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Vascular and Brain Dopamine β-Hydroxylase Activity in Young Spontaneously Hypertensive Rats

Abstract. *Dopamine β-hydroxylase activity was higher in mesenteric vessels, adrenal glands, and serum of 3-week-old spontaneously hypertensive rats but lower in the locus coeruleus than it was in the control Wistar-Kyoto rats. The results support the concept that the nervous system is an important regulator of blood pressure.*

It has long been thought that the sympathetic nervous system plays an important part in the regulation of blood pressure in patients with hypertension, but there is no firm evidence of excessive activity of the sympathetic nervous system in development and maintenance of essential hypertension. Dopamine β-hydroxylase (DBH) (E.C. 1.14.2.1) is secreted by the process of exocytosis from sympathetic nerve endings with norepinephrine (*1*). Therefore, DBH in blood was thought to serve as an index of the activity of the sympathetic nerves (*2*). However, results on the plasma level of DBH in patients with essential hypertension were contradictory—several reports indicated an increase in plasma DBH activity (*3*), whereas others showed that this activity does not correlate with blood pressure (*4*). One difficulty in evaluating blood DBH as an index of sympathetic function is the wide variation of DBH activity in man, since this activity is significantly influenced by hereditary factors (*5*). Lovenberg *et al.* (*6*) also reported wide variations in DBH activity in sympathetically innervated organs and brain in various strains of rats.

Studies of blood DBH may be more meaningful in an animal model of hypertension such as spontaneously hypertensive (SH) rats (*7*), since the genetic variations may be small if Wistar-Kyoto rats (from which the SH strain was bred) are used as controls. In fact, we found that both Wistar-Kyoto and SH rats (both strains supplied by Drs. K. Okamoto and Y. Yamori, University of Kyoto) show only slight variations in serum DBH activity, which is fairly constant after the rats are 14 weeks old and is not significantly different between the two strains. However, young SH rats (3 weeks old) were shown to have higher serum DBH activity than Wistar-Kyoto rats of the same age (*8*). One interesting analogy in the blood level of DBH between our study on SH rats and the human study by Stone *et al.* (*3*) is the high activity of DBH before the onset of hypertension and subsequent decrease after development of hypertension.

Since there is considerable controversy over whether DBH levels in serum can be an index of peripheral sympathetic activity, we examined the activity of DBH in peripheral sympathetically innervated tissues and adrenals of 3-week-old SH rats, in order to prove the increase of the DBH level and to investigate the origin of the elevated serum DBH activity in these young rats.

Some central noradrenergic neurons are supposed to have either inhibitory (*9*) or stimulatory (*10*) roles in the regulation of peripheral sympathetic activities; therefore the DBH activity in some noradrenergic regions of the brain was also examined. Tyrosine hydroxylase (TH), which synthesizes dopa from tyrosine as the initial step of biosynthesis of catecholamines and therefore exists in both noradrenergic and dopaminergic brain regions, was also measured in comparison with DBH.

The SH and Wistar-Kyoto rats of the same age were raised under the same conditions. At 3 weeks of age the rats were killed by decapitation; blood samples were obtained by exsanguination and were put into a test tube kept in ice, and the serum was removed. Mesenteric vessels, vas deferens, adrenals, and brain were quickly removed, frozen on Dry Ice, and stored at −80°C. The regions of catecholaminergic neurons (caudate nucleus, substantia nigra, hypothalamus, and locus coeruleus) were dissected out under a microscope from frozen sections of the brain (*11*). The brain tissues were homogenized in 250 to 380 μl of 0.1*M* potassium phosphate buffer, *p*H 7.5, containing 0.1 percent Triton X-100. Mesenteric vessels, vas deferens, and adrenals were homogenized in 2 to 4 ml of the same buffer; the homogenate was centrifuged at 50,000*g* for 30 minutes, and the supernatant was used for the assay of DBH and TH activities. The DBH activity was determined by dual-wavelength spectro-

Table 1. Dopamine β-hydroxylase activity of Wistar-Kyoto (control) and spontaneously hypertensive (SH) rats at 3 weeks of age. Numbers in parentheses are the number of samples. Abbreviation: S.E.M., standard error of the mean.

Source of enzyme	Dopamine β-hydroxylase activity			
	Nanomoles per minute per gram (wet weight ± S.E.M.)		Nanomoles per minute per milligram of protein ± S.E.M.	
	Wistar-Kyoto rats	SH rats	Wistar-Kyoto rats	SH rats
Serum	1.14 ± 0.08 (4)	1.63 ± 0.07* (5)		
Mesenteric vessels	2.2 ± 0.1 (5)	5.8 ± 0.8* (5)	0.30 ± 0.01 (5)	0.76 ± 0.03* (5)
Vas deferens	8.5 ± 0.9 (4)	7.5 ± 0.9 (5)	0.57 ± 0.05 (4)	0.49 ± 0.04 (5)
Adrenals	31 ± 4 (4)	46 ± 3† (5)	0.82 ± 0.07 (4)	1.12 ± 0.08† (5)

*Differs from control (Wistar-Kyoto rats), $P < .01$. †Differs from control (Wistar-Kyoto rats), $P < .05$.

Table 2. Dopamine β-hydroxylase and tyrosine hydroxylase activities in the brain regions of Wistar-Kyoto (control) and spontaneously hypertensive (SH) rats at 3 weeks of age. Values are expressed in picomoles per minute per milligram of protein ± standard error of the mean. Numbers in parentheses are the number of samples.

