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Norepinephrine Metabolism in Brainstem of Spontaneously Hypertensive Rats

Abstract. *Concentrations of norepinephrine in lower brainstem and hypothalamus of genetically hypertensive rats are significantly lower than in control rats. There is a concomitant reduction (50 percent) in aromatic* L*-amino acid decarboxylase but not in tyrosine hydroxylase activity. A possible relation of this central catecholamine deficiency to the hypertension is discussed.*

Because catecholamines are neurohumoral pressor substances, it has long been suspected that they play a role in the pathogenesis of hypertension. However, generally speaking, investigators have been unsuccessful in demonstrating elevated concentrations of catecholamines in relation to hypertension. The spontaneously hypertensive rat (SHR) developed by Okamoto and Aoki (*1*, *2*) appears to be a suitable model of human essential hypertension and is now available as a genetically pure strain. Using this model we have investigated catecholamine metabolism in the central nervous system.

The blood pressures of male SHR's of F_{19-20} generations, control Wistar (NIH) and Sprague-Dawley rats (Zivic-Miller Lab. P.A.) from 5 to 20 weeks of age were measured without anesthesia by a tail plethysmographic method (*1*). Even in the earliest measurements (5 weeks after birth) the systolic blood pressure in SHR's was slightly higher than that in the controls. It was greatly elevated at 6 weeks of age and thereafter.

The SHR's and controls at 6, 10, and 20 weeks of age and the other rats in experimental groups at 20 weeks of age were killed by decapitation. Studies were done on whole brain (except for olfactory bulbs) and the following brain parts: lower brainstem (medulla oblongata, pons, and midbrain), brainstem (lower brainstem plus diencephalon), hypothalamus, and telencephalon. Various brain parts were dissected out, trimmed carefully, and immediately frozen for norepinephrine assay or chilled for enzyme assay. Mesenteric arteries from the root of superior mesenteric artery to the branches into the intestinal loop were dissected free from fat and connective tissue. The tissues were weighed and homogenized in cold 0.4*N* perchloric acid or in cold distilled water. Supernatant of the former, obtained after centrifugation, was analyzed for endogenous norepinephrine (*3*) and of the latter for aromatic L-amino acid decarboxylase activity (*4*) or for tyrosine hydroxylase activity (*5*). Protein concentration of the supernatant was determined by a modification of the phenol reagent method (*6*), and specific activities of these enzymes were calculated.

We reported recently that the concentration of norepinephrine was slightly, but probably not significantly ($P < 0.2$), decreased in the whole brainstem of the SHR's (*7*) as compared to control Wistar rats. However, looking specifically at the hypothalamus and lower brainstem there is a considerable reduction of norepinephrine in SHR's when compared to NIH control Wistar rats (Table 1). The average concentrations in 10-week-old SHR's in the initial stages of hypertension were about 60 and 70 percent of the control values in lower brainstem and hypothalamus, respectively. In these experiments the concentration of norepinephrine in the whole brain also appeared to be lower. This observation distinguishes this strain of SHR from the strain developed by Smirk and co-workers (*8*), who found their animals to have normal concentrations of norepinephrine in the brainstem but elevated concentrations in the cerebellum.

Aromatic L-amino acid decarboxylase activity in whole brainstem and telencephalon is clearly decreased in SHR's in comparison with that in normotensive control Wistar or Sprague-Dawley rats (Table 2). The enzyme activity in the SHR was about 50 percent of that in normotensive control Wistar or Sprague-Dawley rats. Even in the young, 6-week-old SHR, whose blood pressure was only slightly increased over the control level, the difference was clear-cut with no experimental point from SHR overlapping those of the controls. Animals with renal or deoxycorticosterone (DOC)-salt hypertension did not show any differences in the enzyme activity (Table 2). Consequently, this decrease in the activity did not appear to be due to secondary depression of enzyme activity by the hypertension itself. Since SHR's are considered to have slightly increased adrenocortical function (*2*, *9*), enzyme activity was also examined in adrenalectomized SHR's and control

Table 1. Norepinephrine content of the brain in spontaneously hypertensive and control rats. Values shown are means ± S.D. The numbers in parentheses indicate the number of animals used.

