Catecholamines and hypertension

J. AXELROD
Section on Pharmacology, Laboratory of Clinical Science, National Institute of Mental Health, Bethesda, Maryland, U.S.A.

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Ever since the demonstration that the active principle in the adrenal medulla can raise blood pressure (Oliver & Schafer, 1895), catecholamines have been implicated in the hypertensive diseases. The identity of noradrenaline as the neurotransmitter of sympathetic nerves (von Euler, 1946) and the demonstration that an injection of this catecholamine can produce cardiovascular changes similar to those observed in hypertensive patients (Goldenberg, Piner, Baldwin, Greene & Roh, 1948) pointed to an abnormal sympathetic nervous activity in this disease. Possibly the most compelling evidence of the involvement of the sympathetic nervous system and the adrenal medulla is the demonstration that drugs affecting the adrenergic systems are therapeutically useful in the treatment of hypertensive diseases. These anti-hypertensive drugs act at a variety of sites in the adrenergic system: the uptake, storage, release and metabolism of noradrenaline and on adrenergic receptors. Guanethidine depletes noradrenaline in the peripheral sympathetic nerves and the adrenal medulla is the demonstration that drugs affecting the adrenergic systems are therapeutically useful in the treatment of hypertensive diseases. These anti-hypertensive drugs act at a variety of sites in the adrenergic system: the uptake, storage, release and metabolism of noradrenaline and on adrenergic receptors. Guanethidine depletes noradrenaline in the peripheral sympathetic nerves, reserpine depletes catecholamines peripherally and in the brain; α-methyldopa is metabolized to a false neurotransmitter, α-methylnoradrenaline; propranolol blocks β-adrenoreceptors; clonidine stimulates α-adrenoreceptors in the brain.

The fact that drugs influencing the disposition of the neurotransmitter noradrenaline alleviate hypertension does not necessarily mean that sympathetic nerve dysfunction is the primary cause of hypertension, but it is an important factor. The degree of involvement of sympathetic nerves may vary with different types of hypertension and during different phases of the development of hypertensive diseases. The development of anti-hypertensive drugs that act on the sympathetic nervous system was made possible by the interaction of basic and clinical sciences. These drugs increased the survival time of patients with hypertensive diseases dramatically and their introduction represents a major achievement in modern medicine.

In hypertension produced by phaeochromocytomas there is a marked elevation in the excretion of noradrenaline and its metabolites (Crout, Pisano & Sjoerdsm, 1961). However, attempts to relate abnormal excretion of catecholamines in other types of hypertension were contradictory (de Champlain, 1972). It is likely that in hypertension there is an aberration in catecholamine metabolism in local but critical sites in the peripheral and/or central sympathetic nerves. This then would not be reflected in detectable elevation of catecholamine or its metabolites since urinary excretion represents metabolism of these amines in the entire body.

Storage, release and metabolism of catecholamines

In the past decade or so there have been great advances in our knowledge concerning the pharmacology, physiology and biochemistry of the sympathetic (catecholamine-containing) nervous system (Axelrod, 1973). With our understanding of the action of these nerves, the questions regarding the relationship between hypertension and catecholamine neurotransmitters have become more sophisticated.

There are three catecholamine-containing neurotransmitters: noradrenaline, dopamine and adrena-
416s  J. Axelrod

line. Noradrenaline-containing nerves are present in the peripheral and central nervous system, and dopamine- and adrenaline-containing nerves are found in the central nervous system. The adrenal medulla contains adrenaline and noradrenaline. Catecholamine-containing nerves consist of a cell body, long axon and nerve terminals. Nerve endings are highly branched and contain many thousand varicosities for each cell body. There are four biosynthetic enzymes involved in catecholamine biosynthesis: tyrosine hydroxylase, aromatic L-amino acid decarboxylase, dopamine-β-hydroxylase, phenylethanolamine N-methyltransferase. These enzymes are formed in the nuclei of the cell body and are then transported down the axons to the nerve endings. The catecholamine neurotransmitters are, for the most part, synthesized in the nerve terminals and then stored in dense core vesicles. Dopamine-containing nerves lack dopamine-β-hydroxylase and phenylethanolamine N-methyltransferase, whereas the methylating enzyme is absent in noradrenaline-containing nerves. The adrenal medulla has all four enzymes.

