

Elevated Tyrosine Hydroxylase mRNA Levels in the Adrenal Medulla of Spontaneously Hypertensive Rats

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ABSTRACT—We investigated the expression of tyrosine hydroxylase (TH) mRNA and its activity in the adrenal medulla of spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY). The TH mRNA levels were determined by Northern blot and dot blot analyses. The TH activity and the expression of TH mRNA in the adrenal medulla of SHR were significantly higher than those of WKY ($P < 0.01$). These results suggested that the hypertension of SHR may be related to the high activity of TH due to the high level of TH mRNA, which increases epinephrine and norepinephrine levels in the adrenal medulla.

Keywords: Tyrosine hydroxylase mRNA, Adrenal medulla, Spontaneously hypertensive rat

Elevated sympathetic nervous tone was proposed as the cause of hypertension in spontaneously hypertensive rats (SHR) by Okamoto et al. (1). Ozaki et al. reported that the norepinephrine and epinephrine contents in the adrenal medulla of SHR were significantly higher than those of Wistar Kyoto rats (WKY) (2). The adrenal medulla is important for the synthesis and release of norepinephrine and epinephrine (3, 4). Tyrosine hydroxylase (TH) is a rate-limiting enzyme in the synthetic pathway of catecholamine. Nagatsu et al. (5) reported that the TH activity of the adrenal medulla was increased in SHR. However, the underlying mechanism for this has remained unsolved. The purpose of this study was to determine the TH mRNA level in the adrenal medulla of SHR.

Male SHR and WKY (25–27 weeks old) were used. Systolic blood pressure was measured in conscious rats by the tail-cuff method using an electro-sphygmomanometer (PS-100; Riken Kaihatsu, Co., Tokyo).

Norepinephrine and epinephrine levels were assayed by the method of Lewis et al. (6). The adrenal medulla was homogenized with 0.05 N perchloric acid in a glass tissue grinder. Norepinephrine and epinephrine were extracted with aluminum oxide. Norepinephrine and epinephrine were measured by HPLC-ECD (Waters, Milford, USA; 460 electrochemical detector). The mobile phase was a mixture of 50 mM sodium acetate, 20 mM citric acid, 3.75 mM sodium octyl sulphate, 1 mM di-*n*-butylamine, 0.134 mM EDTA and 5% (V/V) methanol. All separations were performed isocratically at a flow-rate of 0.9

ml/min at 35°C. The detector potential was maintained at +0.65 V.

TH activity was assayed by the method of Nagatsu et al. (7). The adrenal medulla was homogenized with 0.25 M sucrose in a glass tissue grinder. The homogenate was incubated with 1 mM L-tyrosine and 1 mM 6-methyl-5,6,7,8-tetrahydropterine at 37°C for 10 min. DOPA was extracted with aluminum oxide and Amberlite CG50. DOPA was measured by HPLC-ECD (Waters, 460 electrochemical detector). The mobile phase was a mixture of 50 mM sodium acetate, 20 mM citric acid, 12.5 mM sodium octyl sulphate, 1 mM di-*n*-butylamine and 0.134 mM EDTA. All separations were performed isocratically at a flow-rate of 0.7 ml/min at 28°C. The detector potential was maintained at +0.65 V. TH activity was calculated as the amount of DOPA synthesis from tyrosine.

The TH mRNA levels were determined by Northern blot and dot blot analyses (8). The total RNA fraction was extracted from homogenized adrenal medulla into 50% guanidine thiocyanate. The homogenate was centrifuged at 20°C for 15 hr at 100,000×*g*, and the precipitate obtained was dissolved in 10 mM Tris-EDTA, 10% SDS. After the total RNA fraction was purified by ethanol, it was dissolved in sterilized water and quantitated for total RNA at 260 nm.

