LEVELS OF TYROSINE AND TRYPTOPHAN IN THE PLASMA AND BRAIN OF SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—Tyrosine and tryptophan contents in the plasma and brain, and noradrenaline and serotonin contents in the brain were measured in spontaneously hypertensive rats (SHR) and two normotensive strains, Wistar/Kyoto rats (WKR) and Wistar/Carworth rats (WCR) to investigate the quantitative relationship between monoamines and their precursor amino acids in the brain of SHR. WKR is the parent strain of SHR. Tyrosine level in the plasma and brain of SHR was significantly lower than that in WCR, whereas tryptophan level in the plasma and brain of SHR was higher than that of WCR. The levels of these amino acids were the same in SHR and WKR. Noradrenaline and serotonin contents in the brain stem of SHR and WKR were lower than the respective contents of WCR, while no difference was found in noradrenaline and serotonin contents in the telencephalon of these 3 strains. In SHR, 1.0% L-tyrosine ingestion for 5 days produced a slight but significant fall of the blood pressure accompanied by an increase of tyrosine in the plasma and brain. However, noradrenaline in the brain stem did not change following the L-tyrosine feeding. These results suggest that differences in contents of the monoamines and their precursor amino acids in SHR and WKR could be due to genetic factors in a specific closed colony.

The spontaneously hypertensive rats (SHR) developed by Okamoto and Aoki (1) are regarded as a suitable animal model for the study on the pathogenesis of hypertension. From the biochemical study on SHR, Yamori et al. (2) speculated the possible role of a central noradrenergic mechanism in the modulation of blood pressure. Thereafter, a number of studies were documented concerning catecholamine metabolism in the brain of SHR: noradrenaline content (2, 3), tyrosine hydroxylase activity (4), turnover rate of noradrenaline (5) and synthesis of noradrenaline from tyrosine (6). Recently, Yamabe et al. (7) demonstrated that the catecholamine level, turnover rate and in vivo synthesis rate were lower in SHR than in the normotensive Wistar/NHI strain rats but similar to those in the normotensive Wistar/Kyoto rats (WKR), from which the SHR were isolated. Brain serotonin level in SHR over 6 months of age was found to be higher than that of normotensive WKR (8).

Although there are several views concerning the relationship between central monoamine metabolism and blood pressure, there is still a paucity in information concerning precursor amino acids of these monoamines in SHR. In the present experiment, tyrosine and tryptophan levels in the plasma and brain of SHR were compared with those of two normotensive strains, WKR and Wistar/Carworth strain rats (WCR). In addition, effects of L-tyrosine feeding on the blood pressure and contents of tyrosine and noradrenaline were studied in SHR.
MATERIALS AND METHODS

The SHR were derived from an original colony in the Department of Pathology, Kyoto University, and had been inbred by successive matings in our own animal house. Normotensive WKR 4 weeks of age were obtained from a closed colony of Wistar rats kept in the Animal Center of Kyoto University, from which the SHR were isolated. Other normotensive control WCR were purchased from a commercial source (Kitayama Lab. Exp. Animals, Kyoto). These 3 different strains of male rats, each 4 weeks old, were simultaneously housed in separate cages at 22-24°C under a constant day-night rhythm and maintained on a commercial diet (Japan CLEA Co., CA-1) and drinking water ad libitum. Systolic blood pressure of the tail artery was measured using an Automatic Blood Pressure Recorder (Ueda Seisakusho, USM-105-R). The rat was put in a warm chamber at a temp. of 35°C for 10 min, and then the tail was placed on a pick-up connected to a pressure transducer. The systolic blood pressure corresponded to the onset of pulse wave.

