Peripheral and central catecholaminergic neurons in genetic and experimental hypertension in rats

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Summary

1. Activity of peripheral and central catecholaminergic neurons was studied in spontaneously hypertensive rats (SHR) and deoxycorticosterone (DOCA)-salt hypertensive rats.

2. In young SHR (4 weeks) the plasma values of both noradrenaline and dopamine-β-hydroxylase activity were increased compared with those of normotensive rats of the Wistar/Kyoto strain. Total catecholamines (mostly adrenaline) were not significantly different.

3. In the adrenal glands of 2-weeks-old and 4-weeks-old SHR activities of tyrosine hydroxylase, dopamine-β-hydroxylase, phenylethanolamine-N-methyl transferase were decreased, compared to Wistar/Kyoto rats.

4. The adrenaline-forming enzyme was elevated in the A₁ and A₂ regions of the brain stem of 4-weeks-old SHR and in the A₁ region of adult DOCA–salt hypertensive rats.

5. In the adrenal glands of adult DOCA–salt hypertensive rats tyrosine hydroxylase activity was increased.

6. These results implicate peripheral noradrenaline-containing neurons and central adrenaline-containing neurons in the development of genetic and experimental hypertension in rats.

Key words: adrenaline, brain stem, deoxycorticosterone–salt hypertensive rats, dopamine-β-hydroxylase, noradrenaline, phenylethanolamine-N-methyl transferase (noradrenaline-N-methyltransferase), tyrosine hydroxylase.

Introduction

It is well known that peripheral and central catecholaminergic neurons may play a role in the regulation of blood pressure and the expression of some forms of experimental hypertension (de Champlain, Krakoff & Axelrod, 1969; Louis, Spector, Tabai & Sjoerdsma, 1969; de Quattro, Nagatsu, Maronde & Alexander, 1969; Nakamura, Gerold & Thoenen, 1971; Chalmers & Wurtman, 1971). Experiments in rats are usually performed on two forms of hypertension: first in spontaneously hypertensive rats derived from a normotensive Wistar/Kyoto strain (Okamoto & Aoki, 1963); secondly in DOCA (1)-salt hypertensive rats, in which a progressive elevation of systolic blood pressure is induced by combined administration of DOCA and salt to unilaterally nephrectomized animals. In the studies quoted, activity of peripheral and central catecholaminergic neurons was measured by fluorimetric determinations of catecholamines, for example in heart and brain stem, or by investigation of the turnover of noradrenaline.

Controversial results prompted us to reinvestigate the role of the sympatho-adrenal system in the development of experimental hypertension. The activity of the sympatho-adrenal system was examined by measuring noradrenaline and adrenaline in the plasma and adrenal glands. Activities of adrenal tyrosine hydroxylase, dopamine-β-hydroxylase and phenylethanolamine-N-methyltransferase, and plasma dopamine-β-hydroxylase, were examined. In specific regions of the brain stem of SHR and

(1) Abbreviations: DOCA, deoxycorticosterone acetate; SHR, spontaneously hypertensive rats; WK, Wistar/Kyoto strain; WNN, normotensive rats from N.I.H. colony; DBH, dopamine-β-hydroxylase; PNMT, phenylethanolamine-N-methyltransferase.
DOCA-salt hypertensive rats the activity of the adrenaline-forming enzyme (phenylethanolamine-N-methyltransferase) was also investigated.

Methods

Male rats of various substrains (Wistar/Kyoto, SHR, normotensive rats from the N.I.H. colony), were decapitated at 4, 8 or 12 weeks, blood was collected and plasma prepared for catecholamine and dopamine-β-hydroxylase activity as described previously (Roizen, Weise, Grobecker & Kopin, 1975). Plasma total catecholamines (adrenaline and noradrenaline) and noradrenaline were assayed radiometrically according to Coyle & Henry (1973) and Henry, Starman, Johnson & Williams (1975). Dopamine-β-hydroxylase activity was measured by the method of Molinoff, Weinshilboum & Axelrod (1971). The catecholamine content of the adrenals was assayed fluorimetrically (Anton & Sayre, 1962).

Tyrosine hydroxylase, DBH and phenylethanolamine-N-methyltransferase activities in adrenal glands were assayed radiometrically (Nagatsu, Levet & Undenfriend, 1964; Molinoff et al., 1971; Axelrod, 1962). Individual nuclei of the brain stem were dissected according to the method of Palkovits, Brownstein & Saavedra (1974) and PNMT activity was assayed as described by Saavedra, Palkovits, Brownstein & Axelrod (1974). Unilaterally nephrectomized rats were made hypertensive as described by de Champlain et al. (1969). Blood pressure was measured by means of an indirect tail plethysmog-raph (Narco, Dallas, Texas, U.S.A.).

Results

DBH activity and noradrenaline concentrations in plasma of 8–12-weeks-old SHR with an established systolic blood pressure of 200 mmHg were not significantly different from those values in normotensive WK rats.

