Central Action of Drugs in Blood Pressure Regulation

Edited by
Donald S. Davies
and
John L. Reid

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Editors' Foreword

Extensive morphological and physiological studies have been performed to define the role of the nervous system in cardiovascular control. This neural contribution participates not only in short term tonic modification of heart rate and arterial pressure by baroreceptor and chemoreceptor pathways, but also in long term regulation of cardiovascular function.

In the last twenty years several compounds have been proposed as central neurotransmitters and elegant studies have described the distribution and localisation of these compounds.

More recently it has been suggested that several of these neurotransmitter systems participate in blood pressure regulation. Monoamine transmitters, noradrenaline and serotonin in particular, have been demonstrated, in pharmacological preparations, to influence central cardiovascular regulation.

However, much of this evidence is derived from experiments in which pharmacological agents are given into brain tissue, cerebrospinal fluid or the vertebral artery of animals. There has been little direct evidence that central mechanisms contribute to the cardiovascular effects of these agents given by mouth over long periods to hypertensive patients.

This Symposium sponsored by the British Pharmacology Society brought together histologists, physiologists and pharmacologists to review with clinicians the central nervous action of drugs in the regulation of blood pressure and the possible implications of these actions to the aetiology and management of human hypertension.

In addition to invited contributors, several groups submitted volunteer poster presentations. Abstracts of these presentations have been included in the published proceedings.

We would like to thank the Pharmaceutical Industry in Great Britain for their support of the Symposium and Ms E A Davis and B Edinborough for their invaluable assistance in the preparation of these proceedings for publication.

J L Reid
D S Davies

List of Speakers

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Experimental Hypertension and Noradrenergic Nerves

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The relationship between the sympathetic nervous system, the adrenal gland, and blood-pressure regulation has been recognized for many years. Oliver and Shäfer (1895) noted a marked elevation in blood pressure and heart rate after an injection of an extract of the adrenal medulla. The active principle responsible for this effect was soon isolated by Abel (1899) and identified as the catecholamine, adrenaline. von Euler (1946) isolated another catecholamine from peripheral sympathetic nerves and identified it as noradrenaline. It was soon observed by Goldenberg et al (1948) that an injection of noradrenaline raised arterial blood pressure and produced the same cardiovascular changes that were found in hypertensive subjects. These workers also observed that hypertensive patients were supersensitive to an injection of noradrenaline. These observations implicated the catecholamine-containing sympathetic nerves and the adrenal medulla in hypertension. During the subsequent 20 years additional evidence of the involvement of catecholamines in hypertension accumulated. It was found that subjects with adrenaline- and noradrenaline-producing tumours (phaeochromocytomas) were hypertensive. In the past decade or so a number of drugs have been introduced that were therapeutically useful in the treatment of hypertension. Most of these drugs were found to influence the disposition of catecholamines in various ways; reserpine depleted catecholamines in the brain and periphery; guanethidine depleted noradrenaline in the peripheral nerves; phenoxybenzamine and propranolol blocked α- and β-adrenergic receptors; α-methyldopa entered sympathetic nerves and was metabolized to α-methylnoradrenaline which then acted as a false neurotransmitter. With the exception of patients with phaeochromocytomas, earlier findings attempting to relate hypertension to abnormal excretion of catecholamine were contradictory (de Champlain, 1970). Urinary excretion of catecholamines and their metabolites reflected events occurring in the entire body. Thus, an abnormality in catecholamine metabolism occurring in a local, but critical, point in the peripheral tissues or brain was obscured by measuring the total excretion of catecholamines and their metabolic products.

Great progress in our understanding of the biochemistry, pharmacology, and

cell biology of catecholamine-containing nerves and glands has been made during the past 15 years. This was due mainly to the development of sensitive and specific methods for measuring catecholamines in tissues; the use of histofluorimetric techniques that can be used to visualize nerve tracts, the availability of radioactive catecholamines of high specific activity, and the sophisticated use of drugs that can perturb the formation, metabolism and release of catecholamines.

Noradrenaline-containing (sympathetic) nerves are present in the peripheral tissues and in the brain. These nerves consist of a cell body, long axon and nerve terminals. The biosynthetic enzymes for transmitter formation are synthesized in the nuclei of the cell body and are then transported down the axon to the nerve terminals. Terminals of sympathetic nerves are highly branched and contain thousands of swellings or varicosities. The catecholamine neurotransmitters are mainly synthesized in the nerve endings, where they are stored in dense core vesicles.

