Deviation of Central Norepinephrine Metabolism in Hypertensive Rats

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AKIRA OOSHIMA, M.D., AND KOZO OKAMOTO, M.D., D.M.Sc.

\(^3\)H-norepinephrine (NE) disappearance rate after the intraventricular injection of \(^3\)H-NE tended to be delayed in the brainstem but not in the telencephalon of spontaneously hypertensive rats (SHR) in the prehypertensive stage, while these indices were conversely accelerated in the heart and slightly so in the kidney of SHR. A similar tendency was partially noted in the brainstem, telencephalon and heart of SHR, DOC and renal hypertensive rats at the age of 10 weeks which had maintained hypertension for 1 month. The relative delay in the NE turnover of the brainstem compared with telencephalon was seemingly the common deviation of NE metabolism in these rats with genetic or experimental hypertension.

A possible impairment of central norepinephrine (NE) metabolism in hypertension was shown by studies on spontaneously hypertensive rats (SHR)\(^1\) as well as on DOC hypertension. On the other hand, accumulating evidences recently obtained by various pharmacological approaches have indicated the existence of a central noradrenergic sympathoinhibitory mechanism in SHR\(^3\)–\(^6\) and also in normotensive animals\(^7\)–\(^13\). These findings raised the importance of central noradrenergic mechanism in the pathological blood pressure regulation especially in the incipient or early stage of hypertension before adaptive organic changes to hypertension are established. Therefore, in the present study norepinephrine turnover in the central nervous system and peripheral organs was studied by determining the disappearance rate of intraventricularly or intravenously injected \(^3\)H-NE in SHR in the prehypertensive stage as well as in SHR DOC and renal hypertensive rats in the relatively early stage of hypertension.

**Materials and Methods**

Spontaneously hypertensive rats\(^14\)–\(^16\) of F\(_{26}\) generation at the age of 42 and 75 days, respectively, were used with age-matched normotensive rats from a closed colony of Wistar-Kyoto from which SHR were separated, and from the authentic inbred strain of Wistar-Mishima(F\(_{60}\)) which we received from National Institute of Genetics, Mishima, Japan and kept in our laboratory. DOC\(^17\) and renal\(^18\) hypertensions were induced in Wistar-Kyoto rats at the age of 40 days and served for the turnover study at the age of 75 days, 1 month after the development of hypertension, with the age-matched SHR and Wistar-Kyoto. Blood pressure was checked in unanesthetized rats by a tail-water-plethysmographic method\(^14\).

For NE turnover study, \(^3\)H-NE(DL-7,\(^3\)H-NE, 5 C/m mole, New England Nuclear, Boston) was

**Key Words:**
- Spontaneous Hypertension
- DOC Hypertension
- Renal Hypertension
- Norepinephrine Turnover
- Brainstem
- Telencephalon
- Heart
- Kidney

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<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Organs</th>
<th>Norepinephrine (NE) Metabolism</th>
<th>Aromatic L-Amino Acid Decarboxylase (nM/h/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Endogenous NE Level [N] (ng/g)</td>
<td>Rate Constant (k) [hr]</td>
</tr>
<tr>
<td>Spontaneously</td>
<td>Brainstem</td>
<td>513 ± 14</td>
<td>0.086*** ± 0.007</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>Telencephalon</td>
<td>225 ± 6</td>
<td>0.142 ± 0.007</td>
</tr>
<tr>
<td>Rats</td>
<td>Heart</td>
<td>663* ± 27</td>
<td>0.076** ± 0.014</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>117** ± 4</td>
<td>0.105 ± 0.007</td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
<td>Brainstem</td>
<td>524 ± 29</td>
<td>0.099*** ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Telencephalon</td>
<td>206 ± 3</td>
<td>0.144 ± 0.007</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>702** ± 39</td>
<td>0.059 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>110** ± 6</td>
<td>0.081 ± 0.009</td>
</tr>
<tr>
<td>Wistar-Mishima</td>
<td>Brainstem</td>
<td>504 ± 22</td>
<td>0.138*** ± 0.007</td>
</tr>
<tr>
<td></td>
<td>Telencephalon</td>
<td>218 ± 9</td>
<td>0.130 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>570 ± 28</td>
<td>0.057 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>95 ± 7</td>
<td>0.083 ± 0.007</td>
</tr>
</tbody>
</table>