When sympathetic nerves are stimulated, the catecholamine-containing vesicles fuse with the neuronal membrane; an opening is then made, allowing the catecholamine and the soluble contents of the vesicles to be discharged into the synaptic cleft. A similar process of exocytosis takes place in the adrenal medulla. The liberated neurotransmitter then diffuses across the synaptic gap to bind a specific receptor on the post-junctional cell and produce the characteristic response of the cell. Catecholamine neurotransmitters are rapidly inactivated by re-uptake into the neurons, metabolism by monoamine oxidase or catechol-O-methyltransferase or physical removal by the blood stream and ultimate metabolism by the liver and other tissues.

Neurotransmitters are in a dynamic state, constantly being released, metabolized and synthesized. In spite of these rapid changes, the neurotransmitters maintain a steady level in the nerves. This is due to a variety of regulatory mechanisms involving rapid inhibition or disinhibition of tyrosine hydroxylase, increased or decreased synthesis of biosynthetic enzymes, inhibitory α-adrenoreceptors on presynaptic nerve terminals, changes in responsiveness of post-junctional receptors and induction of biosynthetic enzymes in the adrenal medulla by glucocorticoids. Thus abnormalities in catecholamine synthesis, storage, release, metabolism and responsiveness of receptors in the brain, cardiovascular system or adrenal medulla could initiate and/or sustain hypertension.

**Plasma catecholamine and dopamine-β-hydroxylase in hypertension**

Measurement of catecholamine changes in plasma might be a useful index of sympathetic nerve activity. Until recently attempts to relate circulatory catecholamine concentrations in hypertensive diseases were unsatisfactory, mainly due to the lack of specificity and sensitivity in the methods for measuring plasma catecholamines. With the use of the catechol-O-methyltransferase and radioactive methyl donor S-adenosylmethionine (Axelrod, Insoe & Daly, 1965) it is now possible to measure nanogram amounts of catecholamines in blood (Engelman, Portnoy & Sjoerdsma, 1970). In the past 5 years or so several investigators have reported the plasma concentrations of catecholamines in various types of hypertension (Engelman *et al.*, 1970; de Quattro & Chan, 1972; Louis, Doyle & Anavekar, 1973; de Champlain, Farley, Cousineau & van Ameringen, 1976) (Table 1). In almost all cases significant elevation of plasma catecholamines were found in these patients. However, in these experiments the control groups were not matched in age and tended to be younger than the hypertensive population. In a study in which hypertensive subjects were age-matched, there was no difference in plasma noradrenaline when blood was taken in either a sitting or a standing position between the normal and hypertensive groups (Lake, Ziegler, Coleman & Kopin, 1976). Since there is a tendency for a gradual rise in plasma catecholamines with age, it will be important to use age-matched controls in future studies.

Dopamine-β-hydroxylase, the enzyme that converts dopamine into noradrenaline, is present in plasma of man and animals (Weinshilboum & Axelrod, 1971). This enzyme is highly localized in sympathetic nerves and is released on nerve stimulation. Its level in plasma might serve as an index of sympathetic nerve activity. Comparisons of dopamine-β-hydroxylase activity in plasma from normal and hypertensive subjects were reported by several laboratories with contradictory results. Some laboratories found elevated dopamine-β-hydroxylase activity (Wetterberg, Aberg, Ross & Froden,
Catecholamines and hypertension

