Biosynthetic Enzyme Activities and Catecholamines in Adrenal Glands of Genetic and Experimental Hypertensive Rats

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SUMMARY. The activities of catecholamine-synthesizing enzymes in adrenal glands have been investigated during the development of high blood pressure in spontaneously hypertensive rats (SHR) and in rats made hypertensive by administration of DOCA and saline. There was a significant decrease in the activity of tyrosine hydroxylase, dopamine-β-hydroxylase, and phenylethanolamine N-methyltransferase in young spontaneously hypertensive rats at the age of 2 and 4 weeks, when compared with the activity in adrenals of the Wistar-Kyoto substrain. Tyrosine hydroxylase activity was not different between normotensive and hypertensive animals at 8 weeks of age, but was increased in adult SHR. Both dopamine-β-hydroxylase and phenylethanolamine N-methyltransferase activity were still decreased at the age of 8 weeks in SHR, but were not significantly different from the controls at the age of 14 weeks. Blood pressure in SHR was slightly but significantly higher at 4 weeks of age and rose steadily during maturation. In rats made hypertensive by administration of deoxycorticosterone-sodium chloride (DOCA-saline hypertensive rats), tyrosine hydroxylase activity in adrenals was increased after only 1 week of treatment and remained increased after 2 and 4 weeks of treatment. Adrenal epinephrine was increased after 4 weeks of treatment, whereas dopamine-β-hydroxylase, phenylethanolamine N-methyltransferase, and norepinephrine levels were unchanged. Our findings of increased tyrosine hydroxylase in adrenal glands of adult genetically hypertensive and chronic experimentally hypertensive rats indicate a sympathoadrenal activation during the established phase of hypertension in both models. Whereas, in DOCA-saline hypertensive rats, the sympathoadrenal synthesis of catecholamine parallels the development of high blood pressure, in genetically hypertensive rats, the activity of the catecholamine-synthesizing enzymes is decreased early in development. These results suggest the existence of different mechanisms regulating the participation of adrenal catecholamines in both the spontaneously (genetic) and the experimentally induced (DOCA-saline) hypertension in rats.

IN RECENT years, the involvement of the sympathetic nervous system and the adrenal gland during the development and maintenance of high blood pressure in genetic and experimental hypertensive rats has been studied intensively (Axelrod, 1976; de Champlain et al., 1977). Increasing evidence has been accumulated which shows that peripheral noradrenergic nerves, the adrenal medulla, and central noradrenergic and adrenergic neurons may play an important role in the course of development of hypertension in spontaneously hypertensive rats (Nagatsu et al., 1974, 1976; Grobecker et al., 1975; Saavedra et al., 1976; Nagaoka and Loovenberg, 1976; Saavedra et al., 1978; McCarty and Kopin, 1978; Saavedra and Grobecker, 1979; Grobecker et al., 1979). Although the contribution of the adrenal medulla to the regulation of blood pressure in normotensive and DOCA-saline hypertensive rats (de Champlain et al., 1977), as well as in spontaneously hypertensive rats (Aoki, 1964; Ozaki, 1966) has been demonstrated, previous reports on biosynthetic enzyme activities in the adrenal glands of young spontaneously hypertensive rats (SHR) were contradictory (Nagatsu et al., 1971; Grobecker et al., 1975; Saavedra and Grobecker, 1979). Part of these contradictions were possibly related to the study of genetically hypertensive rats of different ages, without a simultaneous comparative study of the sympathoadrenal function during the complete developmental course of the hypertension. In the present study we have investigated the activities of catecholamine-synthesizing enzymes and levels of catecholamines in adrenal glands during the development of high blood pressure in SHR and rats made hypertensive by administration of DOCA and saline.

We report that, in both models of hypertension, there is increased activity of adrenal tyrosine hydroxylase when the animals present established hypertension. The stimulation of the sympathoadrenal system in these models proceeds with different development patterns. Very early in the development of hypertension, DOCA-saline hypertensive rats show increased tyrosine hydroxylase. This increased enzyme activity is maintained during the chronic phase of hypertension. In the spontaneously (genetic) hypertensive rats, catecholamine synthesis is decreased rather than increased at early stages in the development of the hypertension, and only increases later, in adult chronically hypertensive rats.
Methods

Animals

Male spontaneously hypertensive rats (SHR) and normotensive rats of the Wistar-Kyoto substrain (WKY) from which the SHRs derived (Okamoto, 1972) were obtained at different ages (2-14 weeks) from Taconic Farms and were kept under diurnal lighting conditions with the lights on from 6 a.m. to 6 p.m. for at least 3 days before each experiment.

