaptic $\alpha_1$-adrenoreceptors are involved in the hypotension elicited by clonidine is also supported by the finding that the central hypotensive effect of this drug is diminished by central injection of the specific $\alpha_1$-antagonist prazosin (Cavero et al. 1977; Timmermans et al. 1979; Hamilton and Longman 1982). However, the use of yohimbine and its stereoisomers rauwolscine ($\alpha$-yohimbine) and corynanthine as $\alpha$-adrenoreceptor blocking agents led to conflicting results. Of these compounds, yohimbine and rauwolscine are specific $\alpha_2$-receptor blocking agents, while corynanthine preferentially blocks $\alpha_1$-adrenoreceptors (Starke et al. 1975b; Weitzell et al. 1979). In anaesthetized rats, the central hypotensive effect of clonidine is not only diminished by the $\alpha_1$-receptor blocking drug prazosin, but also by yohimbine (Hamilton and Longman 1982). Similar results were obtained in conscious, renal hypertensive cats (Beckett and Finch 1982). On the other hand, in anaesthetized cats, the order of antagonistic potency to the central hypotensive effect of clonidine was found to be rauwolscine > yohimbine > corynanthine, indicating that the action of clonidine is mediated by $\alpha_2$-receptors (Timmermans et al. 1981).

Taken together, the findings indicate that the central cardiovascular effects of clonidine are mediated by $\alpha_1$- and $\alpha_2$-receptors. The $\alpha_2$-adrenoreceptors might be postsynaptically located. However, a presynaptic location of $\alpha_2$-receptors on catecholaminergic neurons cannot be definitively ruled out (see above). Finally, the possibility exists that prejunctional $\alpha_2$-receptors are also located on non-catecholaminergic neurons. Stimulation by clonidine of these $\alpha_2$-receptors could inhibit the release of a still unknown neurotransmitter, thus lowering arterial blood pressure.

In SHR clonidine lowers blood pressure and decreases DBH and PNMT activity in the A1/C1 cell groups of the ventrolateral medulla. Withdrawal of the clonidine treatment increases blood pressure and heart rate and normalizes the PNMT activity, while DBH activity remains reduced (Atkinson et al. 1986). These results, together with the finding that chronic clonidine treatment reduced the adrenaline level in the hindbrain, may indicate that the hypotensive action of clonidine is due to a decreased synthesis of adrenaline in the C1 region.

Some of the difficulties concerning the type(s) of adrenoreceptors which mediate the central cardiovascular effects of clonidine would be eliminated if clonidine would bind to a site separate from adrenoreceptors. It is indeed surprising that very high doses of noradrenaline have to be injected into the *rostral* ventrolateral medulla in order to obtain a depressor response, although the amine possesses high affinities for both $\alpha_1$- and $\alpha_2$-receptors (see Sect. 3.2.2.1). Particularly astonishing is the observation that the $\alpha_2$-adrenoreceptor agonist $\alpha$-methylnoradrenaline is almost ineffective when injected into this region. On the other hand, injections of the potent $\alpha_1$-agonists cirazoline or ST 587 (2-(2-chloro-5-trifluoromethylphenylamino)-imidazoline) elicit a fall in blood pressure. These compounds are, like clonidine, imidazolines. These

observations led to the view that clonidine and clonidine-like substances might act on "imidazoline-preferring" sites within the lateral reticular nucleus of the *rostral* ventrolateral medulla (Bousquet et al. 1984a). On the other hand, a "clonidine displacing substance" (CDS) has been isolated from calf brain. CDS binds specifically to $\alpha_2$-receptors but neither to $\alpha_1$- nor to $\beta$-receptors (Atlas and Burstein 1984a, b). More recently, CDS was isolated from the rat medulla. It was found that CDS displaces [$^3$H]*para*-aminoclonidine (Meeley et al. 1986) which binds to the same membrane sites as [$^3$H]clonidine, but with a greater specific/nonspecific ratio than clonidine (Rouot and Snyder 1979). Clonidine, phentolamine and CDS appear to bind preferentially to a subpopulation of membrane receptors isolated from the ventrolateral medulla. The receptors do not seem to be adrenoreceptors or histamine receptors. The binding sites may be "imidazoline-preferring", because clonidine and phentolamine bind to them with high affinities (Meeley et al. 1986; Ernsberger et al. 1987). The chemical structure of the endogenous ligand CDS is still unknown.

Contrasting results already exist concerning the central cardiovascular effects of the compound. According to Meeley et al. (1986), microinjections of CDS into the lateral reticular nucleus lead to a fall in blood pressure, while a pressor response was observed by Bousquet et al. (1986). Although different effects of CDS on blood pressure have been reported, the existence of an "imidazoline-preferring" binding site may throw new light on the mechanisms involved in the central cardiovascular effects of clonidine and other drugs.

Nevertheless, it should be kept in mind that the following non-catecholaminergic transmitters of the brain also seem to be implicated in the central cardiovascular effects of clonidine:

1. *Histamine.* In rats, infusion of the $H_2$-receptor antagonist metiamide into the lateral ventricle attenuates the hypotensive effect of intravenously administered clonidine (Karppanen et al. 1976; Finch et al. 1978). Similarly, cimetidine, which also blocks $H_2$-receptors, diminishes the antihypertensive effect of clonidine in SHR (Frisk-Holmberg 1980).
2. *Acetylcholine.* Clonidine inhibits the physostigmine-induced increase in blood pressure and diminishes the acetylcholine turnover in the hypothalamus, the pons-medulla and the midbrain. The findings also indicate that the $\alpha$-adrenoreceptors which mediate the hypotensive effect of clonidine are located on hypothalamic and/or medullary cholinergic neurons (Buccafusco et al. 1980).
3. *Vasopressin.* Plasma vasopressin is increased in rats rendered hypertensive by bilateral electrolytic lesions of the nucleus of the solitary tract. Clonidine inhibits both hypertension and high vasopressin levels. The drug also antagonizes the fall in blood pressure evoked by a vasopressin antagonist. It seems likely that the antihypertensive effect of clonidine is partly due to inhibition of the vasopressin release (Sved 1985).

4. *Serotonin.* In rabbits pretreated with the neurotoxins 6-OHDA or 5,6-dihydroxytryptamine (5,6-DHT) to destroy catecholaminergic or serotoninergic nerve terminals, respectively, the clonidine-induced fall in blood pressure and bradycardia are attenuated. Catecholaminergic and serotoninergic pathways seem to be involved in the central cardiovascular effects of clonidine (Head et al. 1983).
5. *Opioids.* In SHR, the cardiovascular effects of intravenously or centrally injected clonidine or α-methyldopa are inhibited or even reversed by peripheral or central injections of naloxone (Farsang and Kunos 1979; Farsang et al. 1980), β-endorphin antiserum (Ramirez-Gonzales et al. 1983), dynorphin antiserum (Xie et al. 1986) or naltrexone (Mosqueda-Garcia et al. 1986). Similar results have been obtained in normotensive rats, in which naloxone inhibits the fall in blood pressure elicited by low, but not high clonidine doses (Eriksson and Tuomisto 1983). Naloxone also attenuates the fall in blood pressure elicited by clonidine microinjection into the nucleus of the solitary tract of SHR (Mosqueda-Garcia et al. 1986) and antagonizes the effect of clonidine in hypertensive patients (Farsang et al. 1982). Most interestingly, the perfusion of the spinal subarachnoid space with clonidine enhances the release of immunoreactive dynorphin in the perfusate. Since intrathecal administration of dynorphin lowers blood pressure and heart rate, the release of dynorphin in the spinal cord may contribute to the depressor effects of clonidine (Xie et al. 1986).

These findings were not confirmed, however, by other authors; in anaesthetized cats, naloxone did not influence the clonidine-induced changes in blood pressure and heart rate (Shropshire and Wendt 1983; Head and de Jong 1984). Naloxone also failed to affect the clonidine-induced cardiovascular changes in normotensive rats (Conway et al. 1984; Mosqueda-Garcia et al. 1986) and SHR (Conway et al. 1984), in normotensive volunteers (Watkins et al. 1980) and in hypertensive patients (Rogers and Cubeddu 1983). The reason for the conflicting results is not clear.

In this connection it is of interest to mention that the fall in blood pressure elicited by injections of α-methylnoradrenaline into the nucleus of the solitary tract (De Jong and Nijkamp 1976) is prevented by the local injection of phentolamine or naloxone, which blocks opioid receptors. Phentolamine also diminishes the fall in blood pressure caused by microinjection of β-endorphin into the nucleus of the solitary tract. It seems that in the nucleus of the solitary tract, the α-methylnoradrenaline-induced fall in blood pressure involves β-endorphin or a β-endorphin-like peptide (Petty and De Jong 1984).

The central cardiovascular effects of clonidine also depend on anaesthesia. In conscious rats, the intracerebroventricular injection of clonidine increases blood pressure (Kawasaki and Takasaki 1986; Imai et al. 1983). This pressor response to clonidine has been attributed to stimulation of suprabulbar centres (Trolin 1975; Kawasaki and Takasaki 1986). Indeed, superfusion of the

posterior hypothalamus with clonidine affects the pressor response to electrical stimulation of the superfused area in a dual way; the pressor response is inhibited by high concentrations of clonidine, while hypothalamic superfusion with low clonidine concentrations enhances it. The attenuation of the stimulation-induced rise in blood pressure has been attributed to a decreased release of noradrenaline via stimulation of presynaptic $\alpha$-adrenoreceptors, and the enhancement of the pressor response by low clonidine concentrations to stimulation of postsynaptic $\alpha$-receptors (Philippou et al. 1974). The involvement of suprabulbar receptors in the pressor response to centrally administered clonidine is also supported by the finding that transection of the brain caudal to the hypothalamus abolishes the initial rise in blood pressure observed on intravenous injection of the drug (Trolin 1975; Henning et al. 1976). However, it was recently reported that in conscious rats microinjection of clonidine into the nucleus of the solitary tract also increases blood pressure and decreases heart rate. Only the lowest dose of clonidine used (20 nmol) led to a subsequent fall in blood pressure (Vlahakos et al. 1985). As mentioned in Sect. 3.2.2.2, noradrenaline applied to the nucleus of the solitary tract of conscious rats also leads to a pressor response. The results demonstrate that in conscious rats stimulation of $\alpha$-receptors of bulbar and suprabulbar centres increases blood pressure. Since in anaesthetized animals injections of adrenoreceptor agonists into the posterior hypothalamus also increase blood pressure (see Sect. 3.2.3), anaesthesia seems to reverse the response of bulbar receptors to clonidine and other $\alpha$-mimetics, without influencing the pattern of response of hypothalamic $\alpha$-adrenoreceptors.

## 4 Serotonin

### 4.1 Mapping of Serotonin-Containing Neurons

The serotonin-containing B1–B9 cell groups are located in various nuclei of the raphe region (Dahlström and Fuxe 1964). Ascending fibres from the mesencephalic and rostral pontine raphe nuclei lie within or outside the medial forebrain bundle innervating the suprachiasmatic nucleus and the hypothalamus, as well as the limbic and cortical areas (Dahlström and Fuxe 1964; Palkovits et al. 1977; Azmitia and Segal 1978; Moore et al. 1978; Steinbusch 1981). Serotoninergic neurons which descend to the intermediolateral cell column of the spinal cord are located in the B1–B3 cell groups (Dahlström and Fuxe 1964; Basbaum et al. 1978; Loewy and McKellar 1981; Steinbusch 1981).

Small cell bodies which contain serotonin are present in the area postrema of the rat (Dahlström and Fuxe 1964; Newton et al. 1983) and the hamster (Yoshida et al. 1982). In the cat, cell bodies immunoreactive for serotonin

were found in the nucleus of the solitary tract (Maley and Elde 1982), but not in the area postrema (Newton et al. 1983). Cell bodies and nerve terminals are also present in the external layer of the ventral medulla oblongata (Smialowska et al. 1985).

The locus coeruleus contains serotonin cell bodies (Sladek and Walker 1977; Léger et al. 1979; Steinbusch 1981), as well as serotonin nerve terminals originating from the cell bodies of the raphe nuclei (Conrad et al. 1974; Bobillier et al. 1976). It is of interest to note that the dorsal raphe nucleus (B7) also contains noradrenergic nerve terminals arising from the locus coeruleus (Fuxe 1965; Loizou 1969; Sakai et al. 1977a, b).

## 4.2 Cardiovascular Effects of Serotonin and Related Drugs

### *4.2.1 Cerebroventricular System*

It has been repeatedly shown in cats and dogs that intracerebroventricular administration of serotonin or its precursor 5-hydroxytryptophan (5-HTP) lowers blood pressure and heart rate (Bogdanski et al. 1958; McCubbin et al. 1960; Dunkley et al. 1972). The cardiovascular effects of serotonin seem to be mediated by 5-$HT_2$-receptors, because the fall in blood pressure and bradycardia are abolished by ketanserin and ritanserin which preferentially block 5-$HT_2$-receptors, while antagonists of 5-$HT_1$-receptors are ineffective (Shvaloff and Laguzzi 1986).

Coote et al. (1985) reported that in the anaesthetized cat injections of low serotonin doses into the lateral ventricle increase blood pressure, while high doses lead to a depressor response. The fall in blood pressure does not occur when access of the drug to the fourth ventricle is prevented, thus indicating that in the cat the depressor response to serotonin is due to its action on sites of the brainstem. Indeed, serotonin applied to the nucleus of the solitary tract decreases blood pressure and heart rate (Coote et al. 1985; Shvaloff and Laguzzi 1986). On the other hand, the pressor response to serotonin might be due to its action on the hypothalamus since in the anaesthetized rat it has been shown that injection of serotonin into the anterior hypothalamus/preoptic area leads to a pressor response (Smits and Struyker-Boudier 1976; Robinson 1982; Sukamoto et al. 1984), while the neurotoxin 5,7-DHT lowers blood pressure (Benarroch et al. 1983). At least one part of the serotonin nerve terminals of the anterior hypothalamus/preoptic area seem to originate from cell bodies located in the dorsal raphe nucleus, because the pressor response to electrical stimulation of the latter nucleus is attenuated by the serotonin antagonist metergoline injected into the anterior hypothalamus/preoptic area (Robinson 1984). Moreover, it seems that a cholinergic link in the posterior hypothalamus is needed for the rise in blood pressure elicited by serotonin ad-

ministered to the anterior hypothalamus, since microinjections of atropine or hemicholinium-3 into the posterior hypothalamus of the rat inhibit the pressor response to serotonin injected into the anterior hypothalamus (Robinson 1982).

Parachlorophenylalanine (PCPA) inhibits the serotonin-synthesizing enzyme tryptophan hydroxylase and depletes the stores of serotoninergic neurons. In the rat, peripheral or central administration of PCPA increases blood pressure (Ito and Schanberg 1972; De Jong et al. 1975), while intracerebroventricular injection of the serotonin precursor 5-HTP leads to a fall in blood pressure (Krstić and Djurković 1980). These findings seem to suggest that, in the rat, serotonin lowers blood pressure. However, intracerebroventricular administration of serotonin itself in conscious or anaesthetized rats elicits a pressor response (Lambert et al. 1976; Krstić and Djurković 1976; Sukamoto et al. 1984). Very recently, Dalton (1986) also reported that in conscious normotensive or hypertensive rats the intracerebroventricular injection of serotonin increases blood pressure and decreases heart rate. However, when high doses of 5-HTP or serotonin are intraventricularly injected in rats, marked and prolonged depressor effects are observed (Krstić and Djurković 1981; Dalton 1986). As in cats and dogs, it is possible that high doses of these compounds, when injected intraventricularly, reach the brainstem, thus lowering blood pressure. It is not clear, however, whether the brainstem structure responsible for the hypotensive action of serotonin is the nucleus of the solitary tract, because it was found that serotonin applied to this structure either decreases blood pressure and heart rate (Laguzzi et al. 1984), or increases (Wolf et al. 1981) blood pressure.

### *4.2.2 Raphe Nuclei*

Electrical stimulation of several serotonin raphe cell groups also leads to a pressor response which is attenuated by central administration of PCPA or 5,7-DHT (Smits et al. 1978; Kuhn et al. 1980; Howe et al. 1983a, Robinson et al. 1985). Similarly, serotonin microinjected into the dorsal raphe nucleus increases blood pressure and heart rate. The cardiovascular effects are blocked by the serotonin antagonist methysergide (Saxena et al. 1985). These findings suggest the involvement of serotoninergic neurons in the pressor response to electrical stimulation. Moreover, the results confirm the existence of a bulbospinal serotoninergic pressor pathway (Ross et al. 1981b; Loewy and McKellar 1981). The existence of this pathway has been directly demonstrated by in vivo dialysis of the spinal cord; in the rat, chemical stimulation of the cell group B3 enhances the release of serotonin in the thoracic spinal cord and increases blood pressure (Pilowsky et al. 1986a).

The role of serotonin as a neurotransmitter in the dorsal raphe nucleus has been directly demonstrated by Echizen and Freed (1984) who studied the

release of the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA). They found that experimentally induced increases in blood pressure are associated with an increase in the release of 5-HIAA, while decreases in blood pressure do not affect the release of the metabolite. Since the increased release of 5-HIAA apparently reflects increased activity of serotoninergic neurons so as to counteract the experimentally induced rise in blood pressure, a depressor function has been ascribed to the dorsal raphe nucleus. This idea is in contrast to the pressor response to injection of serotonin into this nucleus or to its electrical stimulation. Nevertheless, sinoaortic denervation abolishes the effect of the pressor response on the release of the serotonin metabolite, suggesting that serotonin neurons of the dorsal raphe nucleus are responding to increased blood pressure in baroreceptor areas (Echizen and Freed 1984; Freed et al. 1985).

### 4.3 Serotonin in Drug-Induced Hypotension, and in Experimental and Genetic Hypertension

The hypotensive response to intraperitoneally injected $\alpha$-methyldopa is attenuated by the intracerebroventricular administration of 5,7-DHT. The neurotoxin also inhibits the fall in blood pressure elicited by $\alpha$-methyldopa injected into the cell group B3, suggesting that central serotoninergic neurons might contribute to the hypotensive action of this antihypertensive drug (Choy and Chalmers 1984; Minson et al. 1984; Macrae et al. 1986).

Several findings support the idea that central serotoninergic neurons are also involved in hypertension. Intracerebroventricularly injected 5,7-DHT retards the development of hypertension in 6-week-old SHR (Buckingham et al. 1976), but injection of the neurotoxin into the nucleus of the solitary tract enhances their hypertension (Howe et al. 1983b). Furthermore, the accumulation of 5-HTP has been found to be increased in the whole hypothalamus (Smith et al. 1979) and in the periventricular and paraventricular hypothalamic nuclei (Koulu et al. 1986b), indicating an increased synthesis rate of serotonin in the hypothalamus of SHR. The serotonin levels were also found to be increased in hypothalamic nuclei of young SHR. Increases in the 5-HTP accumulation have also been reported in caudally located raphe nuclei, the cell groups A1–C1, the nucleus of the solitary tract and the locus coeruleus (Koulu et al. 1986b, c).

Interesting differences have also been observed in hypertensive animals when serotonin was administered intracerebroventricularly. In conscious DOCA-salt hypertensive rats and SHR the pressor and bradycardic responses to serotonin are much more pronounced than in normotensive animals (Kurumatani et al. 1982; Dalton 1986).

Taken together, these results suggest the involvement of serotonin neurons in α-methyldopa-induced hypotension, as well as in genetic and experimentally induced hypertension.

## 5 Histamine

### 5.1 Mapping of Histamine-Containing Neurons

The development of antisera against histamine (Wilcox and Seybold 1982) or histamine decarboxylase (Watanabe et al. 1983) made possible the identification of histamine cell bodies and fibres in various brain structures. In the rat, cell bodies were found in the lateral hypothalamus (Wilcox and Seybold 1982; Watanabe et al. 1983, 1984) and in various nuclei (posterior nucleus and the magnocellular nucleus) of the posterior hypothalamus (Watanabe et al. 1984), the median eminence (Wilcox and Seybold 1982), the arcuate nucleus, the raphe nuclei, the locus coeruleus (Watanabe et al. 1983, 1984) and the cerebral cortex (Wilcox and Seybold 1982; Watanabe et al. 1983, 1984). Hisntitamine-containing fibres have been identified in the hypothalamus, the cerebral cortex, the medial area of the amygdaloid complex and the mamillary nuclei (Wilcox and Seybold 1982; Watanabe 1984). It is of interest to note that the dorsal raphe nucleus and the nucleus of the solitary tract also contain histamine fibres, but no cell bodies were identified in the latter nucleus. Therefore, histamine-containing cell bodies are found exclusively in the hypothalamus and project to various brain structures (Watanabe et al. 1984; Steinbusch et al. 1986). A very similar distribution of histaminergic neurons has been found previously by lesion studies (for review see Schwartz et al. 1987). Recently, a descending pathway to the spinal cord has been described (Wahlestedt et al. 1985).

In the cat, cell bodies have been found in the posterior hypothalamus and in the supra-, peri- and premamillary regions. Histamine immunoreactive fibres have been detected in the posterior and anterior hypothalamus, as well as in the cortex and the amygdaloid complex (Lin et al. 1986).

### 5.2 Cardiovascular Effects of Centrally Administered Histamine

In anaesthetized cats, the intracerebroventricular injection of histamine elicits a pronounced rise in blood pressure and heart rate (Trendelenburg 1957; White 1961; Sinha et al. 1969). The histamine-induced cardiovascular effects have been attributed to stimulation of central sympathetic centres which in-

creases the outflow of sympathetic impulses to the cardiovascular system (Trendelenburg 1957; White 1961). Similar results were obtained in anaesthetized rats when histamine was centrally injected (Brezenoff and Jenden 1969; Finch and Hicks 1976a, b).

Although the central administration of histamine increases blood pressure in all anaesthetized and conscious animal species studied, the effect of the amine on heart rate depends on anaesthesia and animal species. In conscious rats, the central administration of histamine lowers heart rate (Hoffman and Schmid 1978; Klein and Gertner 1981). In conscious cats, histamine does not influence heart rate (Finch and Hicks 1976b), but, in contrast, the pressor response is accompanied by variable effects on heart rate in the conscious goat (Tuomisto and Eriksson 1980). In conscious rats, inhibition by SKF 91488 (*S*[4-*N*(*N*, *N*-dimethylamino)-butyl]isothiourea) of histamine-*N*-methyltransferase also increases blood pressure and lowers heart rate (Klein and Gertner 1981). These results, together with the existence of histaminergic neurons in the brain (see Sect. 5.1), support the view that histaminergic pathways of the CNS may be involved in blood pressure regulation.

The question arises as to which histamine receptors of the brain mediate the central cardiovascular effects of histamine. In anaesthetized rats, the pressor response and tachycardia following central administration of histamine are antagonized by intracerebroventricular pretreatment with the specific $H_1$-receptor blockers mepyramine and diphenylpyraline, while the $H_2$-antagonist metiamide is ineffective. In conscious cats, the pressor response to histamine is also inhibited by mepyramine but not by metiamide. These results were interpreted as indicating that central $H_1$-receptors mediate the cardiovascular effects of histamine (Finch and Hicks 1976a, b). However, different results were obtained when the effects of $H_1$- and $H_2$-agonists and antagonists were more carefully investigated. Such investigations revealed that the selective $H_2$-receptor agonists dimaprit and 4-methylhistamine, as well as the selective $H_1$-receptor agonist 2-methylhistamine, increased blood pressure and heart rate. The cardiovascular effects of the $H_2$-agonists were antagonized by the $H_2$-antagonist metiamide, but not by the $H_1$-antagonist mepyramine. Mepyramine inhibited the effects of the $H_1$-agonist without influencing those of the $H_2$-agonists. Hence, $H_1$- and $H_2$-receptors of the brain seem to be involved in the central cardiovascular effects of histamine (Hicks 1978).

In anaesthetized rats, the pressor response to histamine, but not its tachycardic effect, is antagonized by the intracerebroventricular injection of the $\alpha$-adrenoreceptor blocking agent phentolamine, and also by 6-OHDA. The $\beta$-adrenoreceptor blocking drug propranolol is ineffective. On the other hand, the tachycardic response to histamine is abolished by atropine. Thus, (nor)adrenergic and cholinergic systems of the brain seem to be involved in the central cardiovascular effects of histamine (Finch and Hicks 1976a).

The central administration of histamine not only increases blood pressure; when the posterior hypothalamus is superfused with histamine through a push-pull cannula, the amine also greatly enhances the pressor response to electrical stimulation of the posterior hypothalamus. Hypothalamic superfusion with the $H_2$-agonist dimaprit also enhances the pressor response, while the $H_1$-agonist 2-methylhistamine is ineffective. Hence, $H_2$- rather than $H_1$-receptors of the hypothalamus seem to be involved in the enhancement of the pressor response to histamine. This effect of histamine is abolished by hypothalamic superfusion with $\alpha$-adrenoreceptor blocking drugs. The effect of histamine is also inhibited by propranolol concentrations which elicit a specific blockade of $\beta$-receptors (Philippu and Wiedemann 1981). The inhibition of the histamine-induced increase in the pressor response by $\alpha$- and $\beta$-adrenoreceptor blocking agents demonstrates the involvement of catecholaminergic systems of the hypothalamus. This view is supported by the observation that hypothalamic superfusion with histamine agonists enhances the release of endogenous catecholamines by acting on histamine receptors localized on catecholaminergic neurons of the hypothalamus. Experiments with various histamine agonists and antagonists have revealed that dopaminergic neurons of the hypothalamus probably possess only $H_1$-receptors, while noradrenergic and adrenergic nerve terminals possess $H_1$- and $H_2$-receptors (Philippu et al. 1984).

The $H_2$-antagonists metiamide and cimetidine applied centrally also increase blood pressure (Finch and Hicks 1976b; Karppanen et al. 1977; Dadkar et al. 1984) and enhance the pressor response to hypothalamic stimulation (Philippu and Wiedemann 1981). The effects of the antagonists seem to be mediated by catecholaminergic systems, because the pressor response to metiamide is inhibited by $\alpha$-adrenoreceptor blocking agents and 6-OHDA (Dadkar et al. 1984). Moreover, it has been shown that metiamide increases the release of endogenous catecholamines in the hypothalamus by a calcium-dependent process (Philippu et al. 1984). It is not certain whether the effects of the antagonist are due to a specific blockade of $H_2$-receptors, because the $H_2$-antagonist ranitidine does not influence the release rates of catecholamines in the hypothalamus (Philippu et al. 1984).

Other transmitters also seem to be involved in the cardiovascular effects of histamine. Histamine is a potent releaser of vasopressin (Blackmore and Cherry 1955; Bhargava et al. 1973; Dogterom et al. 1976; Tuomisto et al. 1980) and pretreatment of rats with the specific vasopressin antagonist [1-($\beta$-mercapto-$\beta$,$\beta$-cyclopentamethylenepropionic acid), 2-(*O*-methyl)tyrosine] arginine-vasopressin reduces the pressor response to centrally administered histamine. Hence, the rise in blood pressure elicited by histamine seems to be due partly to release of vasopressin (Gatti and Gertner 1983).

Further evidence for the involvement of the histaminergic neurons of the brain in blood pressure regulation was provided by the observation that ex-

perimentally induced blood pressure changes influence the rate of release of endogenous histamine in the cat hypothalamus (Philippu et al. 1983). In contrast to catecholamines (see Sect. 3.2.3), both increases and decreases in arterial blood pressure enhance the rate of release of endogenous histamine in the posterior hypothalamus. Another difference in the patterns of response between catecholaminergic and histaminergic neurons concerns the duration of the changes in the release rates. A short-lasting increase in blood pressure elicited by the intravenous injection of noradrenaline or the pressor response immediately after transection of the spinal cord at C1/C2 lead to an enhanced release of histamine which coincides in time with the blood pressure change. Similarly, short-lasting decreases in blood pressure by nitroprusside or by controlled bleeding enhance the release of histamine for approximately the same time period. On the other hand, a long-lasting pressor response to the intravenous injection of tramazoline or long-lasting decreases in blood pressure (by the intravenous injection of chlorisondamine or permanent hypotension after transection of the spinal cord) elicit a rise in the rate of histamine release which is shorter than these blood pressure changes. It seems likely that the short-lasting elevation of the release of histamine represents a first signal for activating or inhibiting the release of other neurotransmitter(s) and/or hormone(s), thus initiating counteracting mechanisms (Philippu et al. 1983).

## 5.3 Histamine in Genetic Hypertension

Since the central administration of histamine increases blood pressure, the possible involvement of the amine in the development of genetic hypertension has been investigated.

Chalmers et al. (1979a) found no significant differences in the histamine levels of various brain areas between SHR and normotensive WKY rats. On the other hand, the histamine concentration was found to be greatly increased in the median eminence of 4-week-old SHR. In 12-week-old SHR, the histamine levels were also elevated in hypothalamic nuclei such as the suprachiasmatic nucleus, the arcuate nucleus, and the ventral premamillary nucleus, while the histamine concentration in brainstem areas was not changed (Corrêa and Saavedra 1981). These findings were largely confirmed by Oishi et al. (1985) who reported increased histamine levels in the telencephalon, hypothalamus and brainstem of SHR. A detailed determination in various nuclei was not attempted by these authors, but the turnover of histamine was estimated by determining the accumulation of tele-methylhistamine after inhibition of monoamine oxidase (MAO) with pargyline, as proposed by Hough et al. (1982, 1984). Surprisingly, the histamine turnover was found to be decreased in those areas in which histamine levels were

elevated (Oishi et al. 1985). Tele-methylhistamine levels were also found to be reduced in the brainstem and the hypothalamus of 5-week-old SHR, indicating that the methylation rate of histamine may be decreased. The decreased tele-methylhistamine concentrations may be due also to the increased MAO activity observed in brain areas of SHR (Yasuhara et al. 1983). Since catabolism and biosynthesis rates of catecholamines are disturbed in SHR (see also Sect. 3.3), it would be of interest to determine the histamine turnover by another method. On the other hand, in SHR the rate of histamine release was found to be increased in superfusates of the posterior hypothalamus (Tuomisto et al. 1983). The causal relationship of the above-mentioned findings with the development and/or maintenance of hypertension is far from established.

## 6 GABA and Other Neuroinhibitory and Neuroexcitatory Amino Acids

### 6.1 Mapping of Amino Acid-Containing Neurons

GABA and its synthesizing enzyme glutamic acid decarboxylase (GAD) are present in all brain areas. High GAD activity is found in the hypothalamus, the amygdaloid nuclei and the limbic system (olfactory tubercle, hippocampus, singulate cortex, dentate gyrus) (Fonnum et al. 1974, 1977; Tappaz et al. 1976). In the hippocampus, GABA immunoreactivity is present in cell bodies, dendrites and nerve terminals (Gamrani et al. 1986). In the hypothalamus, GAD-activity is present in short interneurons which seem to connect the lateral and posterior hypothalamus, as well as medial-basal hypothalamic nuclei (Tappaz and Brownstein 1977).

Recently, GABA neurons have been identified in the ventrolateral medulla. Rostrally, the GABA neurons coincide with and extend beyond the adrenaline C 1 cell group. Dual labelling for GAD and PNMT revealed that GABAergic terminals form synapses with PNMT-containing cell bodies and dendrites. Caudally, the GABAergic neurons partially overlap the noradrenaline A 1 cell group. Furthermore, GABA-containing cells are present in the parvocellular reticular nucleus and the raphe region (Ruggiero et al. 1985; Meeley et al. 1985; Milner et al. 1987). GABA neurons have also been identified in the nucleus of the solitary tract (Hwang and Wu 1984; Maley and Newton 1985; Meeley et al. 1985). However, the inhibitory neurotransmitter of the afferent projection from the nucleus of the solitary tract to the *rostral* ventrolateral medulla does not seem to be GABA, because lesions of the nucleus of the solitary tract do not alter GABA or GAD activity in the *rostral* ventrolateral medulla (Meeley et al. 1985).

## 6.2 Cardiovascular Effects of Amino Acids and Related Drugs

### *6.2.1 Cerebroventricular System*

In dogs (Elliot and Hobbiger 1959; Bhargava et al. 1964), cats (Philippu et al. 1973b; Antonaccio and Taylor 1977; Williford et al. 1980) and rats (Persson and Henning 1980; Yang and Lin 1983), the intracerebroventricular injection of the inhibitory amino acid GABA leads to a decrease in blood pressure and heart rate. Injection of GABA into the third ventricle also inhibits the pressor response to electrical stimulation of the posterior hypothalamus (Philippu et al. 1973b). The cardiovascular effects of GABA and the GABA agonist muscimol are inhibited by bicuculline (Persson 1980), an antagonist of GABA receptors (Curtis et al. 1970, 1971). More recently, it was shown that bicuculline is a specific antagonist of $GABA_A$-receptors (Bowery et al. 1980; Hill and Bowery 1981).

Bucuculline inhibits not only the cardiovascular effects of GABA because, when given alone, it leads to a rise in blood pressure and enhances the splanchnic and renal sympathetic nerve discharges, thus showing that the rise in blood pressure is due to increased sympathetic activity (Antonaccio et al. 1978). The inhibition by intraventricularly applied GABA of the pressor response to hypothalamic stimulation (Philippu et al. 1973b) also indicates the involvement of catecholaminergic systems in the central cardiovascular effects of GABA agonists and antagonists (see Sect. 3.2.3).

Experiments with the lipophilic GABA derivative baclofen (*p*-chlorphenyl-GABA) have shown that the cardiovascular effects of this compound depend on the anaesthetic. In conscious rats, intracerebroventricular administration of baclofen increases blood pressure and heart rate (Persson and Henning 1980), but in anaesthetized cats this compound leads to a fall in blood pressure and bradycardia (Bousquet et al. 1981b). The central cardiovascular effects of baclofen are not inhibited by the $GABA_A$-receptor blocking agent bicuculline. The ineffectiveness of bicuculline may indicate that the cardiovascular effects of baclofen are due to stimulation of $GABA_B$-receptors, which are insensitive to bicuculline (Bowery et al. 1980; Hill and Bowery 1981). Since stimulation of $GABA_B$-receptors inhibits the release of the neuroexcitatory amino acid glutamate (Potashner 1979; Johnston et al. 1980), the baclofen-induced fall in blood pressure has been attributed to inhibition of the glutamate release via stimulation of $GABA_B$-receptors (Bousquet et al. 1981b).

To further characterize the receptors involved in the central cardiovascular effects of GABA agonists, analogues of this amino acid have been given by intracerebroventricular injection to anaesthetized rats. The decrease in blood pressure elicited by muscimol or kojic amine (Atkinson et al. 1979; Sweet et al. 1979, 1980; Bousquet et al. 1984b) is reduced by bicuculline (Bousquet et

al. 1984b), indicating the involvement of $GABA_A$- and perhaps of $GABA_B$-receptors in the depressor response. On the other hand, the bradycardic effect of muscimol is virtually abolished by bicuculline, suggesting that $GABA_B$-receptors are not involved in the decrease in heart rate elicited by this GABA agonist (Bousquet et al. 1984b). Development of specific $GABA_B$-receptor antagonists may help to confirm these conclusions.

Like GABA, other neuroinhibitory amino acids such as glycine (Persson 1980; Bousquet et al. 1981b; Yang and Lin 1983) and taurine (Bousquet et al. 1981d; Yang and Lin 1983) also decrease blood pressure and heart rate when administered centrally. Since the central administration of these three amino acids enhances the adrenaline-induced bradycardia (Yang and Lin 1983), it seems likely that the bradycardic effects of GABA agonists are due to fascilitation of reflex bradycardia mediated through the vagus (Yang and Lin 1983). The inhibition by atropine of the central cardiovascular effects of GABA agonists supports this view (Bousquet et al. 1984b). As might be expected, the intracerebroventricular injection of the excitatory amino acid L-glutamate increases blood pressure and heart rate (Chelly et al. 1979; Bousquet et al. 1981b).

### *6.2.2 Brainstem*

GABA or glycine applied to the exposed ventral surface of the cat medulla cause a fall in blood pressure (Guertzenstein 1973; Guertzenstein and Silver 1974; Yamada et al. 1982). Microinjection of GABA (Ross et al. 1984) or of the GABA agonist muscimol into the *rostral* ventrolateral medulla (lateral reticular nucleus) also lowers blood pressure. The hypotensive action of muscimol is reversed by the $GABA_A$-receptor antagonist bicuculline (Bousquet et al. 1981c), suggesting the involvement of $GABA_A$-receptors in the central cardiovascular effect of muscimol (Bousquet et al. 1985).

The results suggest the involvement of GABAergic neurons of this area in baroreceptor modulation. This view is supported by the finding that GABA or glycine applied to the exposed *rostral* part of the ventral surface of the medulla attenuate the pressor response to carotid occlusion (Feldberg and Rocha e Silva Jr. 1981; Yamada et al. 1984). In this connection it is of interest to note that the depressor response to GABA injected into the *rostral* ventrolateral medulla was found to be attenuated in SHR (Kubo et al. 1986b). Determination of diffusion of locally applied [$^3$H]bicuculline revealed that the GABAergic synapse involved in baroreceptor modulation lies within the first 2 mm of the *rostral* ventrolateral medulla (Yamada et al. 1984).

As might be expected, microinjection of the antagonist bicuculline into the *rostral* ventrolateral medulla increases blood pressure and heart rate (Ross et al. 1984; Willette et al. 1984a) and enhances the pressor response to carotid occlusion (Willette et al. 1984a).

On the other hand, microinjections of muscimol, glycine, or GABA into the *caudal* ventrolateral medulla increase blood pressure and heart rate (Blessing and Reis 1983; Willette et al. 1983), while bicuculline leads to a fall in blood pressure and bradycardia (Willette et al. 1984a). It seems that GABA neurons in the *rostral* and *caudal* ventrolateral medulla exert opposing effects on the homoeostasis of blood pressure and heart rate. Probably, most of these GABAergic neurons belong to an intrinsic neuronal population (see Sect. 6.1). It has been proposed that the pressor response to microinjections of inhibitory amino acids into the *caudal* ventrolateral medulla is due to inhibition of the noradrenergic sympathoinhibitory A 1 cell group of this area (Blessing and Reis 1983).

Opposite cardiovascular effects have also been described for the neuroexcitatory amino acid L-glutamate (Table 8). Injection of this amino acid into the *rostral* ventrolateral medulla increases blood pressure and heart rate (Dampney 1981; Willette et al. 1984a; Kubo et al. 1985b), while injection into the *caudal* ventrolateral medulla elicits a fall in blood pressure and bradycardia (Blessing and Reis 1982; Willette et al. 1984a).

As in the *caudal* ventrolateral medulla, microinjections of GABA or muscimol into the nucleus of the solitary tract lead to hypertension and tachycardia, while the antagonist bicuculline lowers blood pressure and heart rate and inhibits the cardiovascular effects of muscimol (Bousquet et al. 1982). On the other hand, microinjection of the neuroexcitatory amino acids L-glutamate (Talman et al. 1980b, 1984; Kubo and Kihara 1988) or *N*-methyl-*D*-aspartate (Kubo and Kihara 1988) into the nucleus of the solitary tract causes dose-dependent decreases in blood pressure and heart rate (Table 8). The cardiovascular effects of glutamate and *N*-methyl-*D*-aspartate are abolished by the receptor antagonists glutamate diethyl ester (Talman et al. 1984) and 2-amino-5-phosphonovalerate (Kubo and Kihara 1988), respectively. Moreover, vagal stimulation enhances the release of tritium in a push-pull cannula inserted into the nucleus of the solitary tract after preloading the

**Table 8.** Effects of locally applied neuroinhibitory and neuroexcitatory amino acids on blood pressure and heart rate

| | Blood pressure and heart rate | |
|---|---|---|
| | IA | EA |
| RVLM | Decrease | Increase |
| CVLM | Increase | Decrease |
| NTS | Increase | Decrease |

*RVLM, rostral* ventrolateral medulla; *CVLM, caudal* ventrolateral medulla; *NTS*, nucleus of the solitary tract; *IA*, neuroinhibitory amino acids (GABA, glycine, taurine); *EA*, neuroexcitatory amino acid (glutamate, aspartate). For references see text

nucleus with $L[^3H]$-glutamate (Talman et al. 1984). It appears that glutamatergic neurons within or projecting into the nucleus of the solitary tract participate in the baroreceptor reflex. This postulate should be confirmed by determining the release of endogenous glutamate, because the mere determination of tritium release is far from convincing. Kubo and Kihara (1988) reported that it is possible to determine glutamate and aspartate in perfusates of the nucleus of the solitary tract perfused through a push-pull cannula.