Table 1. Plasma catecholamines and human hypertension

<table>
<thead>
<tr>
<th>Hypertension</th>
<th>Age-matched</th>
<th>Conc. of plasma catecholamine</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential</td>
<td>No</td>
<td>Elevated</td>
<td>Engelman et al. (1970)</td>
</tr>
<tr>
<td>Stabilized and labile</td>
<td>No</td>
<td>Elevated</td>
<td>de Quattro &amp; Chan (1972)</td>
</tr>
<tr>
<td>Essential</td>
<td>No</td>
<td>Elevated</td>
<td>Geffen et al. (1973)</td>
</tr>
<tr>
<td>Essential</td>
<td>No</td>
<td>Elevated</td>
<td>Louis et al. (1973)</td>
</tr>
<tr>
<td>Essential</td>
<td>No</td>
<td>Elevated</td>
<td>de Champlain et al. (1976)</td>
</tr>
<tr>
<td>Essential</td>
<td>Yes</td>
<td>No change</td>
<td>Lake et al. (1976)</td>
</tr>
</tbody>
</table>

1972; Geffen, Rush, Louis & Doyle, 1973; Schanberg, Stone, Kirshner, Gunnells & Robinson, 1974) whereas others found no change (Horwitz, Alexander, Lovenberg & Keiser, 1973; Lake et al., 1976). In a study using age-, sex- and race-matched control subjects no difference in plasma enzyme was observed (Lovenberg, personal communication). Plasma dopamine-β-hydroxylase levels remain highly constant in normotensive adults over a period of 3–7 years (Lamprecht, Andres & Kopin, 1975). In four of nine patients who developed high blood pressure there was a decrease in serum dopamine-β-hydroxylase, but there were no significant changes in serum enzyme level in all subjects examined where there was no change in blood pressure over the same period of time.

Abnormal sympathetic and adrenal medullary activity in experimental hypertension

To examine the relationship between sympathetic nerve activity and hypertension the turnover of the noradrenaline was studied in rats (de Champlain, Krakoff & Axelrod, 1967). Experimental hypertension was produced by giving sodium chloride and deoxycorticosterone (DOCA) to unilaterally nephrectomized rats. Normotensive and hypertensive animals were injected with [3H]noradrenaline, which was previously shown to be selectively taken up by sympathetic nerves of tissues. The rate of decline of the specific radioactivity of the neurotransmitter in the rat brain and other tissue was taken as a measure of turnover. There was considerable increase in noradrenaline turnover in the sympathetic nerves, the heart, spleen, kidney, muscle and intestines of hypertensive rats. The higher the blood pressure elevation, the greater the turnover of noradrenaline. The increased turnover of noradrenaline in hypertensive rats was confirmed by other procedures, e.g. more rapid conversion of dopamine into noradrenaline; increased disappearance of noradrenaline after blockade of biosynthetic enzyme. Elevated plasma concentrations of catecholamines were

![Fig. 1. Blood pressure, sodium intake and turnover of noradrenaline in DOCA-salt hypertension in the rat. (a) Percentage changes in blood pressure and (b) percentage changes in retention of [3H]noradrenaline (3HNA) after treatment with various sodium regimens. 3HNA was measured in the rat heart 4 h after an intravenous injection of the radioactive catecholamine. The amount of 3HNA retained is a measure of turnover of the catecholamine neurotransmitter. As blood pressure returned to normal on a salt-restricted diet the retention of 3HNA increased. Each point is the mean of values obtained from six to eight rats. (From de Champlain et al., 1968.)](image-url)
found in rats with DOCA-salt hypertension (de Champlain et al., 1976; Reid, Zivin & Kopin, 1975). The circulatory amounts of catecholamines as well as the turnover of noradrenaline began to rise before the development of hypertension in DOCA-salt-treated rats, suggesting that abnormalities in sympathetic nerve function precedes the rise in blood pressure (de Champlain, Krakoff & Axelrod, 1968).

To study the interrelations between sodium ions, sympathetic nerve activity and blood pressure changes, DOCA-salt hypertensive rats were placed on a sodium-deficient diet and salt-depleting drugs. On such a salt-restricted regimen, blood pressure and catecholamine turnover returned to normal levels in hypertensive rats within 2 weeks (Fig. 1). Increased sympathetic nerve activity was also found in other forms of hypertension: renal encapsulation (Volicer, Scheen, Hilse & Wisweswaram, 1968) and neurogenic hypertension (de Quattro, Nagatsu, Maronde & Alexander, 1969).