Northern blotting was performed as follows. Total RNA (20 µg) was incubated with 6 M glyoxal and DMSO, and then this solution was applied to an agarose gel. After agarose gel electrophoresis, the proteins were blotted to a nitrocellulose membrane over night. This

membrane was baked at 80°C for 60 min, prehybridized in hybridization buffer (50% formamide, 5% SDS, 25×Denhard's solution, 5×SSPE and 0.25 mg/ml salmon sperm DNA) and then hybridized in hybridization buffer with 1.2×10^7 Bq/ml of rat TH cDNA (1.2 kb, derived from the PC12 cell line) (9). The membranes were washed at 40°C with two changes of 5×SSPE for 15 min each and then with two changes of 1×SSPE at 65°C for 30 min each. The membrane was exposed to scientific imaging film (Eastman Kodak Co., Rochester, USA) with an intensifying screen at -80°C for one day. Dot blotting was carried out as follows: Total RNA (2 µg) was incubated with 6 M glyoxal and DMSO, and this solution was then applied to a nitrocellulose membrane. This membrane was baked at 80°C for 60 min, prehybridized in hybridization buffer and then hybridized in hybridization buffer with 1.2×10^7 Bq/ml of rat TH cDNA (1.2 kb, derived from PC12 cell line) (9). The membrane was washed at 40°C with two changes of 5×SSPE for 15 min each and then with two changes of 1×SSPE at 65°C for 30 min each. The radioactivity of the membrane was counted by a scintillation counter.

The statistical difference between mean values was calculated by the Student's *t*-test.

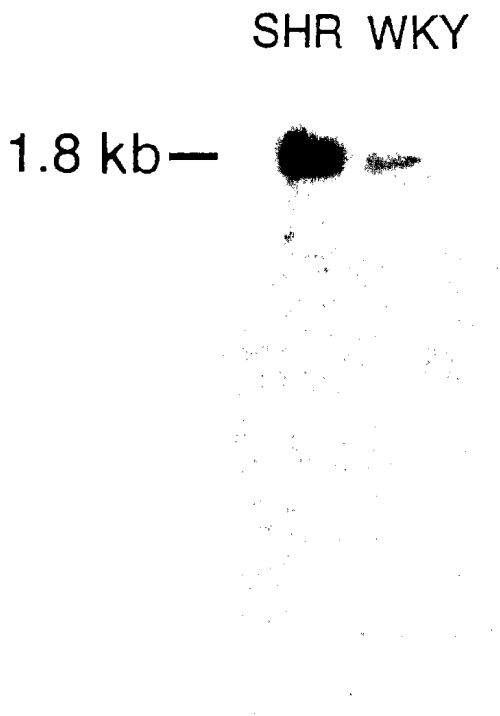


Fig. 1. The expression of TH mRNA in the adrenal medulla of SHR and WKY by Northern blot analysis. Aliquots of total RNA (20 µg) isolated from the adrenal medulla of SHR (left lane) and WKY (right lane) were hybridized with radiolabeled rat TH cDNA.

The TH mRNA expression in the adrenal medulla of SHR was detected by Northern blot analysis as a single band of 1.8 kb. This particular mRNA showed a significantly higher intensity than did that of WKY (Fig. 1). As a matter of fact, when the [32 P]radioactivities of the dots were determined, the TH mRNA level in the adrenal medulla of SHR was found to be as high as 3 times that of WKY (SHR: 1742.4 ± 432.0 Bq/µg RNA, N=6; WKY: 530.4 ± 99.6 Bq/µg RNA, N=6, $P < 0.05$) (Fig. 2). The higher TH mRNA level of SHR was accompanied by higher TH activities (SHR: 401.14 ± 56.78 µg/g tissue/hr, N=6; WKY: 204.39 ± 19.27 µg/g tissue/hr, N=6, $P < 0.01$), higher norepinephrine level (SHR: 68.58 ± 2.77 µg/g tissue, N=3; WKY: 34.91 ± 3.87 µg/g tissue, N=3, $P < 0.01$) and higher epinephrine level (SHR: 419.63 ± 31.53 µg/g tissue, N=3; WKY: 271.43 ± 29.80 µg/g tissue, N=3, $P < 0.05$) in the adrenal medulla as well as higher systolic blood pressure (SHR: 191.1 ± 3.8 mmHg, N=6; WKY: 139.6 ± 2.2 mmHg, N=6, $P < 0.01$).