*Release by Amino Acids of Vasopressin in the Hypothalamus*

The release of vasopressin is inhibited by afferent inputs from the atrial receptors (Gauer and Henry 1963; Shade and Share 1975) and from carotid and aortic baroreceptors (Share and Levy 1962; Clark and Rocha e Silva Jr. 1967). Attenuation by carotid occlusion of this inhibitory mechanism leads to vasopressin release.

Applied to a caudal region B of the ventrolateral medulla (situated at the transition between spinal cord and medulla), the inhibitory amino acids GABA and glycine have virtually no effect on blood pressure (Feldberg and Guertzenstein 1976; Feldberg and Rocha e Silva Jr. 1981). This area does not seem to correspond totally to the *caudal* ventrolateral medulla of the rat, because inhibitory amino acids applied to the latter area increase blood pressure (see Sect. 6.2.2). When applied to this region B, both amino acids greatly reduce the release of vasopressin. When applied to a region A situated rostral to the region B, the amino acids decrease blood pressure and pressor response to carotid occlusion without influencing the vasopressin release (Feldberg and Rocha e Silva Jr. 1981).

Vasopressin may be responsible for the pressor response to the microinjection of the excitatory amino acid L-glutamate into the *rostral* ventrolateral medulla, because L-glutamate increases the release of the peptide. Moreover, the pressor response to the amino acid is abolished by the vasopressin antagonist TMAV which blocks $V_1$ receptors (Kubo et al. 1985b). Similarly, the depressor response to glutamate injection into the *caudal* ventrolateral medulla increases the release of vasopressin so as to counteract the fall in blood pressure (Blessing and Willoughby 1985).

From these findings, and from those described in the preceding sections, the following picture emerges (Fig. 8). The afferent fibres to the nucleus of the solitary tract and/or neurons within the nucleus seem to be glutamatergic. Stimulation of baroreceptors of the aortic arc and carotid sinus by a rise in blood pressure increases the release of the excitatory amino acid, which in turn increases the release of an inhibitory neurotransmitter. The inhibitory neurotransmitter, which does not seem to be GABA (see Sect. 6.2.1), inhibits the sympathoexcitatory neurons in the *rostral* ventrolateral medulla, leading to a fall in blood pressure. The inhibitory neurotransmitter also inhibits the

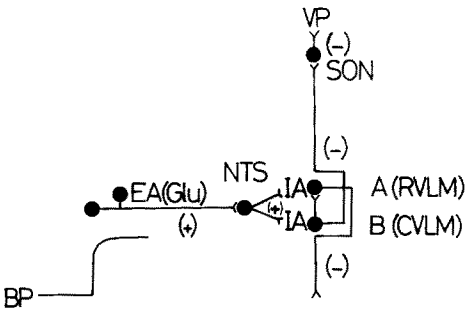


**Fig. 8.** Effects of a rise in blood pressure in the carotid sinus on the release of neurotransmitters in the brainstem. The rise in blood pressure (*BP*) increases the release of an excitatory amino acid (*EA*) in the region B of the caudal ventrolateral medulla (*CVLM*), thus leading to decreased neuronal activities of the ascending pathways and to decreased release of vasopressin (*VP*). Release of the inhibitor amino acid (*IA*) in the rostral region A of the ventrolateral medulla (*RVLM*) decreases the sympathetic tone to blood vessels and heart. The sympathoinhibitory influence of the caudal ventrolateral medulla is mediated through noradrenergic neurons to the rostral ventrolateral medulla. The excitatory amino acid seems to be glutamic acid (*Glu*). *NTS*, nucleus of the solitary tract; *SON*, supraoptic nucleus; +, increased neuronal activity; –, decreased neuronal activity

neurons of the caudally located region B and consequently the release of vasopressin. The activity of the neurons of the *rostral* ventrolateral medulla may also be reduced by the *caudal* ventrolateral medulla, presumably through noradrenergic neurons of the A1 cell group. Carotid occlusion decreases the release of the inhibitory neurotransmitter(s) thus enhancing the release of vasopressin and increasing the neuronal activity in the excitatory *rostral* ventrolateral medulla. The activity of the neurons of the two areas of the ventrolateral medulla is influenced by intrinsic GABAergic neurons. However, the importance of adrenergic and noradrenergic neurons of the *rostral* and *caudal* ventrolateral medulla, respectively, needs to be confirmed.

### *6.2.3 Hypothalamus*

In conscious rats, the GABA agonist muscimol decreases blood pressure and heart rate when applied to the hypothalamus. The GABA-receptor antagonists bicuculline and picrotoxin injected elicit a rise in blood pressure and tachycardia and increase sympathetic nerve activity indicating that the sympathoexcitatory system of the hypothalamus is modulated by its GABAergic neurons (Wible et al. 1988). This is in accordance with the observation that in cats intracerebroventricular injection of GABA decreases the pressor response elicited by hypothalamic stimulation (Philippu et al. 1973b). Surprisingly, superfusion of the posterior hypothalamus with high GABA concentrations through a push-pull cannula gradually increases the release of [$^3$H]noradrenaline in the superfusate and the pressor response to hypothalamic stimulation (Philippu et al. 1973b).

### 6.3 GABA in Genetic Hypertension

In 75-day-old SHR, but not in 30-day-old hypertensive rats, the hypothalamic GABA level is decreased and the muscimol binding sites are reduced (Hambley et al. 1984). The release rate of GABA in hypothalamic superfusates of 60-day-old SHR is not changed (Tuomisto et al. 1983). Thus, GABA changes seem to be established in rats older than 8–10 weeks.

## 7 Acetylcholine

### 7.1 Mapping of Acetylcholine-Containing Neurons

Acetylcholinesterase is present in neurons of the hypothalamus and of the ventral thalamus. It is of interest to note that no cholinergic perikarya are present in the cerebral cortex, the hippocampus and the amygdala, but acetylcholinesterase-containing cell bodies are found in the raphe nuclei and in the central part of the gigantocellular reticular nucleus (Satoh et al. 1983).

Acetylcholine is also found in neuronal and non-neuronal (blood vessel walls) elements of the nucleus of the solitary tract (Lewis and Shute 1967; Gwyn and Wolstencroft 1968). However, no acetylcholinesterase activity was found in the nucleus of the solitary tract by Palkovits and Jacobowitz (1974). There are also conflicting results concerning the presence of the synthesizing enzyme choline acetyltransferase in this nucleus. While Kobayashi et al. (1975) and Helke et al. (1983), using radioimmunoassay, found enzyme activity in this brain structure, Armstrong et al. (1983), using immunocytochemistry, demonstrated the presence of the enzyme in the dorsal vagal complex, but not in the nucleus of the solitary tract. Acetylcholinesterase-stained cells are present in the locus coeruleus. In this area, most of the perikarya seem to contain noradrenaline and acetylcholinesterase (Palkovits and Jacobowitz 1974).

### 7.2 Cardiovascular Effects of Acetylcholine and Related Drugs

#### *7.2.1 Cerebroventricular System*

Species differences and anaesthesia influence the cardiovascular effects of acetylcholine and related compounds (for review see Philippu 1981).

In conscious and anaesthetized rats, the intracerebroventricular administration of acetylcholine elicits a pressor response which is inhibited by atropine (Krstić and Djurković 1978). Inhibition of the acetylcholine degradation by physostigmine also increases blood pressure (Dirnhuber and Cullumbine

1955; Varagić 1955). Most important, the pressor response to physostigmine is abolished by hemicholinium-3 (Varagić and Vojvodić 1962), which, by preventing the uptake of choline into the nerve terminals, leads to acetylcholine depletion (McIntosh et al. 1956; for review see Schueler 1960). The findings with physostigmine and hemicholinium-3 are of particular interest; they show that acetylcholine released from cholinergic nerve terminals of the brain influences the cardiovascular system, thus demonstrating the importance of central cholinergic neurons in cardiovascular control.

In the anaesthetized cat, intracerebroventricular injections of carbachol elicit a fall in blood pressure and bradycardia (Armitage and Hall 1967; Ingenito et al. 1972), which have been attributed to an action of the drug on "the ventral brainstem" (Armitage and Hall 1967). In the conscious cat, acetylcholine and carbachol increase blood pressure and lower heart rate by an action on muscarinic receptors, because the pressor response is inhibited by central administration of atropine (Sinha et al. 1967; Lang and Rush 1973; Day and Roach 1977). However, the central administration of various muscarinic or nicotinic receptor antagonists revealed that nicotinic receptors, as well as muscarinic receptors, are involved in the central cardiovascular effects of acetylcholine (Armitage and Hall 1967; Armitage et al. 1967; Day and Roach 1977). Acetylcholine also increases blood pressure in conscious (Lang and Rush 1973) and anaesthetized dogs (Bhawe 1958; Sinha et al. 1967). Hence, in rats, dogs and conscious cats acetylcholine elicits a rise in blood pressure when applied centrally, while in anaesthetized cats it lowers blood pressure.

The effect on cardiovascular function of acetylcholine and other cholinergic agonists and antagonists depends on the integrity of the catecholaminergic system of the brain. Intracerebroventricular injections of 6-OHDA deplete the brain catecholamines and inhibit the pressor response to centrally applied carbachol (Gordon et al. 1979). The rise in blood pressure effected by carbachol is also inhibited by the central administration of guanethidine or bethanidine (Ozawa and Uematsu 1976; Hoffman 1979) and $\alpha$- and $\beta$-adrenoreceptor blocking agents (Ozawa and Uematsu 1976; Day and Roach 1977; Hoffman 1979), and is enhanced by desipramine (Ozawa and Uematsu 1976), which inhibits the reuptake of catecholamines into the nerve terminals, thus increasing their concentrations at the receptor sites. Moreover, perfusion of the cat hypothalamus through the third ventricle with the nicotinic agonist DMPP (dimethyl-4-phenylpiperazinium) enhances the release of [$^3$H]noradrenaline in the perfusate in a calcium-dependent way. The catecholamine release is also increased by the muscarinic antagonist atropine (Philippu 1970; Philippu et al. 1970). The results suggest that, as in the peripheral sympathetic system (for review see Muscholl 1979), the release of catecholamines in the CNS is modulated by acetylcholine receptors; stimulation of nicotinic and blockade of muscarinic receptors enhance the release of noradrenaline.

Hence, the cardiovascular effects of the central cholinergic neurons seem to be mediated at least partly by acetylcholine receptors localized presynaptically on catecholaminergic nerve endings.

### *7.2.2 Brainstem*

#### *Ventrolateral Medulla*

Carbachol applied to the ventral surface of the anaesthetized cat medulla lowers blood pressure. The same effect is elicited when physostigmine is locally applied, while atropine elicits a pressor response (Guertzenstein 1973; Feldberg and Guertzenstein 1976), thus underpinning the view that centrally applied acetylcholine affects blood pressure by acting on the brainstem.

Like carbachol, nicotine applied to the ventral surface of the cat medulla leads to a fall in blood pressure. The depressor response is associated with an increased level of vasopressin in the blood. The effect has been attributed to activation by nicotine of central projections to the supraoptic and paraventricular nuclei, so as to counteract the fall in blood pressure (Bisset et al. 1975).

In the rat, where central administration of acetylcholine increases blood pressure, microinjections of carbachol or physostigmine into the *rostral* ventrolateral medulla also increase blood pressure and heart rate, while atropine leads to a fall in blood pressure and bradycardia (Willette et al. 1984c). In this connection it is interesting that, in rats, the rise in blood pressure elicited by intravenous injection of physostigmine is abolished when tetrodotoxin, local anaesthetics or the muscarinic receptor antagonist scopolamine are injected into the *rostral* ventrolateral medulla (Punnen et al. 1986). It seems that this area mediates the pressor response to peripherally administered physostigmine. Carbachol also lowers blood pressure and heart rate when microinjected into the rostral raphe nucleus, thus demonstrating the presence of muscarinic receptors in the nucleus (Saxena et al. 1983).

#### *Area Postrema and Nucleus of the Solitary Tract*

Fall in blood pressure and bradycardia are also elicited when physostigmine, choline or nicotine are given by microinjection into the rat area postrema. The cardiovascular effects seem to be mediated by nicotinic receptors, because they are abolished by hexamethonium but not by atropine. Intracerebroventricular administration of 6-OHDA diminishes the hypertensive response to physostigmine and choline. Moreover, the cardiovascular effects of these drugs are attenuated by phentolamine injected into the nucleus of the solitary tract, suggesting that the cardiovascular effects of acetylcholine released from cholinergic nerve terminals are mediated by nicotinic receptors localized on catecholaminergic neurons (Kubo and Misu 1981b,c). However, the fall in

blood pressure and bradycardia elicited by microinjections of acetylcholine or carbachol into the nucleus of the solitary tract of the rat seem to be due to activation of muscarinic receptors, since the effects are abolished by atropine but not by hexamethonium. Atropine given alone elicits a moderate rise in blood pressure. The muscarinic antagonist decreases, while physostigmine increases the bradycardia elicited by an experimentally induced rise in blood pressure. The results seem to indicate that cholinergic mechanisms in the nucleus of the solitary tract modulate the baroreceptor reflex (Criscione et al. 1983). However, it was shown very recently that carbachol microinjected into the nucleus of the solitary tract of cats is ineffective, while microinjections of the drug into the nucleus ambiguus or the dorsal motor nucleus of the vagus lower heart rate without influencing blood pressure (Gurtu et al. 1986). It is possible that, in the rat, carbachol injected into the nucleus of the solitary tract may easily reach adjacent structures, thus eliciting cardiovascular effects.

### *7.2.3 Hypothalamus*

It is now well established (for review see Phillippu 1981) that injections of acetylcholine receptor agonists into the posterior hypothalamus of rats lead to a rise in blood pressure and to variable effects on heart rate (Cho et al. 1962; Hoffman and Phillips 1976). The cardiovascular effects seem to be due predominantly to activation of muscarinic receptors, because atropine abolishes the pressor response to cholinergic agonists, while mecamylamine is ineffective (Buccafusco and Brezenoff 1979). Microinjections of the cholinesterase inhibitors neostigmine or physostigmine into the posterior hypothalamic nucleus of conscious or anaesthetized rats also increase blood pressure and decrease heart rate. Similar effects are elicited by the intrahypothalamic injection of *d*-tubocurarine (Fletscher and Pradhan 1969; Brezenoff 1972; Buccafusco and Brezenoff 1979).

Although these results suggest that release of acetylcholine in the brain influences the cardiovascular system, depletion of the acetylcholine pools by the intrahypothalamic injection of hemicholinium-3 does not affect blood pressure. It seems that the cholinergic system is quiescent under resting conditions, but that it is activated when the concentration of acetylcholine is increased at the receptor sites, as for example on administration of cholinesterase inhibitors. A similar effect is elicited when acetylcholine receptor agonists are microinjected (Buccafusco and Brezenoff 1978; Brezenoff and Caputi 1980). Nevertheless, the cardiovascular effects of intravenously applied cholinesterase inhibitors are not due to their action on the hypothalamus, because decerebration (Varagić 1955) or transection of the brain caudal to the hypothalamus does not modify the pressor effects. The rise in blood pressure elicited by intravenous injection of physostigmine is abolished when the brain

is transected at the rostral pons, indicating that the site of action of cholinesterase inhibitors is localized caudal to the midbrain (Brezenoff and Rusin 1974).

The rise in blood pressure caused by muscarinic agents and cholinesterase inhibitors is not due to release of catecholamines from the adrenal medulla, because adrenalectomy does not affect the pressor response to these drugs (Dirnhuber und Cullumbine 1955; Varagić 1955; Henning and Trolin 1975). Likewise, bretylium and 2,6-xylyl ether bromide almost abolish the pressor response to cholinesterase inhibitors. These drugs block the peripheral noradrenergic neurons without influencing the release of catecholamines from the suprarenals (Lesić and Varagić 1961).

In the cat, superfusion of the posterior hypothalamus through a push-pull cannula with acetylcholine, carbachol or nicotine increases blood pressure, while superfusion with the muscarinic agonists pilocarpine or oxotremorine is ineffective (Bhargava et al. 1978). Superfusion of the posterior hypothalamus with the nicotinic agent DMPP or nicotine also enhances the pressor response elicited by electrical stimulation of the superfused area (Philippu et al. 1974; P. Schartner and A. Philippu, unpublished observations). The muscarinic and nicotinic agonist arecoline (Feldberg and Vartiainen 1935; Von Euler and Domeij 1945) also enhances the rise in blood pressure on hypothalamic stimulation, but the increase in the pressor response is converted to an inhibition of the pressor response after hypothalamic superfusion with hexamethonium to block nicotinic receptors. Moreover, hypothalamic superfusion with muscarine or the muscarinic agonists oxotremorine or AHR 602 (*N*-benzyl-3-pyrolidyl-acetate methobromide) also reduces the pressor response to hypothalamic stimulation (Philippu and Bohuschke 1976). Thus, in the cat, nicotinic and muscarinic receptors are present in the posterior hypothalamus. Stimulation of nicotinic receptors increases blood pressure and enhances the pressor response to hypothalamic stimulation, while activation of muscarinic receptors reduces the rise in blood pressure elicited by hypothalamic stimulation. As may be expected (see Sect. 7.2.1), hypothalamic superfusion with $\beta$-adrenoreceptor blocking drugs abolishes the rise in blood pressure elicited by hypothalamic superfusion with acetylcholine, suggesting the involvement of catecholaminergic systems (Bhargava et al. 1978).

The significance of hypothalamic acetylcholine for blood pressure regulation is underlined by the observation that injections of physostigmine or neostigmine into the posterior hypothalamic nucleus enhance the pressor response to bilateral carotid occlusion. The effect of the cholinesterase inhibitors is suppressed by the intrahypothalamic injection of atropine. It seems that acetylcholine in the posterior hypothalamus is implicated in the modulation of the baroreceptor reflex (Brezenoff et al. 1982).

## 7.3 Acetylcholine in Drug-Induced Hypotension and in Experimental and Genetic Hypertension

As already mentioned (see Sect. 3.4), clonidine inhibits the pressor response to physostigmine and reduces the turnover of acetylcholine in various brain regions. The antihypertensive effect of clonidine is also reduced in rats pretreated intracerebroventricularly with hemicholinium-3 (Squadrito et al. 1985), suggesting that the antihypertensive effect of clonidine depends partially on the integrity of central cholinergic neurons. In contrast, the hypotensive effect of intravenously administered $\alpha$-methyldopa is potentiated by the intracerebroventricular injection of hemicholinium-3. $\alpha$-Methyldopa also inhibits the pressor response to the intracerebroventricularly applied cholinesterase inhibitor echothiophate, while the hypertensive effect of the directly acting agonist carbachol is not affected. This difference may indicate that the antihypertensive drug $\alpha$-methyldopa interferes with the release of acetylcholine (Buccafusco 1984).

Remarkable differences are seen in the central cardiovascular effects of acetylcholine-receptor agonists and antagonists between normotensive and hypertensive rats. The pressor response to intravenously injected physostigmine is much more pronounced in 5- to 10-month-old SHR than in normotensive WKY rats (Kubo and Tatsumi 1979). Similarly, the pressor response to systemic administration of physostigmine is greater in Dahl-salt-sensitive rats than in Dahl-salt-resistant animals (McCaughran et al. 1983). Since no differences exist in the increases in blood pressure between normal rats, on the one hand, and renal hypertensive or DOCA-salt-sensitive rats, on the other hand, the enhanced pressor response to physostigmine in SHR and Dahl-salt-sensitive rats seems to be specific to the genetic hypertension.

As mentioned above, intracerebroventricular administration of hemicholinium-3 does not influence blood pressure. However, central injection of hemicholinium-3 lowers blood pressure in SHR (Brezenoff and Caputi 1980). Moreover, in conscious rats, the intravenous injection of atropine decreases blood pressure in 11- to 20-week-old SHR, but not in normotensive WKY rats (Caputi et al. 1980).

The findings obtained with physostigmine and hemicholinium-3 suggest an increased activity of cholinergic neurons in the brain of SHR. This idea is confirmed by the observations that the activities of choline acetyltransferase and acetylcholinesterase are increased in the brainstem of young (40-day-old) SHR, while the activity of acetylcholinesterase is additionally elevated in brainstems of old (3- to 6-month-old) SHR (Yamori et al. 1972). Choline acetyltransferase activity and acetylcholine level are also increased in the locus coeruleus but decreased in hypothalamic nuclei of SHR. Furthermore, the activity of this enzyme was found to be increased in the nucleus of the

solitary tract, but to be decreased in the dorsal hypothalamic nucleus of DOCA-salt hypertensive rats (Helke et al. 1980a, b).

## 8 Vasopressin

### 8.1 Mapping of Vasopressin-Containing Neurons

The magnocellular and parvocellular neurons of the paraventricular and supraoptic nuclei synthesize vasopressin and oxytocin (Sofroniew and Weindl 1978; Sofroniew 1980; Sofroniew et al. 1981; for review see Swanson and Sawchenko 1983; Silvermann and Zimmerman 1983). Vasopressin-immunostaining cell bodies have also been identified in accessory nuclei of the hypothalamus (Buijs 1978; Sofroniew 1983), as well as in the medial amygdaloid nucleus and the locus coeruleus (Sofroniew 1983; Buijs et al. 1983). Peptide-containing fibres have been identified in the locus coeruleus, the nucleus of the solitary tract, the dorsal motor nucleus of the vagus and the dorsal raphe nucleus (Buijs and Swaab 1979; Sofroniew 1983; Voorn and Buijs 1983). A descending vasopressinergic pathway extends from the paraventricular and supraoptic nuclei to brainstem structures involved in cardiovascular control. In the locus coeruleus, vasopressin seems to be present in noradrenaline cell bodies (Caffé et al. 1985).

### 8.2 Cardiovascular Effects of Vasopressin and Related Drugs

#### *8.2.1 Cerebroventricular System*

The intracerebroventricular or intrathecal administration of arginine-vasopressin to conscious or anaesthetized rats and rabbits increases blood pressure (Matsuguchi et al. 1982; Pittman et al. 1982; Feuerstein et al. 1984; Martin et al. 1985; Riphagen and Pittman 1986), while the heart rate is either increased (low doses) or decreased (high doses) (Feuerstein et al. 1984; Riphagen and Pittman 1986). These cardiovascular effects are elicited by doses lower than those needed when vasopressin is injected intravenously. Hence, an action of centrally applied vasopressin on peripheral receptors is unlikely.

However, contrasting results have been reported by Versteeg et al. (1982), who found that the intracerebroventricular administration of vasopressin does not affect the cardiovascular system of anaesthetized rats. The authors further observed that vasopressin administered intracerebroventricularly inhibits the pressor response elicited by electrical stimulation of the mesenceph-

alic reticular formation (De Jong et al. 1984). In the anaesthetized dog, intracisternal administration of lysine-vasopressin decreases blood pressure without changing heart rate, while oxytocin leads to a pressor response (Tran et al. 1982). The latter finding might indicate that action of vasopressin on brainstem structures decreases blood pressure.

Haemorrhage is a potent stimulus for vasopressin release to restore arterial blood pressure (Ginsburg and Brown 1956; Baratz and Ingraham 1960; Beleslin et al. 1967; Rocha e Silva Jr. and Rosenberg 1969; Laycock et al. 1979; Cowley et al. 1980; Schwartz and Reid 1981; Zerbe et al. 1982). Haemorrhage and osmotic stimulation also enhance the release of vasopressin in perfusates of the lateral ventricle and septum (Demotes-Mainard et al. 1986). Hence, vasopressin seems to play a predominant role in blood pressure regulation.

The influence of vasopressin on the baroreceptor reflex is equally well established, although contrasting results have been reported as to whether vasopressin stimulates or inhibits this reflex. Determination of the baroreflex activity by plotting changes in pulse interval against changes in blood pressure after intravenously administered phenylephrine (Smyth et al. 1969) revealed that in rats and rabbits central administration of vasopressin increases baroreflex sensitivity (Izdebska et al. 1982; Imai et al. 1983; Schmid et al. 1985). Furthermore, in Brattleboro rats with diabetes insipidus and a complete lack of endogenous vasopressin, baroreflex sensitivity to phenylephrine is greatly reduced in comparison with that in normal Long-Evans rats (Imai et al. 1983). On the other hand, it has been reported that in dogs intracisternally applied vasopressin attenuates the fall in blood pressure elicited by stimulation of the carotid sinus (Brattström and Kalkoff 1970). Similarly, blockade of vascular vasopressin receptors by the intracerebroventricular administration of the $V_1$-receptor antagonist TMAV sensitizes the baroreceptor reflex (Unger et al. 1986). Species differences and variation in routes of administration and/or stimulation of various vasopressin receptors by vasopressin (see below) may explain the diversity of the results.

### *8.2.2 Brainstem*

Blessing et al. (1981 b, 1982) reported that in the rabbit electrolytic lesions of the *caudal* ventrolateral medulla elicited hypertension and increased plasma vasopressin. Similar results were obtained more recently by Elliott et al. (1985 b), but Sved et al. (1985) were not able to confirm their earlier results (Blessing et al. 1981 b, 1982); the electrolytic lesion did not increase blood pressure and only slightly increased plasma vasopressin. It is likely that slight differences in the position of electrodes greatly influence the cardiovascular response (Sved et al. 1985).

The rise in blood pressure elicited by bilateral lesions of the nucleus of the solitary tract is also associated with an enhanced release of vasopressin in the hypothalamus (see Sect. 3.2.2). In animals with bilateral lesions of the nucleus of the solitary tract, administration of TMAV, a $V_1$-receptor antagonist, which blocks the vascular effects of vasopressin, inhibits hypertension and increases heart rate (Barnes et al. 1984; Kubo and Amano 1986). Similarly, electrical stimulation of the nucleus of the solitary tract of rats with spinal transection at C1 leads to a rise in blood pressure which seems to be due to release of vasopressin, because stimulation of this nucleus is ineffective in Brattleboro rats (Nakai et al. 1982). Microinjections of vasopressin into the nucleus of the solitary tract of the rat also increase blood pressure and heart rate (Vallejo et al. 1984; Casto and Phillips 1985). These cardiovascular effects are abolished by the $V_1$-receptor antagonist TMAV, while the $V_2$-receptor antagonist 1-desamino-8-D-arginine-vasopressin (DDAV) or oxytocin are ineffective (Vallejo et al. 1984). DDAV possesses a potent antidiuretic activity, but a minimum vascular action (Sawyer et al. 1974). Taken together, the results provide evidence that vasopressin plays a role in cardiovascular control in the nucleus of the solitary tract and that this effect of vasopressin is mediated by $V_1$- rather than by $V_2$-receptors.

In other areas of the brainstem vasopressin also seems to be involved in cardiovascular regulation. The pressor response to electrical stimulation of the locus coeruleus in Brattleboro rats is less pronounced than that in Long-Evans rats, indicating involvement of vasopressin (Berecek et al. 1984; Berecek and Mitchum 1986) (see Sect. 3.2.2). Furthermore, vasopressin is also implicated in the pressor response to electrical stimulation of the fastigial nucleus observed after chemosympathectomy with 6-OHDA, since the rise in blood pressure is antagonized by the $V_1$-receptor antagonist TMAV (Del Bo et al. 1983). Finally, ablation of the area postrema prevents intravenously administered arginine-vasopressin from enhancing the inhibitory influence of the baroreceptor reflex (Undesser et al. 1985) and increases the pressor response to vasopressin administered to the vertebral artery (Michelini et al. 1986). It seems that vasopressin acts on the area postrema or the tissue surrounding it so as to enhance baroreflex activity (Undesser et al. 1985).

### *8.2.3 Hypothalamus*

Microinjections of arginine-vasotocin or arginine-vasopressin into the medial preoptic nucleus of the hypothalamus increase blood pressure and heart rate, while oxytocin is ineffective. The cardiovascular effects are associated with elevated noradrenaline and adrenaline plasma levels (Feuerstein et al. 1984). In rats with intact baroreceptor reflex electrical stimulation of magnocellular or parvocellular regions of the paraventricular nucleus of the hypothalamus has virtually no effect on the cardiovascular system. However, following sino-

aortic denervation, stimulation of parvocellular cells increases blood pressure, but stimulation of magnocellular cells is still ineffective. Thus, the baroreceptor reflex buffers the effects of parvocellular cell activation (Porter and Brody 1986).

### 8.3 Vasopressin in Experimental and Genetic Hypertension

Under normal conditions, vasopressin levels in various brain nuclei have been found to be similar in SHR and normotensive rats, but acute stress seems to increase vasopressin in SHR (Negro-Vilar and Saavedra 1980). In contrast to these results, hypothalamic vasopressin was found to be decreased in SHR (Morris et al. 1981). On the other hand, injections of the vasopressin-receptor inhibitors TMAV and DDAV do not alter either blood pressure or heart rate in SHR, although both antagonists almost completely abolish the pressor response to exogenous arginine-vasopressin (Filep and Fejes-Tóth 1986).

In DOCA-salt hypertensive rats no changes in the hypothalamic level of vasopressin have been found (Morris et al. 1981), suggesting that vasopressin is not involved in DOCA-salt hypertension. The same conclusion was drawn by Okuno et al. (1983), who observed that in DOCA-salt hypertensive rats pretreatment with 6-OHDA lowers blood pressure without decreasing vasopressin levels. Furthermore, TMAV and DDAV do not alter blood pressure and heart rate in malignant two-kidney one-clip Goldblatt hypertension (Filep et al. 1985). The findings demonstrate that vasopressin is not involved in the development of genetic or experimental hypertension.

## 9 Angiotensin

### 9.1 Mapping of Angiotensin-Containing Neurons

Angiotensin II-like immunoreactivity has been demonstrated in cell bodies located in the supraoptic and paraventricular nuclei, as well as in the dorsomedial hypothalamic nucleus, the perifornical area and the ventrolateral part of the lateral hypothalamus (Fuxe et al. 1981).

Angiotensin II-like immunoreactivity is present in numerous axons and nerve terminals of the median eminence and the lateral column of the spinal cord. The dorsomedial hypothalamic nucleus, the ventral hypothalamus, the central amygdaloid nucleus and the locus coeruleus possess a moderate density of angiotensin II-like immunoreactivity, while the density is low in the thalamus, the periventricular hypothalamus, the preoptic region, and the subthalamus, as well as in the locus coeruleus, the nucleus of the solitary tract

and the dorsal motor nucleus of the vagus. Single nerve terminals are present in all levels of the brain (Fuxe et al. 1976).

## 9.2 Cardiovascular Effects of Angiotensin and Related Drugs

### *9.2.1 Cerebroventricular System*

In many animals species, the intracerebroventricular administration of angiotensin II produces a pressor response (Halliday and Buckley 1962; Smookler et al. 1966; Severs et al. 1966; Hoffman and Phillips 1977), which is inhibited by the angiotensin antagonist saralasin (Hoffman and Phillips 1977). Angiotensin III has the same pressor activity as angiotensin II when administered intracerebroventricularly. Moreover, chronic infusion of angiotensin II or III into the lateral ventricle leads to severe hypertension (Fink and Bruner 1985).

The central cardiovascular effects of angiotensin II seem to be mediated partly by the sympathetic system, because adrenalectomy and peripheral administrations of 6-OHDA (Falcon et al. 1978), phenoxybenzamine, pronethalol (Severs et al. 1966) or prazosin attenuate the rise in blood pressure elicited by the intracerebroventricular injection of angiotensin II. The $V_2$-receptor antagonist DDAV also reduces the angiotensin-induced pressor response, but the rise in blood pressure is abolished by a combined pretreatment with DDAV and prazosin (Unger et al. 1981). Hence, stimulation of the sympathetic system and release of vasopressin seem to contribute to the rise in blood pressure elicited by central administration of angiotensin II.

A similar interaction exists between angiotensin II and GABA. Intracerebroventricular injection of GABA or of the GABA-receptor agonist muscimol reduces the pressor response to central administration of angiotensin II (Unger et al. 1983; Brennan et al. 1984). The inhibitory effect of GABA seems to be due to decreased vasopressin release by angiotensin, because GABA inhibits the vasopressin-dependent pressor response to the peptide (Brennan et al. 1984). This observation is in agreement with the interaction between GABA and vasopressin described in Section 6.2.2.

### *9.2.2 Brainstem*

The area postrema has been proposed as a site of angiotensin action. Electrical stimulation of this area leads to a rise in blood pressure and tachycardia. In the dog, ablation of the area postrema lowers blood pressure and heart rate (Ferrario et al. 1979), but in the rat ablation of this region is either ineffective (Zandberg et al. 1977), or it leads to chronic labile hypertension (Ylitalo et al. 1974). It is possible that the contrasting results are due to the

anatomical proximity of the area postrema and the nucleus of the solitary tract, since it is difficult to lesion one of these two regions without damaging the other. In the dog the pressor response to intravenously applied angiotensin II is blunted after ablation of the area postrema (Gildenberg et al. 1973; Ferrario et al. 1979); this attenuation of the pressor response to angiotensin lasts several weeks (Joy and Lowe 1970), becoming normal again 4–7 weeks after ablation (Otsuka et al. 1986).

It seems that the area postrema is not the sole region of the brainstem responsible for the central cardiovascular effects of angiotensin. In anaesthetized rats, microinjection of low doses (1 ng) of angiotensin II into the nucleus of the solitary tract decreases blood pressure and heart rate, while the angiotensin-receptor antagonist saralasin exerts opposite effects. However, moderate doses of angiotensin II (10 ng) lead to biphasic blood pressure changes (Rettig et al. 1986) and high doses (50–500 ng) increase blood pressure without changing heart rate (Casto and Phillips 1984; Rettig et al. 1986). The bradycardic response to low doses of angiotensin II seems to be mediated by cholinergic fibres, because it is abolished by atropine injected intravenously (Rettig et al. 1986). The pressor response to high doses of angiotensin II is reduced by ganglionic blockade with hexamethonium, indicating the involvement of descending sympathetic fibres (Casto and Phillips 1984).

### *9.2.3 Hypothalamus*

Microinjections of angiotension II into the lateral ventricle or into the anterior hypothalamic/preoptic area increase blood pressure (Phillips and Hoffman 1977; Benarroch et al. 1981; Jones 1984). The pressor response to angiotensin is inhibited by central administration of 6-OHDA (Hoffman et al. 1977a; Benarroch et al. 1981) or phentolamine (Phillips and Hoffman 1977; Jones 1984), suggesting the involvement of catecholaminergic systems. This is in apparent contrast to the depressor response to noradrenaline injected into the anterior hypothalamic/preoptic area (see Sect. 3.2.3). On the other hand, treatment with 5,7-DHT also abolishes the rise in blood pressure elicited by angiotensin II applied to the anterior hypothalamic/preoptic area. It has been argued that the pressor response to angiotensin II is mediated by an increased release of serotonin which in turn inhibits the release of noradrenaline (Benarroch et al. 1981). Hence, inhibition of the pressor response to angiotensin II by $\alpha$-adrenoreceptor blocking agents or by the neurotoxin 6-OHDA may be attributed to blockade of the depressor effect of noradrenaline. Additional experiments may help to confirm the interactions between angiotensin, serotonin and noradrenaline in the anterior hypothalamic/preoptic area.

### 9.3 Angiotensin in Experimental and Genetic Hypertension

There is substantial evidence indicating the involvement of angiotensin in hypertension. In SHR, intracerebroventricular administration of angiotensin II (Hoffman et al. 1977 b), or its injection into the nucleus of the solitary tract (Casto and Phillips 1985) leads to a pressor response which is more pronounced than that observed in normotensive WKY rats. Moreover, intracerebroventricular administration of the angiotensin-receptor antagonist saralasin, or of the inhibitor of the converting enzyme, captopril, decreases blood pressure in SHR and renal hypertensive rats (Suzuki et al. 1981, 1986). In DOCA-salt hypertensive rats, centrally applied captopril seems to decrease blood pressure (Basso et al. 1985; Itaya et al. 1986), although an increase has also been reported (Suzuki et al. 1981). The fall in blood pressure caused by saralasin and captopril is in agreement with the elevated receptor sensitivity of septal neurons to angiotensin II in stroke-prone SHR (Felix and Schelling 1982), as well as with the increased angiotensin II fibre staining in hypothalamic areas of SHR (Weyhenmeyer and Phillips 1982). Likewise, increased angiotensin II binding affinity but no change in the binding sites has been found in the nucleus of the solitary tract of SHR (Plunkett and Saavedra 1985).

In the subfornical organ of young and adult SHR the binding sites for angiotensin II are increased, while the binding affinity is decreased (Saavedra et al. 1986). In this connection it is of interest to note that a very low dose (0.1 pg) of angiotensin II injected into this structure (Mangiapane and Simpson 1980) or its electrical stimulation (Ishibashi and Nicolaidis 1981) lead to a pressor response.

Taken together, all these findings point increased activity to the angiotensin II system in the brain of SHR.

## 10 Opioids

### 10.1 Mapping of Opioid-Containing Neurons

Enkephalins are widely distributed in almost all areas of the CNS. Met-enkephalin and Leu-enkephalin are found in fibres in the dorsal motor nucleus of the vagus and the nucleus ambiguus and in fibres and cell bodies in the nucleus of the solitary tract (Elde et al. 1976; Hökfelt et al. 1977; Simantov et al. 1977; Watson et al. 1977; Sar et al. 1978). Met-enkephalin has also been identified in cell bodies and fibres of the area postrema (Newton et al. 1983), dynorphin A and B in the nucleus of the solitary tract (Watson et al. 1977). Dynorphin B immunoreactive cell bodies are present in the central

amygdaloid nucleus and the dorsomedial, lateral and anterior nuclei of the hypothalamus (Weber and Barchas 1983). In the raphe nuclei, enkephalin-like immunoreactivity is found in cell bodies which contain serotonin. The highest density of cell bodies that were immunoreactive for enkephalins and serotonin are present in the raphe nuclei pallidus and obscurus, followed by the nucleus magnus (Léger et al. 1986).

## 10.2 Cardiovascular Effects of Opioids and Related Drugs

### *10.2.1 Cerebroventricular System*

In several animal species, intravenous injections of opiates lower blood pressure and heart rate. These cardiovascular effects have been attributed to a centrally mediated activation of vagal tone and attenuation of sympathetic activity (Evans et al. 1952; Laubie et al. 1973, 1974). The central administration of opiates and endogenous opioids has also been reported to affect blood pressure and heart rate, but the cardiovascular effects of these agents much depend on several factors, such as properties and dosage of the compound, kind of respiration and anaesthesia (Laubie et al. 1973, 1974, 1977a, b; Florez and Mediavilla 1977; Bolme et al. 1978; Schaz et al. 1980; Lang et al. 1982).

In the anaesthetized dog, the intracisternal injection of opiates leads to a fall in blood pressure and bradycardia (Laubie et al. 1974), while $\beta$-endorphin (a selective agonist of $\mu$- and $\delta$-opioid receptors) elicits a biphasic effect; an initial rise in blood pressure and heart rate is followed by hypotension and bradycardia (Laubie et al. 1977b). In anaesthetized rats, $\beta$-endorphin, morphine or the $\delta$-receptor agonist (D-Ala$^2$-Met$^5$)-enkephalinamide (DAME) lower blood pressure and heart rate, but Leu-enkephalin, Met-enkephalin and $\alpha$-endorphin lead to vasopressor effects (Bolme et al. 1978). On the other hand, in anaesthetized and *spontaneously* breathing rats low doses of morphine and DAME have been found to increase blood pressure and heart rate, but when high doses of these compounds are applied, the pressor response is followed by hypotension and bradycardia. The cardiovascular response seems to be dependent on the action of these compounds on respiration, because in *artificially* ventilated rats even high doses of DAME increase blood pressure (Bellet et al. 1980). Anaesthesia seems also to interfere with the cardiovascular response to centrally applied opioid peptides, because injection of DAME into the lateral ventricle of anaesthetized rats lowers blood pressure, while in conscious rats the same dose of this enkephalin analogue increases blood pressure (Lang et al. 1982).