Catecholamine metabolism in the adrenal medulla is also disturbed in DOCA-salt hypertension (de Champlain, Mueller & Axelrod, 1969). The conversion of radioactive tyrosine into catecholamines is increased in the adrenal glands of hypertensive rats. The increased synthesis of catecholamines in experimental hypertension indicates increased splanchnic nerve activity and a compensatory synthesis of catecholamines.

The destruction of peripheral sympathetic nerves with an injection of 6-hydroxydopamine partially reduced blood pressure in rats made hypertensive with DOCA and salt (de Champlain & van Ameringen, 1972). Bilateral removal of the adrenal gland also partially reduced blood pressure in hypertensive rats. Chemical sympathectomy and removal of the adrenal gland resulted in a greater fall in blood pressure in hypertensive rats as compared with normotensive rats.

There is a decreased turnover of noradrenaline in the hearts of genetically spontaneously hypertensive rats (Louis, Spector, Tabei & Sjoerdsma, 1969). In young rats prone to spontaneous hypertension, there is an increase in dopamine-β-hydroxylase levels in plasma (Nagatsu, Kato, Namata, Ikuta, Umezawa, Matsuzaki & Takeuchi, 1974; Nagatsu, Ikuta, Numata, Kato, Sano, Nagatsu, Umezawa, Matsuzaki & Takeuchi, 1976) and in noradrenaline concentrations in blood (Grobecker, Roizen, Weise, Saavedra & Kopin, 1975). The enzyme in blood returns to normal values as blood pressure develops. This indicates that sympathetic nerves might be involved in the initiation of spontaneous hypertension.

**Hypertension and central noradrenergic nerve activity**

When a ganglion-blocking agent was given to rats with DOCA-salt hypertension the turnover of noradrenaline was reduced, and there was no significant difference in the turnover of noradrenaline in the hearts of normotensive and hypertensive rats (de Champlain et al., 1968). Ganglion-blocking agents also reduced blood pressure of hypertensive rats to normal values. These findings indicated that the elevated peripheral sympathetic nerve activity in DOCA-salt hypertension had a central nervous system component. Destruction of central catecholaminergic neurons by an intracerebroventricular injection of 6-hydroxydopamine prevented the DOCA-salt-induced rise in blood pressure (Reid et al., 1975).

Studies on noradrenergic nerve activity in the brain showed a decreased turnover of noradrenaline in the brain stem and hypothalamus but not in other brain regions (Nakamura, Gerold & Theonen, 1971). These findings suggested a reciprocal relationship between noradrenergic nerve activity in certain parts of the brain and the peripheral sympathetic nerves. It would appear that the decreased activity of specific central noradrenergic nerves in DOCA-salt hypertension disinhibits peripheral sympathetic nerve activity and thus blood pressure is increased. Consistent with this is the observation that deafferentation of the arterial baroreceptors results in an increased activity of the bulbospinal noradrenergic nerves (Chalmers & Wurtman, 1971). When noradrenergic nerves in the spinal cord are destroyed by an intracisternal injection of 6-hydroxydopamine the neurogenic hypertension produced by cutting the carotid and aortic nerves is abolished (Chalmers & Reid, 1972). These results demonstrate a reciprocal relationship between brainstem noradrenergic nerves and bulbospinal nerves. These latter nerves are involved in blood pressure regulation via baroreceptor reflexes and peripheral nerves.