On the 12th week after birth, the rats were sacrificed by decapitation between 10 and 12 a.m. following a 5 hr fasting period. Blood samples were collected into a heparinized tube and centrifuged. The brain was rapidly removed and homogenized in ice-cold saline solution. For the assay of noradrenaline and serotonin, the brain was divided into two portions: the telencephalon and brain stem. The contents of noradrenaline were determined fluorometrically by a modified method (9) of Anton and Sayre (10). Serotonin was assayed according to the method of Bogdanski et al. (11). Tyrosine and tryptophan in the plasma and brain were assayed by the method of Waalkes and Udenfriend (12) and Denckla and Dewey (13), respectively. \( \alpha \)-Amino nitrogen in the plasma and brain was estimated colorimetrically as an index of free amino acid according to the method of Lee and Takahashi (14) in which glycine was used as a standard for calculation.

In the next series of experiments, effects of L-tyrosine feeding on the blood pressure and contents of tyrosine and noradrenaline were studied using SHR aged 12-14 weeks. The animals were divided into two groups: one group was used as control and the other was maintained for 15 days on a diet containing 1.0% L-tyrosine. Body weight, blood pressure, tyrosine content in the plasma and brain, and noradrenaline content in the brain were measured on the 5th and 15th days of feeding. The statistical significance of the data was determined by Student's t-test.

RESULTS

1. Noradrenaline and serotonin

Mean systolic blood pressure of WCR, WKR and SHR aged 12 weeks was 110±1 (S.E.), 132±3 and 181±5 mmHg, respectively. Table 1 shows contents of brain noradrenaline and serotonin in these 3 strains. As already reported by Yamabe et al. (7), noradrenaline content in the brain stem of SHR was significantly (\( p<0.05 \)) lower than that of WCR, however, the level of noradrenaline did not differ between SHR and WKR. Serotonin contents in the brain stem of SHR and WKR were slightly lower as compared
with those of the same-aged WCR. Noradrenaline and serotonin contents in the telencephalon were the same in all 3 strains.

2. Tyrosine, tryptophan and α-amino nitrogen

As shown in Table 2, contents of tyrosine in the plasma and whole brain of SHR and WKR were significantly (p<0.01) lower than the respective contents of WCR. On the other hand, tryptophan levels of SHR and WKR were slightly higher than those of WCR in both the plasma and brain. Contents of α-amino nitrogen in the plasma and brain of SHR and WKR were less than those in WCR. However, the differences of α-amino nitrogen contents in the brain among SHR, WKR and WCR were statistically insignificant.

### Table 1. Contents of noradrenaline and serotonin in the brain of WCR, WKR and SHR.

|                | Noradrenaline | Serotonin |
|----------------|---------------|-----------|
|                | (µg/g) (%)    | (µg/g) (%)|
| Brain stem     |               |           |
| WCR            | 0.63±0.04     | 100       |
| WKR            | 0.49±0.02     | 78        |
| SHR            | 0.52±0.02*    | 83        |
| Telencephalon  |               |           |
| WKR            | 0.24±0.02     | 100       |
| SHR            | 0.23±0.03*    | 96        |

Results are mean±standard error of 5 rats.

*P<0.05 (Significantly different from the values of WCR).

### Table 2. Contents of tyrosine, tryptophan and α-amino nitrogen in the plasma and brain of WCR, WKR and SHR.

|                | Tyrosine     | Tryptophan | α-Amino nitrogen |
|----------------|--------------|------------|-----------------|
|                | (µg/ml or g) | (µg/ml or g) | (µmole/ml or g) |
|                | (%)          | (%)        | (%)             |
| Plasma         |              |            |                 |
| WCR            | 19.1±0.2     | 100        | 6.77±0.14       | 100 |
| WKR            | 15.1±0.7*    | 79         | 22.6±1.2        | 118 |
| SHR            | 13.3±1.2*    | 70         | 22.1±3.0        | 115 |
| Brain          |              |            |                 |
| WKR            | 23.4±0.3     | 100        | 5.69±0.46       | 100 |
| SHR            | 18.4±0.5*    | 79         | 7.10±0.61       | 125 |

Results are mean±standard error of 5 rats.

*P<0.01 (Significantly different from the values of WCR).