The varying cardiovascular effects of opioid-receptor agonists according to agent and/or experimental conditions may be due to stimulation of different opioid receptors by the various compounds. Indeed, naloxone (affinity to $\mu$-

receptors/$\delta$-receptors = 10/1) inhibits the pressor response to intraventricular injection of the opioid peptide DAME in conscious animals. On the other hand, the antagonist diprenorphine (equal affinity to $\mu$- and $\delta$-receptors) diminishes the depressor response to this analogue in anaesthetized animals, while naloxone is ineffective (Schaz et al. 1980; Lang et al. 1982). It seems that different receptors mediate the cardiovascular effects of the agonists in anaesthetized and conscious animals.

Laurent and Schmitt (1983) found that stimulation of $\kappa$-receptors by intracisternal administration of ethylketocyclazocine or dynorphin lowers blood pressure and heart rate in anaesthetized rats. In contrast, stimulation of $\mu$- (intracisternal injection of fentanyl or $\beta$-endorphin), $\delta$- (intracisternal injection of DAME or $\beta$-endorphin) or $\varepsilon$- (intracisternal injection of $\beta$-endorphin) receptors leads to hypertension and tachycardia. The existence of multiple opioid receptors in the medulla oblongata (Hökfelt et al. 1977; Atweh and Kuhar 1979) together with the above-mentioned results suggest the involvement of two opioid systems in cardiovascular control; a depressor system which seems to be activated by $\kappa$-receptor agonists and a pressor system which is stimulated by $\mu$-, $\delta$-, and/or $\varepsilon$-receptor agonists. Because of the low selectivity of some of the compounds (Paterson et al. 1983), experiments with specific opiate receptor agonists and antagonists are necessary for the further characterization of the cardiovascular effects mediated by the various receptor subtypes.

The baroreceptor reflex is inhibited by opiates. This effect seems to be mediated by $\mu$-receptors, because baroreceptor sensitivity is reduced by intracisternal administration of the $\mu$-receptor agonists Ty-D-Ala-Gly-MePhe-$NH(CH_2)_2NME_2$ (Petty and Reid 1982a) or D-$Ala^2$-MePhe-$Gly(ol)^5$ (DAGO) (Gordon 1986). Intracisternal administration of the antagonist naloxone was found to either increase the baroreceptor reflex sensitivity (Petty and Reid 1981, 1982a), or to be ineffective (Gordon 1986). Thus, it is still doubtful whether endogenous opioids are involved in baroreflex control. Opioids seem to be involved in the pathophysiology of shock, because naloxone reduces the hypotensive effect of acute haemorrhage (Faden and Holaday 1978; Vargish et al. 1980; Schadt and York 1981; Gurll et al. 1982) and reverses the hypotension induced by endotoxin (Holaday and Faden 1978; Reynolds et al. 1980) or spinal shock (Holaday and Faden 1980). These results are in agreement with the observation that controlled bleeding is associated with a rise in Leu-enkephalin-like immunoreactivity in the CSF, while the levels of noradrenaline and dopamine are decreased (Elam et al. 1984b). More recently, it was shown that naloxone does not antagonize either the fall in blood pressure or the increase in plasma vasopressin level elicited by stepwise haemorrhage. It is probable that naloxone-sensitive opiate receptors are implicated in blood pressure maintenance only in profound shock situations (Rockhold et al. 1986).

In anaesthetized, spontaneously breathing rats, the hypertensive effects of centrally applied morphine or DAME are abolished by bilateral adrenalectomy or pentamethonium (Bellet et al. 1980). On the other hand, it has been shown that enkephalin in vitro inhibits the release of catecholamines by a presynaptic mechanism (Taube et al. 1976). Since injection of noradrenaline into the nucleus of the solitary tract lowers blood pressure (however, see Sect. 3.1.1) it has been proposed that the hypertensive effect of opioids is due to a decreased release of noradrenaline in the medullary cardiovascular sites (Bellet et al. 1980).

A localization of the site of action of opiates has been attempted by the intravenous injection of fentanyl in intact dogs, as well as after lesion of the lateral reticular nucleus of the ventrolateral medulla. The lesion abolishes the fall in blood pressure and heart rate elicited by fentanyl. Likewise, the cardiovascular effects of fentanyl are reduced on microinfusion of naloxone into the lateral reticular nucleus (Laubie and Schmitt 1983).

Similar results have been obtained by Wong et al. (1984) with centrally applied DAME. In anaesthetized rats, intracerebroventricular injection of DAME elicits a fall in blood pressure which is antagonized by naloxone. The vasodepressor response to DAME is also diminished by bilateral lesions of the gigantocellular reticular nucleus. At high doses (approximately ten times higher than those needed to elicit a fall in blood pressure), DAME leads to a consistent hypertension which is not affected either by naloxone or by lesions of the gigantocellular reticular nucleus (Wong et al. 1984). These findings, together with those of Laubie and Schmitt (1983), indicate that structures of the ventral medulla are important for the cardiovascular effects of opiates and opioids.

### *10.2.2 Brainstem*

To investigate the role of the various opioid receptors in cardiovascular regulation, specific agonists of $\mu$- (DAGO), $\delta$- (D-Ala$^2$-D-Leu$^5$-enkephalin; DADLE) and $\kappa$-receptors (MRZ 2549; 5,9-dimethyl-2-hydroxy-2-(2-methoxypropyl)-6,7-benzomorphan) were used.

In order to avoid respiratory depression that might interfere with the cardiovascular effects of opioid receptor agonists, anaesthetized rats were artificially ventilated. Injections of these agonists into the nucleus of the solitary tract or the nucleus ambiguus revealed that in both nuclei *$\mu$- and $\delta$-*receptors mediate pressor responses and tachycardia. $\kappa$-Receptors mediate cardioacceleration in the nucleus of the solitary tract, but decrease blood pressure in the nucleus ambiguus (Hassen et al. 1983). In spontaneously respiring rats low doses of opioids are ineffective, while high doses lower blood pressure without influencing heart rate (Hassen et al. 1984). Injection of the $\mu$- and $\delta$-opiate receptor agonist $\beta$-endorphin into the nucleus of the

solitary tract of anaesthetized, non-ventilated rats also lowers blood pressure and heart rate (De Jong et al. 1983), while injection of the selective $\kappa$-opiate receptor agonist *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide (U 50488H) leads to a rise in blood pressure and mild bradycardia (Carter and Lightman 1985). Hence, in the brainstem several factors also influence the cardiovascular effects of opioids.

Injection of the $\delta$-receptor agonists DADLE or DAME into the *caudal* ventrolateral medulla also increases blood pressure and heart rate. Atropine injected into the *rostral* ventrolateral medulla decreases blood pressure and abolishes these cardiovascular effects, suggesting that they are mediated by cholinergic mechanisms located in the *rostral* ventrolateral medulla. The cardiovascular effects due to stimulation of $\delta$-receptors of the *caudal* ventrolateral medulla are inhibited by intravenous injection of phentolamine; this indicates activation of the sympathetic outflow (Willette et al. 1984b; Punnen and Sapru 1985). In contrast to this, stimulation by DAME of $\delta$-receptors of the *rostral* ventrolateral medulla lowers blood pressure and heart rate (Laubie and Schmitt 1983; Punnen et al. 1984) and reduces the pressor response to carotid occlusion (Punnen et al. 1984). Hence, stimulation of $\delta$-receptors of the *caudal* ventrolateral medulla increases blood pressure and heart rate and enhances the pressor response to carotid occlusion, while stimulation of $\delta$-receptors of the *rostral* ventrolateral medulla exerts the opposite effects.

### *10.2.3 Hypothalamus*

In anaesthetized rats, microinjection of the $\mu$-opiate receptor agonist DAGO into the medial preoptic area of the hypothalamus leads to a fall in blood pressure and tachycardia. The $\delta$-receptor agonist DADLE also lowers blood pressure and increases heart rate but at much higher doses than those of DAGO. Thus, $\mu$- rather than $\delta$-receptors of the hypothalamus mediate the cardiovascular effects of opioids (Faden and Feuerstein 1983). In this area the pattern of the cardiovascular changes to stimulation of opioid receptors seems also to depend on anaesthesia because in conscious rats the opposite effects have been observed (Pfeiffer et al. 1983a, b); the intrahypothalamic administration of DAGO increases blood pressure and decreases heart rate. The hypothalamus seems to possess abundant $\mu$-binding sites (Goodman et al. 1980; Duka et al. 1981; Moskowitz and Goodman 1984), but extremely low densities of binding sites have also been reported (Quirion et al. 1983; Mansour et al. 1986). The cardiovascular effects of the $\mu$-receptor agonist DAGO in conscious animals are associated with increases in the plasma levels of catecholamines which suggest involvement of sympathoadrenomedullary pathways (Pfeiffer et al. 1983b; Appel et al. 1986; Kiritsy-Roy et al. 1986). It seems that stimulation of $\mu$-receptors activates the sympathetic system, thus leading to changes in blood pressure and heart rate.

### 10.3 Opioids in Genetic Hypertension

Dynorphin-(1 – 13)-like immunoreactivity is decreased in the hypothalamus and pituitary gland of SHR (Kouchich et al. 1984), while enkephalin levels and Met-enkephalin binding sites seem to be reduced in the lateral reticular nucleus of 4-week-old SHR (Nakamura et al. 1984). On the other hand, a selective increase of $\kappa$-opioid receptors in the hypothalamus of SHR was recently reported (Bhargava and Das 1986). $\kappa$-Binding sites are present in various hypothalamic nuclei (Morris and Herz 1986).

In this connection interactions between opioids and vasopressin are noteworthy. Several investigators have observed that in normotensive animals the release of vasopressin is inhibited by opioid peptides (Van Wimersma et al. 1979; Knepel et al. 1980, 1982a, b; Summy-Long et al. 1981). In adult (17-week-old) SHR, but not in normotensive WKY rats, naloxone increases the vasopressin level in plasma. Thus, endogenous opioids seem to decrease the release of vasopressin in SHR (Rosella-Dampman et al. 1985).

## 11 Substance P

### 11.1 Mapping of Substance P-Containing Neurons

Substance P-like immunoreactivity is present in cell bodies and fibres located in the amygdaloid complex, in various hypothalamic areas (anterior, medial and posterior hypothalamus) and the thalamus. In the medulla oblongata, cell bodies and fibres which contain substance P immunoreactivity are present in various raphe nuclei and the lateral reticular nucleus (Ljungdahl et al. 1978; Cuello and Kanazawa 1978).

Substance P-like immunoreactivity has also been found in the dorsal vagal complex and in the area postrema (Armstrong et al. 1982b), as well as in the nucleus of the solitary tract (Ljungdahl et al. 1978; Cuello and Kanazawa 1978; Gillis et al. 1980; Helke et al. 1980c; Veening et al. 1984). In the latter nucleus, the dorsal and dorso-lateral subnuclei which receive baroreceptor and chemoreceptor afferents possess substance P-immunoreactive nerve terminals.

The innervation of the nucleus of the solitary tract with substance P terminals derives partially from primary afferent fibres in the glossopharyngeal and vagus nerves (Gillis et al. 1980; Helke et al. 1980c; Kalia et al. 1984). In the caudal part of the nucleus of the solitary tract, synaptic contacts of substance P-immunoreactive axon terminals with catecholaminergic neurons of the cell group A1 were observed (Kubota et al. 1985). Substance P was also detected in the ventrolateral medulla and in at least some of the PNMT-

immunoreactive cell bodies of the adrenaline-containing C1 cell group of this area (Lorenz et al. 1985; Pilowsky et al. 1986b). Substance P cell bodies from the ventral medulla project to the intermediolateral cell columns of the spinal cord (Helke et al. 1982).

## 11.2 Cardiovascular Effects of Substance P and Related Drugs

### *11.2.1 Cerebroventricular System*

Intravenous injection of substance P lowers blood pressure. In anaesthetized and conscious rats administration of substance P to the lateral ventricle increases blood pressure (Haeusler and Osterwalder 1980; Fuxe et al. 1980b; Petty and Reid 1981; Unger et al. 1981) and heart rate (Haeusler and Osterwalder 1980; Fuxe et al. 1980b), while the pressor response is associated with bradycardia when substance P is injected into the cisterna magna of anaesthetized rabbits (Petty and Reid 1981, 1982b). The pressor response to substance P is reversed to a fall in blood pressure after blockade of peripheral $\alpha_1$-adrenoreceptors by prazosin, while the antagonist of vasopressin receptors [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid), 4-valine-D-arginine] (DVAP) is ineffective. Moreover, centrally applied substance P increases noradrenaline and adrenaline levels in the plasma without influencing the plasma level of arginine-vasopressin. Hence, the pressor response to substance P seems to be mediated by the sympathetic nervous system without participation of vasopressin (Unger et al. 1981). Central cholinergic pathways also seem to be of importance for the pressor response to substance P, because intracerebroventricular administration of hemicholinium-3, hexamethonium or atropine attenuates the rise in blood pressure elicited by the peptide (Trimarchi et al. 1986).

The pressor response, but not the tachycardia elicited by substance P is diminished by intracerebroventricular administration of the GABA-receptor agonist muscimol (Unger et al. 1986). A similar dissociation of the two cardiovascular effects of substance P has been previously described by Fuxe et al. (1982b), who observed that the substance P-receptor antagonist [D-Pro$^2$,D-Phe$^7$,D-Trp$^9$]SP inhibits the rise in blood pressure caused by substance P without affecting its tachycardic effect.

### *11.2.2 Brainstem*

Injections of kainic acid into the ventrolateral medulla of the rat lead to a rise in blood pressure which is associated with an increased release of substance P in the superfused spinal cord (Takano et al. 1984). Capsaicin releases substance P from terminals of primary sensory neurons (Gamse et al. 1979).

Applied to the exposed ventral surface of the rat medulla, capsaicin also increases blood pressure without influencing the heart rate. The area more sensitive to capsaicin seems to be the "chemosensitive area S" of the *rostral* ventrolateral medulla (Jancsó and Such 1985). It should be remembered that substance P may not be specific. Subcutaneous injections of capsaicin not only diminish the concentration of substance P in the spinal cord, but also increase noradrenaline and serotonin levels (Virus et al. 1983).

Since the nucleus of the solitary tract is densely innervated with substance P-immunoreactive nerve terminals which originate from primary afferent fibres in the glossopharyngeal and vagus nerves (see Sect. 11.1), it seems likely that, in this nucleus, the peptide is involved in transmission of the baroreceptor reflex. Unfortunately, the existing results are rather conflicting. It was found that in rats and cats substance P applied to the nucleus of the solitary tract lowers blood pressure and heart rate, while the vehicle is ineffective (Haeusler and Osterwalder 1980). Moreover, capsaicin elicits cardiovascular effects identical with those of substance P (Haeusler and Osterwalder 1980). These results suggest the neuromodulatory role of substance P at the first synapse of the baroreceptor reflex in the nucleus of the solitary tract. On the other hand, Talman and Reis (1981), as well as Carter and Lightman (1983), found substance P to be ineffective when microinjected into the nucleus of the solitary tract in untreated rats, while the peptide increased blood pressure in animals pretreated with capsaicin. The latter finding has been interpreted as indicating the involvement of substance P of this region in cardiovascular control (Carter and Lightman 1983), but destruction by capsaicin of primary spinal and medullary substance P afferents neither changes blood pressure, nor influences baroreflex function (Lorez et al. 1983). It is still doubtful whether substance P neurons are implicated in central cardiovascular regulation.

## 12 Neuropeptide Y

### 12.1 Mapping of Neuropeptide Y-Containing Neurons

The neuropeptide Y is widely distributed in the brain. Neuropeptide Y-like immunoreactivity is found in fibres and cell bodies located in the hypothalamus, the cortex, the hippocampus, the preoptic region and in various amygdaloid nuclei (Vincent et al. 1982; Chronwall et al. 1984; Allen et al. 1984; Nakagawa et al. 1985; Bai et al. 1985; Gray and Morley 1986; Ueda et al. 1986). The hypothalamus contains a high concentration of neuropeptide Y.

In the *dorsal* medulla, neuropeptide Y-like immunoreactivity has been shown in the nucleus of the solitary tract (Uhl et al. 1977; Jennes et al. 1982;

Kalia et al. 1984). Almost all adrenaline-containing neurons of the medial part of the nucleus of the solitary tract also contain neuropeptide Y-like immunoreactivity, while the noradrenergic cell bodies (A2) do not. Several neuropeptide-immunoreactive cell bodies are also present which do not contain catecholamines. Neuropeptide Y-like immunoreactivity is also found in the noradrenaline cell bodies of the locus coeruleus (A6), but neither in the subcoeruleus group, nor in the noradrenergic cell bodies of the groups A5 and A7.

In the *ventrolateral* medulla oblongata, neuropeptide Y-like immunoreactivity is present in most catecholamine-containing bodies of the A1 and C2 cell groups (Everitt et al. 1984). Neuropeptide Y-immunoreactive fibres are also present within the dorsal motor nucleus of the vagus and the nucleus of the solitary tract. The nerve terminals of the nucleus of the solitary tract originate partly from cell bodies located in the dorsomedial region of the hypothalamus (Gray and Morley 1986), while neuropeptide-containing neurons of the nucleus of the solitary tract innervate the parabrachial nucleus (Mantyh and Hunt 1984). In the rat, an arcuatoparaventricular system of neuropeptide Y neurons exists, which seems to lack noradrenaline (Bai et al. 1985).

## 12.2 Cardiovascular Effects of Centrally Applied Neuropeptide Y

In rats, neuropeptide Y injected intracisternally lowers blood pressure without influencing heart rate (Fuxe et al. 1983b). The cardiovascular effects of intracisternally applied neuropeptide Y are not influenced by central administration of the $\alpha_2$-adrenoreceptor antagonist idasoxan (Härfstrand et al. 1984).

Injection of the peptide into the nucleus of the solitary tract changes blood pressure in a dose-dependent way; a low dose (470 fmol) increases blood pressure, while a dose ten times higher elicits a depressor response. Furthermore, an ineffective dose of neuropeptide Y reverses the hypotensive effect of a low dose (20 nmol) of noradrenaline injected into this nucleus, thus eliciting a pressor response similar to that caused by a high dose (100 nmol) of the amine (Carter et al. 1985). In this nucleus, a high density of neuropeptide Y binding sites has been demonstrated (Härfstrand et al. 1986).

Injection of neuropeptide Y into the third ventricle, however, leads to a rise in blood pressure and heart rate.

Neuropeptide Y is still able to increase blood pressure in rats pretreated with 6-OHDA. Apparently, release of catecholamines is not essential for the activity of the peptide. However, 6-OHDA prolongs the rise in blood pressure elicited by neuropeptide Y. It seems possible that the prolonged duration of the peptide action after 6-OHDA treatment is due to the denervation super-

sensitivity of adrenoreceptors (Vallejo and Lightman 1986). Indeed, in vitro experiments revealed that neuropeptide Y increases the number of $\alpha_2$-adrenoreceptors in the CNS (Agnati et al. 1983). Nevertheless, other neurotransmitters are also involved in the cardiovascular effects elicited by central application of neuropeptide Y. Injection of the peptide into the posterior hypothalamic nucleus of rats leads to a dose-dependent pressor response which is inhibited by the H1-receptor antagonist chlorpheniramine, but not by the H2-antagonist cimetidine. The pressor response is also decreased by atropine injected into the posterior hypothalamic nucleus, indicating that histaminergic (H1-mediated) and cholinergic neuronal pathways are involved in the rise in blood pressure caused by neuropeptide Y (Martin et al. 1988).

### 12.3 Neuropeptide Y in Experimental and Genetic Hypertension

Renal hypertension does not seem to influence the neuropeptide Y level in the brainstem of rats (Allen et al. 1986), but differences have been observed in brain areas of SHR. In most hypothalamic areas, increased neuropeptide Y levels have been found but not in the lateral preoptic area, in which the neuropeptide Y concentration is decreased. Similarly, the level of neuropeptide Y is decreased in the locus coeruleus (Maccarrone et al. 1986).

## 13 Neurotensin

### 13.1 Mapping of Neurotensin-Containing Neurons

Neurotensin immunofluorescence is present throughout the CNS of the rat. Cell bodies with intense fluorescence occur in several hypothalamic areas, the amygdaloid complex, the locus coeruleus and the dorsal raphe nucleus. Fibres with dense fluorescence are present in the ventral surface of the hypothalamus and the preoptic area (Uhl et al. 1977, 1979). In the arcuate nucleus, dopamine neurons show neurotensin immunoreactivity. Neurotensin-like immunoreactivity has also been described in adrenaline and noradrenaline cell bodies of the nucleus of the solitary tract (Hökfelt et al. 1984b). Neurotensin-containing fibres are present throughout the nucleus, while some fibres are located in the dorsal motor nucleus of the vagus (Uhl et al. 1977; Hökfelt et al. 1984b).

### 13.2 Cardiovascular Effects of Centrally Applied Neurotensin

In conscious rats, the intracerebroventricular injection of neurotensin increases blood pressure (Sumners et al. 1982). In anaesthetized and conscious

rats decreases in blood pressure have also been reported, but the volumes injected intracerebroventricularly were too large for this animal species (Rioux et al. 1981). The neurotensin-induced rise in blood pressure is diminished by $\alpha_1$-(prazosin) or $\alpha_2$-adrenoreceptor (yohimbine) antagonists, suggesting the involvement of central catecholamine neurons (Sumners et al. 1982).

## 14 Atrial Natriuretic Factor

### 14.1 Mapping of Atrial Natriuretic Factor-Containing Neurons

Immunoreactive atrial natriuretic factor-positive cell bodies have been identified in the central and medial amygdaloid nuclei, the base of the hypothalamus, the mamillary body and the ventral parabrachial nucleus. A few cell bodies have been observed in the nucleus of the solitary tract. Nerve fibres are present in those areas in which cell bodies are found. A high density of nerve fibres is present in the anterior-ventral third ventricle (Skofitsch et al. 1985). The concentration of the atrial natriuretic factor in the rat hypothalamus in roughly one-tenth of that in rat atria (Tanaka et al. 1984).

### 14.2 Cardiovascular Effects of Centrally Applied Atrial Natriuretic Factor; Atrial Natriuretic Factor in Genetic Hypertension

When synthetic $\alpha$-human natriuretic peptide is injected into the cerebroventricular system of rats, it affects neither blood pressure nor heart rate (Lappe et al. 1986; Shimizu et al. 1986), but does attenuate the pressor response to centrally administered angiotensin II. Atrial natriuretic factor also enhances the depressor response to intracerebroventricular injection of the angiotensin-receptor antagonist saralasin (Shimizu et al. 1986). It seems, therefore, that an antagonism exists between angiotensin and the atrial natriuretic factor in the brain. Indeed, is has been found that the subfornical organ possesses binding sites for the atrial natriuretic factor (Quirion et al. 1984); the number of binding sites for atrial natriuretic factor (McCarty and Plunkett 1986) and rat atrial natriuretic peptide, which closely resembles atrial natriuretic factor (Saavedra et al. 1986), is decreased in the subfornical organ of young and adult SHR, while the binding sites for angiotensin II are increased in this structure (see Sect. 9.3). Recently, it has been reported that the level of atrial natriuretic factor is increased in the hypothalamus and pons of SHR (Imada et al. 1985).

## 15 General Conclusions

The main bulk of information concerning central cardiovascular effects of drugs has been obtained from anaesthetized animals. Since anaesthesia reverses the cardiovascular effects of many centrally applied neurotransmitters and drugs, it is difficult to evaluate the pattern of blood pressure changes which are elicited by endogenously released neurotransmitters in conscious animals. For example in the conscious rat, noradrenaline and adrenaline increase blood pressure when applied centrally, as do drugs which stimulate $\alpha$-adrenoreceptors. Even clonidine, which may also act on separate imidazoline-receptors, elicits a pressor response when centrally administered in the rat. A re-examination of the cardiovascular effects of agonists and antagonists of various neurotransmitter and neuropeptide receptors in *conscious* animals is necessary for a precise idea of the functions of the released substances.

### 15.1 Brainstem

There is no doubt that catecholaminergic neurons play a predominant role in the brainstem. Furthermore, central cardiovascular effects of several neurotransmitters and neuropeptides are mediated through catecholaminergic neurons. However, the involvement of catecholaminergic neurons of the ventrolateral medulla in cardiovascular control is not certain. Although glutamatergic and GABAergic neurons seem to be involved in the baroreceptor reflex, the nature of the inhibitory neurotransmitter of neurons connecting the nucleus of the solitary tract with the ventrolateral medulla remains to be clarified. Catecholaminergic neurons of the nucleus of the solitary tract also seem to be implicated in the baroreceptor reflex. Direct determination of the release rates of catecholamines revealed that noradrenaline and adrenaline may lead to pressor responses when released in this area.

Changes in blood pressure also alter the activity of catecholaminergic neurons of the locus coeruleus, which influence the release of angiotensin in the hypothalamus. Serotoninergic neurons of the raphe nuclei also seem to be involved in cardiovascular regulation, because the release of the serotonin metabolite 5-HIAA in the dorsal raphe nucleus is altered by experimentally induced blood pressure changes.

The cardiovascular effects of angiotensin applied to the nucleus of the solitary tract seem to be mediated by cholinergic neurons. Neurons containing neuropeptides, as well as receptors of several neuropeptides are present in the brainstem. The co-localization of neurotransmitters and neuropeptides in brainstem neurons which are involved in cardiovascular regulation is indirect

evidence for the importance of neuropeptides in central cardiovascular control. It remains to be clarified whether the release of endogenous peptides influences the cardiovascular system.

## 15.2 Hypothalamus

Catecholaminergic neurons of the hypothalamus seem to be responsible for pressor and depressor responses elicited by the posterior and anterior hypothalamus, respectively. Moreover, experimentally induced blood pressure changes alter the release rates of catecholamines, thus demonstrating the homoeostatic function of catecholaminergic neurons. The pressor response to release of catecholamines may be mediated by angiotensin. Histaminergic neurons may also be involved, although their role in cardiovascular control is still obscure.

Serotoninergic nerve terminals of the anterior hypothalamus/preoptic area originate from cell bodies located in raphe nuclei. Release of serotonin in the hypothalamus increases blood pressure, probably by inhibiting the release of catecholamines in this area. Endogenously released acetylcholine or acetylcholine exogenously applied to the hypothalamus increase blood pressure, enhance the pressor response to hypothalamic stimulation and increase the rise in blood pressure elicited by carotid occlusion. The cardiovascular effects of acetylcholine are mediated through central catecholaminergic pathways, because they are inhibited by $\beta$-adrenoreceptor blocking agents. However, GABAergic systems of the hypothalamus lower blood pressure and suppress vagal reflex bradycardia. As in the brainstem, several neuronal transmitters and peptides in the hypothalamus are involved in the homoeostasis of blood pressure.

## 15.3 Hypertension

Although remarkable changes in catecholamine concentrations and/or turnover rates have been described in genetic and experimental hypertension, the results greatly differ from each other, thus rendering difficult clear-cut conclusions.

Besides these alterations in catecholaminergic neurons, concentration changes of several other neurotransmitters and neuropeptides in brain areas of SHR have been reported in recent years. Results are summarized in Table 9. It is of interest to note that in the hypothalamus of hypertensive animals, concentrations are increased of mainly those neurotransmitters and neuropeptides which, when exogenously administered to this brain region, increase blood pressure. An exception to this seems to be acetylcholine which is de-

**Table 9.** Neurotransmitter and neuropeptide changes in brain areas of spontaneously hypertensive rats

| Transmitter or peptide | Brain area | Concentration | Turnover | Release | References |
|---|---|---|---|---|---|
| Serotonin | Hypothalamus | Increase | | | Koulu et al. (1986b, c) |
| Histamine | Hypothalamus | Increase | Decrease | | Corrêa and Saavedra (1981), Oishi et al. (1985) |
| Histamine | Posterior hypothalamus | | | Increase | Tuomisto et al. (1983) |
| GABA | Hypothalamus | Decrease | | | Hambley et al. (1984) |
| | Posterior hypothalamus | | | No change | Tuomisto et al. (1983) |
| Acetyl-choline | Hypothalamus | Decrease | | | Helke et al. (1980a) |
| | LC | Increase | | | |
| Angiotensin | Hypothalamus | Increase | | | Weyhenmeyer and Phillips (1982) |
| Dynorphin | Hypothalamus | Decrease | | | Kouchich et al. (1984) |
| Enkephalin | LRN | Decrease | | | Nakamura et al. (1984) |
| Neuropep-tide Y | Hypothalamus | Increase | | | Maccarrone et al. (1986) |
| | LPA, LC | Decrease | | | |
| ANF | Hypothalamus, pons | Increase | | | Imada et al. (1985) |

*LC*, Locus coeruleus; *LRN*, lateral reticular nucleus; *LPA*, lateral preoptic area; *ANF*, atrial natriuretic factor

creased in the hypothalamus of SHR. The concentration of GABA, which lowers blood pressure, is decreased, at least in adult animals.

Changes in the concentration of neurotransmitters and neuropeptides are mediocre criteria for what really happens in neurons and their biophases, because concentration changes may be the result of altered biosynthesis, release, uptake or inactivation rates. Thus, the only possible conclusion is that in areas which are involved in central cardiovascular regulation profound changes in the activities of many neurotransmitters and neuropeptides occur. The reason for the concentration alterations, as well as the causal relationships between changed neuronal activities on the one hand, and development and/or maintenance of hypertension on the other hand, remain to be clarified.

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# The Roles of Calcium and Phosphoinositides in the Mechanisms of $\alpha_1$-Adrenergic and Other Agonists

JOHN H. EXTON

**Contents**



Howard Hughes Medical Institute and Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, TN 37232, USA

## 1 Introduction

Activation of the sympathetic nervous system leads to the release of epinephrine and norepinephrine from the adrenal medulla into the blood stream and of norepinephrine from adrenergic nerve endings throughout the body. The effects of these catecholamines are widespread and are mediated by four subtypes of adrenergic receptors. Two of these receptor subtypes ($\beta_1$ and $\beta_2$) are stimulatory to adenylate cyclase, and the physiological responses resulting from their activation are generally attributable to an increase in cellular cAMP and phosphorylation of specific proteins by cAMP-dependent protein kinase (Fig. 1). The other two receptors ($\alpha_1$ and $\alpha_2$) mediate quite dif-

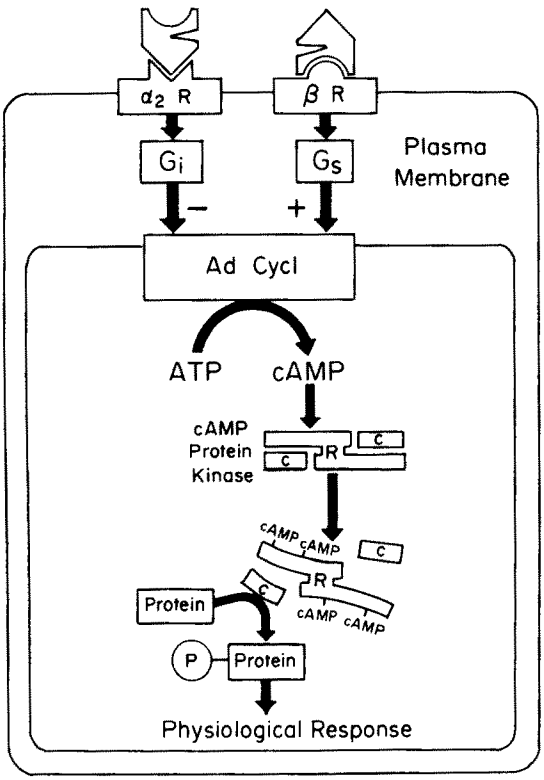


**Fig. 1.** Mechanisms by which $\alpha_2$- and $\beta$-adrenergic agonists produce their physiological responses. $\alpha_2$ *R*, $\alpha_2$-adrenergic receptor; $\beta$ *R*, $\beta_1$- or $\beta_2$-adrenergic receptor; $G_i$, the inhibitory G-protein of adenylate cyclase; $G_s$, the stimulatory G-protein of adenylate cyclase; *Ad Cycl*, the catalytic moiety of adenylate cyclase; *R*, the regulatory subunit dimer of cAMP-dependent protein kinase; *C*, the catalytic subunit of cAMP-dependent protein kinase

ferent responses. $\alpha_2$-Adrenergic receptors are located pre- and post-junctionally, i.e., on the terminal noradrenergic axon and also on some of the effector cells which are the targets for the released norepinephrine. The function of the presynaptic $\alpha_2$-adrenergic receptors is to mediate feedback inhibition of norepinephrine release, whereas the postsynaptic $\alpha_2$-adrenergic receptors mediate such catecholamine responses as platelet aggregation and inhibition of pancreatic insulin secretion and of adipose tissue lipolysis. Activation of postsynaptic $\alpha_2$-adrenergic receptors results in inhibition of adenylate cyclase (Fig. 1), although it is likely that other mechanisms are involved, since not all of the responses can be attributed to a decline in cAMP. For example, in platelets, there is evidence for activation of phospholipase $A_2$, and in neuroblastoma-glioma hybrid cells, there is stimulation of $Na^+/H^+$ exchange.

Activation of $\alpha_1$-adrenergic receptors is linked to an increase in the activity of a phospholipase C that catalyzes the breakdown of polyphosphoinositides in the plasma membrane with the generation of two intracellular messages, namely myoinositol 1,4,5-$P_3$ ($IP_3$) and 1,2-diacylglycerol (DAG) (Fig. 2). The function of $IP_3$ is to release $Ca^{2+}$ from intracellular stores, which are probably located in the endoplasmic reticulum, thereby raising cytosolic $Ca^{2+}$ and altering the activity of $Ca^{2+}$-calmodulin-dependent protein kinases and

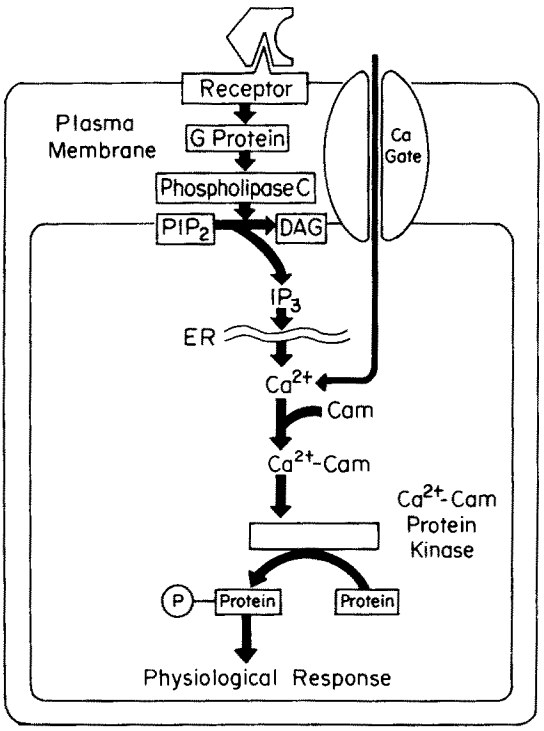


**Fig. 2.** Mechanisms by which $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists produce their physiological responses. *$PIP_2$*, phosphatidylinositol 4,5-$P_2$; *DAG*, 1,2-diacylglycerol; *$IP_3$*, myoinositol 1,4,5-$P_3$; *ER*, endoplasmic reticulum; *Cam*, calmodulin

other proteins (Fig. 2), whereas the function of DAG is to activate a $Ca^{2+}$-phospholipid-dependent protein kinase (protein kinase C). Although activation of phospholipase C is the major response of most cells to $\alpha_1$-adrenergic stimulation, some cells also exhibit activation of phospholipase $A_2$ with production of eicosanoids from the arachidonic acid released. In some tissues, stimulation of $\alpha_1$-adrenergic receptors also leads to changes in cyclic nucleotides, but the mechanism(s) involved are unclear.

The coupling of $\beta$- and $\alpha_2$-adrenergic receptors to adenylate cyclase involves guanine nucleotide-binding regulatory proteins or G-proteins, termed $G_s$ and $G_i$, which have a heterotrimeric ($\alpha\beta\gamma$) subunit structure and are stimulatory and inhibitory to adenylate cyclase respectively (Fig. 1). The coupling of $\alpha_1$-adrenergic receptors and other receptors for $Ca^{2+}$-mobilizing agonists to the phospholipase C catalyzing polyphosphoinositide breakdown also involves a G-protein (Fig. 2). This is designated $G_p$, although it has not yet been identified.

$\alpha_1$-Adrenergic receptors are located in many tissues throughout the body and mediate many responses to catecholamines (Table 1). An important $\alpha_1$-adrenergic response is the contraction of smooth muscle in blood vessels and other tissues such as the uterus, the ureter, and the iris. Other effects are the relaxation of gastrointestinal smooth muscle, secretion of watery saliva,

**Table 1.** $\alpha_1$-Adrenergic target tissues and responses

| Tissue | Response |
|---|---|
| Smooth muscle (vascular, iris, pilomotor, uterus, ureter, trigone, gastrointestinal and bladder sphincters) | Contraction |
| Smooth muscle (gastrointestinal) | Relaxation |
| Liver | Glycogenolysis, gluconeogenesis, ureogenesis, $K^+$ fluxes |
| Heart | Increased force, glycolysis |
| Salivary glands | Secretion ($K^+$, $H_2O$) |
| Adipose tissue (brown) | Thermogenesis |
| Sweat glands (localized) | Secretion |
| Kidney (proximal tubule) | Gluconeogenesis, $Na^+$ reabsorption |
| Brain | Neurotransmission |

and neurotransmission in certain parts of the central nervous system. Activation of $\alpha_1$-adrenergic receptors can also increase glycogen breakdown and gluconeogenesis in liver and the force of contraction in heart, although these responses can also be elicited by activation of $\beta$-adrenergic receptors.

Synthetic analogues of the natural catecholamines such as phenylephrine and methoxamine are able to activate $\alpha_1$-adrenergic receptors, and the activation of these receptors can be blocked by ergot alkaloids and synthetic antagonists such as prazosin, phentolamine, phenoxybenzamine, and tolazoline, the most specific of which is prazosin. These and other agonists and antagonists are used to define whether or not a given catecholamine response is mediated by $\alpha_1$-adrenergic receptors. When radioactively labeled, the agonists and antagonists can also be employed to identify and characterize these receptors. Since many $\alpha_1$-adrenergic agonists and antagonists are nonselective, e.g., epinephrine and dihydroergocryptine, they are usually used in combination with antagonists to other adrenergic receptors in order to enhance specificity.

## 2 The $\alpha_1$-Adrenergic Receptor

### 2.1 Characterization and Purification of $\alpha_1$-Adrenergic Receptors

Radioactive ligands employed to identify the $\alpha_1$-adrenergic receptor include [$^3$H]prazosin, epinephrine, norepinephrine, dihydroergocryptine, phenoxybenzamine, and WB-4101, and some analogues of prazosin, namely [$^{125}$I]HEAT (also called BE-2254), CP65,526, CP63,789, A55453, and

ADPQ. Using these ligands, $\alpha_1$-adrenergic receptors have been identified in brain (cerebral cortex, hippocampus, corpus striatum, hypothalamus, thalamus, caudate nucleus, pons), lung, liver, kidney, heart, uterus, iris, adipose tissue, vas deferens, salivary glands, and certain blood vessels (Bylund and U'Prichard 1983; Graham and Lanier 1986). Studies in a variety of tissues have indicated that $\alpha_1$-adrenergic receptors differ in their pharmacological properties (for references, see Flavahan and Vanhoutte 1986; Morrow and Creese 1986; Johnson and Minneman 1987), and it has been proposed that there are receptor subtypes, termed $\alpha_{1A}$ and $\alpha_{1B}$ (Morrow and Creese 1986). Both bind [$^3$H]prazosin and catecholamines with equal affinity, but one ($\alpha_{1A}$) has a higher affinity for phentolamine and phenylephrine. This type binds [$^3$H]WB4101 in the subnanomolar range, whereas the other ($\alpha_{1B}$) has an affinity for this ligand in the micromolar range and is therefore not usually detected (Morrow and Creese 1986). There is a wide variation in the ratios between the two subtypes in various tissues, and some tissues appear to have only one of the subtypes. Similar findings have been reported by Han et al. (1987) using [$^{125}$I]BE 2254. As described in Sec. 3.4, it has been proposed that the $\alpha_{1A}$ receptor subtype mediates $Ca^{2+}$ influx into cells, whereas the $\alpha_{1B}$ subtype mediates mobilization of internal $Ca^{2+}$.

