NOREPINEPHRINE-SYNTHESIZING ENZYMES IN BRAIN, ADRENALS AND PERIPHERAL SYMPATHETIC NERVES OF SPONTANEOUSLY HYPERTENSIVE RATS

Toshiharu NAGATSU, Takeshi KATO, Yukiko NUMATA(SUDO), Keiko IKUTA*, Masao SANO**, Ikuko NAGATSU**, Hamao UMEZAWA***, Meiki MATSUZAKI*** and Tomio TAKEUCHI***

Laboratory of Cell Physiology, Department of Life Chemistry, Graduate School at Nagatsuda, Tokyo Institute of Technology, Midori-ku, Yokohama 227, Japan

Accepted April 6, 1977

Abstract—Dopamine-β-hydroxylase (DBH) activity in serum, DBH and tyrosine hydroxylase (TH) activities in mesenteric vessels, and DBH and TH activities in locus coeruleus and hypothalamus of brain did not differ significantly between spontaneously hypertensive rats (SHR) and normotensive Wistar-Kyoto rats (WKR) at 16 weeks of age when hypertension of SHR was fixed. In contrast, DBH and TH activities in vas deferens and adrenal glands were significantly higher in SHR than in WKR. These changes in SHR at 16 weeks of age after establishment of hypertension are directly opposite those reported previously in SHR at 3 weeks of age before the onset of hypertension.

We have previously reported (1, 2) that dopamine-β-hydroxylase (DBH) activity in young spontaneously hypertensive rats (SHR) (3) was increased in serum and mesenteric vessels and was decreased in the locus coeruleus area of the brain. These results suggest that the sympathetic nerve activity may be increased in blood vessels due to a low noradrenergic activity in the brainstem of young SHR before the onset of hypertension. During the development and maintenance of hypertension in SHR from 6 to 53 weeks of age, serum DBH activity in SHR was found to be similar to that in control normotensive Wistar-Kyoto rats (WKR) (1), suggesting that the peripheral sympathetic nerve activity may be decreased to the level of normotensive WKR by a compensatory mechanism.

We investigated DBH activity in serum, and DBH and tyrosine hydroxylase (TH) activities in mesenteric vessels, vas deferens, adrenals and catecholaminergic regions of brain in SHR and WKR at 16 weeks of age when the hypertension is fixed, in order to compare the activity of the peripheral sympathetic nerves, adrenal glands and catecholaminergic neurons of the brain.

* Departments of Biochemistry and Anatomy, School of Dentistry, Aichi-Gakuin University, Nagoya.
** Department of Anatomy, School of Medicine, Fujita-Gakuen University, Toyoake, Aichi.
*** Institute of Microbial Chemistry, Tokyo, Japan.
MATERIALS AND METHODS

SHR and WKR were kindly provided by Drs. Okamoto and Yamori (Kyoto Univ., Kyoto) and raised in our laboratory under the same conditions. Blood pressure was measured in unanaesthetized rats using an indirect tail plethysmograph (3).

Rats were decapitated, and mesenteric vessels, vas deferens, adrenal glands, and brain were quickly removed, weighed, frozen on dry ice and stored at -80°C. "Mesenteric vessels" includes superior, inferior and coeliac mesenteric arteries and veins plus connective tissues after removing fat from the mesentery. Blood samples were obtained by exsanguination and were put into a test tube kept in ice, and the serum was removed.

The regions of catecholaminergic neurons (caudate nucleus, substantia nigra, hypothalamus, and locus coeruleus) were excised under a microscope from frozen sections of the brain (4). The brain tissues were homogenized in 250 to 380 μl of 0.1 M potassium phosphate buffer, pH 7.5, containing 0.1% Triton X-100. Mesenteric vessels, vas deferens, and adrenal glands were homogenized in 2 to 4 ml of the same buffer, the homogenate was centrifuged at 50000×g for 30 minutes, and the supernatant was used for the assay of DBH and TH activities.

DBH activity was assayed by dual-wavelength spectrophotometry of Kato et al. (5) based on the photometric assay of Nagatsu and Udenfriend (6). Incubation mixture (total volume 1.0 ml) contained (in final concentrations): enzyme, 0.2 M sodium acetate buffer, pH 5.0, 10 mM N-ethylmaleimide, 1 μM CuSO₄, 1 mM pargyline hydrochloride, 10 mM sodium fumarate, 10 mM ascorbic acid, 50 μg (1500 units) of catalase, and 20 mM tyramine hydrochloride. A sample of boiled enzyme preparation was used as tissue blank. Incubation was carried out for 45 min at 37°C in air with constant shaking.

