A number of reports (1) have been appeared in attempt to explain the pathogenesis of essential hypertension from the pathological, pharmacological and epidemiological points of view.

It has recently been demonstrated that norepinephrine (NE) and serotonin (5HT) are important transmitters of the nervous system. In addition, metabolic pathway of these amines has recently been clarified (2-4).

The present study is primarily concerned with a comparison between normotensive Wistar strain rats and spontaneously hypertensive rats separated from the same strain through the selective inbreedings by Okamoto and his colaborators (5). They mated Wistar strain rats which showed slightly higher blood pressure than the average under normal conditions. Continuously hypertensive first generation rats were selected and mated again in brother-sister breedings, until by serial reperation of this method, a spontaneously hypertensive colony of Wistar rats was obtained.

This hypertensive colony is useful for these experiments, because it is not so difficult to obtain numerous offsprings and they keep an average span of life, and it is not necessary to turn to any particular agent like DOCA or surgical operation to obtain hypertension. They show an almost 100% occurrence of over 150 mmHg of blood pressure after 25 weeks of age. Pathological changes (6) such as heart hypertrophy, thickening of blood vessels, and nephrosclerosis after the maintenance of hypertension being satisfactory for experimental hypertension, and it may be comparable with human essential hypertension.

The purpose of this study is to ascertain whether the metabolism of monoamines is consistently associated with hypertension or not, by means of comparison, on 5HT and NE metabolism has been made between normotensive and hypertensive rats.

MATERIAL AND METHODS

Male Wistar strain rats and hypertensive colony that is F₁ (No 6087 ♂, No. 6093 ♂) (No 6098 ♂, No. 6100 ♂) in Okamoto's lineage which kindly placed at my disposal,
and F₂-F₅ male rats, both groups weighing 200-300 g, were used. In the present study I have compared the responses between these two groups, using, in all cases, male rats older than 14 weeks. Therefore, at the beginning of experiments, the hypertensive group had already been in a state of hypertension for more than three months. Blood pressure measured by Grollman's plethysmograph without anesthesia.

Tissue 5HT levels were measured fluorometrically (Farrand fluorometer), in the manner of Bogdanski, (7) and the μg/g was obtained. Tissue NE levels were measured by alumina adsorption and trihydroxy indole method in the manner of Crout, (8) and the μg/g was obtained. 5-Hydroxy tryptophan decarboxylase activity in various tissue was measured by 5HT formation during 60 minutes incubation with the precursor, by the method of Clark and Udenfriend (9).

Monoamine oxidase activity in various tissues was determined by the 5HT disappearance 40 minutes after the incubation (10). 5-Hydroxy indole acetic acid (5HIAA) in urine was measured by the method of Sjoerdsma (11).

RESULTS

1. Rat blood pressure (weeks of age) (Fig. 1)

The dotted line shows the systolic blood pressure of hypertensive male rats, and the solid line shows the control normotensive group. The blood pressure, exceeding 150 mmHg, of the hypertensive group lasted over 10 weeks period; that of the control group, however, remained lower than 150 mmHg even after 15 weeks of age.

![Fig. 1. Rat blood pressure (weeks of age).](image)

2. 5HT and NE concentration in the tissue (Table 1)

No remarkable differences were noticed between the two groups except 5HT levels of the heart and blood, and the NE levels of the hypertensive rat heart are all higher than those of normotensive rats.
TABLE 1. Serotonin (5HT) and norepinephrine (NE) concentrations in the tissue.

<table>
<thead>
<tr>
<th></th>
<th>Normotensive rat</th>
<th>Hypertensive rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT µg/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.44±0.07</td>
<td>0.49±0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>0.60±0.1</td>
<td>0.87±0.2</td>
</tr>
<tr>
<td>Blood</td>
<td>0.20±0.08</td>
<td>0.29±0.12</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.3±0.4</td>
<td>3.4±0.5</td>
</tr>
<tr>
<td>NE µg/g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>0.29±0.08</td>
<td>0.27±0.1</td>
</tr>
<tr>
<td>Heart</td>
<td>0.58±0.13</td>
<td>0.76±0.15</td>
</tr>
</tbody>
</table>

TABLE 2. 5-Hydroxytryptophan decarboxylase activity (5HT formations µg/g/hr).

<table>
<thead>
<tr>
<th></th>
<th>Normotensive rat</th>
<th>Hypertensive rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>33% tissue homogenate 1.0 ml, phosphate buffer pH 8.0, 0.5 ml, 5HTP 0.5 mg, Iproniazid 10^-3Mol, pyridoxal PO₄, 100 mg.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>34</td>
<td>33 (- 2%)</td>
</tr>
<tr>
<td>Heart</td>
<td>22</td>
<td>18 (- 16%)</td>
</tr>
<tr>
<td>Liver</td>
<td>125</td>
<td>97 (- 21%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>190</td>
<td>216 (+ 14%)</td>
</tr>
</tbody>
</table>

TABLE 3. Monoamine oxidase activity (5HT destructions µg/100 mg/hr).