Molecular studies of the $\alpha_1$-adrenergic receptor have been largely confined to rat liver and smooth muscle cells. Using [$^{125}$I]ADPQ as a photoaffinity probe to specifically label the $\alpha_1$-adrenergic receptor of rat liver plasma membranes, a binding subunit of $M_r$ 78000–85000 has been identified (Leeb-Lundberg et al. 1984). In the absence of protease inhibitors, this binding subunit becomes less prominent and lower $M_r$ species are observed. Photoaffinity labeling with another prazosin analogue ([$^{125}$I]CP65, 526) also identifies a 78-K labeled protein in rat liver membranes which can be similarly degraded by endogenous proteases (Seidman et al. 1984; Lynch et al. 1986a). On the other hand, incubation of the membranes with low concentrations (0.5–1 n$M$) of [$^3$H]phenoxybenzamine has yielded labeled proteins of 80 and 58 K (Kunos et al. 1983) or 45 K (Guellaen et al. 1982). However, no specific precautions were taken to limit proteolysis in these experiments. Although evidence was presented that the ligand selectively labeled $\alpha_1$-adrenergic receptors, it is known to interact with other monoaminergic receptors. [$^{125}$I]ADPQ has been used to label the $\alpha_1$-adrenergic receptor in other tissues, e.g., spleen, lung, brain, and aortic smooth muscle cells (Leeb-Lundberg et al. 1984). In all cases a 78- to 79-K protein is labeled, but in spleen a smaller $M_r$ species is also observed. Radiation inactivation analysis carried out in rat liver membranes indicates that the receptor exists as a dimer with subunits of approximately 85 K (Venter et al. 1984b). In summary, these observations indicate that the native ligand-binding subunit of the $\alpha_1$-adrenergic receptor has an $M_r$ of approximately 80000 but is very susceptible to proteolysis by endogenous proteases.

Several efforts have been made to purify the $\alpha_1$-adrenergic receptor from different tissues. Using a prazosin analogue (CP57, 609) linked to agarose, the 72000-fold purification of a protein which selectively binds [$^3$H]prazosin has been achieved in rat liver (Graham et al. 1982). However, this has an $M_r$ of only 59000, suggesting proteolytic degradation. Leeb-Lundberg et al. (1985) have purified the $\alpha_1$-adrenergic receptor from DDT$_1$MF-2 cells, which are derived from vas deferens smooth muscle, using another prazosin analogue, A55414, linked to Affi-Gel. The purification was approximately 300-fold and the resulting binding subunit had an $M_r$ of 80000. Lomasney et al. (1986) have taken the purification further using the prazosin analogue A55453 linked to Sepharose, followed by chromatography on wheat germ agglutinin-agarose and high-performance steric exclusion ligand chromatography. The binding subunit again had an $M_r$ of 80000 and a single ligand-binding site.

Although some studies with monoclonal antibodies have suggested the existence of common structure determinants in $\alpha_1$-adrenergic, $\alpha_2$-adrenergic, and muscarinic cholinergic receptors (Venter et al. 1984a; Shreeve et al. 1985), peptide maps of $\alpha_1$- and $\alpha_2$-adrenergic receptors reveal little, if any, structural homology (Lomasney et al. 1986). $\alpha_1$-Adrenergic receptors adsorb to wheat germ lectin-Sepharose and are eluted by *N*-acetylglucosamine (Meier et al. 1984; Lomasney et al. 1986) indicating that they contain *N*-acetylneuraminic acid and/or *N*-acetylglucosamine residues.

## 2.2 Regulation of $\alpha_1$-Adrenergic Receptors by Guanine Nucleotides

It is now accepted that $\alpha_1$-adrenergic receptors can exist in more than one agonist-affinity state and that guanine nucleotides influence the equilibrium between these states. There was initially some controversy about this, with some workers reporting that agonist binding to these receptors was unaffected by GTP and its analogues (Hoffman et al. 1980; Stiles et al. 1983). However, many other groups have now observed guanine nucleotide effects of varying magnitude in liver, heart, smooth muscle, and kidney (El-Refai et al. 1979; Yamada et al. 1980; Geynet et al. 1980; Snavely and Insel 1982; Goodhardt et al. 1982; Boyer et al. 1984; Lynch et al. 1985b; Schwartz et al. 1986a; Terman et al. 1987). The probable reason for the discrepancy is provided by the observation that addition of proteases, or omission of metal ion chelators or of protease inhibitors, leads to extensive proteolysis of the $\alpha_1$-adrenergic receptor in liver plasma membranes and to an associated loss of guanine nucleotide effects on agonist binding (Geynet et al. 1980; Lynch et al. 1985b, 1986a). Thus, varying degrees of proteolytic modification may account for the differences in the magnitude of nucleotide effects observed by various groups.

When endogenous proteases are inhibited, $\alpha_1$-adrenergic receptors of rat liver plasma membrane exist mainly in a form with high affinity for agonists

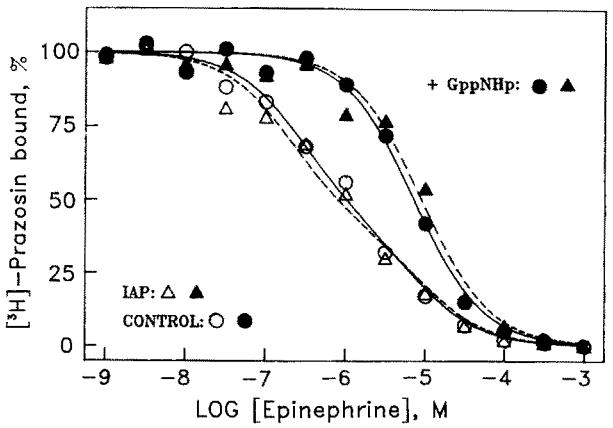


**Fig. 3.** Effects of a GTP analogue (*GppNHp*) and treatment with islet-activating protein (*IAP*) on binding of epinephrine to $\alpha_1$-adrenergic receptors in rat liver plasma membranes. Epinephrine displacement of 1 n*M* [$^3$H]prazosin was assayed without (*open symbols*) or with (*closed symbols*) 0.5 m*M* GppNHp (guanyl-5′-yl imidodiphosphate). Treatment with IAP involved injecting rats with 25 μg IAP/100 g body weight 24 h prior to preparation of membranes. *Triangles* refer to IAP-treated rats and *circles* refer to control rats

– $K_d$ for (−)epinephrine or (−)norepinephrine of 20–30 n*M* – (Lynch et al. 1985b, 1986a). As illustrated in Fig. 3, addition of micromolar or higher concentrations of GTP and its nonhydrolyzable analogues causes the receptors to change to a form with low agonist affinity – $K_d$ for (−)epinephrine or (−)norepinephrine greater than 1 *μM*. These data are similar to those obtained with receptors linked positively or negatively to adenylate cyclase, e.g., *β*- and $\alpha_2$-adrenergic receptors, and provide some of the evidence that $\alpha_1$-adrenergic receptors couple to a G-protein.

In addition to being regulated by guanine nucleotides through a G-protein, $\alpha_1$-adrenergic receptors can be induced to change their agonist affinities by temperature shifts. Thus, the $\alpha_1$-adrenergic agonist affinity of liver plasma membranes or the solubilized receptors is increased approximately 100-fold by lowering the temperature from 25° or 37 °C to 2° or 4 °C (Schwartz et al. 1986a, b; Lynch et al. 1985b). This is thought to involve a change in the receptor per se and may account for some discrepancies between agonist binding to liver plasma membranes and intact hepatocytes (Schwartz et al. 1986b).

## 2.3 $\alpha_1$-Adrenergic Effects on Cyclic Nucleotides

Although $\alpha_1$-adrenergic receptors are linked to $Ca^{2+}$ mobilization in most tissues, they are also coupled to cAMP accumulation in some tissues. For example, in the livers of aging rats, $\beta_2$-adrenergic-mediated cAMP accumula-

tion declines whereas $\alpha_1$-adrenergic receptor-induced cAMP elevation appears (Blair et al. 1979; Morgan et al. 1983a). The $\alpha_1$-adrenergic receptor responsible for the cAMP response shows much similarity to that mediating $Ca^{2+}$ mobilization, but it is more sensitive to phentolamine and WB4101 (Morgan et al. 1983e) and therefore appears to be of the $\alpha_{1A}$ subtype (Morrow and Creese 1986). Calcium depletion of hepatocytes enhances the cAMP accumulation elicited by $\alpha_1$-adrenergic stimulation (Chan and Exton 1977; Morgan et al. 1983a), but the mechanism of the enhancement is unknown. The elevation of cAMP induced by $\alpha_1$-adrenergic agonists in liver is not large, but it probably accounts for reports that these agonists have two mechanisms of action in this tissue (Hernandez-Sotomayor et al. 1984; Pushpendran et al. 1984; Corvera et al. 1984; Garcia-Sainz and Hernandez-Sotomayor 1985). Other $Ca^{2+}$-mobilizing agonists do not induce cAMP accumulation in calcium-depleted hepatocytes or hepatocytes from aging rats (Morgan et al. 1983a).

Elevation of cAMP in response to $\alpha_1$-adrenergic stimulation has also been reported in brain and spinal cord (Perkins and Moore 1973; Schultz and Daly 1973; Davis et al. 1978; Jones and McKenna 1980; Johnson and Minneman 1986, 1987). However, there is clear evidence that the $\alpha_1$-receptors linked to cAMP accumulation in the brain are different from those coupled to phosphoinositide breakdown (Johnson and Minneman 1986, 1987). For example, the alkylating agent chlorethylclonidine inactivates only some of the $\alpha_1$-adrenergic binding sites and partially blocks the increases in cAMP elicited by norepinephrine, but it does not affect the increases in inositol phosphates (Johnson and Minneman 1987). There are also differences between the two responses in different regions of the brain. Interestingly, in pinealocytes, $\alpha_1$-adrenergic stimulation alone does not alter cAMP or cGMP levels, but it markedly potentiates $\beta$-adrenergic stimulation of the accumulation of both nucleotides (Vanacek et al. 1985; Sugden et al. 1986). There is evidence that protein kinase C is involved in this potentiation (Sugden et al. 1985; Ho et al. 1987).

As noted above, activation of $\alpha_1$-adrenergic receptors leads to an increase in cAMP in certain tissues, due apparently to activation of adenylate cyclase. There is also evidence that $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists can decrease cAMP in liver or heart (Assimacopoulos-Jeannet et al. 1982; Morgan et al. 1983c, d; Buxton and Brunton 1985). Since these agonists also inhibit the actions of exogenous cAMP (Assimacopoulos-Jeannet et al. 1982) and antagonize forskolin (Morgan et al. 1983d), and since inhibitors of cyclic nucleotide phosphodiesterase eliminate the effect in cardiac myocytes (Buxton and Brunton 1985), the effect appears to be due to activation of cAMP phosphodiesterase. A similar action of muscarinic cholinergic agonists has been reported (Meeker and Harden 1982; Evans et al. 1985; A. R. Hughes et al. 1984; Masters et al. 1984; Erneaux et al. 1985). It has been proposed that

the mechanism by which cAMP is decreased by $Ca^{2+}$-mobilizing agonists could involve, in part at least, $Ca^{2+}$-calmodulin-activated cyclic nucleotide phosphodiesterase (Erneux et al. 1985).

### 2.4 $\alpha_1$-Adrenergic Activation of Phospholipase $A_2$

Although there is much evidence that $\alpha_1$-adrenergic receptors are linked to a polyphosphoinositide phospholipase C (see Sect. 4), an interesting new development is that these receptors can also stimulate arachidonic acid and eicosanoid release in pineal glands and in some thyroid and kidney cell lines due to the activation of phospholipase $A_2$ (Levine and Moskowitz 1979; Burch et al. 1986a, b; Meier et al. 1985; Slivka and Insel 1987; Ho and Klein 1987). The Madin-Darby cloned renal epithelial (MDCK) cell line expresses both $\alpha_1$- and $\beta$-adrenergic receptors, and activation of the $\alpha$-receptors leads to both phosphoinositide breakdown and prostaglandin $E_2$ production (Meier et al. 1983, 1985; Slivka and Insel 1987). Likewise, in the FRTL-5 thyroid cell line, stimulation of $\alpha_1$-adrenergic receptors causes the release of arachidonic acid which is metabolized mainly to prostaglandin $E_2$ (Burch et al. 1986a, b). In both cell lines, there is strong evidence that the $\alpha_1$-receptors are coupled in parallel to both phospholipase C and phospholipase $A_2$ (Burch et al. 1986a, b; Slivka and Insel 1987). It is clear in the case of the FRTL-5 cells that the responses are mediated by different G-proteins, but whether or not two receptor subtypes are involved is not yet known. In some cells phospholipase $A_2$ activity can be stimulated by phorbol esters via activation of protein kinase C (Parker et al. 1987), but it is not known whether this mechanism is involved in agonist stimulation of the phospholipase.

### 2.5 Long-term Regulation of $\alpha_1$-Adrenergic Receptors and Responses

In addition to being altered by aging, $\alpha_1$-adrenergic responses are influenced in the liver and other tissues by thyroid hormones, glucocorticoids, hepatectomy, cell culture, gender, and chronic exposure to agonists. In the liver, thyroidectomy decreases $\alpha_1$-adrenergic responses but increases $\beta$-adrenergic responses (Malbon et al. 1978; Preiksaitis and Kunos 1979; Preiksaitis et al. 1982; Storm et al. 1984). These alterations are accompanied by corresponding changes in the density of $\alpha_1$- and $\beta$-adrenergic receptors (Malbon 1980; Preiksaitis et al. 1982; cf. Malbon and Lo Presti 1981). In contrast, hypothyroidism decreases $\beta$-adrenergic responses in adipose tissue (Malbon et al. 1978), apparently because of impaired coupling of the $\beta$-adrenergic receptor to $G_s$ (Malbon et al. 1984). However, it does not affect $\alpha$-adrenergic responses in this tissue (Garcia-Sainz and Fain 1980; Garcia-Sainz et al. 1981).

Adrenalectomy also alters $\alpha$- and $\beta$-adrenergic responses in liver. There is an enhancement of $\beta$-responses which can be attributed to an increased number of $\beta$-adrenergic receptors (Chan et al. 1979; Wolfe et al. 1976; Guellaen et al. 1978; Studer and Borle 1984; El-Refai and Chan 1986). On the other hand, $\alpha_1$-adrenergic responses are diminished (Chan et al. 1979; Studer and Borle 1984) due to the loss of high-affinity $\alpha_1$-adrenergic receptors (El-Refai and Chan 1986). Interestingly, adrenalectomy also causes an increase in $\alpha_2$-adrenergic binding sites (El-Refai and Chan 1986).

Hepatectomy causes a marked decrease in $\alpha_1$-adrenergic responsiveness in the liver and an increase in $\beta$-adrenergic responsiveness. This is associated with an increase in $\beta$-adrenergic receptors but apparently no change in $\alpha_1$-adrenergic receptors (Huerta-Bahena et al. 1983). There is also a loss of responsiveness to vasopressin, angiotensin II, and ionophore A23187, although phosphatidylinositol turnover is apparently unchanged (Huerta-Bahena and Garcia-Sainz 1983, 1984). These findings suggest that hepatectomy causes a more general defect in intracellular $Ca^{2+}$ action.

Primary culture of rat hepatocytes leads to a gradual loss of $\alpha_1$-adrenergic responses and to enhancement of $\beta_2$-adrenergic responses (Okajima and Ui 1982; Itoh et al. 1984; Kunos et al. 1984). These changes are associated with a progressive decrease in the ADP-ribosylation of a 41-K membrane protein by islet-activating protein, a *Bordetella pertussis* toxin (Itoh et al. 1984). The changes in this protein, assuming it is $G_i$, could explain the observed increase in $\beta$-adrenergic receptor-mediated cAMP accumulation, but its relationship to the loss of $\alpha_1$-adrenergic responses is uncertain. Kunos et al. (1984) have observed no changes in $\alpha_1$- and $\beta$-adrenergic receptors during hepatocyte culture for 4 h and believe that the altered adrenergic responses are due to increased phospholipase $A_2$ activity. This conclusion is based on studies with two phospholipase-$A_2$ inhibitors (melittin and lipomodulin); however, these agents have rather nonspecific effects.

Livers of female rats display greater $\beta$-adrenergic responses than those of male rats (Studer and Borle 1982, 1983; Morgan et al. 1983b) and also show different cellular $Ca^{2+}$ responses to epinephrine (Studer and Borle 1982, 1983). However, the differences in $Ca^{2+}$ fluxes may be partly due to the difference in the levels of cAMP induced by the catecholamine (Morgan et al. 1983b).

There have been few studies of the effects of chronic agonist exposure on $\alpha_1$-adrenergic receptors. Incubation of Madin-Darby MDCK-D-1 or aortic smooth muscle cells with high concentrations of epinephrine or norepinephrine for 1–2 days caused an 80% loss of $\alpha_1$-adrenergic receptors (Meier et al. 1985; Colucci and Alexander 1986). The loss occurred more slowly than that of $\beta_2$-adrenergic receptors and was due to a decrease in $B_{max}$ without change in $K_d$ for epinephrine or norepinephrine. In the case of the smooth muscle cells, there was a complete loss of norepinephrine-stimulated $^{45}Ca^{2+}$

efflux, implying an additional postreceptor change (Colucci and Alexander 1986). Other in vivo studies of the effects of chemical sympathectomy, epinephrine treatment, or pheochromocytoma have also given evidence of down-regulation of $\alpha_1$-adrenergic receptors (Colucci et al. 1981; Snavely et al. 1983). In contrast to the situation with $\beta$-adrenergic receptors, the mechanisms by which $\alpha_1$-adrenergic receptors are down-regulated have received little attention. In the $DDT_1MF$-2 smooth muscle line, continuous exposure to norepinephrine leads to desensitization of phosphoinositide hydrolysis (Leeb-Lundberg et al. 1987). This is associated with the loss of cell surface $\alpha_1$-adrenergic receptors due to sequestration (Fratelli and DeBlasi 1987) and phosphorylation of the 80-K binding subunit of the receptor (Leeb-Lundberg et al. 1987). A similar phosphorylation is induced by phorbol esters and another $Ca^{2+}$ mobilizing agonist (Leeb-Lundberg et al. 1987). The phosphorylation is probably mediated by protein kinase C, since this can phosphorylate the purified $\alpha_1$-adrenergic receptor, and the phosphorylation is specifically enhanced by agonist occupancy of the receptor (Bouvier et al. 1987).

## 3 Changes in Cell $Ca^{2+}$ Induced by $\alpha_1$-Adrenergic and Other Agonists

### 3.1 Effects of $\alpha_1$-Adrenergic and Other Agonists on Cell $Ca^{2+}$ Fluxes

During the 1970s, evidence began to accumulate that epinephrine and norepinephrine did not always exert their effects by increasing cAMP and that their cAMP-independent actions were mediated by $\alpha$-adrenergic receptors (e.g., Tolbert et al. 1973; Hutson et al. 1976). It also became clear that $\alpha$-adrenergic receptors were comprised of $\alpha_1$- and $\alpha_2$-subtypes (Langer 1974, 1977; Starke 1977; Berthelson and Pettinger 1977). Subsequent work demonstrated that these subtypes were functionally distinct, and that activation of $\alpha_2$-receptors decreased cAMP, whereas the stimulation of $\alpha_1$-receptors altered cellular $Ca^{2+}$ fluxes (reviewed by Exton 1980, 1981, 1985).

### 3.2 Mobilization of Intracellular $Ca^{2+}$

The initial demonstrations of the effects of $\alpha_1$-adrenergic agonists on $Ca^{2+}$ fluxes utilized $^{45}Ca^{2+}$ and were performed in liver and smooth muscle. Both cellular influx and efflux of $^{45}Ca^{2+}$ were stimulated (reviewed by Bolton 1979; Exton 1980, 1981; Williamson et al. 1981; Reinhart et al. 1984c, d). The stimulation of $^{45}Ca^{2+}$ influx led to the view that the agonists opened plasma membrane $Ca^{2+}$ channels. However, studies of agonist-induced cellular

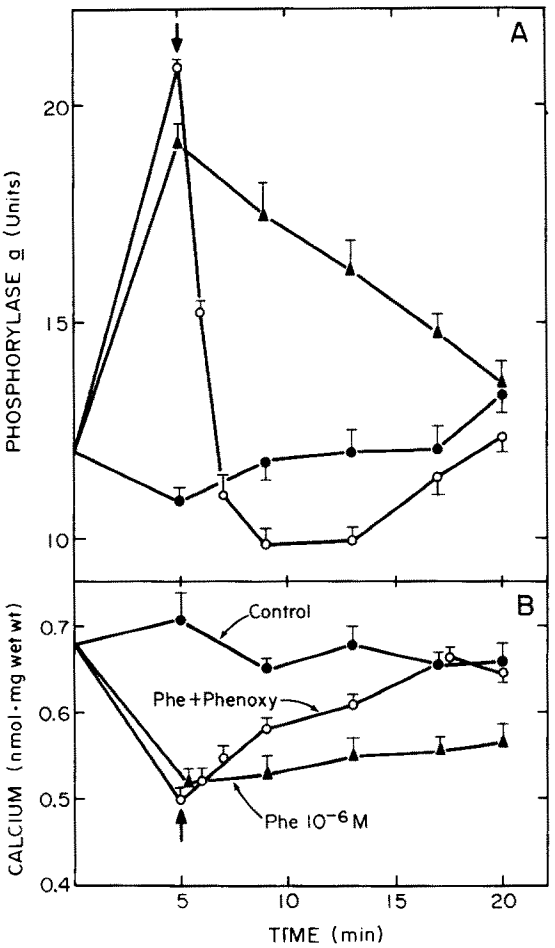


**Fig. 4.** Effects of the $\alpha_1$-adrenergic agonist phenylephrine (*Phe*) on phosphorylase activation and $Ca^{2+}$ mobilization in isolated rat hepatocytes. Hepatocytes were incubated with $10^{-6}$ *M* phenylephrine and the phosphorylase *a* and Ca content were measured at the times shown. *Phenoxy*, $10^{-5}$ *M* phenoxybenzamine. (From Blackmore et al. (1982) by permission of the authors and publisher)

responses known to involve $Ca^{2+}$ – e.g., liver glycogen breakdown, increased $K^+$ permeability in parotid gland, and tonic smooth muscle contraction – showed that they were initially unimpaired by depletion of extracellular $Ca^{2+}$ or by inhibition of its entry (Deth and Van Breemen 1974; Putney 1976; Assimacopolous-Jeannet et al. 1977; Weiss and Putney 1978; Blackmore et al. 1978; Parod and Putney 1978; Casteels and Raeymaekers 1979; Blackmore et al. 1982; Reinhart et al. 1984a). These findings indicated that a functionally important initial change in cell $Ca^{2+}$ induced by $\alpha_1$-adrenergic agonists was the mobilization of $Ca^{2+}$ from intracellular stores, although they did not exclude a role for $Ca^{2+}$ influx. The mobilization of internal $Ca^{2+}$ was confirmed by measurements of Ca (using atomic absorption spectroscopy or a $Ca^{2+}$ electrode) which showed that the agonists caused a rapid loss of $Ca^{2+}$ from hepatocytes or perfused livers (Fig. 4;

Blackmore et al. 1978, 1979, 1982, 1983a; Studer and Borle 1983; Reinhart et al. 1982). It was also supported by observations that $^{45}Ca^{2+}$ previously accumulated into the internal stores of liver, smooth muscle, and other tissues was rapidly released by $\alpha_1$-adrenergic agonists (Assimacopoulos-Jeannet et al. 1977; Casteels and Raeymaekers 1979; Chen et al. 1978; Deth and Casteels 1977; Blackmore et al. 1978; Haylett 1976; Jenkinson et al. 1978; Smith et al. 1984; Ambler et al. 1984; Parod and Putney 1979; Haddas et al. 1979; Miller and Nelson 1977; R.D. Brown et al. 1984; Amitai et al. 1984; Colucci and Alexander 1986). More direct proof of internal mobilization came when measurements of the Ca content of liver subcellular fractions revealed that some of these showed large decreases in response to $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists (Blackmore et al. 1979; Babcock et al. 1979; Murphy et al. 1980; Reinhart et al. 1982).

The concept that $Ca^{2+}$-mobilizing agonists released $Ca^{2+}$ from an intracellular pool was supported by other studies in which livers were perfused with $^{45}Ca^{2+}$ and the $^{45}Ca^{2+}$ content of subcellular fractions was measured (Barritt et al. 1981; Kimura et al. 1982; Studer and Borle 1983) or in which chlortetracycline fluorescence was measured in hepatocytes (Babcock et al. 1979). Although early studies suggested that mitochondria represented the major pool from which $Ca^{2+}$ was mobilized (Blackmore et al. 1979; Babcock et al. 1979; Murphy et al. 1980; Barritt et al. 1981; Studer and Borle 1983; Reinhart et al. 1982), more recent investigations indicate that the source is nonmitochondrial (Althaus-Salzmann et al. 1980; Poggioli et al. 1980; Berthon et al. 1981; Kimura et al. 1982; Shears and Kirk 1984a, b; Kleineke and Soling 1985). It is most likely the endoplasmic reticulum or an associated organelle, as shown by subcellular fractionation (Berthon et al. 1981; Joseph and Williamson 1983) and electron-probe X-ray microanalysis (Bond et al. 1984; Somylo et al. 1985a). Dantrolene, which is an inhibitor of $Ca^{2+}$ release from sarcoplasmic reticulum, has also been reported to inhibit $Ca^{2+}$ mobilization induced by the $\alpha_1$-adrenergic agonist phenylephrine in isolated hepatocytes and the perfused rat liver (Mine et al. 1987). Several studies have indicated that only a functionally discrete portion of the total endoplasmic reticulum is involved (Dawson and Irvine 1984; Joseph et al. 1984b; Prentki et al. 1984b).

The intracellular $Ca^{2+}$ pool that is mobilized by agonists does not refill until the agonists are removed or antagonists are added (Fig. 4; Putney 1977; Morgan et al. 1982; Breant et al. 1981; Aub et al. 1982; Dewitt and Putney 1983; Reinhart et al. 1984b; Joseph et al. 1985). However, refilling does not occur if extracellular $Ca^{2+}$ is absent or its entry is blocked (Marier et al. 1978; Aub et al. 1982; Putney 1976; Weiss and Putney 1978; Reynolds and Dubyak 1985; Joseph et al. 1985). As discussed in Sect. 7, the refilling of the pool is apparently prevented by continuing production of $IP_3$ (Prentki et al. 1985). When this compound declines after agonist removal, $Ca^{2+}$ reac-

cumulates into the stores and readdition of agonists produces further responses (Putney 1977; DenHertog 1981; Parod and Putney 1978; Joseph et al. 1985). During the reaccumulation phase, cytosolic $Ca^{2+}$ levels and the associated physiological responses decline (Fig. 4; Charest et al. 1983; Joseph et al. 1985; Morgan et al. 1982; Poggioli and Putney 1982; Blackmore et al. 1982; Casteels and Droogmans 1981). This suggests that the rate of reuptake of $Ca^{2+}$ by internal organelles exceeds the rate of net $Ca^{2+}$ influx. Alternatively, the internal pool may fill directly from the extracellular space or it may be located sufficiently close to the plasma membrane that incoming $Ca^{2+}$ ions are immediately taken up, and there is no general increase in cytosolic $Ca^{2+}$ (Putney 1986).

### 3.3 Elevation of Cytosolic $Ca^{2+}$

With the introduction of the fluorescent $Ca^{2+}$ probes Quin-2 and Fura-2 by Tsien and co-workers (Tsien 1980; Tsien et al. 1982, 1984), measurements of cytosolic $Ca^{2+}$ have been carried out in many cells. These show a rise in cytosolic $Ca^{2+}$ within a few seconds or less in response to $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists in many cells (Fig. 5; Pozzan et al. 1982;

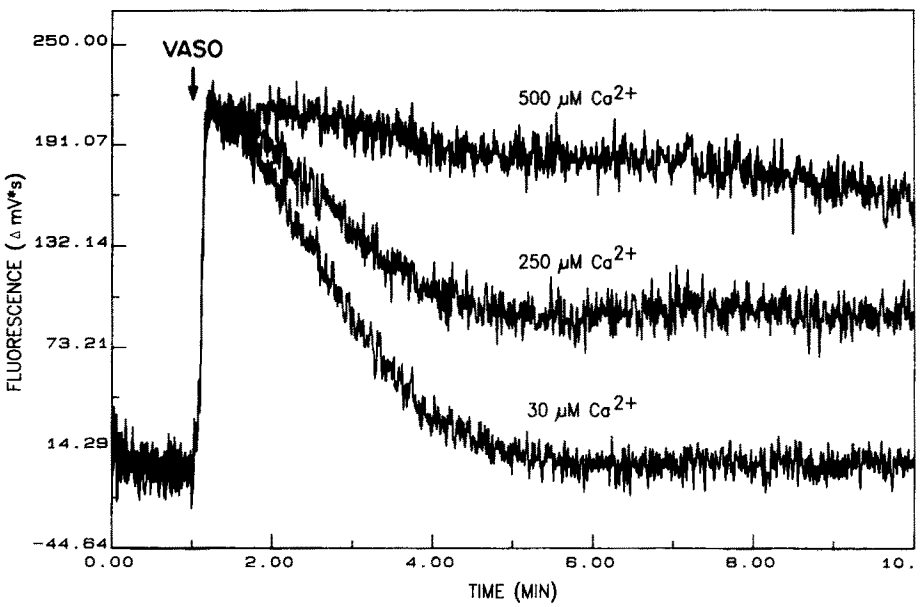


**Fig. 5.** Elevation of cytosolic $Ca^{2+}$ induced by vasopressin (*VASO*) in rat hepatocytes suspended in media of varying $Ca^{2+}$ concentrations. Hepatocytes were loaded with the fluorescent $Ca^{2+}$ indicator Quin-2 and resuspended in media containing 30, 250, or 500 $\mu M$ $Ca^{2+}$. At 1 min, $10^{-7} M$ vasopressin was added and the increases in cytosolic $Ca^{2+}$ were measured fluorimetrically. (From Charest et al. (1985) by permission of the authors and publisher)

Charest et al. 1983; Hesketh et al. 1983; Tsien et al. 1984; Korchak et al. 1984; Capponi et al. 1985; Nabika et al. 1985; Berthon et al. 1984; Smith et al. 1984; Reynolds and Dubyak 1985; Sage and Rink 1986; Rink and Sage 1985; Merritt and Rink 1987).

The reports cited above refer to studies utilizing cell suspensions. When single hepatocytes have been studied, oscillations in cytosolic $Ca^{2+}$ have been observed in response to $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists (Woods et al. 1986, 1987). These have been found in cells microinjected with the photoprotein aequorin, but have not been reported in cells loaded with Quin-2 or Fura-2, perhaps because of the $Ca^{2+}$-buffering properties of these compounds. The frequency of the oscillations, but not the shape or size, was a function of the agonist concentrations. Graf et al. (1987) also observed sustained oscillations in extracellular $Ca^{2+}$ when rat livers were perfused with $Ca^{2+}$-mobilizing agonists and low medium $Ca^{2+}$ (10 $\mu M$). The molecular basis of the oscillations is unknown, but it has been hypothesized that it involves negative feedback via DAG and protein kinase C acting on the receptor or G-protein (Woods et al. 1987).

In confirmation of earlier predictions, the initial increase in cytosolic $Ca^{2+}$ induced by agonists in most cells is largely, but not entirely, independent of extracellular $Ca^{2+}$, but at later times the increase declines unless $Ca^{2+}$ is present in the medium (Fig. 5; Charest et al. 1985; Joseph et al. 1985; cf. Berthon et al. 1984; Binet et al. 1985). In contrast, in pinealocytes, the removal of extracellular $Ca^{2+}$ completely eliminates the increase in cytosolic $Ca^{2+}$ in response to $\alpha_1$-adrenergic stimulation, indicating its total dependence on $Ca^{2+}$ inflow (Sugden et al. 1987).

It should be pointed out that most studies of the relative roles of internal $Ca^{2+}$ mobilization and $Ca^{2+}$ influx in the elevation of cytosolic $Ca^{2+}$ induced by agonists have employed standard fluorimeters. More recent fluorescence measurements using platelets or parotid acinar cells and stopped-flow techniques with millisecond resolution have shown that the increase in cytosolic $Ca^{2+}$ after addition of platelet-activating agents or carbachol occurs more rapidly if $Ca^{2+}$ is present in the medium than if it is absent (Rink and Sage 1985; Sage and Rink 1987; Merritt and Rink 1987). These findings indicate that, in these cells, these agonists induce an extremely rapid influx of $Ca^{2+}$, which occurs before the mobilization of internal $Ca^{2+}$ and may therefore not involve $IP_3$ formation.

### 3.4 Regulation of $Ca^{2+}$ Influx and $Ca^{2+}$ Channels

The intracellular stores of $Ca^{2+}$ in most cells are limited and rapidly become depleted with agonist stimulation (Exton 1985; Charest et al. 1985; Joseph et al. 1985). Calcium released from the stores into the cytosol is extruded from

the cell by the plasma membrane $Ca^{2+}$ pump or the $Na^+/Ca^{2+}$ exchanger, or is taken up by organelles not sensitive to $IP_3$. In the absence of extracellular $Ca^{2+}$, this results in a rapid decline in cytosolic $Ca^{2+}$ and of any $Ca^{2+}$-dependent physiological responses (Charest et al. 1985; Joseph et al. 1985; Binet et al. 1985). However, in the presence of normal levels of extracellular $Ca^{2+}$, agonists cause a persisting increase in cytosolic $Ca^{2+}$ (Fig 5; Charest et al. 1985; Binet et al. 1985) and continuing physiological responses (Exton 1985; Joseph et al. 1985). This implies that $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists also affect a process(es) by which $Ca^{2+}$ is transferred across the plasma membrane.

There have been several reports of agonist effects on both $Ca^{2+}$ uptake and efflux at the level of the plasma membrane in several tissues. Evidence for a stimulation of $Ca^{2+}$ entry is based on measurements of $^{45}Ca^{2+}$ uptake into hepatocytes measured 15–105 s after agonist addition (Mauger et al. 1984, 1985; Poggioli et al. 1985, 1986a; Combettes et al. 1986). Although it is very likely that $Ca^{2+}$ influx is stimulated in such studies, part of the observed increase in cell $^{45}Ca^{2+}$ could be secondary to the mobilization of internal unlabeled $Ca^{2+}$, which occurs within a few seconds (Williamson et al. 1981; Blackmore et al. 1982). A more detailed analysis of $^{45}Ca^{2+}$ fluxes in hepatocytes has been carried out by Barritt and co-workers (Barritt et al. 1981; Parker et al. 1983). These investigators concluded that epinephrine causes both a mobilization of $Ca^{2+}$ from an intracellular compartment and a stimulation of $Ca^{2+}$ influx into the cell.

Additional evidence for agonist stimulation of $Ca^{2+}$ entry in liver comes from studies using $Ca^{2+}$-depleted cells and Quin-2 to measure the influx of extracellular $Ca^{2+}$ into the cytosol (Joseph et al. 1985; unpublished studies by R. Charest, P. F. Blackmore, and J. H. Exton). In addition, high concentrations of $Ca^{2+}$-channel blockers such as diltiazem, nifedipine, and verapamil can block the influx of $Ca^{2+}$ observed in the presence of agonists and accelerate the decline in phosphorylase activity (Joseph et al. 1985; Hughes et al. 1986; unpublished studies by R. Charest, P. F. Blackmore, and J. H. Exton). The molecular mechanisms by which $Ca^{2+}$-mobilizing agonists stimulate the influx of $Ca^{2+}$ into cells are presently unknown, but they appear to involve a G-protein since the influx can be stimulated by $AlF_4^-$, which activates these proteins (Hughes and Barritt 1987; P. F. Blackmore and J. H. Exton, unpublished observations).

There have been numerous other reports of agonist-induced $Ca^{2+}$ influx in other tissues (Reuter 1983). Some of these, as in liver, involve voltage-independent $Ca^{2+}$ channels, e.g., muscarinic cholinergic effects on PC12 pheochromocytoma cells (Pozzan et al. 1986) and ATP effects on arterial smooth muscle (Benham and Tsien 1987), whereas others partly involve voltage-dependent $Ca^{2+}$ channels, e.g., thyrotropin-releasing hormone action on $GH_4C_1$ pituitary cells (Geras and Gershengorn 1982; Albert and Tashjian

1984; Tan and Tashjian 1984). It has been suggested that this latter effect is due to the elevation of DAG and activation of protein kinase C (Albert et al. 1987). This would be in accord with observations that phorbol esters cause an influx of $Ca^{2+}$ into neutrophils, suspended in $Na^+$-free medium, via a pertussis toxin-sensitive process presumably involving a G-protein (Nasmith and Grinstein 1987), and stimulate $Ca^{2+}$ entry into vascular smooth muscle (Gleason and Flaim 1986; Sperti and Colucci 1987). These esters also induce vascular smooth muscle contraction dependent upon extracellular $Ca^{2+}$ (Danthuluri and Deth 1984).

The possibility that inositol polyphosphates could control the plasma membrane $Ca^{2+}$ channel has been raised by many workers. Irvine and Moor (1986) noted that myoinositol 1,3,4,5-$P_4$ ($IP_4$) activated sea urchin eggs when coinjected with myoinositol 2,4,5-$P_3$, provided external $Ca^{2+}$ was present. In contrast, Crossley et al. (1988) found that the effects were independent of external $Ca^{2+}$ and that $IP_3$ was 100-fold more potent than $IP_4$ in activating the eggs. There have also been reports that $IP_3$ activates transmembrane $Ca^{2+}$ channels in T-lymphocytes (Kuno and Gardner 1987) and *Xenopus* oocytes (Parker and Miledi 1987). However, there is now much evidence that $Ca^{2+}$ channels, like $K^+$ channels, are controlled more directly by G-proteins. This is discussed in detail in Sect. 5.3.

Based on the relative potencies of the $\alpha$-adrenergic antagonists WB4101 and benoxathian to block contraction and/or inositol phosphate formation in vas deferens, cerebral cortex, and hippocampus in response to norepinephrine, Han et al. (1987) have proposed that only the $\alpha_{1B}$-subtype of adrenergic receptors is linked to inositol phospholipid hydrolysis (see Sect. 4). They also observed that the addition of the $Ca^{2+}$-channel blocker nifedipine or the removal of extracellular $Ca^{2+}$ markedly reduced norepinephrine-stimulated contractions of the vas deferens, but not of the spleen, and that in the presence of nifedipine, the potency of WB4101 in blocking the contraction of the vas deferens was greatly decreased. Based on these findings, Han et al. (1987) have further proposed that the $\alpha_{1A}$-subtype of adrenergic receptor (with high affinity for WB4101 and benoxathian) is coupled to $Ca^{2+}$ influx. This intriguing proposal clearly requires additional experimental support.