TH activity was determined by measuring the [14C]dopa formed from L-[14C]tyrosine (7, 8). The incubation mixture (total volume 500 μl) contained (in final concentrations) enzyme, 0.2 M acetate buffer to give a final pH of 6.0, 2 mM 6-methylenetetrahydropterin or L-erythro-tetrahydrobiopterin for the assay of peripheral tissues or brain, respectively, 0.1 M mercaptoethanol, 1 mM FeSO₄, and 0.1 mM (for peripheral tissues) or 25 μM (for brain) L-[U-14C]tyrosine (0.07 μCi). Assay blank contained water instead of enzyme. Tissue blank (boiled enzyme) was not necessary for this radiometric assay, since water blank gave a similar blank value. Incubation was carried out at 37°C for 15 min in air with constant shaking. Protein was measured by the method of Lowry et al., using bovine serum albumin as the standard (9).

RESULTS

The blood pressures of SHR and those of control WKR at 16 weeks of age were 186±3 mmHg and 130±3 mmHg, respectively. DBH and TH activities in serum, adrenal glands and sympathetically innervated tissues (mesenteric vessels and vas deferens) are shown in Table 1. DBH activity in serum did not differ significantly between SHR and WKR. DBH and TH activities in adrenal glands and vas deferens were significantly higher than those of WKR. Mean enzyme activities of DBH and TH in mesenteric vessels were higher.
TABLE 1. Dopamine-β-hydroxylase and tyrosine hydroxylase activities in peripheral tissues of control Wistar-Kyoto rats (WKR) and spontaneously hypertensive rats (SHR) at 16 weeks of age

| Source of Enzyme | Enzyme activity |          |          |
|------------------|-----------------|----------|----------|
|                  | Nmol/min/g of wet weight (mean ± S.E.M.) | Prmol/min/mg of protein (mean ± S.E.M.) |
|                  | WKR             | SHR      | WKR      | SHR      |
| Dopamine-β-hydroxylase |              |          |          |          |
| Serum            | 0.37 ± 0.03 (7) | 0.36 ± 0.04 (7) |            |          |
| Mesenteric vessels | 1.44 ± 0.12 (6) | 2.43 ± 0.69 (6) | 410 ± 30 (6) | 590 ± 80 (5) |
| Vas deferens     | 4.75 ± 0.25 (7) | 6.53 ± 0.25 (7) | 360 ± 10 (7) | 460 ± 10 (7) |
| Adrenals         | 47.0 ± 10.8 (4) | 86.8 ± 5.9 (5) | 1460 ± 250 (4) | 2960 ± 220 (7) |
| Tyrosine hydroxylase |              |          |          |          |
| Mesenteric vessels | 0.76 ± 0.08 (7) | 1.06 ± 0.14 (7) | 51 ± 3 (7) | 76 ± 9 (7) |
| Vas deferens     | 6.74 ± 0.27 (7) | 9.21 ± 0.42 (7) | 163 ± 8 (7) | 202 ± 13 (7) |
| Adrenals         | 27.7 ± 2.7 (7)  | 54.2 ± 2.5 (7) | 412 ± 38 (7) | 750 ± 35 (7) |

Numbers in parentheses are the numbers of samples. *Differs from control WKR, p<0.05; †Differs from control WKR, p<0.01

TABLE 2. Dopamine-β-hydroxylase and tyrosine hydroxylase activities in the brain regions of control Wistar-Kyoto rats (WKR) and spontaneously hypertensive rats (SHR) at 16 weeks of age

| Brain Region        | Dopamine-β-hydroxylase* | Tyrosine hydroxylase† |
|---------------------|-------------------------|-----------------------|
|                     | WKR (mean ± S.E.M.)     | SHR (mean ± S.E.M.)   |
| Substantia nigra    | 74.3 ± 9.4 (5)          | 19.6 ± 2.2 (5)        |
| Caudate nucleus     | 286.6 ± 15.3 (5)        | 22.3 ± 1.4 (5)        |
| Locus coeruleus     | 98.2 ± 6.3 (5)          | 97.6 ± 9.6 (5)        |
| Hypothalamus        | 43.2 ± 3.1 (5)          | 31.9 ± 1.2 (5)        |

Numbers in parentheses are the numbers of samples. *Substrate, 20 mM tyramine. †Substrate 25 µM tyrosine; cofactor, 2 mM L-erythro-tetrahydrobiopterin. n.d., not detectable.

in SHR than those in WKR, but the variations were large, and the differences were not statistically significant.