Upon depolarization, the catecholamine neurotransmitter is released from the nerves by exocytosis. In this process the vesicle fuses with the neuronal membrane, an opening is made, and the noradrenaline as well as all the soluble contents of the vesicle are discharged into the synaptic cleft. The neurotransmitter then diffuses across the synaptic gap to interact with an α- or β-adrenergic receptor on the excitable post-junctional cell to produce a biologic response. The neurotransmitter is rapidly inactivated by re-uptake into the nerve terminal. Other less important mechanisms for the inactivation of noradrenaline involve metabolism by catechol-O-methyltransferase or monoamine oxidase, or physical removal into the blood stream followed ultimately by metabolism by enzymes in the liver and other tissues (Axelrod, 1973).

Catecholamines are synthesized by enzymes present in the sympathetic nerves and adrenal glands as follows: tyrosine $\longrightarrow$ dopa $\longrightarrow$ dopamine $\longrightarrow$ noradrenaline $\longrightarrow$ adrenaline. The first step is catalyzed by the enzyme tyrosine hydroxylase, the second by 1-aromatic amino-acid decarboxylase, and the third by dopamine β-hydroxylase (DBH). The formation of adrenaline from noradrenaline takes place mainly in the adrenal medulla, although recent work indicates that it is also formed in the brain and is catalyzed by the enzyme phenylethanolamine N-methyl-transferase (Hokfelt et al, 1973; Saavedra et al, 1974).

The neurotransmitters in nerves are in a constant state of flux due to changes in storage, release, and synthesis. In spite of rapidly altering rates of release and synthesis, the transmitter maintains a constant level in tissues. This is due to a number of regulatory mechanisms involving rapid negative-feedback inhibition of tyrosine hydroxylase, slower induction of all biosynthetic enzymes due to nerve firing, inhibitory α-adrenergic receptors on presynaptic terminals and induction of biosynthetic enzymes in the adrenal medulla by glucocorticoids (Axelrod, 1973).

Experimental Hypertension and Peripheral Sympathetic Nerves

Several years ago, in collaboration with de Champlain and Krakoff (1969a), our laboratory undertook a study on the effect of experimental hypertension on the uptake, storage, release and metabolism of the sympathetic neurotransmitter. The form of experimental hypertension selected for study was that induced by the combined administration of desoxycorticosterone (DOCA) and sodium chloride. The administration of DOCA and salt to unilaterally nephrectomized rats for six weeks produced a progressive elevation of systolic blood pressure to a level of 200 mm Hg. These hypertensive rats showed an increase in heart weight and about a 10 percent reduction in total body weight.

To study the turnover or utilization of noradrenaline in sympathetic nerves in the heart and other tissues, rats were given ^{3}H-noradrenaline and the decline in specific activity of the neurotransmitter in the tissues was measured. There was a marked increase in the turnover of ^{3}H-noradrenaline in the heart, spleen, intestine, muscle and kidney of rats made hypertensive with DOCA and salt. There was a highly significant inverse relationship between turnover and retention of noradrenaline in the heart and the level of blood pressure in hypertensive rats. There was also a significant inverse relationship between the degree of blood-pressure elevation and endogenous levels of noradrenaline. The increased turnover of noradrenaline in hypertensive rats treated with DOCA and salt was confirmed by other experimental approaches; increased disappearance of noradrenaline after blockade of its synthesis by α-methyltyrosine or after its synthesis from radioactive dopamine. Using differential centrifugation techniques to separate various subcellular fractions, it was found that the leakage of noradrenaline from storage granules in nerves of hypertensive rats was markedly accelerated.

DOCA-salt induced hypertension causes a disturbance in the ionic content of vascular tissues, especially an increase in sodium. To reduce the salt content, hypertensive rats were placed on a sodium-restricted diet. Within two weeks from being placed on a sodium-deficient diet, together with a naturetic agent, both the blood pressure and the turnover of noradrenaline returned to normal levels. These results indicated the importance of sodium in altering the metabolism of noradrenaline in DOCA-salt hypertension.

There is also an increase in peripheral sympathetic-nerve activity in other forms of experimental hypertension. There is an acceleration in turnover of noradrenaline in hypertension caused by renal encapsulation (Volicer et al, 1968; Henning, 1969). Neurogenic hypertension resulting from denervation of the carotid sinus and aortic arch leads to an increase in peripheral sympathetic-nerve activity in rabbits (deQuattro et al, 1969; Chalmers and Wurtman, 1971).