### 3.5 Regulation of $Ca^{2+}$ Efflux and $Ca^{2+}$ Pump

The efflux of $Ca^{2+}$ caused initially by $Ca^{2+}$mobilizing agonists in the liver and other tissues is transient (Fig. 6), because the mobilizable intracellular $Ca^{2+}$ pool is limited and there is also a stimulation of $Ca^{2+}$ influx. The increased influx of $Ca^{2+}$ due to the opening of $Ca^{2+}$ channels is sustained, but it becomes balanced by increased efflux of $Ca^{2+}$ since the cytosolic $Ca^{2+}$ concentration stabilizes after a few minutes and there is no net uptake of

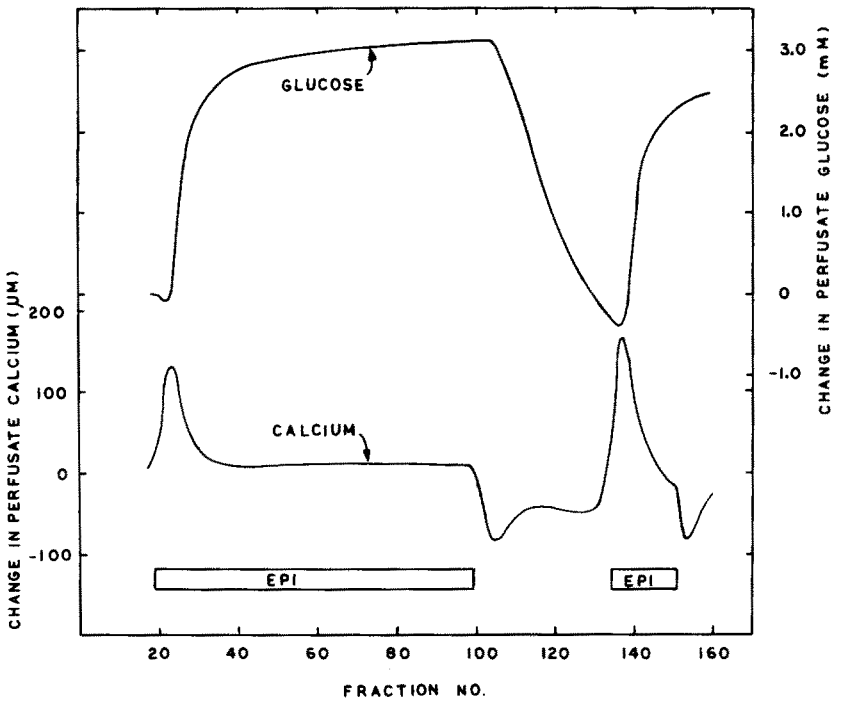


**Fig. 6.** Effects of epinephrine on glucose release and $Ca^{2+}$ fluxes in the isolated perfused rat liver. Livers from fed rats were perfused with nonrecirculating medium containing 1 m*M* $Ca^{2+}$ for 10 min before the commencement of an infusion of epinephrine to give a final concentration of 1 μ*M*. This was continued for 25 min, then withdrawn for 10 min, then recommenced for 5 min, and then withdrawn again. Changes (from pre-epinephrine values) of glucose and calcium in the perfusate leaving the liver are shown. The fraction numbers refer to the samples, which were collected every 18 s. (From Morgan et al. (1982) by permission of the authors and publisher)

$Ca^{2+}$ by the liver as a whole or by its intracellular organelles until agonists are removed (Fig. 6; Morgan et al. 1982; Charest et al. 1983). The increased $Ca^{2+}$ efflux may be simply attributable to stimulation of the plasma membrane $Ca^{2+}$ pump resulting from the elevated concentration of cytosolic $Ca^{2+}$. This would cause increased bidirectional flux of $Ca^{2+}$ across the plasma membrane in the presence of agonists. Reinhart et al. (1984b) have presented some studies of $^{45}Ca^{2+}$ uptake by perfused rat livers which suggest such increased cycling.

Another means of producing a sustained increase in cytosolic $Ca^{2+}$ is to alter the kinetics of the plasma membrane $Ca^{2+}$ pump. Evidence for inhibition of the plasma membrane $Ca^{2+}$ pump by several agonists in liver has been presented by Prpic et al. (1984). In addition, there have been reports of a delay in the release of $Ca^{2+}$ from hepatocytes (Joseph and Williamson 1983) and of an inhibition of the plasma membrane ($Ca^{2+}$ +$Mg^{2+}$)-ATPase

of liver by vasopressin and phenylephrine (Lin et al. 1983) and of myometrium by oxytocin (Soloff and Sweet 1982). The mechanism by which $Ca^{2+}$-mobilizing agonists inhibit the plasma membrane $Ca^{2+}$ pump is unknown, but the inhibition could be due to the changes in phosphoinositides produced by these agonists (Buckley and Hawthorne 1972; Penniston 1983; Prpic et al. 1984; Charest et al. 1985).

### 3.6 Comparison of Effects of $\alpha_1$-Adrenergic Agonists with Those of Other $Ca^{2+}$-Mobilizing Agonists

There have been some reports that the effects of $\alpha_1$-adrenergic agonists on cytosolic $Ca^{2+}$ in hepatocytes differ from those of vasopressin and angiotensin II (Mine et al. 1987; Kleineke and Soling 1987). However, we and others have been unable to confirm these findings using Quin-2 or Fura-2 to measure the cytosolic $Ca^{2+}$ (Lynch et al. 1985a, c; Binet et al. 1985; P.F. Blackmore and J.H. Exton, unpublished observations). It has also been reported by one group that $\alpha_1$-adrenergic agonists produce $Ca^{2+}$ flux responses in perfused rat liver that differ from those induced by vasopressin and angiotensin II (Altin and Bygrafe 1985). For example, the $\alpha_1$-adrenergic agonist phenylephrine was reported to induce $Ca^{2+}$ efflux, but not influx, in the presence of 1.3 m*M* $Ca^{2+}$ in the medium, whereas the other agonists induced $Ca^{2+}$ efflux followed by influx. In contrast, Kleineke and Soling (1987) reported that $Ca^{2+}$ influx can occur with phenylephrine under these conditions. Irrespective of this discrepancy, the possibility exists that these agents could affect $Ca^{2+}$ fluxes secondarily in such a perfusion system because of effects on blood flow and on other cell types. Clearly, more work is required to establish whether or not $\alpha_1$-adrenergic agonists differ from vasopressin or angiotensin II in their actions on hepatocyte $Ca^{2+}$ fluxes.

## 4 Role of Phosphoinositide Changes

### 4.1 Historical Background

Expanding on the pioneering studies of Hokin and Hokin (1953), Michell (1975, 1979) emphasized the association between the changes in $Ca^{2+}$ induced by certain hormones and neurotransmitters and the turnover of phosphoinositides in a variety of tissues. In particular, this was pointed out for $\alpha$-adrenergic agonists in brain, parotid, pineal, iris, liver, vas deferens, aorta, and submaxillary gland (Jones and Michell 1978). Initially, it was demonstrated that these agonists increased both the synthesis and breakdown of

phosphatidylinositol (PI) in labeling studies with $^{32}P_i$ (Jones and Michell 1978). These observations were confirmed using [$^3$H]myoinositol (Tolbert et al. 1980; Prpic et al. 1982). However, it was observed that the turnover of PI induced by $\alpha_1$-adrenergic agonists or other $Ca^{2+}$-mobilizing agents was not fast enough to be responsible for the physiological responses, which occurred within seconds (Canessa de Scarnatti and Lapetina 1974; Kirk et al. 1977, 1981; Billah and Michell 1979; Uchida et al. 1982; Prpic et al. 1982).

## 4.2 Phosphatidylinositol 4,5-$P_2$ Breakdown and Myoinositol 1,4,5-$P_3$ Formation

Early observations by Schacht and Agranoff (1972) and Abdel-Latif et al. (1977) indicated that $Ca^{2+}$-mobilizing agonists stimulated the phosphodiesteratic breakdown of phosphatidylinositol 4,5-$P_2$ ($PIP_2$) in addition to that of PI in neural and smooth muscle tissue. The group of Kirk and Michell then demonstrated that the breakdown of this polyphosphoinositide induced by these agonists in liver occurred much more rapidly than that of PI (Kirk et al. 1981; Michell et al. 1981; Creba et al. 1983). This was later confirmed by others in liver (Rhodes et al. 1983; Thomas et al. 1983; Litosch et al. 1983), parotid (Weiss et al. 1982; Downes and Wusteman 1983), platelets (Billah and Lapetina 1982; Agranoff et al. 1983; Mauco et al. 1983), kidney cortex (Wirthensohn et al. 1984), exocrine pancreas (Putney et al. 1983), neutrophils (Volpi et al. 1983; Yano et al. 1983; Dougherty et al. 1984), and pituitary (Martin 1983; Rebecchi and Gershengorn 1983; MacPhee and Drummond 1984).

The significance of the enhanced breakdown of $PIP_2$ was recognized by Berridge and associates (Berridge 1984; Berridge and Irvine 1984) when they measured the changes in the concentration of one of the products, myoinositol 1,4,5-$P_3$ ($IP_3$), in various tissues stimulated with agonists (Berridge 1983; Berridge et al. 1983) and when Streb et al. (1983) showed that this compound released $Ca^{2+}$ from internal stores in permeabilized pancreatic acinar cells. Since then, $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists have been shown to rapidly increase $IP_3$ in many tissues, including liver (Fig. 7; Thomas et al. 1984; Charest et al. 1985), brain (Berridge et al. 1983), platelets (Agranoff et al. 1983; Vickers et al. 1984; Rittenhouse and Sasson 1985), salivary glands (Berridge et al. 1983; Berridge 1983; Downes and Wustemann 1983; Aub and Putney 1984, 1985; Irvine et al. 1984c, 1985), pituitary (Martin 1983; Rebecchi and Gershengorn 1983; Enjalbert et al. 1986; Morgan et al. 1987), exocrine pancreas (Rubin et al. 1984), endocrine pancreas (Morgan et al. 1985), Swiss 3T3 cells (Berridge et al. 1984), adrenal cortex (Gallo-Payet et al. 1986), endothelial cells (Lambert et al. 1986), smooth muscle cells (Akhtar and Abdel-Latif 1984; Smith et al. 1984), heart

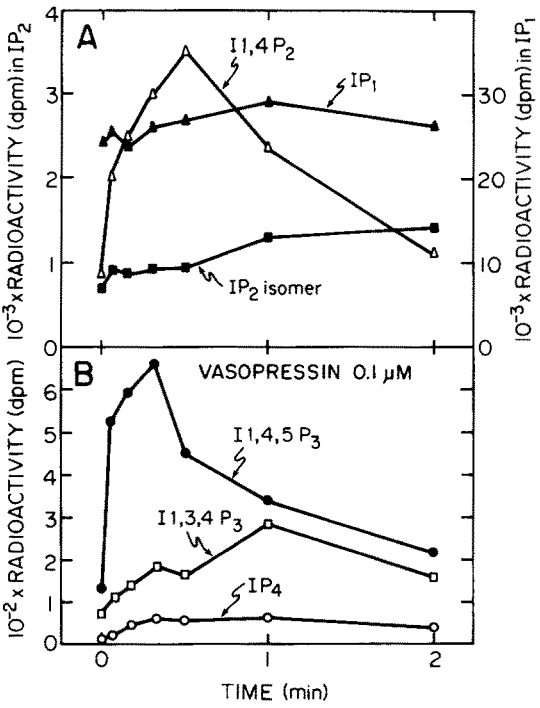


**Fig. 7.** Effects of vasopressin on inositol phosphates in isolated rat hepatocytes. Hepatocytes were incubated for 2 h with [$^3$H]myoinositol to label the inositol phospholipids. They were then washed and incubated with 0.1 μ*M* vasopressin. Samples were removed and deproteinized at the times indicated for measurement of the radioactive inositol phosphates by high-pressure liquid chromatography (Irvine et al. 1985). *IP$_1$*, myoinositol monophosphate(s); *I1,4P$_2$*, myoinositol 1,4-P$_2$; *IP$_2$*, isomer, probably myoinositol 3,4-P$_2$; *I1,4,5P$_3$*, myoinositol 1,4,5-P$_3$; *I1,3,4P$_3$*, myoinositol 1,3,4-P$_3$; *IP$_4$*, myoinositol 1,3,4,5-P$_4$. (Unpublished findings by P.F. Blackmore, S.B. Bocckino, H. Jiang, V. Prpic, and J.H. Exton)

(Poggioli et al. 1986b; Marc et al. 1986), lymphocytes (Imboden and Stobo 1985), gastric mucosal cells (Baudiere et al. 1986; Chew and Brown 1986), astrocytoma cells (Masters et al. 1985b), PC12-pheochromocytoma cells (Vincentini et al. 1985a), and adipocytes (Nanberg and Putney 1986).

The increase in $IP_3$ with agonists is detectable within a few seconds and generally precedes or is coincident with the rise in cytosolic $Ca^{2+}$ (Thomas et al. 1984; Charest et al. 1985; Lew et al. 1986; Trimble et al. 1987; Tilly et al. 1987; Pribluda and Metzger 1987). However, there have been some reports in which an $IP_3$ increase is not detectable early at times when cytosolic $Ca^{2+}$ is elevated by certain agonists (Merritt et al. 1986b; Tashjian et al. 1987; Merritt and Rink 1987). As discussed in Sect. 3.3, this suggests the existence of a very early stimulation of $Ca^{2+}$ influx unrelated to $IP_3$.

The concentrations of agonists which produce half-maximal changes in $PIP_2$ or $IP_3$ are similar to their $K_d$s for binding to their receptors in plasma membranes (Creba et al. 1983; Lynch et al. 1985a). In addition, the maximum generation of $IP_3$ by agonists is proportional to the number of their plasma membrane binding sites (Lynch et al. 1985a). These findings suggest a close relationship between receptor occupancy and phosphoinositide breakdown. However, because of the presence of spare receptors in most cells, the concentrations of agonists required to half-maximally elevate cytosolic $Ca^{2+}$ and elicit physiological responses are usually lower than those that half-maximally

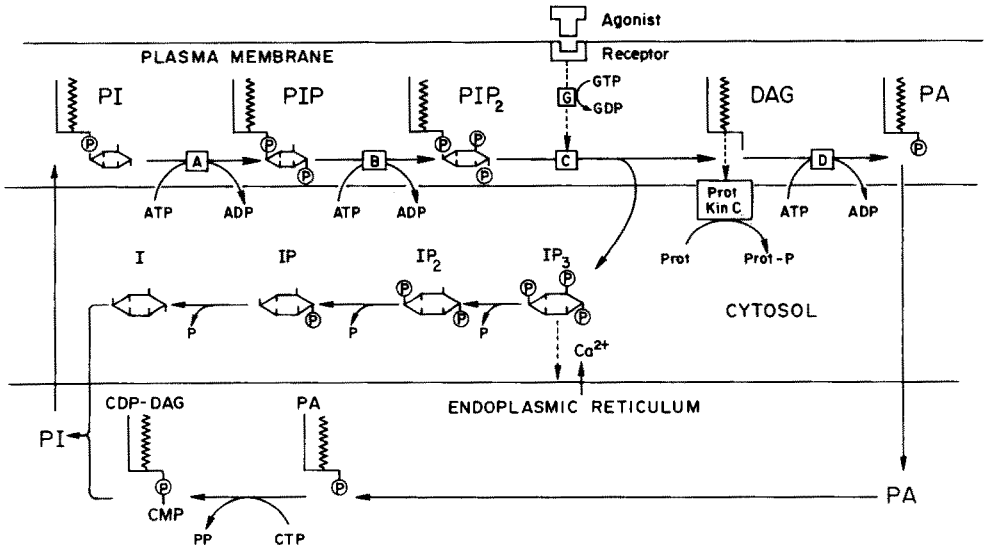


**Fig. 8.** Pathways of cellular phosphoinositide metabolism (with the $IP_3$ kinase and associated pathways omitted for simplicity). Abbreviations not defined in the text are: *G*, G-protein; *A*, PI kinase; *B*, PIP kinase; *C*, $PIP_2$ phospholipase C; *D*, 1,2-diacylglycerol kinase; *$IP_2$*, myoinositol 1,4-$P_2$; *IP*, myoinositol 4-P; *I*, myoinositol

increase $IP_3$ or decrease $PIP_2$. Thus, small increases in $IP_3$ can elicit large physiological responses in most systems (Lynch et al. 1985a; Creba et al. 1983; Rhodes et al. 1983; Charest et al. 1985; Thomas et al. 1984; Aub and Putney 1985; cf. Vincentini et al. 1985a).

As depicted in Fig. 8, it is generally agreed that the reaction primarily stimulated by $\alpha_1$-adrenergic agonists and other $Ca^{2+}$-mobilizing agents is the breakdown of $PIP_2$ to $IP_3$ and DAG, catalyzed by a $Ca^{2+}$-dependent phosphodiesterase commonly termed phospholipase C. There are several forms of phosphoinositide phospholipase C in most cells (Irvine et al. 1984b; Wilson et al. 1984; Rittenhouse 1983; Nakanishi et al. 1985; Low et al. 1986; Deckmyn et al. 1986; Baldassare and Fisher 1986a; Cockcroft 1986; Banno et al. 1986a, b; Ebstein et al. 1987; Manne and Fung 1987; Taylor and Exton 1987; Rock and Jackowski 1987; Ryu et al. 1987a; Bennett and Crooke 1987). However, it is not clear which forms are under hormonal control. As discussed later (Sect. 6), the hormone-sensitive enzyme may also affect phosphatidylinositol 4-P (PIP) but is poorly active or inactive on PI (Uhing et al. 1985, 1986; Aub and Putney 1984; Downes and Wustemann 1983; Martin 1983; Taylor and Exton 1987; Rebecchi and Rosen 1987b). The loss of PI that is observed in most experiments may be due to the accelerated conversion of PI to PIP and then $PIP_2$ to replace $PIP_2$ broken down by $PIP_2$ phospholipase. Alternatively, there may be activation of a PI phospholipase C in some cells (Griendling et al. 1986).

Two different kinases catalyze the conversion of PI to PIP and PIP to $PIP_2$ (Fig. 7). These are located in the plasma membrane and are very active, as are the phosphomonoesterases which reverse their actions (Berridge 1984). The increased conversion of PI to $PIP_2$ induced by agonists which stimulate $PIP_2$ breakdown is thought to be due to the fact that both kinases show product inhibition (Lundberg et al. 1986). However, the possibility that $Ca^{2+}$-mobilizing agonists control $PIP_2$ synthesis by other mechanisms should not be discounted. In this regard, treatment of A431 cells with epidermal growth factor has been shown to cause a rapid increase in membrane PI kinase activity (Walker and Pike 1987) and an increase in PIP in the cells (Pike and Eakes 1987). In chick embryo fibroblast cells transformed by a virus carrying the *erb*B oncogene (which enclodes a truncated form of the epidermal growth factor receptor), the activities of the kinases for PI, PIP, and DAG were also found to be enhanced (Kato et al. 1987). Slower increases in the kinases for PI and PIP have also been observed in Swiss 3T3 cells stimulated by platelet-derived growth factor (MacDonald et al. 1987). Despite earlier reports, it is now believed that the tyrosine kinases associated with certain growth factor receptors and proto-oncogene products do not possess PI kinase activity (for reference, see Walker and Pike 1987). Thus, the effects of growth factors on PI kinase must be indirect.

## 4.3 Metabolism of Myoinositol 1,4,5-$P_3$

Myoinositol 1,4,5-$P_3$ generated from $PIP_2$ is released into the cytosol, where it releases $Ca^{2+}$ from internal stores (Fig. 8). Unless it is continuously generated, its action is short-lived because it is rapidly metabolized. As shown in Figs. 7 and 8, a major pathway of $IP_3$ metabolism is its rapid degradation to myoinositol 1,4-$P_2$ ($IP_2$) by a specific 5-phosphomonoesterase found in the plasma membrane and soluble phase (Downes et al. 1982; Seyfred et al. 1984; Storey et al. 1984; Joseph and Williams 1985; Connolly et al. 1985, 1987; Shears et al. 1987a). $IP_2$ is then sequentially degraded to myoinositol 4-P and myoinositol by other soluble phosphomonoesterases (Joseph and Williams 1985; Storey et al. 1984; Dean and Moyer 1987; Balla et al. 1986; Morgan et al. 1987; Ackermann et al. 1987; Delvaux et al. 1987; Inhorn et al. 1987). The phosphatase that converts myoinositol 1,4-$P_2$ to myoinositol 4-P has been purified and has an $M_r$ of 45000 (Inhorn and Majerus 1987). It is inhibited by $Li^+$ and has been called "inositol polyphosphate 1-phosphatase" (Inhorn and Majerus 1987; Inhorn et al. 1987).

Myoinositol can be reincorporated into PI through the action of CDP-diacylglycerol : inositol transferase in the endoplasmic reticulum (Fig. 8). Synthesized PI is then transferred to the plasma membrane by a specific phospholipid carrier protein (Michell 1975). However, Imai and Gershengorn

(1987) have recently obtained evidence that PI resynthesis can occur in the plasma membrane of $GH_3$ pituitary cells as well as in the endoplasmic reticulum. If this is true for other cells it could account for reports of multiple cellular pools of inositol phospholipids (Monaco and Woods 1983; King et al. 1987), although other explanations can be proposed.

The other main route of metabolism of $IP_3$ is its conversion to myoinositol 1,3,4,5-$P_4$ ($IP_4$) by a 3-kinase (Irvine et al. 1986a; Hansen et al. 1986; Downes et al. 1986; Stewart et al. 1986; Biden and Wollheim 1986; Connolly et al. 1987). $IP_4$ is subsequently converted to myoinositol 1,3,4-$P_3$ by the same 5-phosphomonoesterase that acts on $IP_3$ (Connolly et al. 1987; Erneux et al. 1987). This accounts for the accumulation of these two compounds in response to agonists in several tissues (Fig. 7; Irvine et al. 1984c, 1985, 1986a; Batty et al. 1985; Heslop et al. 1985, 1986; Hawkins et al. 1986; Biden et al. 1987; Downes et al. 1986; Hansen et al. 1986; Dean and Moyer 1987; Balla et al. 1986; Stewart et al. 1986; Morgan et al. 1987; Trimble et al. 1987; Tilly et al. 1987; Merritt et al. 1986b). Myoinositol 1,3,4-$P_3$ can be rephosphorylated to an $IP_4$ isomer (Balla et al. 1987; Shears et al. 1987) which has been shown to be myoinositol 1,3,4,6-$P_4$ (Shears et al. 1987b). Inositol pentakisphosphate and hexakisphosphate have also been found in mammalian cells, but usually they do not change with agonist stimulation (Heslop et al. 1985; Tilly et al. 1987; Stewart et al. 1987; cf. Morgan et al. 1987). Myoinositol 1,3,4-$P_3$ is further hydrolyzed to myoinositol 3,4-$P_2$ by inositol polyphosphate 1-phosphatase in brain (Inhorn et al. 1987; Inhorn and Majerus 1987; Erneux et al. 1987), liver (Shears et al. 1987), and polymorphonuclear leukocytes (Dillon et al. 1987). As noted above, this phosphatase also acts on myoinositol 1,4-$P_2$ (Inhorn et al. 1987; Inhorn and Majerus 1987; Erneux et al. 1987). However, the breakdown of myoinositol 1,3,4-$P_3$ is probably more complex, since myoinositol 1,3-$P_2$ is also found in certain cells stimulated with agonists (Irvine et al. 1987). The conversion of myoinositol 1,3,4-$P_3$ to myoinositol 1,3-$P_2$ and then to myoinositol 1-P by brain extracts has been reported by Bansal et al. (1987), but this probably represents a minor pathway. The 4-phosphatase involved can also degrade myoinositol 3,4-$P_2$ to myoinositol 3-P. The myoinositol 1-P, myoinositol 4-P, and myoinositol 3-P formed during myoinositol 1,4,5-$P_3$ and myoinositol 1,3,4-$P_3$ breakdown are apparently converted to myoinositol by the same inositol monophosphate phosphatase (Ackermann et al. 1987; Delvaux et al. 1987).

The 3-kinase that converts $IP_3$ to $IP_4$ is stimulated by $Ca^{2+}$ in complex with calmodulin (Biden and Wollheim 1986; Ryu et al. 1987b). This may explain the transiency of agonist-stimulated $IP_3$ formation and the delay in myoinositol 1,3,4-$P_3$ formation seen in most systems (Lew et al. 1986). The 5-phosphomonoesterase that degrades $IP_3$ and $IP_4$ has also been reported to be phosphorylated and activated by protein kinase C (Connolly et al. 1986a,

1987). This would explain why activators of this kinase stimulate the conversion of $IP_3$ to $IP_2$ in permeabilized platelets (Molina y Vedia and Lapetina 1986). However, this has not been seen in some other cells (Orellana et al. 1987). Based on the $K_m$s of the 3-kinase and 5-phosphomonoesterase for $IP_3$ and $IP_4$, the preferential metabolism of $IP_3$ to $IP_4$ with subsequent dephosphorylation to myoinositol 1,3,4-$P_3$ observed in most tissues can be explained (Irvine et al. 1986a; Connolly et al. 1987).

The functions, if any, of $IP_4$ and myoinositol 1,3,4-$P_3$ remain unclear. $IP_4$ has been reported to activate sea urchin eggs in the presence of external $Ca^{2+}$, provided it is coinjected with myoinositol 2,4,5-$P_3$ (Irvine and Moor 1986), but critical aspects of these results have not been confirmed (Crossley et al. 1988) and there have been no reports of similar findings in mammalian systems.

Wilson et al. (1985) first pointed out that myoinositol 1,2-cyclic 4,5-$P_3$ ($cIP_3$) can be formed together with $IP_3$ during the action of phospholipase C from sheep seminal vesicles of $PIP_2$ in vitro. This is analogous to early studies which showed that brain phospholipase C formed myoinositol 1,2-cyclic P and myoinositol 1-P from PI (Dawson et al. 1971). The formation of $cIP_3$ during agonist stimulation of platelets and pancreas has been reported (Ishii et al. 1986; Sekar et al. 1987), but this compound rises much more slowly than $IP_3$ in pancreatic lobules (Dixon and Hokin 1987) and platelets (Tauven et al. 1987) and could not be detected in parotid glands stimulated with carbachol, although $IP_3$ was formed and $cIP_2$ added to the extracts was quantitatively recovered (Hawkins et al. 1987).

$cIP_3$ has been reported to have equal or slightly greater potency than $IP_3$ in eliciting responses in several systems (Wilson et al. 1985). It is degraded sequentially to myoinositol 1,2-cyclic P by the same enzymes involved in the hydrolysis of $IP_3$, and then to myoinositol 1-P by a cyclic hydrolase (Connolly et al. 1986b, 1987). Compared with $IP_3$, its rate of degradation by 5-phosphomonoesterase is very slow (Connolly et al. 1987; Hawkins et al. 1987); this has implications for its postulated role as an intracellular signal. It is also not a substrate for the 3-kinase that acts on $IP_3$ (Connolly et al. 1987).

The major postulated physiological role of DAG, the other product of $PIP_2$ breakdown, is activation of protein kinase C at or in the plasma membrane (Fig. 8). Compared with the metabolism of $IP_3$, that of DAG has received little attention. The conventional view is that it is mainly converted to phosphatidic acid (PA) through the action of 1,2-diacylglycerol kinase (Fig. 8). The PA is then transferred to the endoplasmic reticulum to be used for the synthesis of PI, other phospholipids, and triacylglycerol. However, there is some evidence that PI resynthesis can occur in the plasma membrane (Imai and Gershengorn 1987), suggesting that the DAG and PA generated by $PIP_2$ breakdown may not enter the general cellular pools.

The metabolism of DAG is discussed in detail in Sect. 8, where it is shown that $Ca^{2+}$-mobilizing agonists generate DAG from sources other than the inositol phospholipids, and that PA is not derived solely from the phosphorylation of DAG or by de novo synthesis.

## 5 Role of Guanine Nucleotide-Binding Regulatory Proteins

### 5.1 Evidence for a Role of Guanine Nucleotide-Binding Regulatory Proteins in Agonist Regulation of Phosphatidylinositol 4,5-$P_2$ Breakdown

As described in Sect. 2.2, the ability of GTP and its nonhydrolyzable analogues to alter the agonist affinity of the $\alpha_1$-adrenergic receptor and other receptors for $Ca^{2+}$-mobilizing agonists implies that these receptors couple to guanine nucleotide-binding regulatory proteins or G-proteins analogous to those involved in the regulation of adenylate cyclase. Further evidence for the involvement of G-proteins in the actions of these agonists comes from a variety of studies. For example, in permeabilized mast cells and platelets, nonhydrolyzable analogues of GTP elicit $Ca^{2+}$-dependent exocytotic secretion (Gomperts 1983; Haslam and Davidson 1984a, b), and some $Ca^{2+}$-mobilizing agonists stimulate a low $K_m$ membrane GTPase activity (Hinkle and Phillips 1984; Fain et al. 1985; Fitzgerald et al. 1986; Grandt et al. 1986; Higashida et al. 1986; Houslay et al. 1986) or the binding/exchange of a GTP analogue to membranes (Lad et al. 1985). In liver and other cells, NaF stimulates the breakdown of $PIP_2$ to $IP_3$ and DAG with resultant increases in cytosolic $Ca^{2+}$ and responses (Blackmore et al. 1985; Martin et al. 1986a; Hepler and Harden 1986; Guillon et al. 1986; Brass et al. 1986; Strnad et al. 1986; Paris and Pouyssegur 1987; Kienast et al. 1987). These effects are potentiated by $AlCl_3$, implying that $AlF_4^-$ is the active molecule. $AlF_4^-$ is known to modulate the activity of other G-proteins (Sternweis and Gilman 1982; Katada et al. 1984; Kanaho et al. 1985).

More direct evidence for a role of a G-protein in the regulation of $PIP_2$ hydrolysis is provided by studies showing that GTP and its analogues stimulate the breakdown of endogenous or exogenous $PIP_2$ or PIP in isolated plasma membranes or permeabilized cells from liver (Uhing et al. 1985, 1986; Wallace and Fain 1985; Taylor and Exton 1987), polymorphonuclear leukocytes (Cockcroft and Gomperts 1985), salivary glands (Litosch et al. 1985), $GH_3$ or 7315c pituitary cells (Lucas et al. 1985; Martin et al. 1986a, b; Straub and Gershengorn 1986; Aub et al. 1987), astrocytoma cells (Hepler and Harden 1986; Orellano et al. 1987), pancreatic acinar cells (Merritt et al. 1986a), platelets (Baldassare and Fisher 1986a, b; Hrbolich et al. 1987), islets (Dunlop and Larkins 1986), Jurkat T cells (Sasaki and Hase-

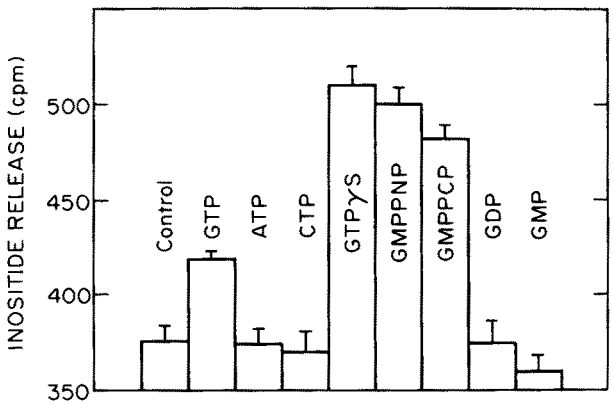


**Fig. 9.** Stimulatory effects of GTP and its analogues on $PIP_2$ breakdown in rat liver plasma membranes. Liver plasma membranes prepared from rats injected 18–20 h earlier with [$^3$H]myoinositol were incubated for 5 min with 100-μ*M* concentrations of the nucleotides shown and the release of radioactive inositides (free myoinositol plus myoinositol tris-, bis-, and monophosphates) was measured. The inositide released initially was $IP_3$ and the inositol phospholipid broken down was $PIP_2$. *GTPγS*, guanosine 5′-0-(thiotriphosphate); *GMPPNP*, guanyl-5′-yl imidodiphosphate; *GMPPCP*, guanyl-5′-yl-(β,γ-methylene)diphosphonate. (From Uhing et al. (1985) by permission of the authors and publisher)

gawa-Sasaki 1987), neutrophils (Cockcroft 1986; Smith et al. 1987), fibroblasts (Rebecchi and Rosen 1987a; Magnaldo et al. 1987), and brain (Litosch 1987). The effect is greater with the nonhydrolyzable analogues of GTP and is mimicked by NaF (Hepler and Harden 1986; Martin et al. 1986a; Rock and Jackowski 1987; Sasaki and Hasegawa-Sasaki 1987; Litosch 1987), but it is not seen with other nucleoside triphosphates or with GDP or GMP (Fig. 9; Uhing et al. 1985; Wallace and Fain 1985; Cockcroft and Gomperts 1985; Litosch et al. 1985; Aub et al. 1987). GTP and its analogues are effective at micromolar concentrations, and their effects are $Mg^{2+}$-dependent and inhibited by GDPβS (Uhing et al. 1986; Martin et al. 1986a; Cockcroft 1986; Baldassare and Fisher 1986a; Litosch 1987; Taylor and Exton 1987; Rebecchi and Rosen 1987a; Aub et al. 1987; Hrbolich et al. 1987). In most tissues, the breakdown of $PIP_2$ is associated with some hydrolysis of PIP, but not of PI, and requires the presence of 100 n*M* or higher free $Ca^{2+}$ (Uhing et al. 1985, 1986; Cockcroft and Gomperts 1985; Rebecchi and Rosen 1987a; Litosch 1987; Taylor and Exton 1987; cf. Wallace and Fain 1985; Melin et al. 1986). Likewise, $IP_3$ is the major product formed initially but is degraded to $IP_2$ by the $IP_3$ phosphatase activity of the membranes (Uhing et al. 1985; Wallace and Fain 1985). However, in platelets, PIP breakdown may predominate (Hrbolich et al. 1987).

Recently, direct effects of $Ca^{2+}$-mobilizing agonists on the breakdown of polyphosphoinositides in membranes from liver, salivary glands, $GH_3$ pituitary cells, platelets, WRK1 mammary tumor cells; astrocytoma cells,

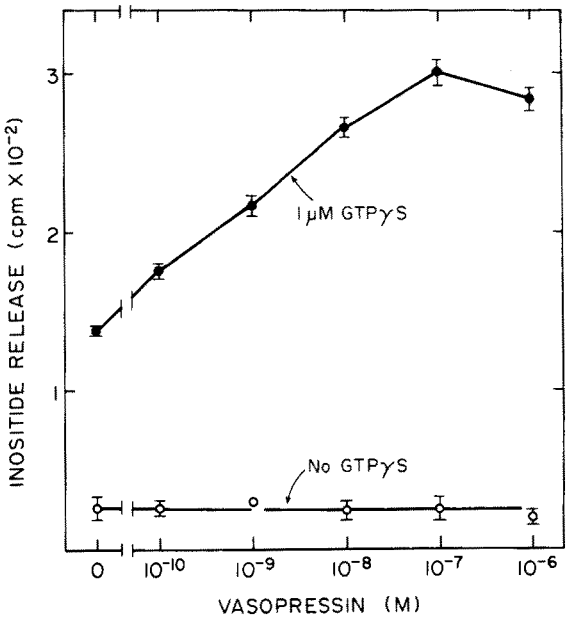


**Fig. 10.** Stimulation of $PIP_2$ breakdown in rat liver plasma membranes induced by vasopressin ($10^{-10}M-10^{-6}M$) in the presence of a low concentration (1 $\mu M$) of GTP analogue. Experimental details are given in the legend to Fig. 9. (From Uhing et al. (1986) by permission of the authors and publisher)

fibroblasts, polymorphonuclear leukocytes, and pancreatic islets have been reported (Lucas et al. 1985; C.D. Smith et al. 1985, 1987; Litosch et al. 1985; Uhing et al. 1986; Baldassare and Fisher 1985a, b; Bradford and Rubin 1986; Guillon et al. 1986; Martin et al. 1986a; Hepler and Harden 1986; Magnaldo et al. 1987; Dunlop and Larkins 1986; Orellano et al. 1987; Rebecchi and Rosen 1987a; Hrbolich et al. 1987; Aub et al. 1987). In all these cases, the effect is dependent upon or is amplified by GTP or its analogues (Fig. 10; cf. Rock and Jackowski 1987). The major inositol phosphate formed initially is $IP_3$ (Uhing et al. 1985; Guillon et al. 1986; Baldassare and Fisher 1986a, b; cf. Hrbolich et al. 1987), and its rate of formation is maximal within 1 min (Uhing et al. 1986). The concentration dependence for agonist-induced inositide formation in liver or salivary gland membranes is similar to that for $IP_3$ formation in the intact tissue or for agonist receptor binding (Uhing et al. 1986; Litosch et al. 1985; Guillon et al. 1986; Martin et al. 1986a; Straub and Gershengorn 1986). These observations differ from reports of direct effects of catecholamines, vasopressin, and thrombin on phosphoinositide breakdown in isolated plasma membranes which have been observed in the absence of guanine nucleotides (Lin and Fain 1981; Wallace et al. 1982, 1983; Harrington and Eichberg 1983; Seyfred and Wells 1984; Rock and Jackowski 1987).

The physical association of a $Ca^{2+}$-mobilizing receptor with a G-protein has been demonstrated by incubating liver plasma membranes with [$^3$H]vasopressin and then subjecting the detergent-solubilized extract to sucrose den-

sity gradient centrifugation, gel filtration, or chromatography on wheat germ agglutinin-agarose (Fitzgerald et al. 1986; Bojanic and Fain 1986). These experiments show the presence of a high-$M_r$ (>200000) complex which binds [$^3$H]vasopressin in a guanine nucleotide-sensitive manner (Fitzgerald et al. 1986; Bojanic and Fain 1986) and which exhibits GTPase and [$\alpha$-$^{32}$P]GDP binding activity (Fitzgerald et al. 1986). The presence in the complex of a 35-K $\beta$ subunit common to several other G-proteins has also been demonstrated (Fitzgerald et al. 1986).

## 5.2 Effects of Pertussis and Cholera Toxins

It is clear that there is more than one type of G-protein involved in polyphosphoinositide breakdown and $Ca^{2+}$ mobilization. In neutrophils, mast cells, mesangial cells, HL-60 leukemic cells, fibroblasts, platelets, and cardiac myocytes, the breakdown of $PIP_2$ and the associated physiological events induced by 48/80, chemotactic peptide, thrombin, angiotensin II, or $\alpha_1$-adrenergic agonists are inhibited by islet-activating protein-pertussis toxin (Okajima et al. 1985; Volpi et al. 1985; Verghese et al. 1985; Nakamura and Ui 1983, 1985; Bokoch and Gilman 1984; Okajima and Ui 1984; Bradford and Rubin 1986; Pfeilschifter and Bauer 1986; Paris and Pouyssegur 1987; Brass et al. 1986; Houslay et al. 1986; Kikuchi et al. 1986; Bruns and Marme 1987; Steinberg et al. 1987). In addition to ADP-ribosylating and inactivating $G_i$, the toxin can act on transducin, a protein involved in coupling rhodopsin to cGMP phosphodiesterase in retinal rod outer segments, and on $G_o$, a G-protein of unknown function isolated from brain and certain other tissues (Manning et al. 1984; Van Dop et al. 1984; Watkins et al. 1984; Sternweis and Robishaw 1984). Thus, the inhibitory effects of the toxin on the above-mentioned cells may be due to the involvement of one of these G-proteins or to a novel G-protein which is also a substrate for the toxin.