The DBH and TH activities in the brain regions of catecholaminergic neurons are shown in Table 2. DBH activity was found only in the regions of noradrenergic neurons, such as locus coeruleus and hypothalamus. DBH activity in the dopaminergic regions, such as substantia nigra and caudate nucleus was less than the limit of sensitivity of the assay. TH activity was found in both dopaminergic and noradrenergic brain regions, although it was much higher in the former. There was no statistical difference in DBH and TH activities in various brain regions between SHR and WKR.
DISCUSSION

We reported that DBH activity was higher in serum and mesenteric vessels but lower in locus coeruleus of 3-week-old SHR than it was in the control WKR (2). This higher DBH activity in serum (units in terms of ml of serum) of young SHR indicates more rapid increase in the sympathetic nerve activity than in control WKR at this young age when the sympathetic nerves in rats develop rapidly. DBH is secreted by the process of exocytosis together with noradrenaline and appears in blood from sympathetic nerve terminals of various sympathetically innervated tissues (10, 11). However, it is not clear from what tissues the enzyme is mainly secreted into blood. We speculate that a significant fraction of the elevated serum DBH in young SHR may be derived from the blood vessels, since the total numbers of sympathetic nerve terminals in peripheral blood vessels of the whole body are considered to be high (2). Lower DBH activity in the locus coeruleus is thought to have some relation to the increased vascular sympathetic nerve activity (2).

We also reported that serum DBH activity did not differ significantly between SHR and WKR after the establishment of hypertension (1).

In the present study, we investigated the DBH and TH activities in the peripheral sympathetically innervated tissues, adrenal glands, and the catecholaminergic regions of brain at 16 weeks of age when hypertension of SHR is fixed. In contrast to the 3-week-old SHR, DBH activity in serum, mesenteric vessels, and locus coeruleus of the 16-week-old SHR did not differ significantly from that of control normotensive WKR. Nagaoka and Lovenberg (12) recently reported a significantly increased plasma DBH activity in SHR at 3 weeks of age, however, they also found a significant increase of plasma DBH activity in SHR even at 15 weeks, although plasma noradrenaline level was significantly high in SHR only at 3 weeks. The reason for the discrepancy in the data is not clear. Although serum DBH activity did not differ between SHR and WKR at 16 weeks, DBH activity in mesenteric vessels still tends to be high in SHR. It is also possible that the degradation rate of DBH in blood during circulation in SHR at 16 weeks of age is more rapid than in WKR.

DBH activity in vas deferens and adrenal glands in SHR at 16 weeks of age was significantly higher than that of WKR. As reported previously, DBH activity in vas deferens of young SHR was similar to that of WKR and DBH activity in adrenal glands of 3-week-old SHR was slightly but significantly higher than that of WKR (2). The present results indicate that DBH activity in adrenal glands progressively increases with the development of hypertension.

TH activity in mesenteric vessels, vas deferens, and adrenal glands is in good parallel with the DBH activity: the TH activity was significantly higher in vas deferens and adrenal glands, but did not differ significantly in mesenteric vessels between SHR and WKR.

The present results suggest that lower DBH activity in the locus coeruleus and higher DBH activity in serum and mesenteric vessels in young SHR may be related with the onset of hypertension, but may not be related to the maintenance of hypertension, since these changes disappear after the onset of hypertension in adult SHR.
Elevated TH activity in vas deferens of SHR after establishment of hypertension is in agreement with the results in studies on humans by DeQuattro et al. (13) who found increased TH activity in the vas deferens with a concomitant elevated systolic blood pressure.

The progressive increase found in adrenal DBH and TH activities during the development of hypertension is in agreement with our previous studies (14–16) and suggests hyperactivity in the adrenal medulla of SHR. As supporting evidence for the possibility of overactivity of the adrenal medulla of SHR, Nakamura et al. (17, 18) confirmed elevation of the spontaneous release of adrenaline and DBH from adrenal medulla and also selective activation of sympathetic ganglia in SHR.

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