In contrast to other forms of hypertension, there is a decrease in the turnover of noradrenaline in the hearts of spontaneously hypertensive rats (Louis et al, 1968). It was concluded that these changes in noradrenaline metabolism do not

play a significant role in the pathogenesis of genetically determined hypertension, but are secondary manifestations of blood-pressure elevation. Spontaneously hypertensive rats show an increased activity of tyrosine hydroxylase and dopamine β-hydroxylase in the adrenal gland. When compared to a normotensive strain from which the spontaneously hypertensive rats were bred, very young hypertensive rats showed increased serum dopamine β-hydroxylase activity (Nagatsu et al, 1974).

Experimental Hypertension and the Adrenal Medulla

To examine whether there are compensatory changes in the rate of biosynthesis of catecholamines in DOCA-salt hypertension, the conversion of ^{14}C-tyrosine to ^{14}C-catecholamines was examined (de Champlain et al, 1969b). The conversion of radioactive tyrosine to catecholamines was normal in the hearts of hypertensive rats but was markedly increased in the adrenal gland. An increase in turnover of noradrenaline in sympathetic nerves of hypertensive rats in the absence of any change in the synthesis rate might explain the reduction of endogenous noradrenaline observed in the tissues of animals made hypertensive by DOCA and salt. The increase in synthesis of catecholamines from tyrosine in the adrenal gland indicates that there is an increase in the firing of the splanchnic nerve which innervates the adrenal as well as compensatory synthesis of the catecholamines.

The relationship between sympathetic nerves and the adrenal medulla was examined by de Champlain and van Ameringen (1972). Experiments were carried out using 6-hydroxydopamine, a compound that selectively destroys sympathetic-nerve terminals without affecting the adrenal medulla. Chemical sympathectomy alone or bilateral removal of the adrenal gland reduced blood pressure in rats made hypertensive with DOCA and salt, but the blood pressure was not reduced to normal. When rats were subjected to both chemical sympathectomy and adrenalectomy there was a greater fall in blood pressure in hypertensive rats than in normotensive animals. Blood pressures recorded after the 6-hydroxydopamine treatment and adrenalectomy were essentially the same in hypertensive and normotensive rats. All these experiments again illustrated that there is a marked increase in activity in sympathetic nerves and especially in adrenal medulla in DOCA-salt hypertension.

Removal of the adrenal gland results in a marked increase in the turnover of cardiac noradrenaline (Landsberg and Axelrod, 1968). On the other hand, chemical sympathectomy with 6-hydroxydopamine results in an increased firing of the splanchnic nerves of the adrenal gland (Thoenen et al, 1969). The increased activity of the sympathetic nerves to adrenal medulla (as indicated by elevated tyrosine hydroxylase activity) can be abolished by cutting the preganglionic or splanchnic nerves. These observations suggest that the central nervous system is involved in the increased activity in the peripheral sympathetic nerves and the adrenal medulla.

Central Noradrenergic Nerves and Hypertension

Increased turnover of noradrenaline in peripheral tissues of hypertensive rats produced by DOCA and salt could originate locally or in the brain. To distinguish between these sites, the effects of ganglionic blocking agents were examined (de Champlain et al, 1968). Within 24 hr of the administration of chlorisondamine, a ganglionic blocking agent, the blood pressure of the hypertensive rats fell to normal limits while normotensive rats became slightly hypotensive. The turnover of noradrenaline in the heart was markedly slowed in hypertensive rats and somewhat diminished in normotensive animals after ganglionic blockade. There was then no significant difference in the turnover of noradrenaline in the DOCA-salt treated and in the normal rats. These results indicated that the central nervous system is involved in the increased blood pressure and noradrenaline turnover in DOCA-salt hypertension.

Nakamura et al (1971) examined the turnover of noradrenaline in various brain areas by observing the rate of disappearance of intraventricularly administered ^{3}H-noradrenaline or by measuring the disappearance of endogenous noradrenaline after blockade of synthesis of the neurotransmitter. These investigators found a reduced turnover of noradrenaline in the hypothalamus and brain stem but not in other brain areas. It was also found that an intraventricular injection of 6-hydroxydopamine prevented the development of DOCA-salt hypertension in newborn animals but did not reverse it, once developed (Finch et al, 1972).

These results suggest that there is a reciprocal relationship between the turnover of noradrenaline in the brain stem and the peripheral tissues. This further indicated that noradrenergic neurons in the brain stem inhibit the peripheral sympathetic nerves. Thus, the decreased activity of certain central noradrenergic nerves produced by DOCA-salt hypertension removes the inhibition of peripheral nerves and allows the blood pressure to become elevated. This would explain the hypotensive actions of clonidine. This drug stimulates inhibitory central α-adrenergic receptors which, in turn, decrease activity of the peripheral sympathetic nerves (Haeusler, 1973). α-methyldopa could exert its blood-pressure-lowering effects centrally because it crosses the blood/brain barrier (Henning and Rubenson, 1970). In noradrenergic nerves in the brain stem the methyldopa is then decarboxylated to α-methyldopamine and then β-hydroxylated to α-methylnoradrenaline. The α-methylnoradrenaline liberated from nerves will then stimulate central adrenergic receptors. This in turn would increase the activity of inhibitory interneurons to the peripheral nerves.

