GENETIC VARIATION OF THE CATECHOLAMINE BIOSYNTHETIC ENZYME ACTIVITIES IN VARIOUS STRAINS OF RATS INCLUDING THE SPONTANEOUSLY HYPERTENSIVE RAT

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NOREPINEPHRINE, the neurotransmitter of the sympathetic nervous system, is synthesised by a well established three step enzymic pathway. These enzymes have been characterised and some of the mechanisms which control their intracellular activity are known. Since the sympathetic system plays a role in regulating the tone of the vasculature and central noradrenergic systems appear to be involved in blood pressure regulation, many investigators have attempted to relate catecholamine metabolism to hypertension. No gross changes in the easily measured parameters of catecholamine metabolism are evident in any type of human hypertension except that resulting from pheochromocytoma; several recent reports, however, indicate that plasma norepinephrine is slightly elevated in certain patients with essential hypertension (ENGELMAN *et al.*, 1970; DEQUATTRO and CHAN, 1972; LOUIS *et al.*, 1973).

The catecholamine metabolism of the spontaneously hypertensive rat (SHR) (OKAMOTO and AOKI, 1963) has also been investigated intensively by our laboratory (LOUIS *et al.*, 1969, 1970; YAMORI *et al.*, 1970, 1972a,b; YAMABE *et al.*, 1973) and by other laboratories (OZAKI, 1966; NAGATSU *et al.*, 1971; NAKAMURA *et al.*, 1971; SPECTOR *et al.*, 1972). No unambiguous relationship of catecholamine metabolism to the pathogenesis of hypertension in these rats has been established.

There is an alternate approach to evaluating cause and effect relationships in hypertension. The results of studies with different species clearly indicate that there is a genetic component in hypertension. For example HANSEN (1972) found in a survey of the blood pressures of inbred rats that considerable interstrain variation exists, although within-strain variation is relatively small. Comparison of factors which are suspected to participate in the development of hypertension in several strains may provide insight into physiologic-biochemical relationships.

In the current study we have measured tissue norepinephrine content and the activities of the three norepinephrine biosynthetic enzymes (tyrosine hydroxylase, aromatic L amino acid decarboxylase, and dopamine β hydroxylase,) in 9 different inbred strains of rats with a wide range of "normal" blood pressures.

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MATERIALS AND METHODS

The animals used in these studies were maintained in inbred colonies in the Animal Production Section of the National Institutes of Health (NIH). The animals were matched as closely as possible for age, sex and size in all the experiments. Deviations from complete matching are noted in the various experiments. The strains of rats used and their abbreviations are as follows: The Kyoto spontaneously hypertensive rat maintained as an inbred strain at the NIH, SHR; Wistar/NIH, W; Wistar/Kyoto, W/Ky; Roman High Avoidance, RHA; Osborne-Mendel, OM; Albany, ALB; ACI, PETH, and M-520. The details of all the physiologic and analytical methods used in this study have been presented previously (YAMABE *et al.*, 1973). Table 1 gives the mean systolic blood pressure for each of these strains.

TABLE 1. SYSTOLIC BLOOD PRESSURE OF 10-WEEK-OLD INBRED RAT STRAINS OF THE NIH*

	Mean blood pressure (mm Hg)		Mean blood pressure (mm Hg)
SHR	182 ± 5	PETH	128 ± 7
OM	160 ± 6	W/K	128 ± 6
RHA	158 ± 11	W	128 ± 6
ALB	143 ± 14	M520	124 ± 9
		ACI	116 ± 6

* Taken from HANSEN, C. T. (1972). Values given are mean systolic pressures ± S.D.

RESULTS AND DISCUSSION

The various parameters of catecholamine metabolism were first examined in brainstem. The level of norepinephrine was relatively constant within any one strain but ranged from a low 455 ng/g in the W/Ky to a high of 739 ng/g in W. All other strains examined were approximately midway between the two extremes.

Table 2 gives the brainstem activity for each of the norepinephrine biosynthetic enzymes. There are several observations of interest in these data. First, the activity of tyrosine hydroxylase varies very little among the different strains. The absence of strain differences is not surprising since this is the rate limiting enzyme in norepinephrine synthesis and since a certain minimal level of norepinephrine is necessary for normal neuronal functions. Conversely, it should be noted that the variation in norepinephrine levels is somewhat greater than that in tyrosine hydroxylase levels and therefore factors other than total tyrosine hydroxylase activity must contribute to amine level. This phenomenon has been discussed elsewhere (YAMABE *et al.*, 1973).

Second, dopamine β hydroxylase which has about 10 times the specific activity of tyrosine hydroxylase shows more variability between strains. There appears to be a partial relationship between dopamine β hydroxylase and norepinephrine content, although insufficient data have been accumulated to determine statistical or physiological significance of such a relationship.