3. L-Tyrosine feeding in SHR

Feeding SHR on a diet supplemented with 1% L-tyrosine for 15 days did not result in a significant body-weight change. Gross behavior remained constant during the experimental period. Blood pressure of the control and L-tyrosine-treated SHR at the start of experiment was 177±3 and 179±3 mmHg, respectively. As shown in Table 3, the
blood pressure of L-tyrosine treated animals on the 5th day was slightly but significantly 
\( p < 0.01 \) lower than that of the non-treated group.

An increase of tyrosine contents in the plasma and brain following L-tyrosine feeding was 
evident, while noradrenaline content in the brain stem of L-tyrosine treated group did not 
differ from that of the control. After continuous administration of L-tyrosine for 15 days, 
the blood pressure of the treated rats was elevated and tyrosine contents in the plasma and 
brain were reduced as compared with the respective values after a 5-day feeding (Table 4).

**DISCUSSION**

In the present study, tyrosine content in the brain of SHR was significantly lower 
than that of normotensive WCR. This result corresponded well with the lower level of 
noradrenaline in the brain stem of SHR as compared with that of WCR. The lower level 
of tyrosine in SHR compared with that in WCR was also found in the plasma. The maximal 
brain to plasma ratio of tyrosine in rats has been reported to be about 1.25 with the 
administration of radioactive tyrosine (15). The lower level of tyrosine in the plasma of 
SHR may explain the low level of this amino acid in the brain. If such is the case, then 
absorption of tyrosine and/or activity of tyrosine transaminase in the liver of SHR would 

differ from those in WCR.

Serotonin content in the brain stem of SHR was also slightly lower than that of WCR, 
while tryptophan content in the SHR brain was higher than that of WCR. The **Km of**
tryptophan hydroxylase is reported to be quite high compared with the level of free tryptophan in the brain and other workers have found that the administration of tryptophan increased the content of serotonin and the excretion of 5-HIAA (16, 17). Recently, Yamabe et al. (7) reported that the uptake of tyrosine into the site of catecholamine synthesis in SHR and WKR brain was lower than that in the Wistar/NIH rats. Thus it may be postulated that serotonin-containing neurons in the brain stem of SHR are less able to take up exogenous tryptophan than are those of the WCR.

The contents of noradrenaline, tyrosine and serotonin in the brain of WKR, the parent strain of SHR, were lower than those in the brain of WCR. In other words, the levels of both monoamines and precursor amino acids were the almost the same in the SHR and WKR. Thus it is suggested that the difference between SHR and WCR regarding central monoamine metabolism could be due to genetic factors in the closed colony. Nevertheless there is evidence that the blood pressure of WKR was always higher than that of WCR and that the aged WKR showed a rather high incidence of hypertension (18). Therefore, whether or not the prehypertensive stage in rats developed from the Wistar/Kyoto colony originates from the disturbances of monoamine metabolism remains obscure.

On a diet containing 1% L-tyrosine for 5 days, there was a significant increase in plasma and brain tyrosine and a slight fall of blood pressure in the SHR. On the 15th day of this diet, the blood pressure of these rats was elevated as compared with the respective values of 5-day feeding, and tyrosine contents in the plasma and brain returned to the control levels, which suggests a correlation between blood pressure and tyrosine contents in the plasma and brain. A lowering of the blood pressure induced by L-tyrosine feeding, however, cannot be attributed to noradrenaline formed from tyrosine, as the rate limiting enzyme of catecholamine synthesis is considered to be tyrosine hydroxylase (19) and noradrenaline content in the brain of STIR did not change after L-tyrosine ingestion. The hypothalamus and cerebellum have a higher concentration of tyrosine than other regions of the brain, and these regional variations in the concentration of the amino acids may be an important factor involved in the regulation of various different functions of the separate brain regions (17). On the other hand, McKean et al. (20) reported that the administration of tyrosine caused cerebral depletions in essential amino acids in rats. All these factors concerning L-tyrosine feeding should be taken into account before making a correlation between blood pressure and tyrosine content in the brain of SHR. The induction of tyrosine transaminase activity may explain the unchanged levels of tyrosine in the brain and plasma on the 15th day of the L-tyrosine treated SHR as this enzyme has a marked diurnal rhythm which limits excessive amounts of tyrosine from entering the body (21).

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