* M ± SE, ( ): Number of rats, Statistically significant differences (*, +; 0.01 < P < 0.05, **, ++; 0.001 < P < 0.01, ***; +++; P < 0.001) from the values in Wistar-Kyoto and Mishima, respectively.
purified on almina column, and its solution in physiological saline was injected into a femoral vein (20 μC/Kg) and subsequently into left lateral ventricle (2 μC/rat) under light ether anesthesia. These rats were sacrificed 2, 7, 12 and 24 hours after the injection. Heart, kidney, brainstem including the portion from the diencephalon in the rear of anterior commissure to the medulla oblongata, and telencephalon were immediately extirpated, weighed, kept frozen until the assay and homogenized in 10 ml of 0.4 N perchloric acid for NE extraction. The supernatant was used for NE assay, and the specific activity of 3H-NE per μg of endogenous NE was determined by counting the radioactivity in a scintillation mixture to estimate ‘rate constant (k)’, ‘NE turnover time (1/k)’, and ‘turnover rate (endogenous NE x k)’. The decay of specific activity of 3H-NE was expressed as percent of the residual activity to the specific

Fig.1. Decay of the specific activity of 3H-norepinephrine after intraventricular (2μC/rat) or intravenous (20μC/Kg) injection in young SHR compared with 2 groups of controls (Wistar-Kyoto and -Mishima). Ordinate shows per cent of the mean specific activity of 3H-norepinephrine per μg of endogenous norepinephrine in the organ 2 hours after the injection.

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<table>
<thead>
<tr>
<th>Blood Pressure (mmHg)</th>
<th>Body Weight</th>
<th>Organs</th>
<th>Organ Weight (g)</th>
<th>NE Content (ng/organ)</th>
<th>Endogenous NE Level</th>
<th>Rate Constant [k] (hr)</th>
<th>NE Turnover Time [1/k] (hr)</th>
<th>Turnover Rate [N-k] (ng/hr/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneously</td>
<td></td>
<td>Brainstem</td>
<td>0.51** ± 0.01</td>
<td>299 ± 8</td>
<td>591 ± 17</td>
<td>0.122 ± 0.016</td>
<td>8.2 ± 0.016</td>
<td>72.1 ± 0.016</td>
</tr>
<tr>
<td>Hypertensive</td>
<td></td>
<td>Telencephalon</td>
<td>0.89** ± 0.02</td>
<td>255 ± 21</td>
<td>285** ± 31</td>
<td>0.122 ± 0.009</td>
<td>8.2 ± 0.009</td>
<td>34.8 ± 0.009</td>
</tr>
<tr>
<td>Rats</td>
<td>205** ± 4</td>
<td>Heart</td>
<td>0.99*** ± 0.03</td>
<td>592* ± 23</td>
<td>598 ± 20</td>
<td>0.065 ± 0.009</td>
<td>15.5 ± 0.009</td>
<td>38.5 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>249 ± 8</td>
<td>Kidney</td>
<td>2.04 ± 0.07</td>
<td>303** ± 23</td>
<td>148* ± 32</td>
<td>0.135 ± 0.018</td>
<td>7.3 ± 0.018</td>
<td>20.0 ± 0.018</td>
</tr>
<tr>
<td>DOC</td>
<td></td>
<td>Brainstem</td>
<td>0.55 ± 0.03</td>
<td>297 ± 8</td>
<td>536 ± 16</td>
<td>0.122 ± 0.009</td>
<td>8.2 ± 0.009</td>
<td>65.4 ± 0.009</td>
</tr>
<tr>
<td>Hypertensive</td>
<td></td>
<td>Telencephalon</td>
<td>0.96 ± 0.01</td>
<td>245 ± 20</td>
<td>255 ± 23</td>
<td>0.143 ± 0.016</td>
<td>7.0 ± 0.016</td>
<td>36.4 ± 0.016</td>
</tr>
<tr>
<td>Rats</td>
<td>200** ± 4</td>
<td>Heart</td>
<td>0.99*** ± 0.02</td>
<td>385*** ± 44</td>
<td>381** ± 42</td>
<td>0.115 ± 0.040</td>
<td>8.7 ± 0.040</td>
<td>44.0 ± 0.040</td>
</tr>
<tr>
<td></td>
<td>258 ± 19</td>
<td>Kidney</td>
<td>2.38*** ± 0.08</td>
<td>119*** ± 26</td>
<td>51*** ± 12</td>
<td>0.145 ± 0.014</td>
<td>6.9 ± 0.014</td>
<td>7.4 ± 0.014</td>
</tr>
<tr>
<td>Renal</td>
<td></td>
<td>Brainstem</td>
<td>0.56 ± 0.01</td>
<td>305 ± 10</td>
<td>539 ± 19</td>
<td>0.142 ± 0.009</td>
<td>7.0 ± 0.009</td>
<td>77.0 ± 0.009</td>
</tr>
<tr>
<td>Hypertensive</td>
<td></td>
<td>Telencephalon</td>
<td>1.08 ± 0.07</td>
<td>254 ± 26</td>
<td>253 ± 28</td>
<td>0.131 ± 0.021</td>
<td>7.6 ± 0.021</td>
<td>33.2 ± 0.021</td>
</tr>
<tr>
<td>Rats</td>
<td>201** ± 9</td>
<td>Heart</td>
<td>1.16*** ± 0.04</td>
<td>373*** ± 30</td>
<td>323*** ± 20</td>
<td>0.143*** ± 0.023</td>
<td>7.0 ± 0.023</td>
<td>46.1 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>234 ± 13</td>
<td>Kidney</td>
<td>2.08 ± 0.07</td>
<td>122*** ± 60</td>
<td>58*** ± 31</td>
<td>0.168 ± 0.016</td>
<td>5.9 ± 0.016</td>
<td>9.7 ± 0.016</td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
<td></td>
<td>Brainstem</td>
<td>0.55 ± 0.01</td>
<td>321 ± 8</td>
<td>580 ± 16</td>
<td>0.164 ± 0.027</td>
<td>6.1 ± 0.027</td>
<td>94.9 ± 0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Telencephalon</td>
<td>0.98 ± 0.02</td>
<td>228 ± 34</td>
<td>233 ± 34</td>
<td>0.120 ± 0.016</td>
<td>8.3 ± 0.016</td>
<td>27.9 ± 0.016</td>
</tr>
<tr>
<td></td>
<td>133 ± 1</td>
<td>Heart</td>
<td>0.84 ± 0.02</td>
<td>518 ± 16</td>
<td>617 ± 20</td>
<td>0.051 ± 0.009</td>
<td>19.7 ± 0.009</td>
<td>31.3 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>256 ± 7</td>
<td>Kidney</td>
<td>1.93 ± 0.06</td>
<td>219 ± 43</td>
<td>115 ± 28</td>
<td>0.122 ± 0.016</td>
<td>8.2 ± 0.016</td>
<td>14.1 ± 0.016</td>
</tr>
</tbody>
</table>