In liver, islet-activating protein is without effect on the stimulation of $PIP_2$ breakdown, $Ca^{2+}$ mobilization, and phosphorylase activation induced by agonists in either intact hepatocytes or isolated liver plasma membranes, under conditions in which $G_i$ is ADP-ribosylated and its functions are blocked (Fig. 11; Uhing et al. 1986; Lynch et al. 1986b). Furthermore, the ability of GTP analogues to decrease high-affinity binding of epinephrine, vasopressin, or angiotensin II to liver plasma membranes is unaffected by treatment with the toxin (Fig. 3; Lynch et al. 1986b). Likewise, the toxin does not affect muscarinic cholinergic effects on phosphoinositide hydrolysis in cardiac myocytes, pancreatic acinar cells, or Flow 9000 pituitary cells (Masters et al. 1985a; Merritt et al. 1986a; Lo and Hughes 1987a), bradykinin stimulation of $IP_3$ formation in aortic endothelial cells (Lambert et al. 1986), thrombin action on inositol release in 3T3 fibroblasts (Murayama and Ui

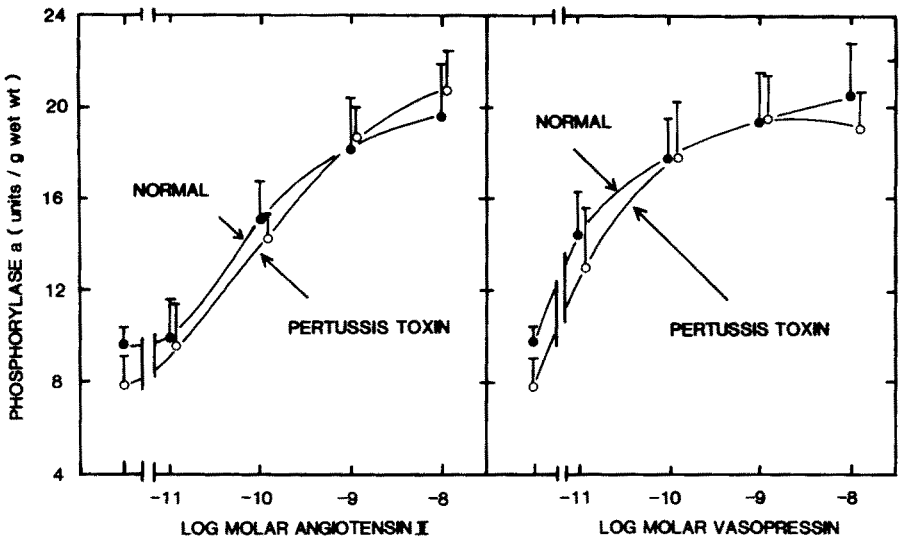


**Fig. 11.** Failure of islet-activating pertussis toxin to inhibit the activation of phosphorylase by angiotensin II and vasopressin in isolated rat hepatocytes. Rats were injected with 25 μg pertussis toxin per 100 g body weight and hepatocytes were prepared 24 h later. Hepatocytes from normal and pertussis toxin-treated rats were incubated for 5 min with the concentrations of the agonists shown, and phosphorylase *a* was then assayed. (From Lynch et al. (1986b) by permission of the authors and publisher)

1985), thyrotropin elevation of cytosolic $Ca^{2+}$ in FRTL-5 thyroid cells (Corda and Kohn 1986), angiotensin II stimulation of $IP_3$ formation in adrenal glomerulosa cells (Kojima et al. 1986), thyrotropin-releasing hormone action on $GH_3$ pituitary or 7315c cells (Martin et al. 1986b; Aub et al. 1986), $\alpha_1$-adrenergic agonist binding to membranes from kidney cortex or cloned kidney or smooth muscle cell lines (Boyer et al. 1984; Terman et al. 1987), carbachol binding to 1321N1 astrocytoma cells (Martin et al. 1985), or $\alpha_1$-adrenergic agonist stimulation of respiration in brown adipocytes (Schimmel et al. 1985) and of inositol phosphate production in FRTL-5 thyroid cells and heart (Burch et al. 1986a; Schmitz et al. 1987). It also does not inhibit agonist-stimulated $PIP_2$ hydrolysis in plasma membranes from $GH_3$ pituitary cells (Martin et al. 1986a), astrocytoma cells (Hepler and Harden 1986), and islet cells (Dunlop and Larkins 1986), or inhibit bradykinin-stimulated GTPase or phosphoinositide hydrolysis in neuroblastoma-glioma hybrid cells (Grandt et al. 1986; Hepler et al. 1987).

To make the situation even more confusing, there have been some reports that cholera toxin inhibits the $IP_3$ response to agonist stimulation in some cell lines, e.g., Jurkat malignant human T cells (Imboden et al. 1986), Flow 9000 pituitary cells (Lo and Hughes 1987b), and A10 smooth muscle cells, in which the response is also inhibited by pertussis toxin (Xuan et al. 1987).

Another interesting point is that pertussis toxin blocks the PI response to $\alpha_1$-adrenergic agonists in cardiac myocytes but not to muscarinic cholinergic agonists in these cells (Steinberg et al. 1987; Masters et al. 1985a). However, the inhibition of the $\alpha_1$-adrenergic response is incomplete (Steinberg et al. 1987) and has not been observed in vivo (Schmitz et al. 1987). Other examples of the differential effects of the toxin on agonist responses apparently mediated by the same signaling system include the blockade of the phospholipase $A_2$ response to norepinephrine, but not to thyrotropin, in FRTL-5 thyroid cells (Corda and Kohn 1986) and the different effects of the toxin on angiotensin II and platelet-activating factor responses in mesangial cells (Schlondorff et al. 1986) and on the actions of thrombin and thromboxane analogue in platelets (Brass et al. 1987). One explanation for these data is that different G-proteins mediate the same response in a given cell type and that these are differentially affected by pertussis toxin. Alternatively, the same G-protein may be involved, but the ADP ribosylation induced by the toxin may affect its interaction with different receptors to different extents. This explanation seems less likely, since it implies that different receptors interact at different sites on the protein.

As alluded to in Sect. 2.4, pertussis toxin blocks norepinephrine stimulation of arachidonic acid release, but not inositol phosphate formation in FRTL-5 thyroid cells (Burch et al. 1986a). A similar dissociation between the toxin effects on actions mediated by phospholipase $A_2$ and phospholipase C is seen in platelets (Fuse and Tai 1987). There is also evidence that separate G-proteins mediate these actions in Madin-Darby kidney cells (Slivka and Insel 1987) and in 3T3 fibroblasts (Murayama and Ui 1985). An interesting question relating to these data is whether the same receptor can be coupled to different G-proteins, or whether different receptor subtypes are involved. Similar considerations apply to the effects of muscarinic cholinergic agonists, $P_2$-purinergic agonists, thrombin, and angiotensin II on phosphoinositide metabolism and adenylate cyclase in several cell types (Lynch et al. 1986b; Houslay et al. 1986; Murayama and Ui 1985; Masters et al. 1985a; Okajima et al. 1987; Hepler et al. 1987).

These findings indicate that at least three different types of G-protein are involved in the actions of agonists on $PIP_2$ breakdown. Since none of these proteins has been unequivocally identified or purified, the molecular basis for the differences remains unknown. The site of ADP ribosylation induced by islet-activating protein in the $\alpha$-subunit of transducin is a cysteine located in a nonspecific sequence at the carboxyl terminus (Hurley et al. 1984; West et al. 1985), and a highly homologous sequence is present in $G_i$ (Nukada et al. 1986; Michel et al. 1986; Itoh et al. 1986) and in $G_o$ (Itoh et al. 1986). It therefore seems likely that the $\alpha$-subunits of the G-proteins involved in regulating $PIP_2$ phospholipase C have different sequences at their carboxyl termini.

As described in Sect. 6.4, $G_i$ and $G_o$ have been effectively reconstituted with a platelet polyphosphoinositide phospholipase C (Banno et al. 1987), but other $G_p$ candidates were not tested and the selectivity of the two G-proteins was not great. Some other potential $G_p$s have been isolated from various tissues. Molecular cloning studies have revealed a G-protein in the U937 monocyte line with an $\alpha$-subunit with marked homology (90%) to $G_{i\alpha}$, but with a different pertussis toxin ADP-ribosylation site (Didsbury and Snyderman 1987). Differentiation of U937 cells to monocyte-like cells is associated with increased transcription of mRNA for this protein as well as with increased $G_p$ activity. Neurophils also contain high levels of a G-protein with a 40-K pertussis toxin substrate (Gierschik et al. 1987; Dickey et al. 1987). This is immunologically distinct from $G_i$ and $G_o$ (Gierschik et al. 1986, 1987). A similar protein has been identified in brain (Katada et al. 1987). Human leukemic (HL-60) cells also have a G-protein that is a pertussis toxin substrate (Oinuma et al. 1987; Uhing et al. 1987). This has a 40-K $\alpha$-subunit and a 36- or 35-K $\beta$-subunit, and it can be distinguished from $G_i$ and $G_o$ immunologically and also on the basis of GTP analogue binding and partial chymotryptic proteolysis. Another GTP-binding, pertussis toxin substrate with an $\alpha$-subunit of 43 K has been found in membranes from erythrocytes, brain, $GH_4C_1$ pituitary cells, and liver (Iyengar et al. 1987).

Brain also contains a G-protein which is not ADP-ribosylated by pertussis or cholera toxins (Waldo et al. 1987). However, the GTP-binding subunit has an $M_r$ of only 25000 and appears to be similar to a GTP-binding protein in placenta and platelets (Evans et al. 1986).

## 5.3 Role of Guanine Nucleotide-Binding Regulatory Proteins in Agonist Regulation of Ion Channels

As noted in Sect. 3.4, there is much evidence that G-proteins are involved in the regulation of plasma membrane ion channels (Rosenthal and Schultz 1987). For example, there have been several recent reports indicating that G-proteins mediate the stimulatory and inhibitory effects of muscarinic cholinergic and other agonists on $K^+$ channels in atrial cells (for references see Rosenthal and Schultz 1987; Birnbaumer 1987; Logothetis et al. 1987; Yatani et al. 1987) and in *Aplysia* ganglion cells (Sasaki and Sato 1987) and $GH_3$ pituitary cells (Codina et al. 1987). There is also evidence that G-proteins mediate the inhibitory effects of norepinephrine and $\gamma$-aminobutyric acid on voltage-dependent $Ca^{2+}$ channels in dorsal root ganglion neurons (Holz et al. 1986; Scott and Dolphin 1986), and of somatostatin and opiate peptides on these channels in AT-20 pituitary cells (Lewis et al. 1986) and neuroblastoma-glioma cells (Hescheler et al. 1987a, b). For example, the effects were mimicked by application of G-proteins or GTP analogues and were

blocked by pertussis toxin or a GDP analogue. The stimulatory effects of angiotensin II on a slowly inactivating $Ca^{2+}$ current in Y1 adrenal cortical cells (Hescheler et al. 1987c; Rosenthal and Schultz 1987) likewise probably involve a G-protein, since they are inhibited by pertussis toxin and are unaffected by either cAMP or cGMP. However, it must be recognized that, in all these instances, the putative G-proteins might not couple directly to the ion channels, but may act through another protein or factor.

### 5.4 Effects of *ras* Proto-oncogene Products on Phosphoinositide Metabolism

The 21-K proteins encoded by the *ras* proto-oncogenes, which are the cellular counterparts of the transforming genes of certain murine sarcoma viruses, possess certain similarities to G-proteins, e.g., GTP-binding and GTPase activities and the ability to activate adenylate cyclase in yeast but not in mammalian cells (for references see Berridge 1986; Lacal et al. 1987). Evidence is accumulating that certain *ras* proteins exert a stimulatory control on $PIP_2$ breakdown to $IP_3$ and DAG. Examples are the increased ability of acetylcholine to stimulate $IP_3$ formation in BALB/3T3 cells transformed with Ha-*ras* (Chiarugi et al. 1985), the increased inositol phosphate response to growth factors in NIH 3T3 cells containing high levels of p21 N-*ras* protein (Wakelam et al. 1986), and the increased turnover of phosphoinositides or increased levels of DAG in various cells chronically transformed with Ki-*ras* or Ha-*ras* (Fleischman et al. 1986; Preiss et al. 1986; Wolfman and Macara 1987). More direct proof for a role of the *ras* p21 proteins in the regulation of phosphoinositide metabolism has come from recent studies involving the injection of the Ha-*ras* p21 product into *Xenopus* oocytes (Lacal et al. 1987). Injection of transforming *ras* p21 protein caused rapid increases in inositol phosphates and DAG and also changes in the inositol phospholipids, whereas the normal *ras* p21 protein was without effect. These findings indicate that an early effect of *ras* p21 protein is the activation of a phospholipase C acting on inositol phospholipids. There is evidence that the p21 oncogene product can also activate a phospholipase C selective for phosphatidylcholine and phosphatidylethanolamine (Lacal et al. 1987b).

## 6 Role of Polyphosphoinositide Phospholipase C

### 6.1 Phospholipases C Active on Phosphoinositides

Mammalian tissues contain a variety of phospholipase C activities with different substrate specificities. Several phospholipases C active on phosphoino-

sitides have been described in soluble and particulate fractions from various tissues. The first of these were assayed using PI as a substrate (for references see Shukla 1982), but it is now clear that the phospholipases C involved in the actions of $Ca^{2+}$-mobilizing agonists hydrolyze $PIP_2$ and PIP rather than PI. Phospholipases C active on the polyphosphoinositides have been found in both the soluble and plasma membrane fractions of a variety of cells and tissues. Soluble activities have been reported in platelets (Rittenhouse 1983; Low et al. 1986; Deckmyn et al. 1986; Baldassare and Fisher 1986a; Banno et al. 1986a; Manne and Kung 1987; Ebstein et al. 1987), brain (Irvine et al. 1984b; Nakanishi et al. 1985; Deckmyn et al. 1986; Kozawa et al. 1987; Rebecchi and Rosen 1987b; Ryu et al. 1987a), seminal vesicles (Wilson et al. 1984), lymphocytes (Carter and Smith 1987), coronary artery smooth muscle (Sasaguri et al. 1985), and uterus (Bennett and Crooke 1987). Some of these reports have shown that the activities can be resolved into several forms (Wilson et al. 1984; Low et al. 1986; Banno et al. 1986a, b; Nakanishi et al. 1985; Carter and Smith 1987; Ebstein et al. 1987; Ryu et al. 1987a; Rebecchi and Rosen 1987b; Bennett and Crooke 1987). Some of these are immunologically distinct, but some may have arisen through proteolysis (Low et al. 1984, 1986). The relationship of the soluble polyphosphoinositide phospholipases C to their membrane counterparts remains unclear, although it is likely that some forms are identical. There has been one report of the stimulation of a soluble enzyme from platelets by guanine nucleotides, presumably via a G-protein (Deckmyn et al. 1986), but it is not known whether other soluble forms can be regulated by this mechanism.

## 6.2 Guanine Nucleotide Regulation of Phosphoinositide Phospholipases C

As alluded to in Sect. 5, there have been many reports of the regulation of membrane-associated polyphosphoinositide phospholipase C by GTP analogues. Polyphosphoinositide phospholipase C activities have been reported in the particulate fraction or plasma membranes of iris smooth muscle (Akhtar and Abdel-Latif 1978), erythrocytes (Allan and Michell 1978; Downes and Michell 1981; Harden et al. 1987), liver (Wallace and Fain 1985; Uhing et al. 1985, 1986; Melin et al. 1986; Guillon et al. 1986a; Taylor and Exton 1987), platelets (Baldassare and Fisher 1986a, b; Rock and Jackowski 1987; Hrbolich et al. 1987), brain (Kozawa et al. 1987; Litosch 1987), parotid (Taylor et al. 1986), lymphocytes or a T-cell line (Carter and Smith 1987; Sasaki and Hasegawa-Sasaki 1987), neutrophils or polymorphonuclear leukocytes (Cockcroft et al. 1984; C. D. Smith et al. 1985; Cockcroft and Gomperts 1985; Cockcroft 1986; Volpi et al. 1985), islet cells (Dunlop and Larkins 1986; pituitary (Lucas et al. 1985; Martin et al. 1986a; Straub and Gershengorn 1986; Aub et al. 1987), fibroblasts (Magnaldo et al. 1987;

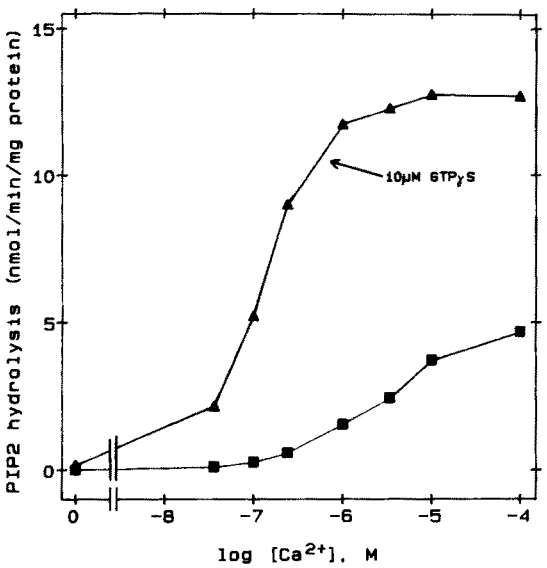


**Fig. 12.** Effect of a GTP analogue on the $Ca^{2+}$ sensitivity of the $PIP_2$ phospholipase C of rat liver plasma membranes. Membranes were assayed with 0.2 m*M* [$^3$H]$PIP_2$, presented as a mixture with phosphatidylethanolamine and phosphatidylserine in a molar ratio of 1:2:2. The concentration of free $Ca^{2+}$ was varied between 0 and $10^{-4}$ *M* using $Ca^{2+}$/EGTA buffers. GTP$\gamma$S, when present, was 10 $\mu$*M*. The product of the assay was shown to be [$^3$H]$IP_3$. (From Taylor and Exton (1987) by permission of the authors and publisher)

Rebecchi and Rosen 1987a), WRK1 cells (Guillon et al. 1986b), and astrocytoma cells (Hepler and Harden 1986; Orellano et al. 1987). Most of these activities have been shown to be stimulated by guanine nucleotides. An important exception is the mammalian erythrocyte, which, in contrast to the turkey erythrocyte, contains a polyphosphoinositide phospholipase C which is stimulated by $Ca^{2+}$ but not by GTP$\gamma$S or NaF (Harden et al. 1987).

In almost all cases, the membrane-associated phospholipase hydrolyzing $PIP_2$ and PIP is completely dependent on $Ca^{2+}$ (0.1 $\mu M - 1$ m*M*) for activity. GTP analogues activate the enzyme by increasing its sensitivity to $Ca^{2+}$ and also by enhancing its activity at high $Ca^{2+}$ (1 $\mu M$) (Fig. 12; Lucas et al. 1985; Taylor and Exton 1987; Uhing et al. 1985, 1986; Rebecchi and Rosen 1987a; Magnaldo et al. 1987; Litosch 1987; Smith et al. 1987; cf. Cockcroft 1986). In contrast, these nucleotides have little or no effect on the membrane enzyme that hydrolyzes PI, which generally requires higher $Ca^{2+}$ (Taylor and Exton 1987; Litosch 1987). Deckmyn et al. (1986) found similar results for the soluble platelet phospholipases C acting on $PIP_2$ and PI.

## 6.3 Agonist Regulation of Phosphoinositide Phospholipases C

Hormonal or agonist activation of membrane polyphosphoinositide phospholipase C has been reported for a number of tissues. These are listed under Sect. 5. In almost all cases, the effect is dependent upon or amplified by GTP or its analogues, and the primary product is $IP_3$ (Uhing et al. 1986; Guillon et al. 1986; Baldassare and Fisher 1986a; Rebecchi and Rosen 1987a;

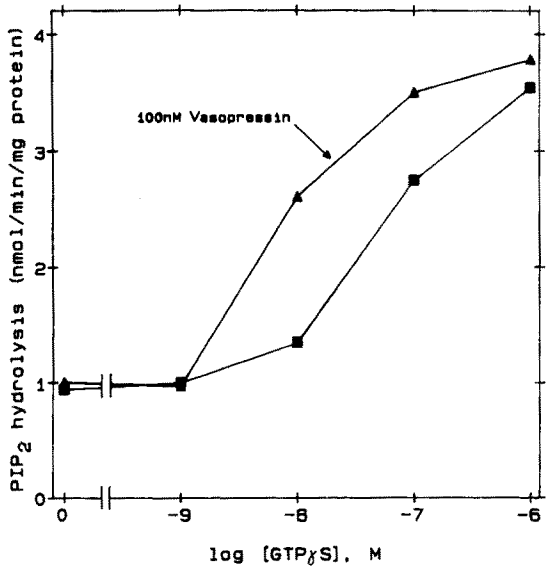


**Fig. 13.** Enhancement by vasopressin of the stimulatory effect of GTPγS on the $PIP_2$ phospholipase C of rat liver plasma membranes. Experimental details are given in the legend to Fig. 12. Vasopressin was 100 n*M*. (From Taylor and Exton (1987) by permission of the authors and publisher)

Jackowski et al. 1986; Taylor and Exton 1987; Magnaldo et al. 1987), although there is evidence that it is $IP_2$ in platelets (Hrbolich et al. 1987). The action of the agonists is to decrease the concentration of GTP or its analogue required for activation of the enzyme (Fig. 13; Litosch and Fain 1985; Litosch et al. 1985; Uhing et al. 1986; Baldassare and Fisher 1986b; Straub and Gershengorn 1986; Taylor and Exton 1987; Rebecchi and Rosen 1986a; Hepler and Harden 1986; Aub et al. 1987). This is presumably because the agonists enhance the binding of these nucleotides to the putative G-protein involved. As expected from these results, the combination of an agonist with GTP or its analogues increases the $Ca^{2+}$ sensitivity of the enzyme more than the nucleotides do alone (Martin et al. 1986a; Taylor and Exton 1987; Magnaldo et al. 1987; Rebecchi and Rosen 1987a; Aub et al. 1987). In vitro and in vivo findings indicate that, in the presence of cytosolic $Mg^{2+}$ concentrations and in the absence of agonists or guanine nucleotides, the enzyme shows little or no activity at basal cytosolic $Ca^{2+}$ concentrations (100–200 n*M*), and a maximal increase in cytosolic $Ca^{2+}$ produces little stimulation (Uhing et al. 1986; Taylor and Exton 1987; Renard et al. 1987; Litosch 1987; Smith et al. 1987). However, when activated by agonists and/or guanine nucleotides, the enzyme shows a very large increase in activity at resting $Ca^{2+}$ concentrations (Uhing et al. 1986; Taylor and Exton 1987; Renard et al. 1987; Litosch 1987; Smith et al. 1987).

## 6.4 Purification of Multiple Phosphoinositide Phospholipases C

Several reports of the partial or complete purification of phospholipases C active on polyphosphoinositides have appeared. In most cases, multiple forms have been identified or isolated (Hofmann and Majerus 1982; Wilson et al. 1984; Nakanishi et al. 1985; Banno et al. 1986a; Low et al. 1986; Ebstein et al. 1987; Carter and Smith 1987; Rebecchi and Rosen 1987b; Ryu et al. 1987a; Bennett and Crooke 1987). Some forms are active toward PI, but these generally require higher than micromolar $Ca^{2+}$ (Nakanishi et al. 1985; Banno et al. 1986a; Deckmyn et al. 1986; Kozawa et al. 1987; Manne and Kung 1987; Bennett and Crooke 1987) and are not regulated by guanine nucleotides (Deckmyn et al. 1986; Taylor and Exton 1987) or are activated equally well by GTP and ATP (Ryu et al. 1987a). The most highly purified forms have been isolated from seminal vesicles (Wilson et al. 1984), platelets (Low et al. 1986; Banno et al. 1986a; Manne and Kung 1987), brain (Nakanishi et al. 1985; Ryu et al. 1987a; Rebecchi and Rosen 1987b), lymphocytes (Carter and Smith 1987), and uterus (Bennett and Crooke 1987). As stated above, the purifications generally yield more than one activity. The purified forms are able to hydrolyze all three phosphoinositides. The enzymes from seminal vesicle and uterus have affinities in the order $PI > PIP > PIP_2$ and maximal hydrolysis rates in the order $PIP_2 > PIP > PI$ (Wilson et al. 1984; Bennett and Crooke 1987). The hydrolysis of all three phosphoinositides is stimulated by micromolar $Ca^{2+}$. There is no activity against phosphatidylcholine, phosphatidylserine, or phosphatidylethanolamine (Hofmann and Majerus 1982; Rebecchi and Rosen 1987b; Bennett and Crooke 1987). As found for other phosphoinositide phospholipases C, phosphatidylethanolamine, phosphatidylserine, and DAG are stimulatory to PI hydrolysis, whereas phosphatidylcholine is inhibitory (Hofmann and Majerus 1982). Studies with unilamellar vesicles indicate that these effects are probably due to a combination of effects, e.g., phosphatidylcholine inhibiting PI interaction with the enzyme, phosphatidylserine increasing the negative charge at the vesicle surface, and phosphatidylserine promoting lateral-phase separation of phosphatidylcholine and PI (Hofmann and Majerus 1982).

Two forms of phosphoinositide phospholipase C have been identified in seminal vesicles by Hofmann and Majerus (1982) and in uterus by Bennett and Crooke (1987). These are immunologically distinct and are unevenly distributed among various tissues, e.g., liver contains almost entirely one form and brain and platelets the other (Hofmann and Majerus 1982). One form has a subunit $M_r$ of 62000 and 65000 by SDS polyacrylamide gel electrophoresis and of 70000 by gel filtration. This is present in both cytosol and membranes of uterus (Bennett and Crooke 1987). The other form has not been purified to homogeneity but contains a protein with an $M_r$ of 85000–90000 which is comparable to an $M_r$ 88000 form found in brain by Rebecchi and Rosen

(1987b). Other workers have purified polyphosphoinositide phospholipases C from platelet cytosol (Banno et al. 1986a; Low et al. 1986; Manne and Kung 1987). There are three different forms with subunit $M_r$s ranging between 67000 and 140000. All forms are $Ca^{2+}$ dependent, and hydrolysis of PI requires higher $Ca^{2+}$ concentrations than does hydrolysis of $PIP_2$. Two forms have been purified from brain and liver cytosol (Nakanishi et al. 1985). These differ in their activities towards the different phosphoinositides depending on the $Ca^{2+}$ concentration. One form is most active against $PIP_2$ and hydrolyzes PI only at millimolar $Ca^{2+}$, whereas the other is most active against PIP. Lymphocytes also contain two forms of the enzyme, one of which is inactive against $PIP_2$ (Carter and Smith 1987).

There has been one report that a polyphosphoinositide phospholipase C partially purified from platelet membranes is stimulated by $G_o$, $G_i$, and another G-protein isolated from brain (Banno et al. 1987). There is also a report of a partially purified soluble platelet phospholipase that responds to GTP analogues (Deckmyn et al. 1986). However, these studies have not tested $G_p$, the G-protein specifically involved in signal transduction for $Ca^{2+}$-mobilizing agonists in platelets and other cells. The successful reconstitution of pure preparations of this G-protein with a purified $PIP_2$ phospholipase C remains a major goal in this research area.

## 7 Role of Myoinositol Trisphosphate and $Ca^{2+}$ Release

### 7.1 Specificity of Myoinositol 1,4,5-$P_3$ in Releasing Intracellular $Ca^{2+}$

As described in Sect. 4.2, the increase in $IP_3$ induced by agonists in a variety of cells is sufficiently rapid to account for the mobilization of internal calcium. However, the hypothesis rests largely on the demonstration that $IP_3$ causes the release of $Ca^{2+}$ from a nonmitochondrial store in permeabilized cells. This was originally shown in saponin-treated pancreatic acinar cells by Streb et al. (1983). Since that time, $IP_3$ has been shown to release internal $Ca^{2+}$ in permeabilized liver cells (Fig. 14; Burgess et al. 1984a, b; Joseph et al. 1984a), insulin-secreting cells (Joseph et al. 1984b; Biden et al. 1984; Prentki et al. 1985; B. A. Wolf et al. 1985), smooth muscle cells (Suematsu et al. 1984; Somlyo et al. 1985b), vesicles from platelets (O'Rourke et al. 1985; Authi and Crawford 1985; Brass and Joseph 1985), neutrophils (Prentki et al. 1984b), 3T3 fibroblasts (Irvine et al. 1984a), macrophages (Hirata et al. 1985), pituitary cells (Gershengorn et al. 1984; Biden et al. 1986), leukocytes (Burgess et al. 1984c), N1E-115 neuronal cells (Chueh and Gill 1986), adipocytes (Delfert et al. 1986), kidney cortex cells (Thevenod et al. 1986), and adrenal chromaffin and glomerulosa cells (Stoehr et al. 1986; Rossier et al.

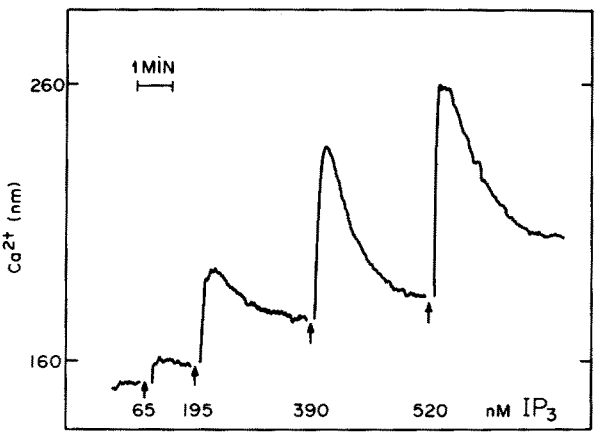


**Fig. 14.** Release of $Ca^{2+}$ from intracellular stores induced by $IP_3$ in permeabilized hepatocytes. Boluses of $IP_3$ of increasing concentrations were added to digitonin-permeabilized hepatocytes and the release of internal $Ca^{2+}$ was monitored by Quin-2 fluorescence. (Unpublished data of P. Thiyagarajah, R. Charest, P.F. Blackmore, and J.H. Exton)

1987). The action of $IP_3$ is extremely rapid and is observed with submicromolar concentrations (Fig. 14). This is the range calculated or measured to exist intracellularly (Charest et al. 1985; Thomas et al. 1984; Rittenhouse and Sasson 1985). The effect is transient, due to the rapid metabolism of $IP_3$ (Streb et al. 1985; Prentki et al. 1985). Both rapid action and rapid removal are desirable properties for a molecule involved in the regulation of intracellular $Ca^{2+}$. Myoinositol 2,4,5-$P_2$ and myoinositol 4,5-$P_2$ also release intracellular $Ca^{2+}$ but are, respectively, approximately 10 and 100 times less potent than $IP_3$. Myoinositol 1,4-$P_2$ and myoinositol 1-P are ineffective (Burgess et al. 1984b; Streb et al. 1983; Irvine et al. 1984a; B.A. Wolf et al. 1985). $cIP_3$ has a potency similar to that of $IP_3$, whereas myoinositol 1,3,4-$P_3$ is about 30 times less potent and myoinositol 1,3,4,5-$P_4$ is ineffective (Irvine et al. 1986b). Although myoinositol 1,3,4,5-$P_4$ is ineffective by itself, it does prolong the effect of $IP_3$ (Joseph et al. 1987) by blocking its breakdown (Joseph et al. 1987; Connolly et al. 1987). It is possible that myoinositol 1,3,4-$P_3$ may sometimes reach concentrations sufficient to mobilize $Ca^{2+}$ (Daniel et al. 1987).

## 7.2 Site and Mechanism of Action of Myoinositol 1,4,5-$P_3$

The intracellular pool from which $Ca^{2+}$ is released by $IP_3$ cosediments with mitochondria and microsomes during centrifugation of tissue homogenates (Dawson and Irvine 1984; Prentki et al. 1984b; Streb et al. 1984; Delfert et al. 1986). However, there is much evidence that it is not mitochondrial (Streb et al. 1983, 1984; Gershengorn et al. 1984; Joseph et al. 1984a, b; Thevenod et al. 1986; Biden et al. 1986; Rossier et al. 1987). It is probably a component of the endoplasmic reticulum, based on studies with uncouplers and other in-

hibitors of mitochondrial energy production and with ruthenium red, an inhibitor of mitochondrial $Ca^{2+}$ transport (Streb et al. 1983; Dawson and Irvine 1984; Gershengorn et al. 1984; Joseph et al. 1984a, b; Somlyo et al. 1985b). Enzyme measurements in subcellular fractions of rat exocrine pancreas and platelets indicate codistribution of NADPH cytochrome C reductase and RNA with the $IP_3$-sensitive pool (Streb et al. 1984; Authi and Crawford 1985), which is consistent with its location in the rough endoplasmic reticulum. However, several studies have shown that only a fraction of the endoplasmic reticulum responds to $IP_3$ (Prentki et al. 1984a; Joseph et al. 1984b; Dawson and Irvine 1984; Taylor and Putney 1985; Biden et al. 1986). It has been proposed that the $IP_3$-sensitive pool is contained in a novel organelle termed a "calciosome" (Volpe et al. 1988).

Addition of $IP_3$ to microsomal fractions isolated from insulinoma cells or liver rapidly releases $Ca^{2+}$ (Prentki et al. 1984a; Dawson and Irvine 1984; Muallem et al. 1985; Joseph et al. 1984b). Similar effects are obtained with membrane vesicles from platelets thought to correspond to the endoplasmic reticulum (O'Rourke et al. 1985; Authi and Crawford 1985). The action of $IP_3$ appears to be exerted on $Ca^{2+}$ efflux rather than on $Ca^{2+}$ uptake, but the mechanism remains unknown. It is relatively insensitive to temperature (J. B. Smith et al. 1985; Chueh and Gill 1986; Henne and Soling 1986; Joseph and Williamson 1986), suggesting that it involves a $Ca^{2+}$ channel rather than a carrier. It requires the countermovement of $K^+$ or another monovalent cation (Muallem et al. 1985; Joseph and Williamson 1986) and is inhibited by high concentrations of anions. These findings indicate that the release process is electrogenic. It is unlikely to involve anion exchange or cation/anion cotransport since it is not inhibited by DIDS or furosemide (Joseph and Williamson 1986). It is also unaffected by dantrolene, TMB-8, or agonists or antagonists of voltage-dependent $Ca^{2+}$ channels (Biden et al. 1984; Henne and Soling 1986; Rossier et al. 1987).

There have been recent reports of $IP_3$ binding to subcellular fractions in liver, adrenal cortex, anterior pituitary, and brain (Baukal et al. 1985; Spat et al. 1986a, b; Guillemette et al. 1987; Worley et al. 1987). Some of these binding sites are of very high affinity ($K_d$, $1-10$ n*M*) and a low capacity, and it has not been convincingly demonstrated that they mediate $Ca^{2+}$ mobilization. Others in brain membranes have a $K_d$ of 40 n*M*, are more abundant, and are very selective for $IP_3$ (Worley et al. 1987).

## 7.3 Comparison with Effects of GTP

In general, the effects of $IP_3$ on isolated organelles are small relative to those observed in permeabilized cells or require higher concentrations (see, e.g., Joseph et al. 1984b). Dawson (1985) has reported that GTP enhances the ef-

fect of $IP_3$ on $Ca^{2+}$ release from liver microsomes, but the enhancement depends upon the presence of polyethylene glycol. Other workers have observed GTP stimulation of intracellular $Ca^{2+}$ release in a variety of cell types (Chueh and Gill 1986; Henne and Soling 1986; Ueda et al. 1986; Jean and Klee 1986; Wolf et al. 1987; Chueh et al. 1987; Mullaney et al. 1987). These effects of the nucleotide are not observed with its nonhydrolyzable analogues, which suggests that they are not mediated by a typical G-protein. In general, the effects of GTP are slower than those of $IP_3$, are more temperature dependent, and are more influenced by the concentration of $Ca^{2+}$ (Chueh and Gill 1986; Henne and Soling 1986; Jean and Klee 1986). There is also evidence that GTP can act on another $Ca^{2+}$ pool in addition to that affected by $IP_3$ (Henne et al. 1987; Chueh et al. 1987). From these observations, and based on the fact that $IP_3$ does not require polyethylene glycol, it has been concluded that GTP and $IP_3$ release $Ca^{2+}$ by different mechanisms. The physiological significance, if any, of the GTP effect remains unresolved at present. Based on an analysis of the effects of GTP in the presence and absence of oxalate, Mullaney et al. (1987) have proposed that the nucleotide promotes the movement of $Ca^{2+}$ across intracellular membranes and between organelles, i.e., from an oxalate-impermeable pool to one which is permeable to oxalate and releasable by $IP_3$.

## 7.4 Other Effects of Myoinositol 1,4,5-$P_3$

In addition to mediating the effects of certain hormones and neurotransmitters, $IP_3$ has been postulated to act as a chemical messenger between transverse (T)-tubular membrane depolarization and $Ca^{2+}$ release from sarcoplasmic reticulum in skeletal muscle (Vergara et al. 1985; Volpe et al. 1985; Nosek et al. 1986; Thieleczek and Heilmeyer 1986). However, much more work is needed to establish this. There is also evidence that it is involved in light-induced excitation and adaptation in *Limulus* or *Loligo* photoreceptors (Fein et al. 1984; J.E. Brown et al. 1984, 1987; Brown and Rubin 1984; Vandenberg and Montal 1984; Szuts et al. 1986) and in fertilization in sea urchins and *Xenopus* (Whitaker and Irvine 1984; Oron et al. 1985; Busa et al. 1985; Slack et al. 1986; Nadler et al. 1986; Ciapa and Whitaker 1986). Patch-clamp studies with T-lymphocytes have also provided evidence that $IP_3$ activates a voltage-insensitive transmembrane $Ca^{2+}$ channel (Kuno and Gardner 1987).

## 8 Role of Diacylglycerol and Protein Kinase C

### 8.1 Regulation and Cloning of Protein Kinase C

With the discovery of the $Ca^{2+}$ phospholipid-dependent protein kinase now commonly known as protein kinase C (for references see Nishizuka 1984), a second mechanism of intracellular signaling for $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists was revealed. This enzyme has a requirement for $Ca^{2+}$ and a phospholipid for activity (Fig. 15). Phosphatidylserine is the most effective phospholipid, but phosphatidylinositol, phosphatdylethanolamine, and phosphatidic acid are also active, whereas phosphatidylcholine is inactive by itself and inhibitory in the presence of phosphatidylserine (Takai et al. 1979a; Kaibuchi et al. 1981). The enzyme is present in several isozymic forms in the particulate and soluble fractions of all tissues examined. It is widely distributed but is highest in brain, spleen, platelets, and lymphocytes (Kikkawa et al. 1982; Kuo et al. 1980). As will be discussed later, the distribution of the enzyme between membrane and cytosol phases is apparently under the control of $Ca^{2+}$ and diacylglycerol and of hormones which alter their concentrations.

Protein kinase C cDNA from rat, bovine, rabbit, and human brain has been cloned (Ono et al. 1986, 1988; Parker et al. 1986; Coussens et al. 1986; Knopf et al. 1986; Ohno et al. 1987). Sequencing of these clones has revealed the existence of seven isozymic forms of the enzyme. This conclusion has been reinforced by the detection of two mRNA species in rat brain using a cDNA clone partially encoding the enzyme (Makowske et al. 1986) and by the observation that three mRNAs complementary to three cDNA sequences for the enzyme

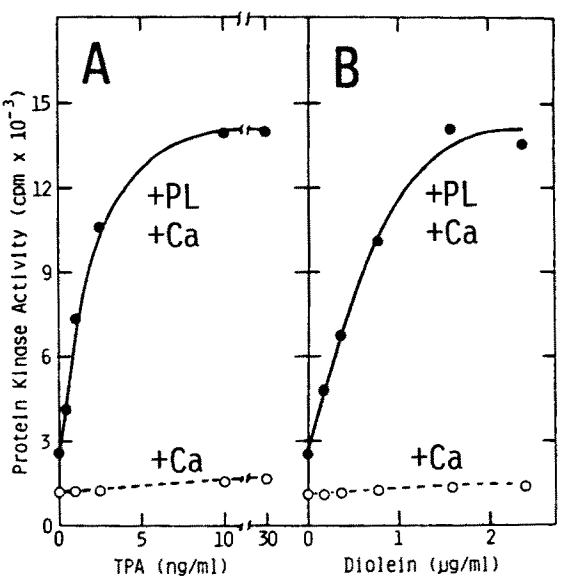


**Fig. 15.** Regulation of protein kinase C by $Ca^{2+}$, phospholipids (*PL*), diolein, and phorbol ester (*TPA*). Protein kinase C was assayed by measuring the incorporation of $^{32}P$ from [$\gamma$-$^{32}P$]ATP into H1 histone in the presence of 20 μg/ml of bovine brain phospholipids, 10 μ*M* $Ca^{2+}$, and the indicated concentrations of diolein and TPA (12-O-tetradecanoylphorbol-13-acetate, also known as PMA). (From Castagna et al. (1982) by permission of the authors and publisher)

are differentially expressed in different rat tissues (Brandt et al. 1987). Furthermore, immunological and other evidence for three forms of protein kinase (approx. 80-K) in rat brain has been presented (Huang et al. 1986; Woodgett and Hunter 1987a, b), and three types of the enzyme have been purified from rabbit brain utilizing hydroxylapatite chromatography (Jaken and Kiley 1987). Two of these can also be distinguished using polyclonal antibodies, and the three forms show different degrees of stimulation by $Ca^{2+}$. It remains to be determined whether or not the various forms of protein kinase C have different roles in signal transduction.