Uninephrectomized or sham-operated male Sprague-Dawley rats (120 g) were obtained from Zivic-Miller Labs and kept under the same conditions as the spontaneously hypertensive rats. The rats received a pellet diet and tap water ad libitum. Hypertension was induced in groups of 10-15 rats by a weekly subcutaneous injection of 25 mg/kg of deoxycorticosterone pivalate (DOCA) (Percorten, Ciba-Geig Corp.) and substituting 1% saline for drinking water.

Blood Pressure Recording

Rats were prewarmed in a plastic cage at 36°C for 10 minutes and habituated to the pressure measurement procedure before the start of the experiment. Systolic blood pressure was measured by a tail cuff plethysmograph method using a pneumatic pulse transducer and a programmed electrophysymonometer (Narco Biosystems, model PE-500). Cuff pressure and pulsatile volume changes were recorded simultaneously on a Grass model 5 Polygraph. The average of at least three measurements for each rat were used for calculations of blood pressure.

Preparation of Adrenal Glands

Animals were killed by decapitation between 9 a.m. and 12 noon. After decapitation of the animals, adrenal glands were rapidly removed, weighed and homogenized in 1.0 ml cold isotonic sucrose. An aliquot (100 μl) of the sucrose homogenate was added to 0.9 ml of 0.4 N perchloric acid for assay of catecholamines. A second aliquot (100 μl) was added to 900 μl 0.005 M Tris-HCl buffer, pH 7.4, containing 0.1% Triton-X for assay of dopamine-β-hydroxylase activity. Another aliquot was taken for protein assay. The remainder of the homogenate was centrifuged in a Sorvall centrifuge at 26,000 g for 10 minutes, and aliquots (100 μl) of the clear supernatant were assayed for tyrosine hydroxylase activity and phenylethanolamine-N-methyltransferase.

Assays for Enzyme Activities and Catecholamines

Tyrosine hydroxylase activity was determined by the method of Nagatsu et al. (1964) by incubation with 3,5-ditritio tyrosine and isolation of the tritiated product by passage over a Dowex 50W-X4 (H⁺) column (Nagatsu et al., 1964).

Dopamine β-hydroxylase activity was assayed by the method of Molinoff et al. (1971) using tyramine as a substrate, and based on the sequential conversion of the product of the β-hydroxylation reaction (octopamine) to a radioactively labeled N-methyl derivative by reaction with partially purified bovine adrenal phenylethanolamine-N-methyltransferase in the presence of S-adenosyl-L-methionine-¹⁴C. The N-methyl derivatives are separated by solvent extraction and their radioactivity is determined by liquid scintillation counting (Molinoff et al., 1971).

Phenylethanolamine-N-methyltransferase activity was measured according to the method of Axelrod (1962) by the use of phenylethanolamine as substrate in the presence of S-adenosyl-L-methionine-methyl-¹⁴C. The radioactive product formed, N-methyl-phenylethanolamine, was separated by organic solvent extraction and the radioactivity determined by liquid scintillation counting (Axelrod, 1962).

Catecholamines (epinephrine and norepinephrine) were assayed according to the method of Anton and Sayre (1962), based on the analysis of the fluorescent spectra of each catecholamine after formation of fluophors by treatment with the oxido-trihydroxyindole procedure (Anton and Sayre, 1962).

Protein Assay

Proteins were measured by the method of Lowry et al. (1951).

Statistical Treatment of Results

Results were expressed as mean ± sem. Significance of the difference between two means was assessed with Student's t-test; a level of significance of P < 0.05 was used.

Results

Development of Blood Pressure in the Spontaneously Hypertensive Rat (SHR)

The systolic blood pressure of groups of 10-12 spontaneously hypertensive rats (SHR) and the respective controls of the Wistar-Kyoto substrain (WKY) are shown in Figure 1. At the age of 4 weeks, the systolic blood pressure in SHRs was slightly but significantly increased, compared with WKY rats. The blood pressure of SHRs steadily rose to 150 to 200 mm Hg at 8 and 14 weeks, respectively. Blood pressure in WKY rats increased only slightly, reaching 120 mm Hg at 14 weeks of age. A significant difference between the systolic blood pressure of WKY and SHR rats at the age of 4 weeks was also observed when blood pressure was measured directly by means of a Statham pressure transducer in the tail artery (McCarty and Grobecker, unpublished results).