M ± SE. ( ) : Number of rats. Statistically significant differences (**: 0.01 < P < 0.05, ***: 0.001 < P < 0.01, ****: P < 0.001) from the values in Wistar-Kyoto.
activity of the organ 2 hours after $^3$H-NE injection, because the decay during the initial 2 hours mainly reflects the removal of extraneurally bound $^3$H-NE according to the results obtained from the difference of NE release between innervated and denervated kidneys.

Aromatic L-amino acid decarboxylase activity in the brainstem and telencephalon was assayed by the method previously reported.

**RESULTS**

1. Norepinephrine metabolism in young SHR in the prehypertensive stage (Table I, Fig. 1)

SHR at the age of 42 days showed a significantly higher blood pressure (144 ± 3 mmHg) than two kinds of normotensive rats, Wistar-Kyoto and -Mishima. Although they were in the prehypertensive stage according to the criteria of hypertension being over 150 mmHg, they were rather in the incipient stage of developing spontaneous hypertension.

Endogenous NE level of the whole brainstem or telencephalon showed no significant difference among three groups of rats examined, but those of the heart and kidney in SHR were increased in comparison with Wistar-Mishima.

Various indices of NE turnover in the brainstem of SHR showed a tendency to be delayed in comparison with those in two control groups, while NE turnover in the telencephalon was almost equal to each other among three groups. A significant difference of rate constant was noted between SHR and Wistar-Mishima ($p<0.001$) and also between SHR and Wistar-Kyoto ($p<0.05$). Moreover, residual specific activity of $^3$H-NE in the brainstem of SHR was significantly higher than those in two groups of controls 24 hours after $^3$H-NE injection as shown in Fig. 1. Conversely, NE turnover of the heart in young SHR was clearly accelerated compared with two groups of controls as shown in a significant increase in rate constant as well as in a significant decrease in the residual specific activity of $^3$H-NE 24 hours after the injection. NE turnover of the kidney in SHR showed a slight acceleration and the residual specific activity of $^3$H-NE 24 hours after the injection was significantly lower than those in two groups of controls.