## 8.2 Control by Diacylglycerols and Sphingosine

Protein kinase C is activated by sn-1,2-diacylglycerols (DAGs) (Fig. 15), the forms containing at least one unsaturated fatty acid being more effective than the saturated forms unless the latter contain symmetrically two $C_6-C_{10}$ saturated fatty acids (Takai et al. 1979a, b; Kishimoto et al. 1980; Mori et al. 1982; Lapetina et al. 1985). The sn-1,3- and sn-2,3-DAG isomers are inactive (Boni and Rando 1985). The naturally occurring DAGs can be replaced by synthetic DAGs or by tumor-promoting phorbol esters (Fig. 15), which have a structure similar to that of DAG (Castagna et al. 1982; Davis et al. 1985; Ebeling et al. 1985; Niedel et al. 1983). The phorbol esters appear to bind to the same "receptor" on protein kinase C as the DAGs (Kikkawa et al. 1983; Ebeling et al. 1985; Sharkey et al. 1984). DAGs and phorbol esters increase the activity of protein kinase C at maximal $Ca^{2+}$ concentrations but, more importantly, decrease the concentration of $Ca^{2+}$ for half-maximal activity down to the submicromolar range found in the cytosol (Takai et al. 1979b; Kishimoto et al. 1980; Kuo et al. 1980). In the absence of phospholipid, DAGs and phorbol esters have little effect (Fig. 15). Although it is often assumed that protein kinase C is the sole cellular target of DAGs and phorbol esters, other possible mechanisms of action should be kept in mind (see, e.g., Gonzatti-Haces and Traugh 1986) and it should be noted that DAGs and phorbol esters do not always produce the same results (Kolesnick and Paley 1987; Ways et al. 1987). In addition, there is evidence that the priming of the neutrophil respiratory burst by 1-oleoyl-2-acetylglycerol (OAG) does not involve protein kinase C (Bass et al. 1987). This is based on the failure of a protein kinase C inhibitor to alter this effect of OAG, and also on the inability of OAG to induce protein kinase C translocation at concentrations effective in priming.

Using a mixed micellar assay, it has been shown that a single molecule of 1,2-dioleoylglycerol and of $Ca^{2+}$ and four molecules of phosphatidylserine are required to activate monomeric protein kinase C (Hannun et al. 1986a; Ganong et al. 1986; Hannun and Bell 1986). The four phospholipid molecules

are believed to bind $Ca^{2+}$ through the four carboxyl groups in the serine headgroups, and protein kinase C binds to this surface structure but is inactive (Hannun et al. 1985; Ganong et al. 1986). The complex then binds active phorbol esters or DAGs, resulting in activation of the kinase (Hannun et al. 1985; Ganong et al. 1986). The DAG or phorbol ester is thought to have at least three attachment points to the complex including the kinase and $Ca^{2+}$ (Ganong et al. 1986; Hannun and Bell 1986). This model also explains the translocation of the enzyme to membranes induced by phorbol esters and $Ca^{2+}$. The lipid-binding, regulatory domain of the enzyme has been shown to be contained entirely in a 32-K tryptic fragment (Lee and Bell 1986). The catalytic domain is in a 50-K fragment (Inoue et al. 1977) that is located at the carboxyl terminal on the basis of sequence homology with other protein kinases (Parker et al. 1986).

Sphingosine, a component of ceramide from which sphingomyelin and sphingoglycolipids are synthesized, is a potent inhibitor of protein kinase C in vitro (Hannun et al. 1986b). It also blocks thrombin-induced secretion, second-phase aggregation, and phosphorylation of a 40-K protein in platelets (Hannun et al. 1986b; 1987). Sphingosine and sphinganine also block the effects of phorbol esters on the adherence and growth of human promyelocytic leukemia (HL-60) cells (Merrill et al. 1986) and inhibit the effects of chemotactic peptide, DAG, and phorbol ester on the oxidative burst of neutrophils (Wilson et al. 1986). Sphingosine apparently acts to prevent the formation of an active protein kinase-lipid complex by displacing the activator (DAG or phorbol ester) from the complex (Hannun et al. 1986b). The possibility that sphingolipids play a role in the regulation of the enzyme in vivo is under active investigation.

Since the phosphoinositides contain predominantly stearic acid at the sn-1 position of glycerol and arachidonic acid at the sn-2 position (Holub and Kuksis 1978), their hydrolysis by phospholipase C yields stearoyl arachidonoylglycerol, which would activate protein kinase C. Thus, $PIP_2$ breakdown induced by $Ca^{2+}$-mobilizing agonists is associated with protein kinase C activation (Nishizuka 1984). However, all the major phospholipids contain some unsaturated fatty acids, predominantly in the sn-2 position of glycerol (Holub and Kuksis 1978), and their breakdown by phospholipase C could therefore yield DAGs capable of activating protein kinase C.

## 8.3 Agonist Effects on Diacylglycerol Accumulation

$\alpha_1$-Adrenergic and other $Ca^{2+}$-mobilizing agonists have been shown to increase DAG in liver, platelets, exocrine pancreas, vascular smooth muscle cells, and HL-60 promyelocytic cells (Fig. 16; Rittenhouse-Simmons 1979; Bocckino et al. 1985; Banschbach et al. 1981; B. P. Hughes et al. 1984; Kawa-

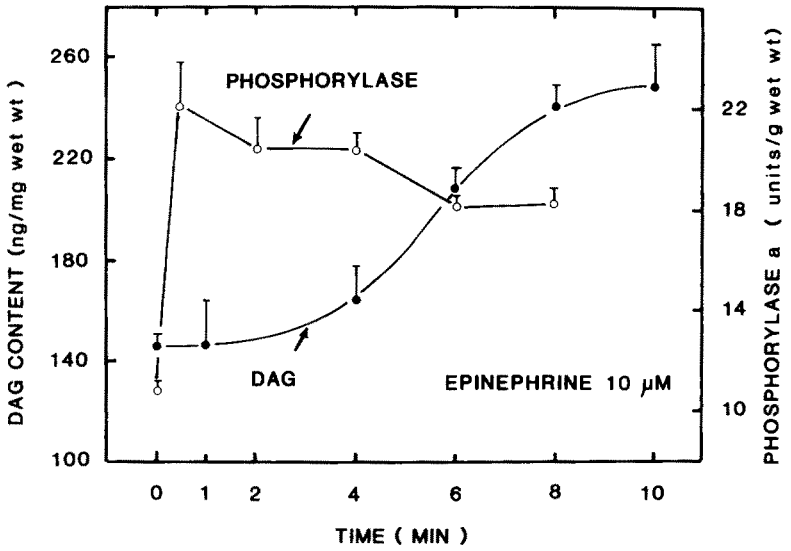


**Fig. 16.** Time course of the effects of epinephrine on 1,2-diacylglycerol accumulation and phosphorylase activation in isolated rat hepatocytes. Hepatocytes were incubated with 10 μ*M* epinephrine and samples taken at the times shown for measurement of phosphorylase *a* and 1,2-diacylglycerol. (From Bocckino et al. (1985) by permission of the authors and publisher)

hara et al. 1980; Rink et al. 1983; Thomas et al. 1983; Haslam and Davidson 1984a; Preiss et al. 1986, 1987; Griendling et al. 1986; Pandol and Schoeffield 1986). In platelets labeled with [$^3$H]arachidonic acid, the increase in [$^3$H]DAG in response to activating factors is very rapid and transient (Kawahara et al. 1980; Rink et al. 1983; Rittenhouse-Simmons 1979), but chemical measurements of DAG in hepatocytes and other cells show a slower and more stable increase (Fig. 16; Bocckino et al. 1985; Griendling et al. 1986; Preiss et al. 1986, 1987). The time course of DAG generation in hepatocytes, vascular smooth muscle cells, HL-60 cells, and pancreatic acini differs markedly from that for $IP_3$ and associated physiological responses (Fig. 16; Bocckino et al. 1985; Griendling et al. 1986; Preiss et al. 1986; Pandol and Schoeffield 1986), consistent with the idea that DAG is formed from other sources besides $PIP_2$.

## 8.4 Sources of Diacylglycerol

High-pressure liquid chromatographic analysis of the DAG generated by stimulation of hepatocytes by $Ca^{2+}$-mobilizing agonists indicates that there are at least two fractions (Fig. 17; Bocckino et al. 1985). One is enriched in stearic and arachidonic acids, suggesting that it is derived from inositol phospholipids, while the other is composed predominantly of palmitic,

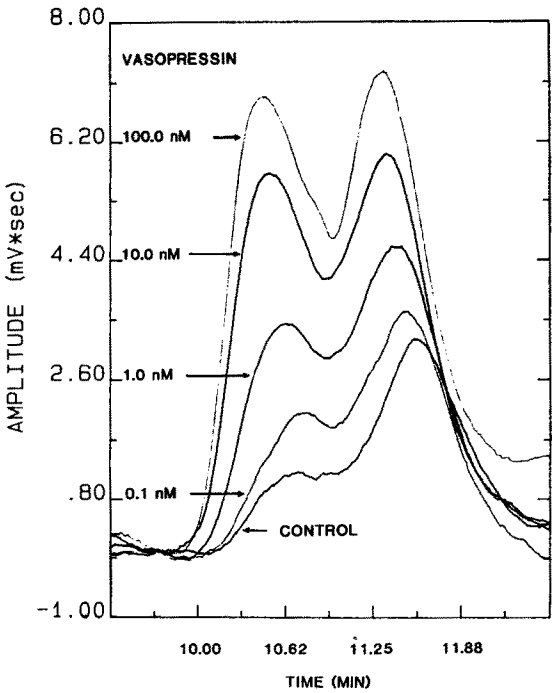


**Fig. 17.** High-pressure liquid chromatographic (HPLC) analysis of the 1,2-diacylglycerol species generated by incubation of isolated rat hepatocytes with increasing concentrations of vasopressin. Hepatocytes were incubated for 8 min with 0.1 – 100 n*M* vasopressin and neutral lipid extracts were then prepared for analysis by HPLC. (From Boccking et al. (1985) by permission of the authors and publisher)

stearic, oleic, linoleic, and arachidonic acids, suggesting another origin. A similar conclusion was reached earlier by Banschbach et al. (1981), who measured the fatty acid composition of the DAG accumulated in pancreas in response to cholinergic stimulation. Isotopic studies using various labeled fatty acids also indicate other precursors for DAG in vasopressin-stimulated hepatocytes (Pickford et al. 1987). A further indication that DAG comes from another source besides inositol phospholipids in this system is the observation that the accumulation of DAG is at least one order of magnitude greater than that of the myoinositol phosphates and myoinositol (Boccking et al. 1985; Preiss et al. 1986; Charest et al. 1983; Prpic et al. 1982).

A likely source of the additional DAG in stimulated cells is phosphatidylcholine (Irving and Exton 1987; Ragab-Thomas et al. 1987; Besterman et al. 1986a). This has a high content of palmitic, stearic, oleic, linoleic, and arachidonic acids (Holub and Kuksis 1978) and thus resembles the second DAG fraction generated by agonists in liver (Boccking et al. 1985). Other evidence for DAG formation by phospholipase C cleavage of phosphatidylcholine comes from measurements of the $^{14}C/^{3}H$ ratio in the lipids of endothelial cells prelabeled with [$^{3}$H]palmitic acid and [$^{14}$C]arachidonic acid and then exposed to thrombin (Ragab-Thomas et al. 1987). More direct proof comes from experiments in which incubation of liver plasma membranes with GTP analogues and $P_2$-purinergic agonists causes breakdown of phospha-

tidylcholine with the appearance of DAG, P-choline, and choline (Irving and Exton 1987). These studies indicate that some receptors can couple to a phosphatidylcholine phospholipase C through a G-protein.

Whole-cell studies indicate that activation of phosphatidylcholine phospholipase C can also occur through a mechanism involving protein kinase C. For example, treatment of liver, 3T3-L1, HL-60, Swiss 3T3, uterine decidua, REF52 and Madin-Darby kidney cells with phorbol esters causes a large increase in DAG (Bocckino et al. 1985; Daniel et al. 1986; Besterman et al. 1986a; Takuwa et al. 1987; Schrey et al. 1987; Cabot et al. 1988). This occurs without a detectable change in inositol phosphates (Lynch et al. 1985c; Takuwa et al. 1987) and is accompanied by generation of choline or P-choline (Daniel et al. 1986; Besterman et al. 1986a; Schrey et al. 1987; Cabot et al. 1988). The involvement of protein kinase C is suggested by the fact that down-regulation of the enzyme by phorbol esters greatly inhibits the response (Besterman et al. 1986a). In vascular smooth muscle, angiotensin II induces a transient increase in DAG, followed within 5 s by a sustained increase (Griendling et al. 1986). Changes in phospholipids indicate that the first phase involves breakdown of $PIP_2$ and PIP and release of $IP_3$ and $IP_2$, whereas the second is associated with a decrease in PI and a sustained increase in $IP_1$. Phorbol esters diminish the first phase changes, but do not significantly alter the second phase.

## 8.5 Sources of Phosphatidate

DAG produced in the plasma membrane is believed to be further metabolized to phosphatidic acid due to the action of diacylglyglycerol kinase, since phosphatidic acid rises rapidly following phosphoinositide breakdown. Translocation of diacylglycerol kinase from the cytosol to the membrane has been reported to be induced by DAG, but not by $Ca^{2+}$, in brain and liver homogenates (Besterman et al. 1986b). A similar translocation is induced by TPA, DAG, and chemotactic peptide in neutrophils (Ishitoya et al. 1987). Other possible routes of DAG metabolism are hydrolysis by diacylglycerol and monoglycerol lipases. Diacylglycerol lipase is present in the plasma membrane of some cells (Mauco et al. 1984; Authi et al. 1985), but it is unclear to what extent membrane-associated DAG is metabolized by this enzyme. Phosphatidic acid can be reconverted to DAG by phosphatidate phosphohydrolase, but it is not known whether this enzyme is present in the plasma membrane. The major fate of phosphatidic acid generated in the plasma membrane is considered to be its transfer to the endoplasmic reticulum for phospholipid and triacylglycerol synthesis. This transfer involves a phospholipid exchange protein.

Phosphatidic acid rises more rapidly than DAG in hepatocytes stimulated by $Ca^{2+}$-mobilizing agonists (Fig. 18; Bocckino et al. 1987; Pickford et al.

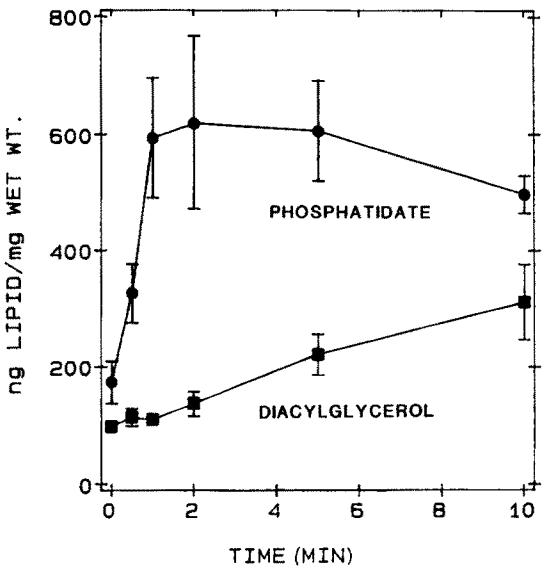


**Fig. 18.** Changes in phosphatidate and 1,2-diacylglycerol induced by vasopressin in isolated rat hepatocytes. Hepatocytes were incubated with $10^{-8}$ *M* vasopressin and the neutral lipid extracts from samples taken at the times shown were assayed for phosphatidate and 1,2-diacylglycerol by thin-layer chromatography. (From Bocckino et al. (1987) by permission of the authors and publisher)

1987), and a two- to threefold increase is observed at early stages, when no increase in DAG can be detected. Changes in the fatty acid composition of phosphatidate also precede those in DAG (Bocckino et al. 1987). These observations are not consistent with the view that most of the phosphatidate accumulating in response to agonists is formed from DAG in this tissue.

Incubation of washed liver plasma membranes with GTP analogues in the presence and absence of agonists causes an increase in phosphatidate in the absence of ATP (Bocckino et al. 1987). This provides evidence for the formation of phosphatidate by mechanisms not involving diacylglycerol kinase. A probable major source of the phosphatidate is phosphatidylcholine, since this is the only phospholipid that decreases significantly during incubation of the membranes with GTP analogues. Furthermore, there is an associated release of choline and P-choline, reported by Bocckino et al. (1987), in agreement with Irving and Exton (1987). The fatty acid composition of the phosphatidate that is produced during incubation of hepatocytes with vasopressin also resembles that of phosphatidylcholine (Bocckino et al. 1987). These results suggest that a major mechanism by which phosphatidate is produced during $Ca^{2+}$-mobilizing agonist action in liver is by the G-protein-mediated activation of a phospholipase D, the major substrate of which is phosphatidylcholine. There is also evidence for a phorbol ester-stimulated breakdown of phosphatidylcholine to choline in NG108-15 cells, which may also be due to activation of a phospholipase D (Liscovitch et al. 1987). It remains to be seen whether similar mechanisms operate in other cell types and whether the large amount of phosphatidate produced has biological functions.

## 8.6 Activation and Translocation of Protein Kinase C

There have been no direct demonstrations that $Ca^{2+}$-mobilizing agonists activate protein kinase C in cells. However, there have been several reports showing that these agonists increase the phosphorylation of several substrates in platelets, liver cells, and mast cells which are also selectively affected by active phorbol esters or synthetic DAGs (Kaibuchi et al. 1983; Katakami et al. 1984; Haslam and Davidson 1984a; Garrison et al. 1984). Some of these substrates have been shown to be phosphorylated by protein kinase C in vitro (Kawahara et al. 1980; Sano et al. 1983; Cooper et al. 1984).

There have been reports showing that phorbol esters or $Ca^{2+}$-mobilizing agonists induce the translocation of protein kinase C from the soluble phase to the plasma membrane in many cells (e.g., Kraft and Anderson 1983; Kraft et al. 1982; Drust and Martin 1985; Wooten and Wrenn 1984). The data indicate that protein kinase C present in the soluble phase is inactive due to the absence of lipid. However, it is postulated that when agonists induce a rise in DAG in the plasma membrane and in cytosolic $Ca^{2+}$, the enzyme becomes associated with the membrane, where it becomes activated by the accumulated DAG (M. Wolf et al. 1985; May et al. 1985). Phorbol ester-induced binding of protein kinase C to isolated membranes differs from that induced by $Ca^{2+}$ in that it is stable, temperature dependent, saturable, and relatively selective for plasma membranes and requires the presence of membrane protein(s) and phospholipid micelles (Gopalakrishna et al. 1986). Translocation has been demonstrated in intact $GH_3$ pituitary and Swiss 3T3 cells treated with phorbol ester, using either [$^{35}$S]methionine-labeled protein kinase C and antisera to the enzyme (Ballester and Rosen 1985) or digitonin-induced release of cytoplasmic proteins (Pelech et al. 1986). The observations on translocation suggest that soluble protein substrates for protein kinase C can be phosphorylated only at the plasma membrane or at other membranes where there is a rise in DAG.

## 8.7 Substrates of Protein Kinase C

Protein kinase C has been shown to phosphorylate a large number of proteins in vitro, but it is unclear to what extent these serve as substrates in intact cells. Addition of active phorbol esters to liver cells increases the phosphorylation of several soluble proteins of unknown function (Garrison et al. 1984; Cooper et al. 1984). It also causes inactivation of glycogen synthase in these cells (Fig. 19; Roach and Goldman 1983; Blackmore et al. 1986; Bouscarel et al. 1988). The inactivation of this enzyme caused by $Ca^{2+}$-mobilizing agonists is better correlated with changes in DAG than in cytosolic $Ca^{2+}$ (Bouscarel and Exton 1986) and is also seen in the absence of changes in cell $Ca^{2+}$ (Blackmore et

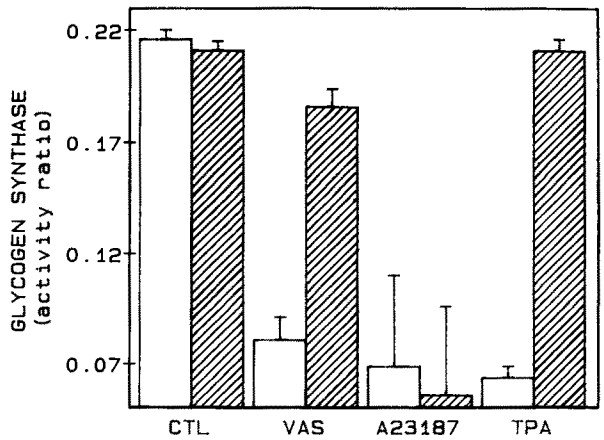


**Fig. 19.** Effects of down-regulation of protein kinase C on the inactivation of glycogen synthase by vasopressin, A23187 ionophore, and phorbol ester (*TPA*) in cultured rat hepatocytes. Rat hepatocytes in primary culture were incubated with 1% dimethylsulfoxide (untreated, *open bars*) or with TPA (12-0-tetradecanoylphorbol-13-acetate) (treated, *shaded bars*) for 18 h to reduce protein kinase C activity to approximately 10% of untreated. The treated or untreated hepatocytes were then incubated for 15 min with 50 m*M* glucose to activate glycogen synthase. They were then incubated for 15 min with 100 n*M* vasopressin, 1 μ*M* A23187, or 1 μ*M* TPA and the glycogen synthase activity ratio (-Glc6-P/+10 m*M* Glc6-P) was measured. (From Bouscarel et al. (1988) by permission of the authors and publisher)

al. 1986). Furthermore, in cultured liver cells in which protein kinase C has been down-regulated by prolonged treatment with phorbol esters, the ability of $Ca^{2+}$-mobilizing agonists to inactivate glycogen synthase is significantly inhibited (Fig. 19; Bouscarel et al. 1988). However, it seems that the inactivation is due to a mechanism(s) other than a direct effect of protein kinase C on the enzyme (Imazu et al. 1984; Nakabayashi et al. 1987). Phorbol esters and synthetic DAGs induce the phosphorylation of a 40- to 47-K protein in platelets (Kawahara et al. 1980; Sano et al. 1983; Kaibuchi et al. 1983). This protein appears to be the same as that phosphorylated in response to platelet-activating factors.

Protein kinase C phosphorylates a large number of neuronal and muscle proteins in vitro. These include tyrosine hydroxylase, GABA-modulin, myelin basic protein, MAP-2, an 87-K protein that is widely distributed in brain, a 48-K brain membrane protein, phospholamban, troponin T, and smooth muscle myosin light chains (Nairn et al. 1985b). Some of these phosphorylations are associated with functional changes, e.g., activation of tyrosine hydroxylase, and some can be observed after depolarization in intact tissue, e.g., 87-K protein (Nairn et al. 1985b). As described below, there is also indirect evidence for the control of ion channels by protein kinase C. Protein kinase C can also inactivate myosin light chain kinase in vitro (Nishikawa et al. 1985;

Ikebe et al. 1985), but it is not known whether this is a regulatory mechanism for smooth muscle contraction in vivo.

## 8.8 Actions of Protein Kinase C on Receptors and Certain Other Cell Responses

There is evidence that phorbol esters induce phosphorylation and/or alter the function of several plasma membrane receptors, including $\alpha_1$-adrenergic receptors (Corvera and Garcia-Sainz 1984; Labarca et al. 1984; Danthuluri and Deth 1984; Lynch et al. 1985c; Cooper et al. 1985; Baraban et al. 1985a; Van de Werve et al. 1985; Leeb-Lundberg et al. 1985; Corvera et al. 1986), epidermal growth factor receptors (Lee and Weinstein 1978, 1979; Shoyab et al. 1979; Moon et al. 1984; Davis and Dzech 1984; Davis et al. 1985; Cochet et al. 1984; Beguinot et al. 1985), insulin receptors (Jacobs et al. 1983; Thomopoulos et al. 1982; Grunberger and Gorden 1982), somatostatin receptors (Matozaki et al. 1986), and transferrin receptors (May et al. 1984). Inhibition by phorbol esters of the actions of other agonists has been reported, e.g., the chemotactic peptide fMet-Leu-Phe in neutrophils (White et al. 1984; Naccache et al. 1985), thyrotropin-releasing hormone in pituitary cells (Albert and Tashjian 1985), muscarinic cholinergic agonists in hippocampus, astrocytoma, and pheochromocytoma cells (Labarca et al. 1984; Orellana et al. 1985; Vincentini et al. 1985b), and several activating factors in platelets (MacIntyre et al. 1985). Although phosphorylation of membrane receptors probably underlies the inhibitory effects of phorbol esters in most cases, there is also evidence that they may affect G-proteins (Blackmore and Exton 1986; Jakobs et al. 1985; Katada et al. 1985).

Prolonged exposure of hepatocytes to phorbol esters, vasopressin, and angiotensin II induces refractoriness to $\alpha_1$-adrenergic agonists (Garcia-Sainz et al. 1986). Evidence that this effect involves protein kinase C is suggested by the fact that it is blocked by inhibitors of the enzyme, namely W-7 and H-7 (Garcia-Sainz and Hernandez-Sotomayor 1987). Further support comes from the observations that the orders of potency and efficacy of phorbol esters for inhibiting $\alpha_1$-adrenergic actions parallel those for activating protein kinase C (Corvera and Garcia-Sainz 1984; Corvera et al. 1986).

In addition to their inhibitory actions on some agonist responses, evidence is accumulating that phorbol esters can increase the responses to other agonists. For example, they increase $\beta$-adrenergic or adenosine responses in brain, S49 lymphoma cells, and pinealocytes (Hollingsworth et al. 1985; Bell et al. 1985; Sugden et al. 1985). The effects of the esters may be exerted at the level of G-proteins (Bell et al. 1985). This may also be true in part for the receptor systems which are inhibited by DAG and its analogues (Blackmore and Exton 1986).

Phorbol esters and synthetic DAGs have been shown to have effects on cells which are not directly related to the modification of receptor functions. For example, they can alter ion channels and pumps in various cell types, as discussed below. They can also induce serotonin secretion in platelets (Yamanishi et al. 1983; Rink et al. 1983), stimulate amylase secretion in pancreatic acini (Wooten and Wrenn 1984), induce superoxide generation or $O_2$ consumption in neutrophils (Dale and Penfield 1984; De Virgilio et al. 1984; Sha'afi et al. 1983), stimulate protein secretion in parotid gland (Putney et al. 1984), stimulate insulin release from islets (Hutton et al. 1984; Malaisse et al. 1985; Zawalich et al. 1983), stimulate prolactin release by pituitary cells (Osborne and Tashjian 1981; Delbeke et al. 1984), induce contraction in certain smooth muscles (Baraban et al. 1985a; Rasmussen et al. 1984), stimulate the $Na^+/H^+$ antiporter in several cells (Besterman and Cuatrecasas 1984; Volpi et al. 1985), cause histamine release from mast cells (Katakami et al. 1984), and increase the phosphorylation and activity of tyrosine hydroxylase and catecholamine secretion in adrenal chromaffin cells (Pocotte et al. 1985; Pocotte and Holz 1986). These observations and others support the view that the effects of $Ca^{2+}$-mobilizing agonists on these various cellular processes are mediated partly or wholly through activation of protein kinase C.

## 8.9 Actions of Protein Kinase C on Ion Channels and Pumps

Several recent reports suggest that activation of protein kinase C can regulate $Ca^{2+}$ and other ion channels in several cell types (Kaczmarek 1987). In the bag cell neurons of the abdominal ganglion of *Aplysia*, the addition of active phorbol esters or synthetic DAGs or the microinjection of protein kinase C causes a striking enhancement of $Ca^{2+}$ action potentials evoked by depolarization (De Riemer et al. 1985). This occurs through recruitment of covert $Ca^{2+}$ channels (Strong et al. 1987). Phorbol esters and/or DAGs also evoke an increase in $Ca^{2+}$ influx in aorta (Gleason and Flaim 1986), $A_7r_5$ vascular smooth muscle cells (Sperti and Colucci 1987), neutrophils (Nasmith and Grinstein 1987), pituitary cells (Albert et al. 1987), and UMR-106 osteosarcoma cells (Yamaguchi et al. 1987) and in a voltage-dependent $Ca^{2+}$ current in *Hermissenda* photoreceptors (Farley and Auerbach 1986). In the latter, there are also decreases in a transient voltage-dependent $K^+$ current and a $Ca^{2+}$-activated $K^+$ current (Farley and Auerbach 1986; Alkon et al. 1986). However, in some systems, phorbol esters and DAGs decrease voltage-dependent $Ca^{2+}$ influx, e.g., aortic smooth muscle (Galizzi et al. 1987) and PC-12 pheochromocytoma cells (Harris et al. 1986). Furthermore, in hippocampal pyramidal neurons, phorbol esters have little or no effect on $Ca^{2+}$ action potentials or the voltage-dependent $K^+$ current, although they abolish the $Ca^{2+}$-associated $K^+$ current and associated late hyperpolarization

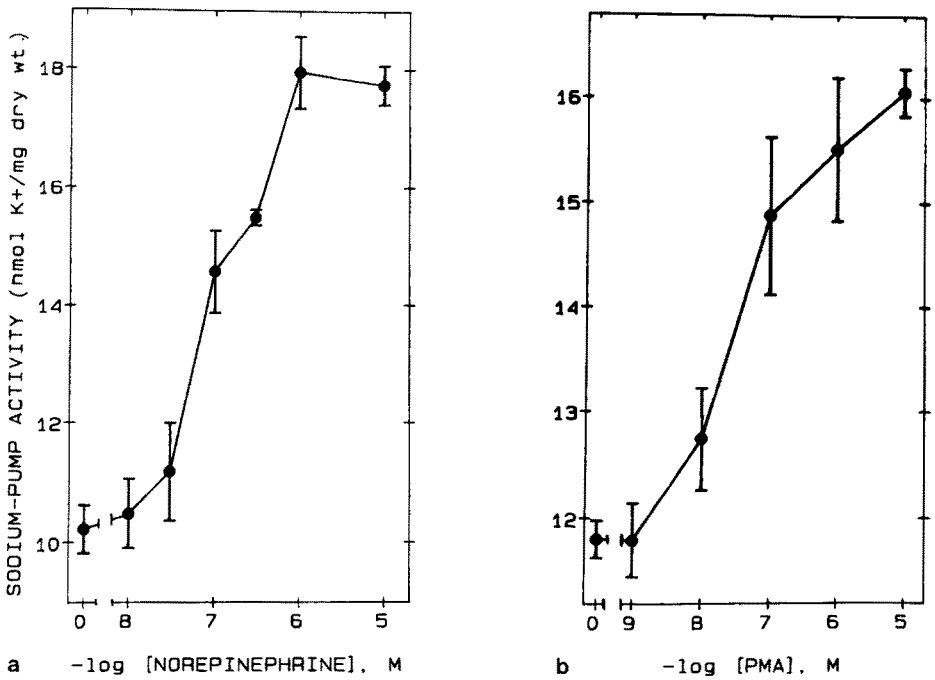


**Fig. 20a,b.** Activation of the hepatic $Na^+$ pump by norepinephrine (**a**) and phorbol ester (*PMA*) (**b**). Ouabain-sensitive $^{86}Rb^+$ uptake during 5 min was used as a measure of $Na^+/K^+$ ATPase-pump activity in isolated rat hepatocytes. The uptake was measured in the presence of the shown concentrations of norepinephrine and PMA (4$\beta$-phorbol 12$\beta$-myristate 13$\alpha$-acetate, also known as TPA). (From Lynch et al. (1986c) by permission of the authors and publisher)

(Malenka et al. 1986; Baraban et al. 1985b). The different effects of protein kinase C activators on the ion channels of these various cells presumably relate to functional and regulatory differences.

In addition to effects on ion channels, activation of protein kinase C may exert actions on ion pumps. $\alpha_1$-Adrenergic agonists and other $Ca^{2+}$-mobilizing agonists activate the $Na^+/K^+$-ATPase-mediated transport of $K^+$ in hepatocytes (Fig. 20; for references see Lynch et al. 1986c). This effect is mimicked by the addition of phorbol esters and other activators of protein kinase C (Fig. 20) and cannot be attributed to the increase in cytosolic $Ca^{2+}$ (Lynch et al. 1986c). The effect is transient due to rapid heterologous desensitization of the pump, also apparently mediated by protein kinase C (Lynch et al. 1987). The possibility that phorbol esters and thrombin stimulate $Ca^{2+}$ efflux from platelets, perhaps via protein kinase C stimulation of a plasma membrane $Ca^{2+}$ pump, has also been raised (Pollock et al. 1987). This is based on changes in cytosolic $Ca^{2+}$ induced by phorbol ester and thrombin in the presence of ionomycin (which blocks the reuptake of $Ca^{2+}$ by internal organelles).

## 8.10 Effects of Protein Kinase C on Proto-oncogene Expression

The c-*myc* and c-*fos* genes are the cellular counterparts of the transforming genes of the avian myelocytomatosis and the FBJ murine osteosarcoma viruses. The proteins they encode are located in the nucleus and are believed to be important in the regulation of the cell cycle, although this is controversial. In several cell types (e.g., several 3T3 fibroblast cell lines, A431 epidermal carcinoma cells, lymphocytes, and 1321-N1 astrocytoma cells) certain growth factors (e.g., platelet-derived growth factor, fibroblast growth factor, epidermal growth factor) can activate the induction of c-*myc* and c-*fos* mRNA (for references see Berridge 1986; Moore et al. 1986; Blackshear et al. 1987). This response can also be elicited by phorbol esters or DAGs, either alone or in combination with A23187 $Ca^{2+}$ ionophore (Kelly et al. 1983; Greenberg and Ziff 1984; Kruijer et al. 1984; Coughlin et al. 1985; Moore et al. 1986; Kaibuchi et al. 1986; Stumpo and Blackshear 1986; Blackshear et al. 1987). Since the growth factors can elicit inositol phospholipid turnover and the activation of protein kinase C in some of the cell lines in which they induce c-*myc* and c-*fos* (for references see Berridge 1986; Blackshear et al. 1987), it seems likely that their effects on the expression of these proto-oncogenes are mediated in part through the kinase. However, the induction is still seen in cells in which protein kinase C has been down-regulated (Kaibuchi et al. 1986; Coughlin et al. 1985; Stumpo and Blackshear 1986; Blackshear et al. 1987) or in which the growth factors fail to increase $IP_3$ or to activate the kinase (Magnaldo et al. 1986; Blackshear et al. 1987). This indicates that protein kinase C-independent pathways must also be involved. This conclusion is supported by the fact that addition of phorbol esters or down-regulation of protein kinase C affects the transcription of only some (c-*myc* and c-*fos*, but not JE and KC) of the genes stimulated by platelet-derived growth factor in BALB/c/3T3 cells (Hall and Stiles 1987).

## 8.11 Interactions Between the $Ca^{2+}$- and DAG-Signaling Systems

In many cases, the effects of the DAG analogues on cellular processes are synergistic with those of $Ca^{2+}$ ionophores, and the addition of both types of agent is necessary to completely mimic the effects of natural agonists (Nishizuka 1984). However, some agonist effects are mediated by an increase in $Ca^{2+}$ or DAG alone (Blackmore et al. 1986; Lynch et al. 1986c; Cooper et al. 1985; cf. Fain et al. 1984; Kimura et al. 1984). Although synergistic interactions of $Ca^{2+}$ and DAG are frequently observed, the molecular mechanisms involved have not been defined. They could be due to the effects of these agents on protein kinase C per se, but this explanation seems inadequate in some cases. Alternative explanations are that some responses require the

phosphorylation of a single protein by both DAG- and $Ca^{2+}$-sensitive protein kinases, that some processes require the separate phosphorylation of two or more proteins by these kinases, and that some effects involve a phosphorylation cascade in which protein kinase C phosphorylates a $Ca^{2+}$-dependent protein kinase or vice versa.

## 9 Role of $Ca^{2+}$-Calmodulin-Regulated Enzymes and Other Proteins

### 9.1 Properties of Calmodulin

An important aspect of the mechanism of action of $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists is the definition of the intracellular targets of the mobilized $Ca^{2+}$ ions. Although troponin C has been known for a long time as the $Ca^{2+}$-responsive protein involved in skeletal muscle contraction, most of the proteins involved in other $Ca^{2+}$ actions were unknown until the discovery of the 17-K $Ca^{2+}$-dependent regulatory protein calmodulin by Kakiuchi, Cheung, Wang and their associates (for reviews see Cheung 1980;

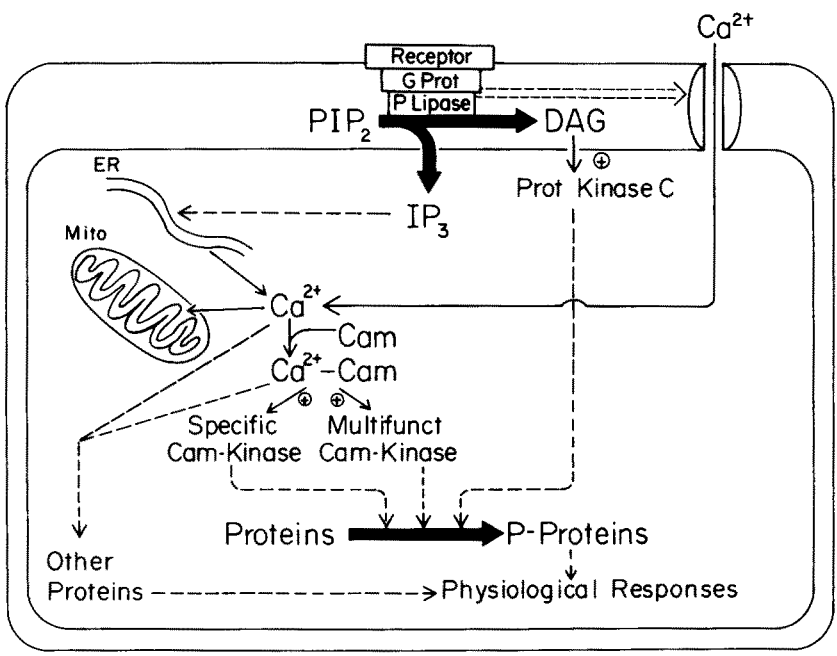


**Fig. 21.** Generalized scheme of the roles of $Ca^{2+}$, calmodulin, $Ca^{2+}$-calmodulin-dependent protein kinases, and protein kinase C in the actions of $Ca^{2+}$-mobilizing agonists. Abbreviations not already given are: *G Prot*, G-protein; *P lipase*, $PIP_2$ phospholipase C; *ER*, endoplasmic reticulum; *Mito*, mitochondrion; *Cam*, calmodulin

Klee and Vanaman 1982). This protein was soon shown to be involved in a large number of $Ca^{2+}$-mediated cellular responses (Fig. 21) and to be distributed widely in various tissues from animal and plant species as well as in protozoa.

Vertebrate calmodulin is a 148-residue protein that is homologous to troponin C and has four nonidentical, but homologous, $Ca^{2+}$-binding sites of high affinity ($K_d$ between $10^{-7}$ and $10^{-5}$ M). The molecule has a dumbbell-like structure with two calcium-binding domains at each end connected by a region of $\alpha$-helical structure. A rise in cytosolic $Ca^{2+}$ within the physiological range leads to increased formation of $Ca^{2+}$-calmodulin complexes (Fig. 21). Binding of $Ca^{2+}$ results in a conformational change in calmodulin which increases its reversible interaction with certain target proteins, thereby altering their activities. These proteins include a form of cyclic nucleotide phosphodiesterase, a form of adenylate cyclase, a plasma membrane $Ca^{2+}$-ATPase, and a specific phosphoprotein phosphatase termed "calcineurin" (Klee and Vanaman 1982). In addition to these proteins, the $Ca^{2+}$-calmodulin complex activates certain specific and multisubstrate protein kinases, leading to the phosphorylation of diverse proteins (Fig. 21; Stull et al. 1986).