<table>
<thead>
<tr>
<th></th>
<th>Normotensive rat</th>
<th>Hypertensive rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>127±17</td>
<td>156±15 (- 9%)</td>
</tr>
<tr>
<td>Heart</td>
<td>180±19</td>
<td>176±14 (2%)</td>
</tr>
<tr>
<td>Liver</td>
<td>516±22</td>
<td>345±27 (-33%)</td>
</tr>
<tr>
<td>Kidney</td>
<td>495±25</td>
<td>490±29 (-11%)</td>
</tr>
</tbody>
</table>

20% tissue homogenate 10 ml, phosphate buffer pH 7.4, 0.5 ml, 5HT 0.1-0.5 mg.

3. 5-Hydroxytryptophan (5HTP) decarboxylase activity (Table 2)

The next studies were performed in order to clarify the reason for high amine levels in these tissue. First of all, tissue enzyme activities of 5HT and NE formation and destruction were compared.

The activity of the hypertensive rat heart and liver was found to be slightly inhibited as compared to that of the normotensive group, although the activity of the kidney was somewhat accelerated.

4. Monoamine oxidase activity (Table 3)

The activity of the hypertensive rat liver was noticeable inhibited to about 70% of the normotensive rat liver activity.

In addition to this, catechol-O-methyl transferase activity in the liver and kidney was also examined by the method of Axelrod, and no remarkable differences were observed between these groups.

5. Counts of platelet and erythrocyte (Table 4)

As mentioned above the blood 5HT levels of the hypertensive rats were higher than those of the normotensive rats. It is well known fact that most of the 5HT in blood are contained in platelet. Table 4 shows counts of platelets and red blood cells. Values are numbered by the cubic mm, as usual. There are no big differences between the two groups.
6. 5-Hydroxyindole acetic acid (5HIAA) excretion in urine (Table 5)

The hypertensive rats excreted less 5HIAA than the normotensive group even after 1 mg/kg intraperitoneal injection of reserpine.

7. 5HT concentration in heart after the injection of 5-hydroxytryptophan (50 mg/kg i.p.) (Fig. 2)

The animals were sacrificed at 20 minutes intervals, and the 5HT was measured after the heart was carefully washed with saline. The results are recorded in percentages measured against the 0 time of each group. 5HT levels in the heart of the hypertensive rats were 20 to 30% higher than those of the controls, when measured at 60, 80 and 100 minutes after the injection.

8. The effect of nephrectomy and adrenalectomy on the blood pressure and the blood level of 5HT (Table 6)

In view of the fact that the hypertrophy of adrenal glands and nephrosclerosis were observed in pathological studies by Okuda (6) and others, I have examined the effect of bilateral nephrectomy and adrenalectomy on the blood pressure and blood 5HT levels in normotensive rats. The Table shows the results of the 20 hours after the operation. In the case of nephrectomy the blood pressure and blood 5HT rose after the operation. In the case of adrenalectomy, however, the blood pressure fell and low level of the 5HT was observed after the operation.

![Graph showing 5HT concentrations in heart after the 5HTP injection (50 mg/kg i.p.)](image)

**Fig. 2.** 5HT concentrations in heart after the 5HTP injection (50 mg/kg i.p.)

<table>
<thead>
<tr>
<th>B.P. mmHg</th>
<th>Nephrectomized rat</th>
<th>Adrenalectomized rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>144±14</td>
<td>150±5</td>
</tr>
<tr>
<td>after</td>
<td>164±12</td>
<td>126±14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5HT µg/ml</th>
<th>Nephrectomized rat</th>
<th>Adrenalectomized rat</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>0.25±0.12</td>
<td>0.25±0.12</td>
</tr>
<tr>
<td>after</td>
<td>0.36±0.03</td>
<td>0.15±0.10</td>
</tr>
</tbody>
</table>

**Table 6.** Blood pressure and 5HT in blood 20 hours after nephrectomy or adrenalectomy.
We understand the blood pressure is as the general answer of the heart activity, dilatation, constriction of peripheral vessels and increase, decrease of the circulating blood volume and others. We come, then to the question of the pathogenesis of hypertension. One of the explanation might be an increase in the peripheral resistance due to the vasoconstriction accompanied with neurochemical-transmitter release.