Group	Systolic blood pressure	Norepinephrine (μg/g)		
		Whole brain	Lower brainstem	Hypothalamus
		SHR		
10-weeks old (10)	176 ± 9*	0.268 ± 0.061†	0.453 ± 0.015*	1.220 ± 0.144*
20-weeks old (14)	214 ± 12*	0.235 ± 0.020*		
		Control Wistar (NIH)		
10-weeks old (10)	129 ± 4	0.333 ± 0.022	0.726 ± 0.072	1.725 ± 0.196
20-weeks old (14)	132 ± 5	0.323 ± 0.035		

* Significant difference from the control ($P < .001$). † Significant difference from the control ($.005 < P < .01$).

Wistars treated with cortisone, but neither a diminution nor an increase in adrenocortical hormones affected enzyme activity. No sex differences were observed.

The supernatants of some of the samples from SHR and controls were dialyzed overnight in 0.01M phosphate buffer, pH 7.6, containing $10^{-4}M$ pyridoxal phosphate. The dialysis had no effect on the enzyme activity in the SHR or in the controls. Consequently the decreased activity in SHR is not due to a deficiency of cofactor, to dialyzable inhibitors, or to feedback inhibition by the reaction product. The difference in decarboxylase enzyme activity was also observed when either o-tyrosine or 5-hydroxytryptophan was used as substrate. Although there was also a somewhat lower enzyme activity in the kidney in the adult SHR than in the control Wistar rats, individual variation was large and no difference was noted between adult SHR and Sprague-Dawley or between young SHR and young control animals (Table 2).

Tyrosine hydroxylase activity was not significantly different in the brainstem of SHR compared with the control groups (Table 3). Recently, a decreased activity of this enzyme in the mesenteric arteries of SHR was reported (*10*). In the present studies tyrosine hydroxylase activity in the mesenteric arteries was slightly lower in young SHR than in young Wistars, but no significant difference was noted between adult SHR and adult control rats.

These findings are consistent with the recent observation that the apparent synthesis rate of norepinephrine from tyrosine is decreased in the brain of the SHR (*7*). In this, as in all such studies, however, the question of the appropriate control animal is important and places certain limitations on the conclusions that can be drawn since various normotensive animals show different biochemical and pharmacological characteristics (*11*). It is significant, however, that the SHR has lower rather than higher central stores of the pressor amines. It is also difficult to relate decreased concentrations of norepinephrine in the brainstem to the decrease in decarboxylase because the rate-limiting enzyme is considered to be tyrosine hydroxylase (*12*). It should be pointed out, however, that while the gross maximum velocities of the enzymes favor decarboxylase by as much as 40:1 when assayed under optimum conditions in vitro, the conditions in vivo may not allow such a favorable ratio to exist. For example, the Michaelis constant (K_m) of tyrosine hydroxylase is on the order of $10^{-5}M$ which is substantially below the concentration of tyrosine in the tissue. On the other hand, the K_m of aromatic L-amino acid decarboxylase for dopa (dihydroxyphenylalanine) is on the order of $10^{-4}M$ which is at least an order of magnitude higher than the concentrations of dopa in the tissues. Therefore these two enzymes may normally be more balanced in vivo, and a 50 percent reduction in decarboxylase might shift the rate-limiting role to this enzyme.

While the importance of central regulation of blood pressure is well known, the biochemical basis of this control is not yet obvious. It would appear that catecholamines, while being pressor peripherally, may participate in a central depressor system. The current findings and those of others are consistent with this proposal. In the SHR the lower concentrations of catecholamines in discrete areas of the brain suggests a possible relation to the hypertension. If this is so, then administration of the catecholamine precursor L-dopa should result in reduction in blood pressure. Initial experiments show this to be true in SHR (*13*) and the normotensive rat (*14*) when the animals are treated with peripheral decarboxylase inhibitors that prevent the peripheral formation of pressor substances. Furthermore, ad-