Adrenal Gland Weight and Protein Content

There were no differences in adrenal weight and adrenal protein content between SHR and WKY rats at the age of 2 weeks and 4 weeks (mg wet weight/adrenal: WKY: 2.5 ± 0.2, SHR: 2.3 ± 0.3 (2 weeks old); WKY: 8.4 ± 0.6, SHR: 7.3 ± 0.7 (4 weeks old):
mg protein/adrenal: WKY: 0.61 ± 0.04, SHR: 0.62 ± 0.05 (2 weeks old), WKY: 1.61 ± 0.11, SHR: 1.61 ± 0.09 (4 weeks old). Also, in adult rats, no differences in the weight or protein content of adrenal glands between WKY and SHR rats could be observed.

Adrenal Medullary Enzymes in Genetic Hypertension

A significant decrease in the activity of all catecholamine-synthesizing enzymes was observed in the adrenal glands of young SHR, compared with their age-matched controls (Fig. 2).

Decreases in tyrosine hydroxylase activity were found in 2- and 4-week-old SHR (Fig. 2); dopamine-β-hydroxylase and phenylethanolamine N-methyltransferase were decreased in SHR at 2, 4, and 8 weeks of age (Fig. 2).

Conversely, adult, 14-week-old SHR showed statistically significant increases in tyrosine hydroxylase when compared with age-matched controls (Fig. 2).

Adrenal Medullary Enzymes and Catecholamine Content in DOCA-Saline Hypertensive Rats

After only 1 week of treatment with DOCA (25 mg/kg) and 1% saline, tyrosine hydroxylase-activity was significantly higher than it was in controls treated either with water or saline (control: 39 ± 1.7; saline: 38 ± 1.5; DOCA-saline: 47 ± 2.2**, nmol product/gland per hour; n = 8–9, **P < 0.01 when compared with control or saline). After 2 weeks of treatment with DOCA and saline, tyrosine hydroxylase activity was as high as 60 ± 2.1 compared to control values of 36 ± 3.1 nmol product/gland/h (n = 10, P < 0.001). After 2 weeks of treatment with DOCA-saline, the blood pressure was already significantly higher (135 ± 2 mm Hg) than in controls (120 ± 3 mm Hg).

Further treatment with DOCA-saline for 4 weeks increased the blood pressure to 158 ± 5 mm Hg compared to 120 ± 6 mm Hg systolic blood pressure of the control group. At this stage of hypertension, the tyrosine hydroxylase-activity was still increased compared to the activity in the adrenal glands of normotensive animals (Table 1). Both adrenal dopamine-β-hydroxylase and phenylethanolamine N-methyltransferase-activities showed a small but not significant increase in hypertensive animals (Table 1). Also norepinephrine levels were slightly higher, but not significantly different from those in control ani-
Discussion

We report a decrease in the activity of catecholamine synthesizing enzymes in the adrenal gland of young spontaneously hypertensive rats. The decrease in tyrosine hydroxylase activity was evident at 2 weeks of age, and persisted at 4 weeks of age; dopamine-β-hydroxylase and phenylethanolamine N-methyltransferase were decreased from 2 to 8 weeks of age. The activity of another catecholamine-synthesizing enzyme, dopa-decarboxylase, had been also reported to be decreased in young (7-week-old) SHR, when compared to normotensive age-matched WKY controls (Lovenberg et al., 1973). In addition, the epinephrine levels in the adrenal glands of 4-week-old SHR had also been reported to be lower than those of age-matched WKY controls (SHR: 1.70 ± 0.09; WKY 2.15 ± 0.15 µg epinephrine per gland; Grobecker et al., 1975).

These changes represent decreased synthesis of adrenal catecholamines early in the development of the spontaneous (genetic) hypertension in rats, and may not be an expression of the "prehypertensive" stage, since the systolic blood pressure of SHR is already higher than that of age-matched WKY rats at the age of 4 weeks. Neither are these changes related to differences in body weight, adrenal gland weight, or adrenal protein content between SHR and WKY rats (c.f. Results, p 743; Morisawa, 1968).