Region	Dopamine β-hydroxylase*		Tyrosine hydroxylase†	
	Wistar-Kyoto rats	SH rats	Wistar-Kyoto rats	SH rats
Substantia nigra	0‡ (8)	0‡ (8)	51 ± 7 (8)	65 ± 6 (8)
Caudate nucleus	0‡ (8)	0‡ (8)	136 ± 7 (8)	141 ± 7 (8)
Locus coeruleus	249 ± 15 (8)	186 ± 19§ (7)	13 ± 2 (8)	12 ± 2 (8)
Hypothalamus	54 ± 4 (8)	47 ± 4 (8)	15 ± 2 (8)	22 ± 3 (8)

*Substrate, $2 \times 10^{-2}M$ tyramine. †Substrate, $2.5 \times 10^{-3}M$ tyrosine; cofactor, $2 \times 10^{-3}M$ L-*erythro*-tetrahydrobiopterin. ‡Less than the limit of sensitivity of the assay. §Differs from control (Wistar-Kyoto rats), $P < .05$.

photometry with tyramine as the substrate, based on the photometric micromethod (*12*). Tyrosine hydroxylase activity was determined by measuring the formation of [^{14}C]dopa from L-[^{14}C]tyrosine (*13*).

Table 1 shows DBH activity in serum, mesenteric vessels, vas deferens, and adrenals of SH rats and the control Wistar-Kyoto rats. The DBH activity in serum, mesenteric vessels, and adrenals of SH rats was significantly higher than those of the controls, but that in vas deferens was not statistically different in these two strains of rats. Serum DBH is known to arise from sympathetic nerve terminals and not from adrenals (*14*), and a significant fraction of circulating norepinephrine is considered to be derived from blood vessels (*15*). The results, therefore, suggest that the elevated DBH activity in serum of young SH rats is derived mainly from the blood vessels, reflecting the activated peripheral sympathetic nerves. This also agrees with our previous results that serum DBH activity and DBH and TH activities in mesenteric vessels in 10-week-old SH rats increase significantly when blood pressure is increased rapidly by administration of NaCl for 4 weeks (*16*).

The DBH and TH activities in the brain regions of catecholaminergic neurons are given in Table 2; DBH activity was found only in the regions of noradrenergic neurons, such as the locus coeruleus and hypothalamus. Very low DBH activity in the dopaminergic regions, such as caudate nucleus and substantia nigra, may be due to accurate dissection of the anatomical region. Low DBH activity in the caudate nucleus has been reported (*17*). In contrast, TH activity was found in both dopaminergic and noradrenergic brain regions, although it was much higher in the former.

In the locus coeruleus DBH activity of SH rats was significantly lower than that of Wistar-Kyoto rats, but in the hypothalamus there was no statistical difference in DBH activity between the SH and the control rats.

The TH activity in the substantia nigra, caudate nucleus, and locus coeruleus was similar in SH and Wistar-Kyoto rats; the activity in the hypothalamus of SH rats was higher than that of Wistar-Kyoto rats but does not differ significantly ($P < .1$). Since Lamprecht *et al.* (*18*) also reported that after 4 weeks of immobilization stress to rats there was a significant increase in the activity of hypothalamic TH and in the activity of serum DBH, the significance of a slightly higher TH value in the hypothalamus remains to be investigated further.

The present findings on the abnormality of central noradrenergic neurons of SH rats may be significant, since such central changes were detected in young SH rats whose peripheral sympathetic nerve activity, as judged by DBH activity in blood vessels and serum, appears to be increased. After the development of hypertension, the peripheral sympathetic tone of SH rats appears to be decreased, probably by a compensatory mechanism (*8, 19*). The noradrenergic neuron in the brainstem is believed to play a role in depression of peripheral sympathetic nerves through an alpha receptor to decrease the blood pressure (*9*), whereas that in the hypothalamus may play a role in activation of peripheral sympathetic nerves through a beta receptor to increase the blood pressure (*10*). Probable implication of the central noradrenergic inhibitory center in hypertension of SH rats had first been suggested by Yamori *et al.* (*20*). However, we need evidence to show whether there is a relation between changes in catecholamine synthesizing enzymes in the brain and their increase in peripheral tissues.

Although care is necessary in relating the present results to hypertension, they do suggest that central and peripheral sympathetic nerve function may be changed before the onset of essential hypertension, and, therefore, should be examined at this early period, and they support the concept that the nervous system is an important regulator of blood pressure.

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Attachment and Penetration of Miracidia Observed by Scanning Electron Microscopy

Abstract. *Scanning electron microscopy can be utilized to understand more clearly many aspects of the parasite-host relationships of schistosome miracidia and their molluscan intermediate hosts. Specialized structures on the apical papilla of the miracidium, used for attachment and penetration, become visible in greater detail.*

Few biologists, including those working with parasites, have observed actual penetration of a snail by the motile and nonfeeding larval stage of a digenetic trematode called the miracidium. The small size of the parasite, the relatively short penetration time, and the continual movement of the snail intermediate host make this parasite-host contact difficult to witness.

Recent developments with scanning and transmission electron microscopes have made possible more extensive research on the mechanisms of miracidial attachment and penetration. Penetration and devel-