The inhibitory action of certain central noradrenergic neurons on peripheral blood pressure regulation would explain the anti-hypertensive action of centrally acting drugs. Clonidine stimulates
the inhibitory α-adrenoreceptors in the brain, which then reduces the activity of peripheral sympathetic nerves (Haeseusler, 1973). Another anti-hypertensive drug acting in the central nervous system is α-methyl-dopa (Henning & Rubenson, 1971). Like dopa, this amino acid can cross the blood–brain barrier. Once in the brain it is taken up by noradrenergic nerves and decarboxylated to α-methyl-dopamine and then β-hydroxylated to α-methylnoradrenaline. The α-methylnoradrenaline can be liberated from nerve terminals and stimulate inhibitory postsynaptic α-adrenoreceptors. An area of the brain stem involved in the regulation of blood pressure is the nucleus tractus solitarius. Lesions in this area of the brain cause a rapid and lethal hypertension (Doba & Reis, 1974). Hypertension caused by lesions of the nucleus tractus solitarius can be abolished by the administration of adrenoceptor-blocking drugs and an intracisternal injection of 6-hydroxydopamine. Such injections have been shown to destroy the noradrenergic nerves in the bulbo-spinal tract. Injection of 6-hydroxydopamine directly into the hypothalamus does not reduce hypertension caused by lesions of the nucleus tractus solitarius. Destruction of noradrenergic tracts of the nucleus tractus solitarius by 6-hydroxydopamine causes a brief elevation of blood pressure. These observations suggest that noradrenergic tracts in the brain have opposing actions, depending on their localization. Noradrenergic tracts in the spinal cord elevate blood pressure and these catecholamine tracts in the nucleus tractus solitarius inhibit blood pressure elevation.

Adrenergic nerves in the brain and hypertension

Workers in our laboratory have described microdissection procedures to isolate specific nuclei in the hypothalamus and other brain areas, and enzymatic assays to measure noradrenaline and dopamine in picogram amounts (Palkovits, Brownstein, Saavedra & Axelrod, 1974). This enabled us to measure the catecholamine content in about 150 nuclei in the brain. In view of the important role that noradrenergic nerves in the brain play in the regulation of blood pressure, its pathology and treatment, we suspected that the availability of these microprocedures might prove to be of value in blood pressure research.

Recently the presence of adrenaline-containing neurons has been demonstrated in the brain by several techniques. Phenylethanolamine N-methyltransferase, the enzyme that converts noradrenaline into adrenaline (Axelrod, 1962), has been found and quantitatively measured in several brain nuclei (Saavedra, Palkovits, Brownstein & Axelrod, 1974) by the microdissection procedure. This enzyme has been visualized in similar brain nuclei by immunological techniques employing an antibody directed toward phenylethanolamine N-methyltransferase (Hökfelt, Fuxe, Goldstein & Johansson, 1973). By direct measurement, adrenaline has also been detected in similar brain regions (Koslow & Schlumpf, 1974). The highest concentration of phenylethanolamine N-methyltransferase-containing nerves has been found in the A₁ region of the brain stem, in the lateral reticular nucleus of the medulla oblongata between the pyramidal tract and the tractus solitarii (Saavedra et al., 1974). Smaller amounts of the adrenaline-forming enzyme were present in the locus coeruleus, a region containing noradrenergic cell bodies. Phenylethanolamine N-methyltransferase activity was detected in all of the hypothalamic nuclei examined but was irregularly distributed. The largest concentration was present in the basal hypothalamus (median eminence, arcuate and para ventricular nuclei); the activity could not be detected in cortical areas, caudate, amygdala and olfactory tubercles.

The A₁ and A₂ areas are involved in blood pressure regulation and contained highest concentrations of the adrenaline-forming enzyme. This association led us to examine phenylethanolamine N-methyltransferase activity in these and other nuclei in rats with DOCA-salt hypertension and spontaneous hypertension (Saavedra, Grobecker & Axelrod, 1976). A considerable increase in plasma dopamine-β-hydroxylase activity in plasma and blood vessels was found in spontaneously hypertensive rats at 4 weeks of age at a time when blood pressure is not elevated (Nagatsu et al., 1974, 1976). Blood levels of dopamine-β-hydroxylase fell to normal values in adult rats when hypertension was fully developed. A marked elevation (60%) of phenylethanolamine N-methyltransferase activity was observed in A₁ and A₂ regions of 4-weeks-old spontaneously hypertensive rats compared with their controls (Kyoto/Wistar rats) (Saavedra et al., 1976); the enzyme’s activity in the locus coeruleus remained unchanged. We are in the process of examining this enzyme activity in the brains of adult spontaneous hypertensive rats. In adult DOCA-salt hypertensive
TABLE 2. Brain phenylethanolamine N-methyltransferase activity and hypertension

