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Epinephrine Contents of Sympathetic Ganglia and Brain Regions of Spontaneously Hypertensive Rats of Different Ages[1] (40136)

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An alteration in the functioning of central or peripheral catecholaminergic neurons may play a role in the development of experimental hypertension (1). Most studies have focused on the role of central and peripheral norepinephrine neurons (2, 3), but recent reports have considered the possible roles of central epinephrine containing neurons. The A_2 region of the rat medulla (similar to the C_2 region of Hökfelt *et al.* (4)) contains a high content of phenylethanolamine *N*-methyltransferase (PNMT), the enzyme that catalyzes the conversion of norepinephrine to epinephrine. Using immunohistochemical techniques Hökfelt *et al.* (4) demonstrated that this region contained cell bodies and terminals of PNMT-positive (epinephrine-containing) neurons. This region contains the caudal parts of nucleus tractus solitarius, considered to play a role in blood pressure regulation (5).

Saavedra *et al.* (6) demonstrated an elevated activity of PNMT in the A_2 region of the medualla of four but not of 14-week-old genetically hypertensive rats, and suggested that epinephrine containing neurons may be involved in the development of hypertensive disease. Versteeg *et al.* (7) found a higher concentration of epinephrine in the A_2 region of the medulla and the nucleus paraventricularis of the hypothalamus in 16-week-old spontaneously hypertensive rats compared with appropriate normotensive controls.

The purpose of the present investigation was to look at developmental changes in the concentrations of catecholamines in the brain and sympathetic ganglia in a genetic strain of hypertensive rats. Furthermore, since adrenal steroids are involved in the maintenance of activity of PNMT in peripheral tissues (8, 9), the effects of dexamethasone on brain PNMT and ganglionic catecholamines were also examined.

Materials and methods. Animals. Spontaneously hypertensive rats (SHR) and corresponding age-matched normotensive Wistar-Kyoto (WKY) controls of both sexes were obtained from Laboratory Supply, Inc., Indianapolis. The rats were caged in groups and were allowed at least one week of acclimation to their new surroundings before use. Systolic blood pressure was measured in unanesthetized rats for four consecutive days by means of a tail plethysmograph and the average of three recordings of pressure taken on day 4 was taken as representing the blood pressure of each age group.

Dissection. At appropriate ages, rats were sacrificed by completely severing them with scissors just caudal to the front paws. The brain was rapidly removed and placed on moist filter paper on a glass plate resting on ice. At the same time that the brain regions were being dissected superior cervical ganglia were isolated and removed bilaterally. The pair of ganglia were homogenized in 75 μl of ice-cold 0.4 *N* perchloric acid containing 10 mg% EGTA. Brain dissections were carried out after removal of the cerebellum. A frontal cut was made at the level of the obex and another 3 mm rostral to the obex. The region containing the nuclei tractus solitarii (NTS) was dissected from this section by making cuts 2 mm lateral to either side of the midline and another cut 1.5 mm from the dorsal surface. This piece was divided at the midline; one piece was homogenized in 50 μl of perchloric acid for the determination of catecholamines while the other was homogenized in 100 μl of 0.005 *M* Tris HCl buffer (pH 8.6) for the determination of PNMT activity. These pieces are designated "NTS" in the Results. In younger animals (less than 4 weeks old), a section containing NTS was obtained by making a frontal cut at the obex

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and another 2 mm rostral to the obex; the dorsal half of this slice was homogenized in the appropriate medium. Finally, the hypothalamus was dissected free from the ventral surface of the brain as described by Glowinski and Iversen (10) and homogenized in 5 vol of the appropriate solution for the analysis of PNMT activity or catecholamines. After centrifugation, the supernatants were assayed as described below while the pellet was assayed for protein as described by Lowry *et al.* (11).

Assays. The content of catecholamines (dopamine, epinephrine, norepinephrine) was assayed by the radioenzymatic methods of Cuello *et al.* (12) as modified and described in detail by Moore and Phillipson (13). Briefly, the supernatant or a standard amine sample is incubated with a crude preparation of catechol-*O*-methyltransferase and *S*-adenosyl-L-[methyl-^{3}H]-methionine (New England Nuclear). The resulting radioactive *O*-methylated products are extracted and then separated by paper chromatography. The sensitivity of the assay was 100–200 pg norepinephrine, 40–50 pg epinephrine and 80–100 pg dopamine; these quantities gave CPMs that were twice blank values (see (13) for details of calculations of appropriate blanks). A correction, varying from 5–12% depending on the assay, was made for contamination of dopamine in the epinephrine spot; there was no cross-contamination in dopamine or norepinephrine spots.

The activity of PNMT was estimated as described previously (13) with the exception that an additional acid extraction was used to lower the blanks (14). Briefly, the radioactive product of the reaction (N-methyl-^{3}H-phenylethanolamine) was extracted from the incubation medium with 1.5 ml toluene:isoamyl alcohol (97:3). The radioactive product in a 1.25 ml aliquot of this organic phase was extracted into 50 μl in 1 *N* HCl and then back extracted into a mixture of 0.45 ml 0.5 *M* borate buffer, pH 10, and 3.5 ml toluene:isoamylalcohol. The organic phase was transferred to scintillation vials, evaporated to dryness and counted in 0.5% PPO in toluene/ethanol (70/30).