## 9.2 Myosin Light-Chain Kinase and Phosphorylase *b* Kinase

A major target of $Ca^{2+}$-calmodulin in smooth muscle and platelets is myosin light-chain kinase. The smooth muscle form of this enzyme has an $M_r$ of 130000–160000 and phosphorylates the regulatory 20-K light chains of myosin. This increases the actin-stimulated myosin ATPase activity and the increased cross-bridge cycling associated with contraction in smooth muscle (Chacko et al. 1977; Dabrowska et al. 1978; Adelstein and Eisenberg 1980; Driska et al. 1981; Ruegg 1982) or shape change in platelets (Adelstein and Conti 1975; Daniel et al. 1981, 1984). The enzyme has a very high substrate specificity and is also present in brain, heart, and skeletal muscle. In the last-mentioned tissue, it does not play a role in the initiation of contraction but augments force generation (Stull et al. 1980). The calmodulin-binding domain of the enzyme lies distal to the catalytic domain and represents the carboxyl terminus (Stull et al. 1986).

Another $Ca^{2+}$-dependent protein kinase with high substrate specificity is phosphorylase *b* kinase. This differs from other calmodulin-responsive enzymes in that it contains calmodulin as a subunit (Cohen et al. 1978; Chan and Graves 1984). It has an $M_r$ of approximately 1.3 million and consists of a tetramer of $\alpha$ or $\alpha'$, $\beta$, $\gamma$, and $\delta$ subunits (Chan and Graves 1984). The $\alpha$ and $\beta$ subunits are regulatory and undergo autophosphorylation or can be phosphorylated by cAMP-dependent protein kinase, whereas the $\gamma$ subunit

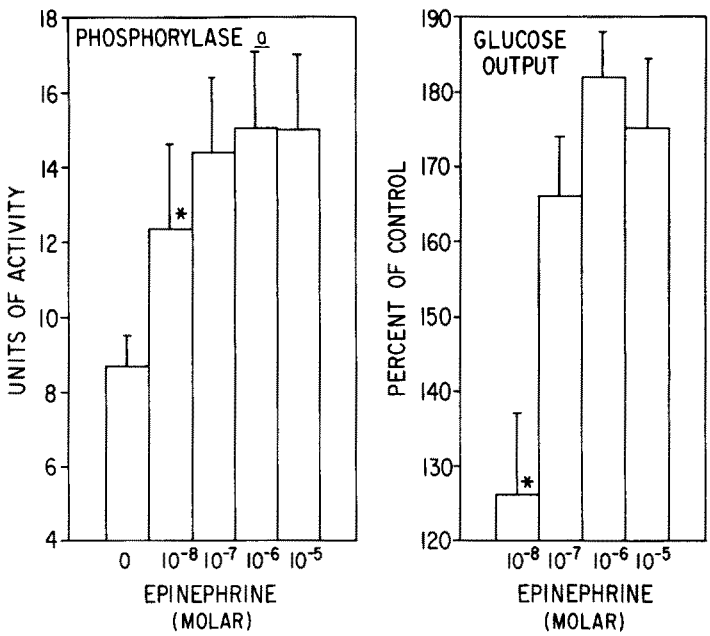


**Fig. 22.** Effects of epinephrine on phosphorylase activation and glycogenolysis in isolated rat hepatocytes. Hepatocytes from fed male rats were incubated with epinephrine at the concentrations shown. Phosphorylase *a* was measured at 1 min and glucose output over 15 min. Under these conditions, epinephrine acts primarily through $\alpha_1$-adrenergic receptors. (Unpublished data of N. J. Hutson, F. T. Brumley, and J. H. Exton)

contains the catalytic domain. The $\alpha/\beta$ subunits are inhibitory to the $\gamma$ subunit, and the inhibition is less when the subunits are phosphorylated (Paudel and Carlson 1987). The $\delta$ subunit is virtually identical with calmodulin, which means that $Ca^{2+}$ interacts directly with the enzyme (Shenolikar et al. 1979). In addition, the $\alpha$ and $\beta$ subunits of most forms of the enzyme can bind additional $Ca^{2+}$-calmodulin, leading to increased activity (Picton et al. 1980; Cohen 1980). Thus, a common response to a rise in cytosolic $Ca^{2+}$ induced by $\alpha_1$-adrenergic agonists and other hormones or neurotransmitters in many tissues is activation of phosphorylase *b* kinase. This leads to phosphorylation of phosphorylase *b*, converting it to the more active form, phosphorylase *a* (Fig. 22). Since this enzyme is rate limiting for glycogen breakdown, its activation leads to enhanced formation of glucose-6-P for energy production via glycolysis in most tissues. In the case of liver, there is also production of glucose (Fig. 22) due to the presence of glucose 6-phosphatase. Phosphorylase *b* kinase has also been shown to phosphorylate and inactivate liver and muscle glycogen synthase (Roach et al. 1978), but it is unclear whether this is important in the inhibition of hepatic glycogen synthase by $Ca^{2+}$-mobilizing agonists (Strickland et al. 1983).

## 9.3 $Ca^{2+}$-Calmodulin-Dependent Protein Kinases

Another protein kinase of major importance in the actions of $Ca^{2+}$-mobilizing agonists is the multifunctional $Ca^{2+}$-calmodulin-dependent protein kinase, which is found widely distributed in mammalian tissues. This kinase is not as selective in its substrate specificity as myosin light-chain kinase or phosphorylase *b* kinase, and it exists in several isozymic forms exhibiting different structural, immunological, and enzymatic properties (Shenolikar et al. 1986). The enzyme was originally discovered as a $Ca^{2+}$-dependent protein kinase in brain (Schulman and Greengard 1978a, b) and as a glycogen synthase kinase in liver (Payne and Soderling 1980), and it has now also been purified from skeletal muscle (Campbell and MacLennan 1982; Woodgett et al. 1983). There is evidence that it is present in adipose tissue (Landt and McDonald 1984), *Torpedo* electric organ (Palfrey et al. 1983), *Aplysia* neurons (DeRiemer et al. 1984), pancreatic islets (Landt et al. 1982), and mammary gland (Brooks and Landt 1985). The kinases from various tissues are composed of either two subunits (50- to 55-K and 60- to 75-K) or the single lower $M_r$ subunit (Stull et al. 1986). The relative subunit compositions and, hence, the $M_r$s of the native enzymes are quite variable.

The $Ca^{2+}$calmodulin-dependent protein kinases of brain have been subdivided into three isozymic forms. Type I is found mainly in the cytosolic fraction of brain and other tissues and phosphorylates two neuron-specific proteins called synapsin 1 and Protein III (Kennedy and Greengard 1981; Nairn et al. 1985b). Its activity toward other substrates is very low, and it phosphorylates synapsin 1 on a single site (I) which is also a site for cAMP-dependent protein kinase (Nairn et al. 1985b). Protein III is also phosphorylated by both enzymes at a single site.

Type-II $Ca^{2+}$-calmodulin-dependent protein kinase exists in several isozymic forms and is more abundant than type I. It has a wider substrate specificity and is present in both soluble and particulate functions of the brain (Nairn et al. 1985b). It is very rich and widely distributed in brain, representing as much as 0.4% of total brain protein (Bennett et al. 1983; McGuinness et al. 1983). Its major substrate in brain is synapsin 1, which it phosphorylates on site II, located in the tail region. This phosphorylation reduces the binding of synapsin 1 to synaptic vesicles and may be involved in neurotransmitter release (Llinas et al. 1985). The principal type-II isozyme from brain is closely related to, but not identical with, the $Ca^{2+}$-calmodulin-dependent glycogen synthase kinase of skeletal muscle (McGuinness et al. 1983; Woodgett et al. 1984; Yamauchi and Fujisawa 1986) and liver (Schworer and Soderling 1983). It is a 550- to 650-K polymer containing both 50- and 60-K subunits which undergo autophosphorylation (Bennett et al. 1983; McGuinness et al. 1985; Kuret and Shulman 1984; Nairn et al. 1985b). The isozymes from various tissues and different regions of the brain contain dif-

ferent ratios of the subunits (McGuinness et al. 1983, 1985). Consequently, they show significant differences in $M_r$. Type-III $Ca^{2+}$-calmodulin-dependent protein kinase was purified first from pancreas, utilizing its specific 100-k substrate (Nairn et al. 1985a). It and its substrate are present in many other tissues, including skeletal muscle, adrenal, brain, and liver. Other proteins are poor substrates for this enzyme (Nairn et al. 1985a).

## 9.4 Substrates of $Ca^{2+}$-Calmodulin-Dependent Protein Kinases

A large number of in vitro substrates of type-II $Ca^{2+}$-calmodulin-dependent protein kinase have been identified. These include glycogen synthase, synapsin 1, microtubule-associated protein 2 (MAP-2), tau-protein, myelin basic protein, myosin light chains, tyrosine hydroxylase, phenylalanine hydroxylase, tryptophan hydroxylase, ATP-citrate lyase, acetyl-CoA carboxylase, and pyruvate kinase (Schworer and Soderling 1983; McGuinness et al. 1983; Woodgett et al. 1983, 1984; Doskeland et al. 1984; Vuillet et al. 1984; Schulman 1984a, b; Nairn et al. 1985b). Many of these proteins are phosphorylated when neuronal and other cells are stimulated by nervous or hormonal signals which increase cytosolic $Ca^{2+}$ (Nestler et al. 1984; Exton 1987; Nestler and Greengard 1983; Schulman 1984a, b; Garrison and Wagner 1982; Blackmore and Exton 1985; Garrison et al. 1984; Nairn et al. 1985b). However, although it is likely that a $Ca^{2+}$-calmodulin-dependent protein kinase is responsible for most of these in vivo phosphorylations, this has not been clearly established, because many of the proteins are also substrates for protein kinase C and/or cAMP-dependent protein kinase.

Phosphorylation of the synaptic vesicle-associated protein synapsin 1 induces neurotransmitter release in the giant squid synapse, and there is evidence that it produces a similar effect in the mammalian nervous system (Nestler et al. 1984). Tyrosine hydroxylase converts tyrosine to dihydroxyphenylalanine (dopa) and is rate controlling for epinephrine and norepinephrine synthesis in adrenal medulla and presumably brain. Phosphorylation of this enzyme increases its activity when assayed in the presence of an "activator protein" (Yamauchi et al. 1981). Type-II $Ca^{2+}$-calmodulin-dependent protein kinase phosphorylates MAP-2, $\alpha$- and $\beta$-tubulin, and $\tau$ factor from brain (Burke and Lorenzo 1981; Yamamoto et al. 1983; Schulman 1984a, b). This suggests that microtubule function (state of polymerization, treadmilling, or interaction with other cell components) may be regulated by $Ca^{2+}$-mobilizing agonists through this enzyme.

Glycogen synthase was utilized initially to identify $Ca^{2+}$-calmodulin-dependent protein kinase in liver (Payne and Soderling 1980; Ahmad et al. 1982; Payne et al. 1983). The kinase phosphorylates this enzyme on site 2, which is serine 7 near the amino terminus, and also on site 1b toward the carboxyl

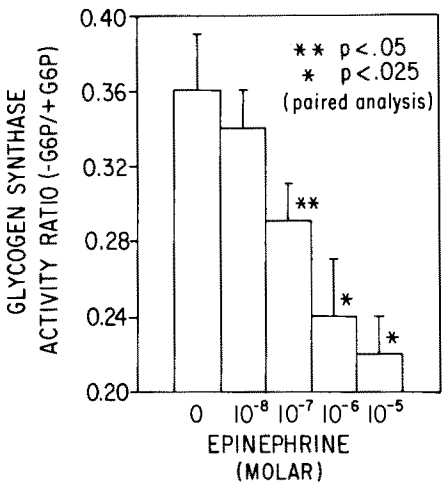


**Fig. 23.** Inactivation of glycogen synthase induced by epinephrine in isolated rat hepatocytes. Hepatocytes from fasted male rats were incubated for 2 min with epinephrine at the concentrations shown and the activity ratio (-Glc6-P/+10 m*M* Glc6-P) of glycogen synthase was measured. (Unpublished data of N. J. Hutson, F. T. Brumley, and J. H. Exton)

terminus (Payne et al. 1983; Juhl et al. 1983). Site 2 is also phosphorylated by cAMP-dependent protein kinase and phosphorylase *b* kinase (Juhl et al. 1983) and is associated with inactivation of the enzyme. Although $Ca^{2+}$-calmodulin-dependent protein kinase is a good candidate for mediating the inhibitory effects of $Ca^{2+}$-mobilizing agonists on glycogen synthase in liver (Fig. 23; Strickland et al. 1980), the synthase is also a substrate for phosphorylase *b* kinase and protein kinase C (Imazu et al. 1984; Ahmad et al. 1984), which are also involved in the actions of these agonists (see Sect. 8).

There is much evidence that $\alpha_1$-adrenergic agonists and other $Ca^{2+}$-mobilizing hormones induce phosphorylation and inactivation of pyruvate kinase in liver and that this contributes to the stimulation of gluconeogenesis by these agents (Chan and Exton 1978; Garrison et al. 1979; Nagano et al. 1980). The kinase involved is not phosphorylase *b* kinase, since the phosphorylation occurs in animals lacking this enzyme, or protein kinase C, since phorbol esters do not induce phosphorylation of pyruvate kinase (Garrison et al. 1984). It is probably type-II $Ca^{2+}$-calmodulin-dependent protein kinase, since this can phosphorylate and inactivate the enzyme in vitro (Schworer et al. 1985) and produces the same phosphopeptide pattern as seen with $Ca^{2+}$-mobilizing agonists in vivo (Connelly et al. 1987). Phenylalanine hydroxylase converts phenylalanine to tyrosine and is controlled by both cAMP- and $Ca^{2+}$-dependent stimuli in liver (Fisher and Pogson 1984; Fisher et al. 1984). Phosphorylation and activation of the enzyme occurs in hepatocytes exposed to $Ca^{2+}$-mobilizing agonists (Garrison and Wagner 1982; Garrison et al. 1984; Fisher et al. 1984), and there is evidence that neither phosphorylase *b* kinase nor protein kinase C is involved (Garrison et al. 1984). On the other hand, the enzyme is phosphorylated and activated in

vitro by type-II $Ca^{2+}$-calmodulin-dependent protein kinase (Doskeland et al. 1984).

## 9.5 Other Targets of $Ca^{2+}$-Calmodulin and $Ca^{2+}$

Although the focus of the preceding paragraphs has been on the specific and multifunctional $Ca^{2+}$-calmodulin-dependent protein kinases, other proteins are sensitive to $Ca^{2+}$-calmodulin. Microtubules, which are key cytoskeletal elements associated with cell movement, flagellar and ciliary motility, chromosome movement, and axonal transport, are targets of $Ca^{2+}$-calmodulin (Means and Dedman 1980). Polymerization of $\alpha\beta$ tubulin to form microtubules is inhibited by $Ca^{2+}$-calmodulin (Marcum et al. 1978; Kamagai and Nishida 1979), and there is evidence that nucleation rather than elongation may be inhibited (Berkowitz and Wolff 1981). In addition to its direct effects, $Ca^{2+}$-calmodulin also influences microtubule assembly/disassembly through phosphorylation of microtubule components by type-II $Ca^{2+}$-calmodulin-dependent protein kinase, as noted above.

$Ca^{2+}$-calmodulin can activate a form of cyclic nucleotide phosphodiesterase found in brain, heart, liver, and most other tissues (Klee and Vanaman 1982). $Ca^{2+}$-calmodulin binds stoichiometrically to a specific site on the enzyme to form a complex which hydrolyzes cGMP with a low $K_m$ ($5-10\,\mu M$) and cAMP with a high $K_m$ (approximately $100\,\mu M$). Despite the well-demonstrated effects of $Ca^{2+}$-calmodulin on this enzyme in vitro, there are no clear-cut examples of $Ca^{2+}$ regulation of cAMP or cGMP concentrations by this mechanism in intact cells. This may relate to the high $K_m$s of the enzyme for its two substrates relative to their cellular concentrations.

$Ca^{2+}$-calmodulin activates a form of adenylate cyclase present in brain, pancreatic islets, adrenal medulla, and kidney cells (Klee and Vanaman 1982). The effect does not involve a G-protein and is exerted directly on the catalytic subunit of the enzyme (Coussen et al. 1985). There are presently no unequivocal examples of regulation of the enzyme under physiological conditions, although this would be difficult to demonstrate in the intact brain.

The plasma membrane $Ca^{2+}$-pump ATPase is calmodulin sensitive in most tissues, with the exception of the liver (Carafoli 1984). This pump is responsible for most of the $Ca^{2+}$ extruded from nonexcitable cells and from excitable cells during rest. In the latter, the lower-affinity $Na^+/Ca^{2+}$ exchanger is responsible for most of the $Ca^{2+}$ ejected during excitation. Addition of $Ca^{2+}$-calmodulin to the ATPase lowers its $K_m$ for $Ca^{2+}$ and increases its $V_{max}$ (Niggli et al. 1979; Waisman et al. 1981). As found for other calmodulin-responsive proteins, the 138-K ATPase has a specific $Ca^{2+}$-calmodulin-binding domain which is approximately 25 K in size, as defined by proteolytic fragmentation (Zurini et al. 1984).

In contrast to the plasma membrane $Ca^{2+}$-pump ATPase, that of the endoplasmic (sarcoplasmic) reticulum is not *directly* controlled by $Ca^{2+}$-calmodulin. In heart, this $Ca^{2+}$-ATPase is regulated by phospholamban, a 22-K proteolipid which can be phosphorylated by both cAMP-dependent and $Ca^{2+}$-calmodulin-dependent protein kinases, leading to increased uptake of $Ca^{2+}$ by the sarcoplasmic reticulum (Tada et al. 1979; LePeuch et al. 1979; Tada and Katz 1982; Davis et al. 1983). $Ca^{2+}$-calmodulin-dependent phosphorylation of phospholamban increases the maximum rate of $Ca^{2+}$ transport by isolated cardiac sarcoplasmic reticulum vesicles, with a small decrease in $K_m$ for $Ca^{2+}$ (Davis et al. 1983). However, efforts to demonstrate that physiological increases in cytosolic $Ca^{2+}$ increase phospholamban phosphorylation in intact myocardium have not been successful. $Ca^{2+}$-calmodulin-dependent protein kinase and phosphorylase *b* kinase phosphorylate several proteins in skeletal muscle sarcoplasmic reticulum (Varsanyi and Heilmeyer 1981; Campbell and MacLennan 1982). The functional significance of these phosphorylations is uncertain, but it has been suggested that the phosphorylation/dephosphorylation cycle of a 60-K protein may control the $Ca^{2+}$-release channel of sarcoplasmic reticulum (Campbell and MacLennan 1982).

$Ca^{2+}$ ions also regulate cellular processes by interacting with proteins other than calmodulin. As alluded to above, troponin C is a major target in skeletal and cardiac muscle. Other $Ca^{2+}$ targets are a group of proteins which alter aspects of actin filament assembly and severance, and thus may be important in cell architecture, cytoplasmic flow, and exocytosis (Stossel 1984). These include gelsolin, profilin, villin, and fragmin, which act on actin in various ways, e.g., by sequestering actin monomers and by nucleating, end-blocking, and severing actin filaments. $Ca^{2+}$ binds to gelsolin with high affinity and this causes shortening of actin filaments, contributing to the collapse of their three-dimensional lattice (Yin and Stossel 1982). This gel-sol transformation may be involved in the regulation of cell motility. Other gelsolin-related proteins bind $Ca^{2+}$ and may contribute to the changes in actin filament assembly/disassembly.

## 10 Role of Mitochondrial Changes

### 10.1 $Ca^{2+}$ Activation of Mitochondrial Dehydrogenases

Denton, McCormack, and others (reviewed by Denton and McCormack 1981, 1985; Hansford 1985) have identified another group of hormonally controlled, $Ca^{2+}$-responsive, but calmodulin-independent enzymes in liver, heart, and adipose tissue. These are all located in mitochondria and include

pyruvate dehydrogenase phosphate phosphatase, α-oxoglutarate dehydrogenase, and $NAD^+$-isocitrate dehydrogenase. The pyruvate dehydrogenase complex is under elaborate control by allosteric effectors and phosphorylation/dephosphorylation mechanisms. Phosphorylation of a specific serine residue in the α-subunit of the pyruvate decarboxylase moiety by pyruvate dehydrogenase kinase causes inactivation, whereas dephosphorylation by pyruvate dehydrogenase phosphate phosphatase leads to activation. Low concentrations ($0.1-10\,\mu M$) of $Ca^{2+}$ stimulate the phosphatase (Denton et al. 1972; McCormack 1985a) and activate pyruvate dehydrogenase in isolated mitochondria (McCormack et al. 1982; McCormack and Denton 1984). Thus, $Ca^{2+}$ has been implicated in the stimulatory effects of $\alpha_1$-adrenergic agonists, glucagon, angiotensin II, vasopressin, and A23187 ionophore on pyruvate dehydrogenase in liver (Hems et al. 1978; Assimacopoulos-Jeannet et al. 1983, 1986; McCormack 1985b, c) and of inotropic agents on the enzyme in heart (McCormack and Denton 1981a, 1984; McCormack et al. 1982). These stimulatory effects can be observed in tissue extracts (Assimacopoulos-Jeannet et al. 1983; Blackmore et al. 1983b; Sies et al. 1983; Oviasu and Whitton 1984; McCormack and Denton 1981a) or in mitochondria isolated from livers or hearts exposed to the agonists (McCormack 1985b, c; McCormack and Denton 1984).

There is some controversy regarding the effects of $Ca^{2+}$-mobilizing agonists on pyruvate dehydrogenase activity in intact liver when this is assayed indirectly by measuring $CO_2$ production from isotopically labeled pyruvate. However, this approach is complicated by intracellular changes in precursor specific radioactivity (due to glycogen breakdown) and by entry of the label into the citric acid cycle via pyruvate carboxylation. Thus, some workers have reported that $\alpha_1$-adrenergic agonists and vasopressin decrease the production of $^{14}CO_2$ from [1-$^{14}$C]pyruvate in isolated hepatocytes or the perfused rat liver (Sies et al. 1983; Fisher et al. 1985).

Increased α-oxoglutarate dehydrogenase activity has been observed in liver mitochondria from rats treated with epinephrine or glucagon (McCormack 1985b, c). It has also been deduced from measurements of $^{14}CO_2$ and [$^{14}$C]glucose production from labeled glutamine, glutamate, or proline. It is also consistent with the decrease in α-oxoglutarate levels in livers perfused with glucagon (Ui et al. 1973) or $\alpha_1$-adrenergic agonists (Haussinger and Sies 1984; Ochs 1984), or in hepatocytes incubated with vasopressin (Staddon and McGivan 1985). Evidence that the increase in enzyme activity is due to increased intramitochondrial $Ca^{2+}$ has been presented by McCormack (1985a, b, c). Thus, when $Ca^{2+}$ influx into mitochondria is prevented during their isolation, and when $Ca^{2+}$ efflux is minimized by the use of $Na^+$-free media, the hormone effect is preserved (McCormack 1985b, c). Furthermore, manipulation of the extramitochondrial $Ca^{2+}$ concentration and examination of the effects of ruthenium red (an inhibitor of mitochondrial $Ca^{2+}$ up-

take) and of $Na^+$ and diltiazem (an inhibitor of $Na^+$-induced mitochondrial $Ca^{2+}$ efflux) strongly implicate intramitochondrial $Ca^{2+}$ as a major regulator of both pyruvate and $\alpha$-oxoglutarate dehydrogenases in liver (McCormack 1985a). There is strong evidence of a similar regulation in heart (Denton et al. 1980; McCormack and Denton 1981b, 1984).

Activation of the three mitochondrial dehydrogenases is probably largely responsible for the stimulation of respiration induced by $\alpha_1$-adrenergic agonists, vasopressin, and angiotensin II in perfused rat liver or isolated hepatocytes (Jakob and Diem 1975; Sugano et al. 1980; Dehaye et al. 1981; Reinhart et al. 1982; Taylor et al. 1983; Blackmore et al. 1983a) and the increased reduction state of NAD(P) (Fig. 24; Sugano et al. 1980; Balaban and Blum 1982; Buxton et al. 1982; Blackmore et al. 1983a). Their activation may also account for the stimulation of fatty acid oxidation to $CO_2$ and inhibition of ketogenesis exerted by $Ca^{2+}$-mobilizing agonists in hepatocytes (Sugden et al. 1980; Williamson et al. 1980; Sugden and Watts 1983), since both effects can be attributed to increased citric acid cycle activity.

## 10.2 Agonist Regulation of Mitochondrial $Ca^{2+}$

The hypothesis that a rise in intramitochondrial $Ca^{2+}$ is responsible for the effects of $\alpha_1$-adrenergic agonists, vasopressin, and angiotensin II on pyruvate dehydrogenase and $\alpha$-oxoglutarate dehydrogenase was initially not compatible with observations that these agonists caused a loss of $Ca^{2+}$ from mitochondria-enriched subcellular fractions of liver (for references see Williamson et al. 1981; Exton 1981; Reinhart et al. 1984a, b). However, recent work indicates that $Ca^{2+}$ is mobilized from components of the endoplasmic reticulum rather than mitochondria (see Sects. 3.2 and 7.2). Thus, it is now accepted that mitochondria take up $Ca^{2+}$ in response to the elevation of cytosolic $Ca^{2+}$ induced by $Ca^{2+}$-mobilizing agonists (Fig. 21) and are not the site from which $Ca^{2+}$ is released, in contrast to what was originally postulated (Exton 1980, 1981; Williamson et al. 1981; Reinhart et al. 1984a, b). Furthermore, the idea that the mitochondrial $Ca^{2+}$ cycle controls the concentration of cytosolic $Ca^{2+}$ within the physiological range (Nicholls 1978; Nicholls and Akerman 1982) is unlikely, now that it is known that the cytosolic $Ca^{2+}$ level in unstimulated cells is approximately 0.2 $\mu M$ (Charest et al. 1983, 1985; Murphy et al. 1980; Joseph et al. 1985) and that the mitochondrial $Ca^{2+}$ content is low in situ (Bond et al. 1984; Somlyo et al. 1985a; Hansford 1985). The function of the mitochondria in regulating cell $Ca^{2+}$ now appears to be to take up cytosolic $Ca^{2+}$ when this rises above 0.5 $\mu M$ and thus to help protect the cell from damage.

Consistent with the view that mitochondria take up $Ca^{2+}$ in response to $Ca^{2+}$-mobilizing agonists in liver are the observations that the increases in

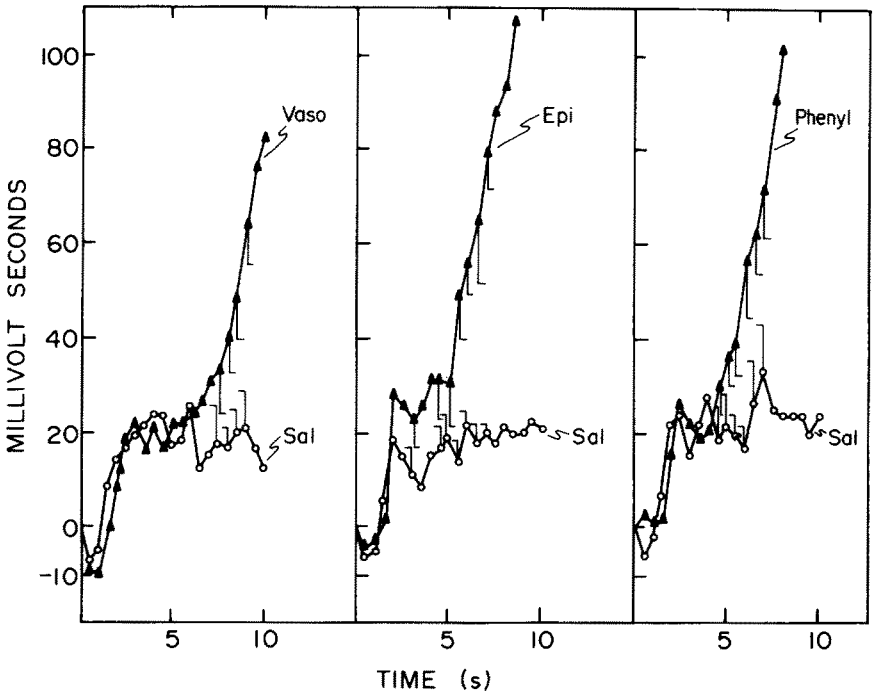


**Fig. 24.** Effects of vasopressin (*Vaso*, $10^{-8}$ *M*), epinephrine (*Epi*, $10^{-6}$ *M*), and phenylephrine (*Phenyl*, $10^{-5}$ *M*) on the reduction of NAD(P) in isolated rat hepatocytes. The reduction of NAD(P) was followed fluorimetrically after addition of saline (*Sal*) or the agonists shown. (From Blackmore et al. (1983a) by permission of the authors and publisher)

respiration, NAD(P) reduction state, and pyruvate dehydrogenase activity induced by these agonists lag significantly (5–20 s) behind the increase in cytosolic $Ca^{2+}$ and associated activation of phosphorylase and initiation of $Ca^{2+}$ efflux (Fig. 24; Blackmore et al. 1983a, b; Charest et al. 1983, 1985). Stable increases in $Ca^{2+}$ uptake by mitochondria isolated from livers perfused with $\alpha_1$-adrenergic agonists or glucagon have been reported (Taylor et al. 1980), and similar effects have been observed with mitochondria from hearts exposed to $\alpha_1$-adrenergic agonists (Kessar and Crompton 1981). However, it is unclear whether or not these stable changes occur in the intact cell.

## 11 Future Directions for Research

Although the foregoing account indicates that much is now known about the biochemical reactions underlying $\alpha_1$-adrenergic phenomena, it should be noted that the molecular bases of many of the effects of $\alpha_1$-adrenergic stim-

ulation remain to be defined. These include the increase in plasma membrane $K^+$ permeability and other ion fluxes in salivary and lacrimal glands, the increase in $K^+$ efflux and thermogenesis in brown adipose tissue, the stimulation of $K^+$ fluxes, ureogenesis, and pyruvate carboxylation in liver, the stimulation of gluconeogenesis in kidney, the alterations in contractility and glycolysis in heart, and the hyperpolarization and relaxation of gastrointestinal muscle (Exton 1985). In each case, the specific enzymes or other proteins that are the targets of $Ca^{2+}$ or $Ca^{2+}$-calmodulin, or of the specific or multisubstrate $Ca^{2+}$-calmodulin-dependent protein kinases or protein kinase C need to be defined.

In addition to this lack of knowledge concerning the enzymes and other proteins involved in these specific responses, there is still much to be learned concerning the general mechanisms by which $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists raise cytosolic $Ca^{2+}$ and elevate DAG in their target cells. The $\alpha_1$-adrenergic receptor has been purified but it has not been sequenced, nor have its physicochemical characteristics been defined. This is also true for other receptors for $Ca^{2+}$-mobilizing agonists. The G-proteins involved in signal transduction for the $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing receptors have not been identified unequivocally, and the molecular bases for their interaction with the receptors and the $PIP_2$-specific phospholipase have not been defined. The G-protein-activated phospholipase has also not been identified unequivocally and its physicochemical characteristics have not been determined. The precise intracellular target of $IP_3$ also has not been defined, and the mechanism by which it releases $Ca^{2+}$ remains unclear. The possible function of the various products of $IP_3$ metabolism, in particular $IP_4$, need to be defined.

The origins of the DAG and phosphatidic acid that accumulate in response to agonists in cells and the mechanisms involved in their formation need to be clarified. There is much evidence that they arise from other phospholipids besides $PIP_2$, in particular phosphatidylcholine, through activation of novel phospholipases. The further metabolism and possible functions of DAG and phosphatidate also need to be explored, in view of the fact that they can achieve very high cellular concentrations during agonist stimulation.

Another area of ignorance relates to the plasma membrane $Ca^{2+}$ channel(s) regulated by $Ca^{2+}$-mobilizing agonists. The physicochemical nature of this channel is completely unknown and the mechanism by which it is regulated is obscure. There is some evidence that a G-protein is involved, but this may be coupled directly to the channel or indirectly via a second messenger or another protein.

Finally, the roles of the changes in phosphoinositides and other phospholipids and of protein kinase C in the actions of growth factors and other agonists regulating cell growth remain obscure. There is evidence that inositol phospholipid turnover and protein kinase C activation play some part in the

actions or induction of certain proto-oncogenes, but the relationship of these changes to mitogenesis is unclear.

## 12 Summary

$\alpha_1$-Adrenergic receptors mediate many actions of epinephrine and norepinephrine, which are the transmitters of information in the sympathetic nervous system. Some important $\alpha_1$-adrenergic responses are the contraction of smooth muscle in vascular and other tissues, the secretion of certain glands, alterations in carbohydrate metabolism in certain tissues, and neurotransmission. $\alpha_1$-Adrenergic receptors have a ligand-binding subunit of approximately 80 K and can exist in low and high agonist-affinity states. The interconversion between these states is controlled by GTP and its analogues, implying that the receptors are coupled to a guanine nucleotide-binding regulatory protein (G-protein).

As illustrated in Fig. 14, the primary effect of $\alpha_1$-adrenergic receptor activation is the breakdown of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) in the plasma membrane to yield myoinositol 1,4,5-trisphosphate ($IP_3$) and 1,2-diacylglycerol (DAG). There is much additional evidence that the coupling of the receptor to the phospholipase C (or phosphodiesterase) responsible for the breakdown involves a G-protein. For example, the stimulation of $PIP_2$ breakdown and formation of $IP_3$ by $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists in isolated plasma membranes is dependent upon GTP and its nonhydrolyzable analogues, and micromolar concentrations of GTP analogues can stimulate $IP_3$ formation in an $Mg^{2+}$-dependent manner. In addition, $AlF_4^-$, which activates several G-proteins, stimulates the breakdown of $PIP_2$ to $IP_3$ in intact cells and plasma membranes. Islet-activating protein (a pertussis toxin), which ADP-ribosylates several G-proteins, can inhibit agonist-induced $PIP_2$ breakdown in some tissues but not others. The G-proteins specifically involved in the regulation of $PIP_2$ phospholipase C have not yet been identified for certain.

The formation of $IP_3$ in response to $Ca^{2+}$-mobilizing agonists occurs within a few seconds and is proportional to receptor occupancy. $IP_3$ rapidly releases $Ca^{2+}$ from nonmitochondrial stores in permeabilized cells and from microsomal preparations (Fig. 14). It appears to act by stimulating $Ca^{2+}$ efflux from a component of the endoplasmic reticulum, and not by inhibiting $Ca^{2+}$ uptake. $IP_3$ is rapidly metabolized to myoinositol 1,3,4,5-tetrakisphosphate by a 3-kinase and is hydrolyzed to other myoinositol phosphates and eventually to myoinositol by phosphomonoesterases present in the soluble phase or plasma membrane. This leads to a cessation of $Ca^{2+}$ efflux from the endoplasmic reticulum unless $IP_3$ generation continues. Another isomer

of $IP_3$ (myoinositol 1,3,4-trisphosphate) is slowly formed from myoinositol 1,3,4,5-tetrakisphosphate by phosphomonoesterase action, but its function is unclear. $IP_3$ is almost certainly the intracellular messenger responsible for $Ca^{2+}$ mobilization.

The formation of DAG in response to $Ca^{2+}$-mobilizing agonists is of slower onset and greater magnitude than that of $IP_3$. This is because DAG is also formed by the breakdown of phosphatidylcholine and perhaps other phospholipids. The accumulation of DAG appears to cause the translocation of the $Ca^{2+}$-phospholipid-dependent protein kinase C from the cytosol to the plasma membrane. There it is activated by unsaturated DAG (Fig. 14), which reduces its $Ca^{2+}$ requirement for activity to the cytosolic range. Protein kinase C is presumed to be the cellular target of tumor-promoting phorbol esters, which activate the enzyme in a manner analogous to that of DAG. Although many enzymes and other proteins have been shown to be phosphorylated by protein kinase C in vitro, few of the intracellular targets of the enzyme have been characterized. Protein kinase C phosphorylates the $\alpha_1$-adrenergic receptor and certain other membrane receptors, thereby inhibiting agonist binding. This may be involved in some forms of agonist desensitization.

In addition to promoting the release of intracellular $Ca^{2+}$ through $IP_3$ generation, $\alpha_1$-adrenergic agonists stimulate $Ca^{2+}$ influx and inhibit $Ca^{2+}$ efflux across the plasma membrane. These effects are responsible for maintaining the elevation of cytosolic $Ca^{2+}$ and thereby prolonging the physiological responses to these agonists. This is because the intracellular $Ca^{2+}$ stores are limited and rapidly become depleted by agonists. The stimulation of $Ca^{2+}$ influx is presumably due to the opening of $Ca^{2+}$ channels, and the inhibition of $Ca^{2+}$ efflux is due to altered kinetics of the plasma membrane $Ca^{2+}$-ATPase/pump. The molecular mechanisms responsible for these changes are unknown, but there is evidence that the $Ca^{2+}$ channels are regulated directly or indirectly by G-proteins.

The $Ca^{2+}$-dependent regulatory protein calmodulin is a major target of intracellular $Ca^{2+}$ (Fig. 14) and is involved in many physiological responses. It has four high-affinity binding sites for $Ca^{2+}$ and is present in all mammalian tissues. It is a subunit of phosphorylase *b* kinase and mediates the stimulatory effects of increased cytosolic $Ca^{2+}$ on the enzyme. This leads to enhanced glycogen breakdown through the phosphorylation and activation of glycogen phosphorylase. More commonly, calmodulin exists in a free form, i.e., not as the subunit of an enzyme. As a result of an increase in cytosolic $Ca^{2+}$, there is increased binding of $Ca^{2+}$ to calmodulin. This leads to a conformational change in the protein, which increases its binding to a variety of enzymes and other proteins, thereby altering their function. $Ca^{2+}$-calmodulin interacts with myosin light-chain kinase, leading to increased phosphorylation of the regulatory 20-K light chains of myosin in smooth muscles and platelets. This

promotes actin-stimulated myosin ATPase and increased cross-bridge cycling between the two proteins, resulting in contraction and shape change of the cells.

Another major target of $Ca^{2+}$-calmodulin is a calmodulin-dependent protein kinase which exists in isozymic forms. One isozyme is distributed widely and acts on many substrates (Fig. 14), and thus is importantly involved in many $Ca^{2+}$-mediated physiological responses. Its substrates include glycogen synthase, synapsin 1, tubulin, microtubule-associated proteins, tyrosine hydroxylase, phenylalanine hydroxylase, and pyruvate kinase. The phosphorylation of these proteins probably controls such functions as synaptic neurotransmitter release, motility, chromosome movement and axonal transport, catecholamine synthesis, and gluconeogenesis.

Cells contain many other targets of $Ca^{2+}$-calmodulin which are not protein kinases but may be involved in the actions of $\alpha_1$-adrenergic and other $Ca^{2+}$-mobilizing agonists. These include microtubules, whose assembly is inhibited by $Ca^{2+}$-calmodulin.

$Ca^{2+}$ can regulate cellular processes by binding to other proteins. It can interact directly with troponin C to initiate contraction in skeletal and cardiac muscle, and with gelsolin and other proteins which alter actin filament assembly/disassembly and thus affect cell architecture, cytoplasmic flow, and perhaps exocytosis. An increase in cytosolic $Ca^{2+}$ also leads to an increase in mitochondrial $Ca^{2+}$ in liver, heart, and probably other tissues (Fig. 14). This results in stimulation of the citric acid cycle and respiration because of increased activity of $\alpha$-oxoglutarate dehydrogenase and $NAD^+$-isocitrate dehydrogenase, and activation of pyruvate dehydrogenase due to stimulation of its phosphatase.

Other $\alpha_1$-adrenergic responses have been shown to be $Ca^{2+}$ dependent, but the mechanisms involved are obscure. These include (a) altered plasma membrane fluxes of $K^+$ and other ions and related membrane potential changes in salivary and lacrimal glands, liver, and brown adipose tissue, (b) stimulation of gluconeogenesis in liver and kidney, (c) alterations in contractility, glucose uptake, and glycolysis in heart, and (d) hyperpolarization and relaxation of gastrointestinal muscle.

It is clear that many details of the mechanisms by which $\alpha_1$-adrenergic agonists generate their intracellular signals ($IP_3$ and DAG) are unclear. This is also true for the mobilization of intracellular $Ca^{2+}$ and the regulation of $Ca^{2+}$ fluxes across the plasma membrane. The specific enzymes and other proteins involved in many of the physiological responses mediated by $Ca^{2+}$ and DAG also remain obscure. All of these areas provide many fruitful research topics.

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# Subject Index