Table 2. Aromatic L-amino acid decarboxylase activity in spontaneously hypertensive rats, renal and DOC-salt hypertensive rats, and controls. Wistar rats with renal infarction hypertension (*17*) and DOC-salt hypertension (*18*) [two 20 mg deoxycorticosterone acetate pellets (Geigy) were implanted bilaterally in psoas muscle without nephrectomy] were maintained on 1 percent salt water for 3 weeks after treatment and were used for enzyme assay after hypertension was confirmed. Wistar rats treated with cortisone [5 mg of cortisone acetate (Merck) injected intramuscularly twice daily for 1 week] were used after the effectiveness of the treatment was checked by measuring decrease in adrenal weight. Adrenalectomized SHR were maintained on 1 percent salt water only for the first week after treatment and were used at the third week. The completeness of bilateral adrenalectomy was confirmed at autopsy. The young and adult rats used were 6 and 20 weeks old, respectively. The numbers in parentheses indicate the number of rats used. Enzyme activity is expressed as the number of nanomoles of serotonin formed per hour per milligram of protein. Values shown are means ± S.D.

Group	Blood pressure (mm-Hg)	Aromatic L-amino acid decarboxylase activity		
		Brainstem	Telencephalon	Kidney
SHR				
Young (11)	145 ± 5*	12.0 ± 1.8*	6.3 ± 1.4*	123.9 ± 20.2
Adult (15)	217 ± 8*	11.5 ± 1.1*	7.1 ± 1.2*	119.5 ± 25.5†
Wistar (NIH)				
Young (11)	121 ± 7	22.4 ± 3.5	12.8 ± 1.8	140.9 ± 37.5
Adult (11)	133 ± 5	24.3 ± 3.2	12.6 ± 1.9	149.5 ± 17.7
Sprague-Dawley				
Adult (6)	133 ± 4	21.2 ± 2.6	13.3 ± 1.5	127.8 ± 11.1
Renal hypertension (5)	205 ± 17*	27.7 ± 1.0	15.7 ± 1.5	158.6 ± 24.1
DOC-hypertension (3)	174 ± 2*	23.2 ± 1.2	13.9 ± 1.2	184.2 ± 30.0
Cortisone-treated Wistar (3)	144 ± 25	24.1 ± 3.7	12.1 ± 1.1	167.4 ± 3.8
Adrenalectomized SHR (3)	182 ± 8*	13.5 ± 1.6*	7.1 ± 0.3*	143.7 ± 28.9

* Significant difference from control values of age-matched Wistar rats ($P < .001$). † Significant difference from control values of age-matched Wistar rats ($.01 < P < .02$).

Table 3. Tyrosine hydroxylase activity and systolic blood pressure in spontaneously hypertensive and control rats. The young and adult rats were 6 and 20 weeks old, respectively. The enzyme activity is expressed as the number of nanomoles of tyrosine hydroxylated per milligram of protein per hour (mean ± S.D.). The numbers in parentheses indicate the number of animals used.

Subject	Blood pressure (mm-Hg)	Tyrosine hydroxylase	
		Brainstem	Mesenteric artery
	SHR		
Young (6)	146 ± 5*	0.91 ± 0.20	0.85 ± 0.19†
Adult (6)	214 ± 12*	0.82 ± 0.16	0.92 ± 0.25
	Wistar (NIH)		
Young (6)	125 ± 4	0.76 ± 0.15	1.29 ± 0.26
Adult (5)	130 ± 3	0.84 ± 0.18	1.20 ± 0.29
	Sprague-Dawley		
Adult (4)	134 ± 2	0.84 ± 0.30	0.97 ± 0.25

* Significant difference from the control value of the age-matched Wistar rats ($P < .001$). † Significant difference from the control value of the age-matched Wistar rats ($.025 < P < .05$).

ministration of L-dopa to parkinsonian patients has been observed to cause a hypotensive response (*15*). It has also been pointed out by Henning (*16*) that the acute hypotensive effects of alpha-methyl-dopa are mediated by a central mechanism requiring the decarboxylation of this amino acid. This action might be due to the replenishment of catecholamine-deficient fibers with the alpha-methylated analogs. The hypotensive action of the monoamine oxidase inhibitors could also be rationalized by relating the buildup of central catecholamines to drug action. Catecholamine-depleting compounds such as reserpine may be hypotensive simply because they also deplete the peripheral amine stores which are required for the maintenance of the hypertensive state.

It can be concluded that catecholamine mechanisms in the central nervous system may play an important role in the regulation of blood pressure, and that genetic hypertension in the rat and possibly in man may be related to a deficiency of catecholamines in certain areas of the brain.