Drug treatments. Thirteen day old SHR and WKY rats were injected subcutaneously with dexamethasone (0.1 mg/kg/day) diluted with saline so that 10 ml/kg was injected. The rats received eight daily injections and were sacrificed 2 days after the last injection. Other rats from the same litters received daily injections of the same volume of saline to serve as controls.

Results. The blood pressure of SHR used in this study increase progressively as the animals age; at 9 and 15 weeks the mean and range of the systolic pressures were 182 (160–200) mmHg and 204 (174–224) mmHg, respectively. The systolic pressures of the older rats (20–30 weeks) were essentially the same as those at 15 weeks. On the other hand, the systolic pressures of appropriate age-matched WKY controls remained fairly constant (118–140 mmHg) at these ages. The SHR consistently weighted less than the WKY controls; for example, at 15 weeks the body weights of SHR were 20–25% less than the WKY.

PNMT activity in the NTS (A_2) region of the medulla from SHR was higher than it was in WKY controls. As illustrated in Fig. 1, the increased PNMT activity develops around the fourth week of life and continues into adulthood.

The norepinephrine and epinephrine concentrations in NTS and hypothalamus of SHR and WKY rats of different ages are summarized in Fig. 2. There were no significant differences between catecholamine concentrations in the NTS of SHR and WKY rats. In the hypothalamus the epinephrine concentration was significantly greater in SHR at 9 and 15 weeks of age; this difference

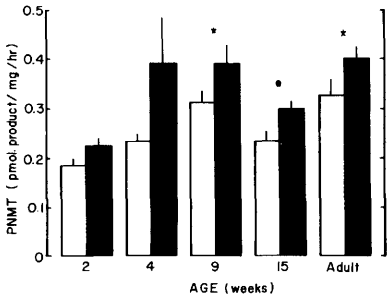

FIG. 1. PNMT activity in NTS region of brain from SHR and WKY rats of different ages. The height of each column (open, WKY; solid, SHR) represents the mean and the vertical line 1 SE as determined in 7–14 animals. *, values in SHR which are significantly greater ($P < 0.05$) than appropriate WKY controls.

was just short of significance in the older adults. There were no significant differences in the hypothalamic concentrations of norepinephrine in the two groups of animals. Similarly, the dopamine concentrations in the hypothalamus of SHR were not significantly different from values in WKY controls at any of the times examined (dopamine concentrations are not presented).

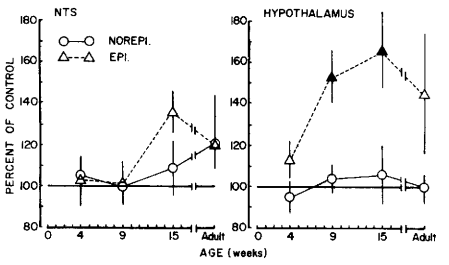

FIG. 2. Norepinephrine and epinephrine contents in brain regions of SHR and WKY rats of different ages. Each symbol represents the mean and vertical lines 1 SE of the contents of norepinephrine (○) and epinephrine (△) in SHR expressed as a percentage of values in WKY controls (n = 7–14). 100% represents 12.9 ± 1.3 ng norepinephrine/mg protein and 0.7 ± 0.1 ng epinephrine/mg protein in NTS, and 17.6 ± 1.2 ng norepinephrine/mg protein and 1.0 ± 0.2 ng epinephrine/mg protein in hypothalamus. Solid symbols indicate those values in SHR which are significantly different ($P < 0.05$) from WKY controls.

The norepinephrine, dopamine and epinephrine contents of the superior cervical ganglia were elevated in SHR when compared to WKY controls (Fig. 3). This effect was noted in 4-week and older rats, except for the increase in norepinephrine which was not significant until 9 weeks of age.

Injection of normal neonatal rats with dexamethasone results in a substantial elevation in PNMT activity and epinephrine content of sympathetic ganglia (8, 13, 15). This response subsides as the rat matures. Nevertheless, both PNMT activity and epinephrine concentrations in the hypothalamus of both young and adult rats can be increased if very large doses of dexamethasone are administered (13). As depicted in Table I, young SHR appear to be more sensitive to the stimulatory effect of dexamethasone than WKY rats. An

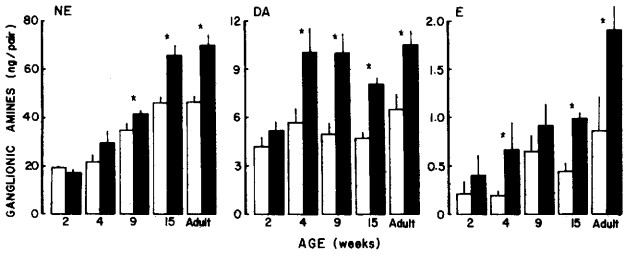

FIG. 3. Catecholamine content of superior cervical ganglia from SHR and WKY rats of different ages. The height of each column (open, WKY; solid, SHR) represents the mean and the vertical line 1 SE as determined in 7–14 animals. *, values in SHR which are significantly greater ($P < 0.05$) than appropriate WKY controls.