Aromatic L-amino acid decarboxylase activity in SHR, assayed as the whole brainstem and telencephalon, was markedly decreased compared with Wistar-Mishima, but almost at the same level as the activity in Wistar-Kyoto.

2. Norepinephrine metabolism in various hypertensive rats (Table II, III, Fig. 2)

Blood pressure in SHR, DOC and renal hypertensive rats which maintained hypertension for 1 month were significantly higher than in the age-matched Wistar-Kyoto.

Among the organ weights examined, a significant decrease in the weights of brainstem and telencephalon was noted in SHR compared with Wistar-Kyoto with or without experimental (DOC or renal) hypertension. As clearly indicated by the significant increase in the weight, cardiac hypertrophy was the common finding in three groups of hypertensive rats, and DOC hypertensive rats showed also renal hypertrophy.

A significant increase in NE content per organ was observed in the heart and kidney of SHR compared with controls, but NE level per g organ weight was not so in the heart because of the cardiac hypertrophy. Conversely, NE content and level in the heart and kidney of renal and DOC hypertensive rats were significantly lower.
than in controls. No significant difference in NE content and level in the brainstem as a whole or in the telencephalon in hypertensive rats was noted in comparison with the controls except for a significant increase in NE level of the telencephalon in SHR.

So far as norepinephrine metabolism was indicated by rate constant, turnover time and turnover rate, brainstem NE metabolism in hypertensive rats showed a slight tendency to decrease, i.e., a decrease in rate constant and especially in turnover rate and a reciprocal increase in turnover time, and the residual specific activity in the brainstem 12 hours after the intraventricular injection of $^3$H-NE was significantly higher in SHR and DOC hypertensive rats, while all such indices showed no difference in the telencephalon of hypertensive rats. Therefore, NE metabolism in the brainstem was compared to that in the telecephalon in each group of rats, and brainstem to telencephalon ratios of various indices were tentatively calculated. As shown in Table III, the brainstem to telencephalon ratio of NE turnover rate was commonly decreased in young SHR and also in three groups of hypertensive rats in comparison with the age-matched controls. This finding was supported by the significant increase in the brainstem to telencephalon ratio of the residual specific activity of $^3$H-NE 12 or 24 hours after the intraventricular injection in hypertensive rats. In contrast to the findings on the brainstem, NE turnover in the heart was increased in hypertensive rats, although this tendency was rather inconspicuous in SHR at this age as previously reported. Some indices of NE turnover of the kidney in hypertensive groups showed a slight increase but not obvious difference from those in normotensive controls.

**DISCUSSION**

One of the most conspicuous quantitative biochemical findings in SHR was a clear decrease in aromatic L-aminoacid decarboxylase activity in the brain in comparison with authentic Wistar rats (Wistar-NIH -Mishima) and allied strains of rats such as Sprague-Dawley. However, it was later noted that this decrease in decarboxylase activity was, when compared with Wistar-Kyoto, rather unobvious except for a portion of midbrain as reported previously. As Wistar-Kyoto rats, from which ancestors of SHR were derived, show a slight skew of blood pressure distribution to hypertension and still include at

**Table III**

<table>
<thead>
<tr>
<th>Brainstem to Telencephalon Ratio of Various Hypertensive Rats</th>
<th>SHR (10 week old)</th>
<th>Wistar-Kyoto (10)</th>
<th>Wistar-Mishima (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Rat (4 week old)</td>
<td>1.75</td>
<td>2.46</td>
<td>3.04±0.09</td>
</tr>
<tr>
<td>NE Turnover Rate</td>
<td>1.23±0.07</td>
<td>1.23±0.07</td>
<td>1.19±0.06</td>
</tr>
<tr>
<td>Residual $^3$H-NE</td>
<td>3.04±0.07</td>
<td>1.23±0.07</td>
<td>1.19±0.06</td>
</tr>
<tr>
<td>(g) 12hr or (g) Mhr. after 1st hr. injection</td>
<td>±0.27</td>
<td>±0.19</td>
<td>±0.06</td>
</tr>
</tbody>
</table>