A number of reports have been published in attempt to explain the mechanism of essential hypertension which is the most important one among the hypertensive diseases. Polypeptides and amines are quite noteworthy as the vasoconstrictor substances. However, as yet, no pathognomonic biochemical pharmacological abnormality has been identified by these. Gitlow (12) measured the disappearance of tritiated NE in the blood of patients, and observed no changes. Mangler (13) reported no differences in the plasma level of catecholamine in patient and normal subject.

Up to the present, pheochromocytoma is the only case where attacks can be precisely mimicked by an injection of NE or epinephrine and excessive amounts of these amines are circulating in blood and excreted in urine. In the extensive search for the factors causing hypertension 5HT has also been considered.

Page (14) observed that the patients with essential hypertension respond to 5HT infusion with the sustained rise of blood pressure, but that the determination of 5HT or 5HIAA in the urine does not increase (15). Wooley (16) observed the antimetabolites of 5HT, BAS, lowers the blood pressure in hypertensive patients. However, it is possible that BAS itself metabolizes to the hypotensive substances or effects by a mechanism un-related to 5HT antagonism.

The monoamine oxidase inhibitors increased the level of tissue 5HT and showed hypotensive effects (17). This is contradictory to the idea showing excessive 5HT production is a fact or in essential hypertension. However, hypotensive agents like reserpine, (18) and \( \alpha \)-methyl-DOPA (19) and guanethidine, which cause the low level of NE and 5HT in the central or peripheral nervous tissues, are the mechanism of lowering blood pressure by these compounds.

While applying these agents, we should consider the whole story of the amine metabolism during the hypertensive condition beforehand. In this study the present author has found that 5HT levels in heart and blood and NE of the hypertensive rat heart are all higher than those of the normotensive subject.

When the 5HTP was given and compared between hypertensive and normotensive rats, evidently 5HT levels of the hypertensive rat heart were higher than those of controls at 60, 80 and 100 minutes after the injection.

Among the enzyme activities in various tissue of amine formation and destruction, only liver monoamine oxidase activities are noticeably inhibited, while on the other hand, enzyme activities in heart itself is not remarkably different from the controls observed. Accordingly, if there are any influence to the heart amine concentration by the liver enzyme activity, there may be indirect effect through blood circulation.
In the meantime, the author has also confirmed the hypertrophy of the heart in hypertensive rat, so it is conceivable that hypertensive rat heart has relatively stronger ability to take up the NE and 5HT from the blood stream. Actually, unpublished data of mine have demonstrated that disappearance of exogenous NE in blood of hypertensive subject at 0.5, 1 and 2 minutes after the injection has been shown little quicker than that of normotensive controls.

On the mechanism of the high blood 5HT level in hypertensive rat, it is possible point out the following. (i) 5HT destruction process of liver MAO activity inhibited. (ii) 5HT excretion process, kidney dysfunction due to the pathological changes, actually the amount of 5HT and 5HIAA excretion in urine are smaller than normotensive rat. In addition, for example, as to kidney dysfunctions utmost, the author has tried bilateral nephrectomy and observed an increase in blood 5HT level and blood pressure rise. (iii) In the case of adrenalectomy, however, decreased 5HT levels and lowering blood pressure. This is a paradoxical explanation, that is hypertrophy of the adrenal gland has been observed in pathological point of view by Aoki et al. (20), thus it may be under hyperfunction. These observations shows that hypertensive rat has high amine levels in several tissue after the sustained high blood pressure.

In the above mentioned series of experiments I have examined the changes in the metabolic pathway of the amines in the post hypertensive stage, but I think I ought to go back to pre-hypertensive stage and henceforth make further studies to ascertain what sort of alteration occur in parallel with and in relation to the beginning of hypertension.

SUMMARY

In order to clarify whether the metabolism of monoamines is consistently associated with hypertension or not, the study has been made by comparing spontaneously hypertensive rats with normotensive rats. With hypertensive rats the levels of 5HT in blood and heart tissue and of NE in the heart were elevated by 25% from the controls. When 5-hydroxytryptophan was injected, 5HT levels in the heart of hypertensive rats were 20-30% higher than those of control as determined after 60, 80 and 100 minutes.

Among the activities of aromatic amino acid decarboxylase, monoamine oxidase (MAO) and catechol-O-methyl transferase in brain, heart, kidney, and liver, only liver MAO activity showed noticeably decreased in hypertensive rat.

The effect of nephrectomy and adrenalectomy of normotensive rats was also examined. An elevation in blood 5HT level and high blood pressure occurred 20 hours after the bilateral nephrectomy, whereas a low 5HT level and low blood pressure ensued from the adrenalectomy.

REFERENCES

2) Axelrod, J.: Pharmacology of Cholinergic and Adrenergic Transmission, Edited by Koelle, G. B.,
MONOAMINES IN HYPERTENSIVE RAT

13) MANGER, W.M.: Ibid. 9, 731 (1962)