TABLE I. EFFECTS OF DEXAMETHASONE ON THE ACTIVITY OF PNMT IN THE BRAIN AND THE CONTENT OF CATECHOLAMINES IN THE SUPERIOR CERVICAL GANGLIA OF SHR AND WKY RATS.[a]

	WKY		SHR	
	Saline	Dex	Saline	Dex
PNMT (*p*mole product/mg wet weight/hr)				
NTS	0.164 ± .013	0.170 ± .006	0.204 ± .018	0.304 ± .038*
Hypothalamus	0.092 ± .002	0.095 ± .003	0.088 ± .003	0.103 ± .004*
GANGLIONIC CATECHOLAMINES (ng/ganglion pair)				
Norepinephrine	18.4 ± 1.8	15.0 ± .8*	21.2 ± 1.8	15.0 ± 1.3*
Dopamine	5.0 ± .5	5.4 ± .9	7.4 ± 1.2	5.1 ± .4
Epinephrine	0.21 ± .06	0.20 ± .05	0.25 ± .05	0.54 ± .08*

[a] Dexamethasone (Dex; 0.1 mg/kg) or saline was injected subcutaneously daily to SHR or WKY rats on days 13–20. The rats were sacrificed on day 22. Values represent means ± SE as determined from 7 to 8 animals. *, values in dexamethasone-treated animals that are significantly different ($P < 0.05$) from saline-treated animals.

8 day treatment with dexamethasone, beginning on day 13 of life, increased PNMT activity in NTS and hypothalamus of SHR but not of WKY rats. In addition, dexamethasone treatment increased the epinephrine content in the superior cervical ganglia of SHR but not WKY controls. Dexamethasone did, however, reduce the norepinephrine concentration in both groups, an effect noted previously in Sprague–Dawley rats (13, 15).

Discussion. Saavedra *et al.* (6) noted that PNMT activity in the A_2 region of 4 but not of 14 week old SHR was significantly increased when compared to controls. On the other hand, the results of the present study show that differences in PNMT activity extend into adulthood. PNMT in the medulla is contained in cell bodies of epinephrine neurons which descend into the lower brain stem and spinal cord, and ascend into the hypothalamus (4). Versteeg *et al.* (7) found an increased concentration of epinephrine in both NTS and hypothalamus of 16-week SHR whereas in the present study a significant increase was noted only in the hypothalamus. Our inability to find elevated epinephrine concentrations in the medulla may be related to the fact that the amount of this amine in the medulla is near the limits of sensitivity of our assay. We have previously reported on increases of epinephrine in hypothalamus but not in the medulla by other treatments (13), and we and others have reported a lack of correspondence between PNMT activity and epinephrine content in the brain (13, 16, 17); these differences may be asssociated with changes in turnover rates of epinephrine which are not reflected in steady-state concentrations.

In addition to the changes in the brain, the catecholamine contents of sympathetic ganglia in SHR were also significantly greater than in control rats. All the changes noted in the SHR rats appeared between the third and ninth week of life and continued into adulthood. These developmental biochemical changes roughly correspond to the reported development of hypertension in SHR (3, 18). Furthermore, regions of the brain containing epinephrine neurons have been shown to play a role in the development of hypertension. For example, lesions of NTS produce a rise in systemic blood pressure (5). Similarly, stimulation of the posterior hypothalamus increases the blood pressure and this effect is greater in SHR (19). The significance of the changes in the catecholamine content of sympathetic ganglia is less apparent. Nevertheless, both dopamine (20) and epinephrine (21) are believed to modulate ganglionic transmission processes and it is possible that changes in the amine concentrations may be related to the high sympathetic activity in SHR (22, 23).

Finally, the enhanced sensitivity of SHR to the effect of dexamethasone on PNMT activity deserves further consideration. It may be important to consider the possibility that an enhanced sensitivity to endogenous adrenal steroids may be responsible for the observed differences in PNMT of SHR and WKY rats and this may be related to the development of hypertension.

Summary. The activity of phenylethanolamine *N*-methyltransferase (PNMT), the enzyme that catalyzes the synthesis of epinephrine from norepinephrine, was higher in a section of the medulla containing nucleus tractus solitarius from a genetic strain of spontaneously hypertensive rats (SHR) than in appropriate Wistar Kyoto (WKY) control rats. The concentration of epinephrine, but not of other catecholamines, was higher in the hypothalamus of SHR. These differences appeared between the fourth and ninth week of life and continued into adulthood. The contents of norepinephrine, dopamine and epinephrine in the superior cervical ganglia of SHR were also higher than in WKY; these differences were first noted at 4 weeks of age. During the first 3 weeks of life, SHR were more sensitive to the dexamethasone-induced increases in both the PNMT activity of the brain and the epinephrine content of the ganglia.

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