M ± SE, ( ): Number of rats, Statistically significant differences (‡: 0.01 < $p$ < 0.05, §: 0.001 < $p$ < 0.01) from the values in Wistar-Kyoto and Mishima, respectively.
present some mild hypertensive offspring sporadically, they seem to have some hypertensive traits which manifest the phenotypical effect in combination with some other more potent hypertensive genes than ones controlling decarboxylase activity. Our previous studies showed that a relatively small number of major genes are involved in the additive mode of inheritance in spontaneous hypertension and the importance of such interactions between the genes regulating decarboxylase activity and additional hypertensive genes were suggested by our analytical studies on F₂ segregate generation obtained from SHR and Wistar-Mishima, which showed a relatively slight inverse correlation of brainstem decarboxylase activity with blood pressure level. The present study showed that NE turnover in the brainstem of SHR with low decarboxylase activity was significantly delayed compared with that in Wistar-Mishima and confirmed the former data on the delay of NE synthesis from labelled tyrosine in the brainstem of SHR. However, such a delay in NE turnover was not observed in the telencephalon of SHR which also showed a clear decrease in decarboxylase activity. Although this difference between brainstem and telencephalon may be due to the distribution of catecholaminergic neurons and the decreased decarboxylase in the telencephalon, which mainly contain catecholaminergic nerve endings not cell bodies may not affect NE turnover, the finding of the present study indicates also a possible existence of at least one more additional genetic factor which is concerned with the specific delay in NE turnover itself of the brainstem in SHR. It remains for further study to determine whether this additional genetic factor is the gene controlling other enzymes regulating NE metabolism or some kind of the regulator gene which influences the gene controlling decarboxylase activity to intensify the relative impairment of the activity locally in a certain discrete noradrenergic mechanism important for blood pressure regulation in the brainstem. These two possibilities are also applicable to the explanation for a tendency to a delay in NE turnover of the brainstem of SHR compared with that of Wistar-Kyoto, the decarboxylase activity of which as a whole is nearly as low as that in SHR.

Generally speaking, NE turnover is obviously a better index of NE metabolism than endogenous NE level, which is labile depending on the balance between the synthesis and destruction.

As seen in this study, endogenous NE level in the brainstem as a whole in SHR was not statistically different from that of normotensive rats but NE turnover, determined by the disappearance curve of the specific activity from 2 to 12 hours after ³H-NE injection, was delayed in the brainstem of SHR. Since the initial disappearance rate during 2 hours after the injection does not properly reflect the release of neurally-bound ³H-NE as previously reported. NE turnover should be determined by the second phase of NE disappearance following the initial decline of the activity and should also be observed for a longer duration in order to detect a minute difference. Such small differences in experimental designs for the determination of NE turnover as well as strain differences in control rats such seen between Wistar-Mishima and --Kyoto may account for the inconsistency of the results on NE turnover studied by different laboratories.

The sympathoinhibitory role of central noradrenergic mechanism was speculatively proposed by Yamori et al. from their observation on an impairment of NE metabolism in the brainstem of SHR, and the validity of this speculation was confirmed by our further observations that an increase in NE or NE analogue of the brainstem or stimulation of central α receptors by exogenous NE, NE analogues or α stimulant like clonidine as well as by endogenous NE released from nerve endings consistently decreased the blood pressure in SHR and also in normotensive control. Moreover, as such depressor effects were always greater in SHR than in controls the intrinsic activity of central noradrenergic inhibitory mechanism seemed to be decreased in the hypertensive state of SHR. Similar concepts on the importance of central noradrenergic mechanism for blood pressure regulation were almost simultaneously developed by Henning and Rubenson as well as by Andén et al. and Kroneberg et al from their observations that the depressor effect of NE precursors or α stimulants was centrally mediated. In spite of these identical findings indicating that the stimulation of central noradrenergic mechanism results in the lowering of blood pressure, extensive destructions of central noradrenergic nerve terminals by the intraventricular injection of 6-hydroxydopamine were not so effective for the induction of hypertension except for a few sporadic cases. However, rats treated with the intraventricular injection of minute doses of 6-hydroxydopamine, which

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showed about 50 per cent depletion of central NE level, exhibited a greater increase in systolic blood pressure and heart rate when exposed to immobilization stress. Folksow et al. reported that SHR showed a greater cardiovascular response to stress than normotensive rats. Consequently, central NE depletion by 6-hydroxydopamine under a certain condition made it possible to simulate this characteristics of SHR in normotensive rats, and this finding provides us with further evidences suggestive of an inhibitory role of central noradrenergic mechanism in cardiovascular regulation. Such a 'hyperreactivity' to stress due to a lack or impairment of the central inhibitory mechanism may be one of the bases for the development of hypertension as an initiation factor of structural vascular changes.