On the other hand, there was a highly significant difference in plasma DBH activity between SHR and WK rats compared with WNN rats (Roizen et al., 1975). Because it is well known that DBH activity is genetically determined (Axelrod, 1972), the use of DBH activity in plasma as an index of sympathetic neuronal activity in rats and man appears highly questionable. However, measurements of both DBH activity and circulating noradrenaline or total catecholamines in plasma of rats or man with specific and highly sensitive radiometric methods do allow conclusions to be drawn about the sympathetic tone (Louis, Doyle & Anavekar, 1973; Geffen, Rush, Louis & Doyle, 1973; Planz, Wiethold, Appel, Böhmer, Palm & Grobecker, 1975; Grobecker, Roizen & Kopin, 1975).

Plasma total catecholamines (adrenaline and noradrenaline) were significantly higher at 12 weeks of age than in the control strains. At the age of 4 weeks (the age at which the blood pressure of SHR begins to rise: Jones & Dowd, 1970; Hansen, 1972) both

| TABLE 1. Catecholamine concentrations and biosynthetic enzyme activities in young spontaneously hypertensive rats and DOCA-salt hypertensive rats |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | WK rats         | SHR (4 weeks old) |
|                                 | Control rats (BP 122±4 mmHg) | DOCA-salt hypertensive rats (BP 180±9 mmHg) |
| Plasma:                        |                 |                 |                 |
| Total catecholamines           | 11.9±0.07       | 13.2±0.8        |
| Noradrenaline                  | 1.8±0.14        | 2.93±0.35**     |
| Dopamine-β-hydroxylase         | 7.84±0.24       | 13.4±0.6***     |
| Adrenal glands:                | (4 weeks old)   |                 |
| Tyrosine hydroxylase           | 11.9±0.48       | 8.67±0.24**     |
| Dopamine-β-hydroxylase         | 25.6±2.6        | 17.7±0.92**     |
| Phenylethanolamine-N-methyltransferase | 8.43±0.18 | 5.13±0.12***   |
| Adrenaline                     | 2.15±0.15       | 1.70±0.09*      |
| Noradrenaline                  | 0.85±0.10       | 0.75±0.04       |
|                                 |                 |                 |                 |
|                                 |                 |                 |                 |
| Adrenal glands:                |                 |                 |                 |
| Tyrosine hydroxylase           | 29.2±2.1        | 47.9±4.5**      |
| Dopamine-β-hydroxylase         | 217±15          | 231±27          |
| Phenylethanolamine-N-methyltransferase | 16.8±1.3 | 19.2±1.1      |

DBH activity and noradrenaline were increased over those of the WK rats (Table 1), but the total catecholamines (mostly adrenaline) were not significantly different. The enzyme activities of tyrosine hydroxylase, DBH and PNMT in the adrenals of 4-weeks-old SHR were decreased (Table 1). Also the adrenaline
content of the adrenals of the SHR were lower than those of the WK rats. Enzyme activities of tyrosine hydroxylase, DBH and PNMT in the adrenals of 2-weeks-old SHR were also significantly decreased as in 4-weeks-old SHR (Saavedra Grobecker, & Axelrod, 1976b). Tyrosine hydroxylase activity was increased over the control values in the adrenal glands of DOCA-salt hypertensive rats with a blood pressure of 180 mmHg (Table 1). No significant change of DBH and PNMT activities could be found.

Compared with WK rats, activity of the adrenaline-forming enzyme (PNMT) was markedly elevated (+a%) in the A1 and A2 regions of the brain stem of 4-weeks-old SHR but not in the locus coeruleus (A8). In adult SHR no significant change of PNMT activity could be observed. In adult DOCA-salt hypertensive rats the activity of PNMT in the A1 region was twice as high as in normotensive control rats. There was no significant change in enzyme activity in the A2 region or A8 region (Saavedra, Grobecker & Axelrod, 1976a).

Discussion

Our experiments have shown that at 4 weeks sympathetic neuronal activity in SHR is increased, whereas adrenal medullary activity is depressed. It is likely that this depressed adrenal medullary activity is secondary to the increased sympathetic neuronal activity, since a reciprocal change was observed when sympathetic nerves were destroyed by 6-hydroxydopamine (Thoenen, Mueller & Axelrod, 1969). The exaggerated sympathetic neuronal activity at this age may be an essential factor in the course of development of hypertension in SHR.

Increased plasma noradrenaline concentrations were observed also in DOCA-salt hypertensive rats (Reid, Zivin & Kopin, 1975). Moreover, our investigations showed an increase of tyrosine hydroxylase activity in the adrenal gland of DOCA-salt hypertensive rats. This indicates also an increase in sympatho-adrenal activity in this form of experimental hypertension.

The elevation of PNMT in specific regions of the brain stem indicates that adrenaline production in these neurons is increased during the development of hypertension. This enhanced activity of adrenaline-containing neurons (cf. Hökfelt, Fuxe, Goldstein & Johansson, 1973; Saavedra et al., 1974; Koslow & Schlumpf, 1974) during the early phase of the hypertension may be a compensatory mechanism in response to the increased activity of peripheral sympathetic nerves, or a central mechanism for the initiation of the hypertensive disease. Our results are compatible with earlier suggestions of a possible involvement of central catecholaminergic neurons in hypertension (cf. Chalmers, 1975).

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