The major findings of the present study were the increased expression of TH mRNA in SHR, along with increases in TH activity, epinephrine and norepinephrine levels of the adrenal medulla and hypertension. The adrenal medulla is known to contain large amounts of epinephrine and norepinephrine (3). The circulating norepinephrine originates from the adrenal medulla and postganglionic sympathetic nerve endings. On the contrary, the circulating epinephrine originates almost exclusively from the adrenal medulla (10). Because the TH is

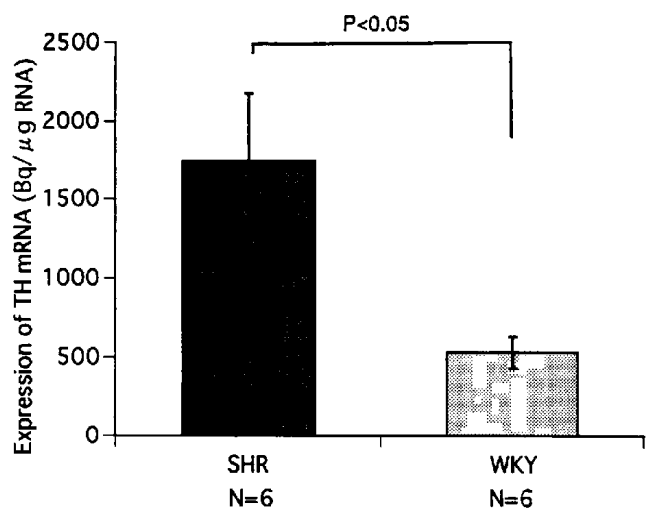


Fig. 2. The expression of TH mRNA in the adrenal medulla of SHR and WKY by dot blot analysis. Aliquots of total RNA (2 µg) isolated from the adrenal medulla of SHR (left column) and WKY (right column) were hybridized with radiolabeled rat TH cDNA. The TH mRNA level of the adrenal medulla in SHR (left column) was significantly higher than that in WKY (right column) ($P < 0.05$). Mean \pm S.E.

rate-limiting in catecholamine synthesis, an increase in its level would be reflected by a concomitant increase in the circulating level of catecholamine. Our data strongly suggest that the increased expression of adrenomedullary TH mRNA results in the increases of TH activity and epinephrine and norepinephrine levels.

The present results show that the TH activity in the adrenal medulla of SHR is significantly higher than that of WKY, which confirms the results of Nagatsu et al. (5). Furthermore, Nagatsu et al. indicated that the amount of TH protein in the adrenal medulla of SHR has been shown to be significantly higher than that of WKY (11). Thus, the increased expression of TH mRNA in the adrenal medulla in the present study may indicate that the enhancement of transcription of TH mRNA increases the synthesis of TH protein, resulting in the higher activity of TH in the adrenal medulla of SHR.

In the adrenergic neuron, TH is soluble at its site of synthesis in cell bodies, and it is transported to nerve endings through the axon (12). There are close similarities in the mode of synthesis, storage and release of chemical messengers between chromaffin cells of the adrenal medulla and adrenergic neurons (13). Therefore, it is possible that an excess of TH protein will be synthesized by the elevated TH mRNA in the adrenal medulla of SHR.

The synthesis of TH is influenced by many factors, of which some appear to act at the level of mRNA transcription. Lewis et al. (9) reported that glucocorticoid and cAMP act by altering the rate of transcription of the TH gene. Further studies will be needed to assess the mechanism for this high expression of TH mRNA in the adrenal medulla of SHR.

In conclusion, the present results clearly indicate that the transcription of the genomic DNA of TH is significantly increased in SHR. Whether the rate of transcription of genomic TH DNA is accelerated in the adrenal medulla of SHR still remains to be studied. The elevated activity of TH due to the high level of TH mRNA that results in the increased epinephrine and norepinephrine levels in the adrenal medulla may play a crucial role in the pathogenesis of hypertension in the SHR.

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