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Trail Odors: Recognition by Insects Parasitic on Cocoons

Abstract. *Female parasitic insects in the genera* Pleolophus, Endasys, *and* Mastrus (*Hymenoptera: Ichneumonidae*) *search the ground cover for hosts and avoid areas they have already inspected. Females respond to their own trail odor, and recognition occurs also between conspecific, congeneric, and intergeneric individuals. This is the first direct evidence for recognition between parasitoids at sites other than on the host itself, and between females of different species.*

Discrimination between parasitized and unparasitized hosts by parasitic insects results in the optimum survival of their progeny. The ability to discriminate has been well documented (*1*) and is considered to be a common attribute of parasitoids (*2*). Here I report observations showing that parasitoids also discriminate between presearched and unsearched areas. This is adaptive, since searching becomes more efficient when presearched areas can be avoided. Also, intraspecific recognition of trails could cause a more even dispersion of individuals throughout the habitat.

Discrimination at the site of the host can result from detection of a previous parasitoid by its odor (*3*). Salt (*3*) found that females of *Trichogramma evanescens* could detect the odor of another female left on a host egg 2 days earlier. Discrimination also resulted when a water solution, made by washing glass over which the females had walked, was painted on a host egg. Ullyett (*4*) suggested that female *Bracon hebetor* retrace their trails by using olfactory stimuli. Many species of Ichneumonidae emit easily detected, pungent odors thought to be protective (*5*). These may also serve as the odors that other parasitoids can detect and avoid. I found that female *Pleolophus basizonus* avoided an area they had previously searched (*6*).

Two experiments were performed to test the ability of female parasitoids to

Table 1. Probability of parasitoids showing apparent recognition of odors in the treatment as frequently as in the control (*7*).

Second species present	First species present			
	Pleolophus basizonus	*Pleolophus indistinctus*	*Endasys subclavatus*	*Mastrus aciculatus*
Pleolophus basizonus	0.005*	0.025*	0.050*	0.010*
Pleolophus indistinctus	.750	.050*	.005*	.500
Endasys subclavatus	.250	.005*	.025*	.025*
Mastrus aciculatus	.250	.050*	.025*	.050*

* Significant difference at 5 percent level or less.

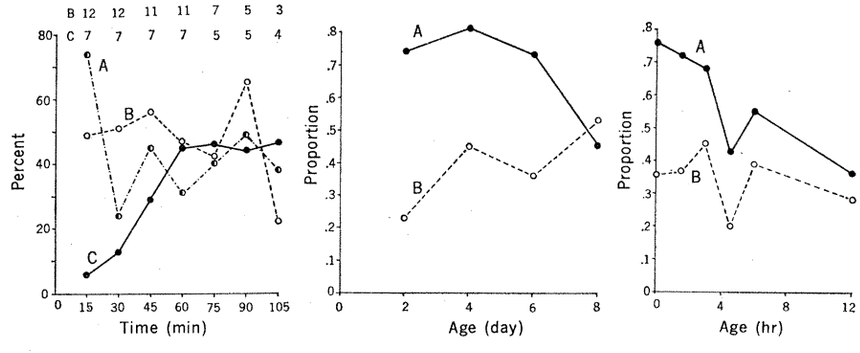

Fig. 1. (Left) Percentage of time spent by a female parasitoid in the half of the observation arena it had searched before, in successive 15-minute periods (experiment 1). *A*, Control in which one female was placed in a fresh tray; *B*, means for females that showed no discrimination; *C*, means for females that showed discrimination. Note that this discrimination was reduced with time as the fresh side was gradually contaminated with the odor. Numbers of replicates for *B* and *C* are shown above. (Middle) The proportion of parasitoid approaches which showed apparent odor recognition in treatment (*A*) and control (*B*), as a function of parasitoid age (experiment 2). Each point is the mean of four replicates, $P_{A=B} < .005$, $< .025$, $< .005$, and $< .750$, respectively. (Right) The proportion of parasitoid approaches which showed apparent odor recognition in treatment (*A*) and control (*B*), as a function of age of the odor (experiment 2). Each point is the mean of 12, 2, 3, 2, 2, and 4 replicates, $P_{A=B} < .005$, $< .050$, $< .050$, $< .050$, $< .500$, and $< .750$, respectively.