Third, aromatic L amino acid decarboxylase activity which is about 100 times higher than tyrosine hydroxylase shows marked variation between strains. This enzyme is thought to be present in neuronal cells in substantial excess. In this limited study there appeared to be two strain-types. The SHR, RHA and W/Ky being a

TABLE 2. NOREPINEPHRINE BIOSYNTHETIC ENZYME ACTIVITY IN THE BRAINSTEM OF INBRED RAT STRAINS

Strain	Enzyme activity* (nmole/hr/mg protein)		
	Tyrosine hydroxylase	Aromatic L amino acid decarboxylase	Dopamine-β-hydroxylase
SHR	0·55 ± 0·01	38 ± 1	5·7 ± 0·3
OM	0·52 ± 0·02	68 ± 2	6·7 ± 0·1
RHA	0·49 ± 0·02	45 ± 1	7·0 ± 0·2
ALB	0·51 ± 0·06	83 ± 1	5·9 ± 0·1
PETH		64 ± 1	5·4 ± 0·2
W/K	0·47 ± 0·02	41 ± 1	4·5 ± 0·1
W	0·55 ± 0·03	71 ± 3	6·4 ± 0·3
M520	0·50 ± 0·02	66 ± 2	5·2 ± 0·3
ACI	0·54 ± 0·02	62 ± 1	5·4 ± 0·1

* The values given are the mean ± S.E.M. for at least four individual animals analysed in duplicate. Tyrosine hydroxylase and aromatic L amino acid decarboxylase were measured in 5-week-old animals. The age of the animals for dopamine-β-hydroxylase was 5–7 weeks.

low activity type and the other six strains a high activity type. When additional strains are examined, however, the types may merge into a continuous spectrum.

The activities of the norepinephrine biosynthetic enzymes have also been measured in the adrenal glands of each of these inbred strains (Table 3). Since there are significant strain differences in adrenal weights and amounts of cortical tissue, the activities are reported per pair of glands. As in the brainstem, strain differences are evident in all three enzymes. The variation in tyrosine hydroxylase is more marked whereas aromatic L amino acid decarboxylase activities fall into a much tighter range. It is also notable that the relative proportions of the various enzymes are quite different when the brainstem is compared to adrenal tissue. There appears to be no relationship between any of the enzymes and blood pressure.

The levels of each of the norepinephrine biosynthetic enzymes appear to be under independent genetic regulation (Tables 2 and 3). This is apparent in both the central nervous system and adrenal gland. Furthermore the relationships that occur in one organ are not present in the other organ. Using a completely different experimental approach THOENEN (1972) also showed that these enzymes are independently regulated.

BARCHAS and coworkers (CIARANELLO *et al.*, 1972; KESSLER *et al.*, 1972) have measured tyrosine hydroxylase in the adrenal gland and brainstem of a number of inbred mouse strains. They found greater variation in tyrosine hydroxylase than we observed among the rat strains, however, their conclusions were similar to ours; i.e., there are significant genetic variations in the catecholamine biosynthetic enzymes.

In the current study, the nine inbred rat strains with a wide range of normal blood pressures showed marked variations in norepinephrine biosynthetic enzymes in the brainstem and adrenal glands. No correlation between any of the enzymes and blood pressure was apparent. This divergence was exemplified by a comparison of aromatic L amino acid decarboxylase and blood pressure in W, W/Ky and SHR (Table 4). All three of these strains are of the Wistar type, however the SHR is genetically similar to the W/Ky, being separated from that strain about 30 generations previously.

TABLE 3. NOREPINEPHRINE BIOSYNTHETIC ENZYME ACTIVITIES IN THE ADRENAL GLAND OF INBRED RAT STRAINS

Strain	Enzyme activity* (nmole/hr/gland)		
	Tyrosine hydroxylase	Aromatic L amino acid decarboxylase	Dopamine-β-hydroxylase
SHR	11·7 ± 0·5	319 ± 20	210 ± 5
OM	8·1 ± 0·2	310 ± 29	396 ± 10†
RHA	7·7 ± 1·0	455 ± 20	313 ± 17
ALB	12·3 ± 0·7		573 ± 52†
PETH		363 ± 14	303 ± 30†
W/Ky	12·3 ± 0·4	405 ± 37	295 ± 7†
W	9·9 ± 0·3	336 ± 25	275 ± 26
M520	8·1 ± 0·9	321 ± 18	551 ± 25
ACI	9·0 ± 0·8	329 ± 16	355 ± 22

* The values given are the mean ± S.E.M. for at least four individual animals. Tyrosine hydroxylase and aromatic L amino acid decarboxylase in 5-week-old animals and dopamine-β-hydroxylase in 5–7 week old animals.

† 7-weeks-old.

The decarboxylase activity of brainstem and heart is similar in the two Kyoto strains, but is 2 to 3 times higher in the Wistar from the NIH. In addition to these organs, other peripheral tissues show significant differences in many parameters of catecholamine metabolism when the W is compared with the two strains developed in Kyoto, Japan (YAMABE and LOVENBERG, to be published).

TABLE 4. AROMATIC L AMINO ACID DECARBOXYLASE AND BLOOD PRESSURE IN 3 RAT STRAINS*

Rat strain	Adult systolic blood pressure (mm Hg)	Decarboxylase (nmole/hr/mg protein)	
		Heart	Brainstem
Wistar/NIH	135 ± 2	5·9 ± 0·1	71 ± 3
Wistar/Kyoto–NIH	132 ± 1	1·9 ± 0·1	41 ± 1
SHR/NIH	187 ± 1	1·9 ± 0·2	38 ± 1

* Data is taken from male animals 10–12 weeks of age for blood pressure and 5 weeks of age for the enzyme studies. Decarboxylase activity is measured as described by YAMABE *et al.* (1973).

CONCLUSIONS

(1) There is significant genetic variation in the level of catecholamine biosynthetic enzymes in various inbred rat strains.

(2) The genetic variation of catecholamine synthesis in the brainstem and adrenal glands does not appear to relate to the"normal" blood pressure of individual inbred strains.

(3) Each of the three norepinephrine biosynthetic enzymes appear to be under independent genetic regulation.

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