The sympathoneuronal activity of young, 4-week-old SHR, however, is probably higher than that of the normotensive age-matched controls, as reflected by elevated circulating levels of norepinephrine and dopamine-β-hydroxylase (Nagatsu et al., 1974; Grobecker et al., 1975; Nagaoka and Lovenberg, 1976; Grobecker et al., 1977). It is likely that the depressed adrenal medullary activity reported here in young SHR might be secondary to the increased sympathoneuronal activity. Conversely, adult SHR present an increase in adrenal tyrosine hydroxylase activity, compared to their normotensive age-matched controls. This is in agreement with an earlier report (Nagatsu et al., 1971). However, in contrast to prior observations (Nagatsu et al., 1976), we found that dopamine-β-hydroxylase activity was not increased significantly nor was phenylethanolamine N-methyltransferase activity changed in these animals. Notwithstanding, the enhanced activity of tyrosine hydroxylase in adult SHR may be considered sufficient to determine increased synthesis and secretion of catecholamines into the circulation (Morisawa, 1968). This assumption is supported by the finding of increased circulating total catecholamines (mainly epinephrine) (Roizen et al., 1975; Kopin, 1979) and by the marked decrease in blood pressure after adrenalectomy (Aoki, 1963) in adult SHR. Thus, in adult genetically hypertensive rats, an enhanced sympathoadrenal activity could be a factor in the maintenance of an enhanced blood pressure. This is supported by the decrease in systolic blood pressure which occurs in adult SHR after subacute administration of an inhibitor of phenylethanolamine N-methyltransferase, devoid of α-adrenergic blocking effects (Saavedra, 1979).

The temporal correlation between changes in sympathoadrenal and sympathoneuronal activities during development in SHR is of interest. The early depression of sympathoadrenal activity in SHR, as noted by the decreased adrenal catecholamine synthesis, ends at about the same time (8 weeks of age) as the enhanced sympathoneuronal activity in these animals, as reflected by a return to control levels of circulating norepinephrine and dopamine-β-hydroxylase (Nagaoka and Lovenberg, 1976) in adult SHR. These changes in peripheral catecholamines also correlate temporally with changes in central catecholamines; the activity of the central adrenergic neurons is increased in young SHR and not changed in adult animals (Saavedra et al., 1978). These temporal correlations may suggest a linkage of the sympathoadrenal and sympathoneuronal activities, and the possibility that a disbalance between these two systems, of peripheral or central (Saavedra et al., 1978) origin, might play a role in the pathomechanism of genetic hypertension.

In previous reports from this laboratory, the contribution of the adrenal medulla to an elevated blood pressure in rats made hypertensive by DOCA and saline has been demonstrated (de Champlain et al., 1967, 1969). After chemical sympathectomy by 6-hydroxydopamine and subsequent adrenalectomy, a rapid and marked fall in blood pressure in both normotensive and hypertensive animals occurred (de Champlain and van Ameringen, 1972). Sympathoadrenal as well as sympathoneuronal hyperactivity may account for elevation of blood pressure in DOCA-saline hypertensive rats, as indicated by increased circulating norepinephrine and epinephrine levels in plasma (Reid et al., 1975; Grobecker et al., 1977; Franco-Morselli et al., 1979). Consistent with an increase in catecholamine synthesis in the adrenal medulla of hypertensive rats (de Champlain et al., 1976), tyrosine hydroxylase activity in adrenal glands of DOCA-saline hypertensive rats was also increased. In addition, a small, but significantly increased, epinephrine level was found 4 weeks after induction of hypertension with DOCA and saline. The increased activity of tyrosine hydroxylase is also demonstrable at a very early stage of hypertension; i.e., after only 1 or 2 weeks of treatment with DOCA and saline. These results indicate an elevated sympathoadrenal activity in this form of experimental hypertension, which parallels the development of high blood pressure.

Our results demonstrate that the sympathoadrenal system plays a role in genetic as well as in experimentally induced (DOCA-saline) hypertension in the rat, and that different mechanisms may modulate the expression of the sympathoadrenal system in each
model of hypertension. In both animal models, increased activity of the adrenal tyrosine hydroxylase is evident when the hypertension is established, after 4 weeks of DOCA-saline treatment and in adult genetically hypertensive rats. However, both models exhibit different developmental patterns regarding their sympathoadrenal activity. In the DOCA-saline hypertensive rats, the activation of this system parallels the development of high blood pressure. In the spontaneously hypertensive rats, however, there is an early stage of decreased sympathoadrenal activity, only followed by activation later in the development of the disease. These changes in the spontaneously hypertensive rat occur in spite of continuously increasing hypertension, and may be inversely related to the activity of the sympathoneural system.

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