Unilateral nephrectomized Sprague-Dawley rats were given 25 mg of deoxycorticosterone/kg weekly and a 1% solution of sodium chloride ad libitum for 4 weeks. SK & F 7698 (200 mg/kg) was administered to five rats orally twice daily throughout the fourth week of DOCA–salt treatment, until blood pressure was elevated to 170 mmHg in a group of five control rats. The brains were removed, frozen, specific nuclei dissected and examined for the enzyme. Results are expressed as mean values ± SEM. * P < 0.01, compared with control group; ** P < 0.02, compared with non-drug-treated group. (From Saavedra et al., 1976.)

<table>
<thead>
<tr>
<th>Activity (units)</th>
<th>Control DOCA–salt</th>
<th>+ SK &amp; F 7698</th>
<th>DOCA–salt + SK &amp; F 7698</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain region</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>40.9 ± 4.5</td>
<td>89.9 ± 13.3*</td>
<td>30.6 ± 0.7**</td>
</tr>
<tr>
<td>A2</td>
<td>119 ± 2.8</td>
<td>119 ± 10.3</td>
<td>80.2 ± 3.2**</td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>17.7 ± 0.9</td>
<td>25.2 ± 4.4</td>
<td>15.5 ± 0.3</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>120</td>
<td>170</td>
<td>110</td>
</tr>
</tbody>
</table>

Rats (170 mmHg) activity was more than doubled in the A1 region (Table 2). There were no changes in enzyme activity in the A2 region or locus coeruleus. No changes in noradrenaline and dopamine concentration and tyrosine hydroxylase activity in the A1 or A2 regions in the two types of hypertension were observed.

The increase in the adrenaline-forming enzyme in the rat brain stem suggests that the pharmacological manipulation of the enzyme might affect blood pressure. Several years ago we found that tranylcypromine (trans-DL-2-phenylcyclopropylamine) and related compounds inhibit adrenal phenylethanolamine N-methyltransferase in vitro (Krakoff & Axelrod, 1967). Recently SK & F 7698 (3-methyl-1,2,3,4-tetrahydro-(1-benzothieno)-3,2-c pyridine hydrochloride) was shown to inhibit the enzyme in vivo, and lower the amount of adrenaline in the adrenal gland by 80% (Pendleton, Kaiser, Gessner, Finlay & Greene, 1974). We then examined the effect of SK & F 7698 on brain phenylethanolamine N-methyltransferase activity and blood pressure in DOCA–salt hypertensive rats (Saavedra et al., 1976). The oral administration of SK & F 7698 for 7 days reduced phenylethanolamine N-methyltransferase activity to below normal levels in the A1 and A2 regions, but not in the locus coeruleus of hypertensive rats. The administration of the enzyme inhibitor reduced the blood pressure of DOCA–salt hypertensive rats from 170 mmHg to normal pressure, 120 mmHg (Table 2). Because of the limited supply of SK & F 7698 we were unable to examine the effect of this drug in spontaneously hypertensive rats. Inhibitor SK & F 7698 inhibits the enzyme in the adrenal medulla and also has some α-adrenoceptor-blocking action and thus the site and specificity of its effects remain to be determined. However, the development of the inhibitor with selective central or peripheral action may provide a new type of drug to treat hypertension and to clarify its site and mechanism of action.

The increase in brain-stem phenylethanolamine N-methyltransferase activity in experimental and genetic hypertension indicates that formation of adrenaline is increased during the development of hypertension. The increased activity of the enzyme in A1 and A2 regions of the brain during the early phase of hypertension might involve a central mechanism for the initiation of hypertensive disease or it might be a compensatory mechanism in response to increased peripheral sympathetic nerve activity.

References

CHALMERS, J.P. & WURTMAN, R.J. (1971) Participation of
Catecholamines and hypertension

421s


Kraffok, L.R. & Axelrod, J. (1967) Inhibition of phenyl-