Chalmers and Wurtman (1971) have observed that when arterial baroreceptors are de-afferentated there is an increase in the activity of bulbospinal noradrenergic neurons. Intercisternal injection of 6-hydroxydopamine destroys noradrenergic nerves in the spinal cord. This procedure abolishes neurogenic hypertension produced by cutting the carotid and aortic nerves (Chalmers and Reid, 1972). Thus, there appears to be a reciprocal relationship between the brain stem

noradrenergic nerves and bulbospinal nerves. These latter nerve fibres connect with the baroreceptor reflexes which in turn regulate the blood pressure via peripheral nerves.

Doba and Reis (1974) reported that bilateral lesions in the nucleus tractus solitarius (NTS) in the medulla oblongata cause a rapid and lethal arterial hypertension. This presumably results from central de-afferentation of inhibitory baroreceptors. The hypertension produced by lesions of the NTS can be blocked by the administration of α-adrenergic blocking agents and by the intracisternal injection of 6-hydroxydopamine. Intracisternal injection of 6-hydroxydopamine specifically destroys noradrenaline containing bulbospinal tracts. These findings implicate the bulbospinal noradrenergic neurons in the expression of the NTS hypertension.

Local injection of 6-hydroxydopamine into the lateral hypothalamus does not diminish NTS hypertension, while injection of this amine into the NTS tract results in a transient elevation of blood pressure. According to Reis, it appears that noradrenergic tracts in the brain have opposing central actions on arterial blood pressure, depending on the site of origin and release. Noradrenaline in tracts in NTS inhibits an elevation in blood pressure but in tracts in the spinal cord it facilitates a rise in blood pressure.

It has been observed that phenylethanolamine N-methyltransferase (Saavedra et al, 1974) and a fluorescent antibody to this enzyme (Hokfelt et al, 1973) are highly localized to the NTS. This would suggest that this area of the brain stem, which is very much involved in blood-pressure regulation, has high numbers of adrenaline-containing nerves.

It is becoming increasingly apparent that specific catecholamine-containing nuclei in the brain stem and the hypothalamus are involved in regulating blood pressure. Some of these nuclei control inhibitory neuronal systems while others affect stimulating nerves. Recent reports from our laboratory have described microdissection techniques for the removal of individual nuclei in the brain stem, hypothalamus and limbic areas (Palkovits et al, 1974), as well as very sensitive enzymatic assays for noradrenaline and other biogenic amines and their biosynthetic enzymes. These microdissection procedures together with the sensitive assays have made it possible to map the content of biogenic amines and other biosynthetic enzymes in about 130 brain nuclei. It would be of interest to see which specific brain nuclei are affected in the various forms of hypertension.

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The Topography of Central Catecholamine Pathways in Relation to their Possible Role in Blood Pressure Control

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Summary

It is proposed that the A pathways innervating autonomic centres, especially those in the medulla oblongata, represent an important vasodepressor system, and that the clonidine-induced hypotension is mediated via direct stimulation of A receptors. The α-methyldopa and dopa-induced hypotension might be mediated via increased A receptor activity. It is speculated that the central NA pathways innervating autonomic centres at various levels may represent a vasopressor system. The possible role of the mesolimbic and nigro-neostriatal DA pathways in blood pressure control remains to be elucidated.

Introduction

About ten years after the original mapping of the central catecholamine (CA) cell bodies and their terminal networks (Anden et al, 1966; Carlsson et al 1962; Dahlström and Fuxe, 1964; Dahlström and Fuxe, 1965; Fuxe, 1965a; Fuxe, 1965b; Fuxe et al, 1969b; Fuxe et al, 1970) a large number of papers have appeared, which give additional important information concerning the distribution of CA in the brain and the spinal cord. These studies have been performed with the following techniques: the Falck-Hillarp technique (Battista et al, 1972; Di Carlo et al, 1973; Hubbard and Di Carlo, 1973, 1974a, 1974b; Jacobowitz and Palkovits, 1974; Palkovits and Jacobowitz 1974); a modification of the Falck-Hillarp technique involving the glyoxylic-acid fluorescence method combined with Vibratome sections (Hökfelt and Ljungdahl, 1972; Lindvall and Bjorklund, 1974a; Lindvall and Bjorklund, 1973); immunohistochemical techniques for the demon-