Another candidate for the central inhibitory mechanism of cardiovascular regulation is a serotonergic one because an increase in serotonin level by the intraventricular injection of serotonin or 5-hydroxytryptophan mainly decreased blood pressure in SHR, while depletion of serotonin by parachlorophenylalanine increased blood pressure in SHR as well as in normotensive rats. However, we have not yet obtained any data definitely indicating the alteration of serotonin metabolism in the brain of SHR in spite of the low decarboxylase activity.

In contrast to these sympathoinhibitory or depressor mechanisms, cholinergetic mechanisms seem to play a sympathoexcitatory or pressor role in central blood pressure regulation. So far as observed, pressor responses to intraventricularly injected cholinergetic agents were greater in SHR than in normotensives and the activity of enzymes of cholinergetic system was partially increased in the brainstem of SHR whether primarily or secondarily. These findings suggest that such metabolic imbalance between central pressor and depressor mechanisms might be involved in the pathogenetic mechanisms of hypertension in SHR and the aforementioned additional genetic factors to the gene controlling decarboxylase activity might be the gene that induces antagonistic activation of central cholinergetic mechanisms.

Since Nakamura et al. detected a delay in NE turnover in DOC hypertensive rats secondary involvement of central noradrenergic mechanisms in experimental hypertension has become the focus of our interest, because a bulk of studies on renal or DOC hypertension showed the participation of neurogenic component especially in the chronic stage of these hypertensions. A tendency to a delay in NE turnover in the brainstem in DOC hypertension also detected in this study might be a secondary impairment of the central noradrenergic inhibitory mechanisms, although the detailed process how primary pressor factors or hypertension itself influence such central functions is not clear at all. The interaction of the primary pressor factor in renal hypertension, angiotensin, with central nervous structure has been recently disclosed by various studies. The importance of central noradrenergic mechanisms for pressor response to angiotensin was suggested by Smookler et al. and our recent observation showed a clear diminution of pressor response to the intravenously injected minute dosis of angiotensin in central norepinephrine-depleted rats following the intraventricular injection of 6-hydroxydopamine. This finding, indicating that central noradrenergic mechanism is necessary for the central pressor effect of angiotensin, suggests the possible existence of central noradrenergic pressor mechanisms as well, but it is not yet definite whether this central action of angiotensin is a stimulation of the central noradrenergic pressor mechanism or an inhibition of the central noradrenergic depressor mechanism. The secondary involvement of central noradrenergic mechanisms in renal and DOC hypertension and its similarity to the central NE metabolism in SHR with primary genetic hypertension seem to be a clue for clarifying the pathogenetic role of central nervous system in hypertension, because a deviation of central NE turnover in the brainstem was also reported on the cerebral and neurogenic hypertensions in rabbits, although compensatory activation of central noradrenergic depressor mechanism following deafferentation can not be eliminated in the latter experiment.

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Discussion:

Chairman: TERUO OMAE, Kyushu Univ.

Dr. TERUO OMAE (Chairman, Kyushu Univ.): The paper is now open for discussion.

Dr. AKIRA ITO (Kyushu Univ., Fukuoka): I would like to ask you the following questions: 1) Intraventricular administration of norepinephrine has reportedly revealed pressor response to its lesser doses and depressor to its larger doses. But a pressor reaction could be elicited even by the latter procedure when endogenous store of norepinephrine was depleted by reserpine or other drugs. Therefore, is there any possibility that overdosage of norepinephrine results in the inhibitory reaction in your study? 2) How do you think of the correlation between central and peripheral adrenergic mechanisms? 3) Do you know any evidence to show some qualitative changes in norepinephrine metabolism in these hypertensive rats? and 4) according to our observation of intracranially applied adrenergic receptor blockades, we suppose two different and probably competing adrenergic mechanisms existing in the medullary vasomotor area. As you know, however, an augmentation both in synthesis and turnover of norepinephrine in the peripheral sympathetic nervous system has recently been observed in the animals treated with the blockades, in spite of the suppressed synaptic transmission. Considering these observation and obscurity in the central receptor mechanisms, it appears difficult to impute your present results as well as ours to either excitation or inhibition of the supposed central adrenergic receptors. 

Dr. YAMORI: 1) It is true that an extremely small dose of intraventricular norepinephrine could elicit a pressor response. But the response to intraventricularly injected norepinephrine could also be varied by the distance of the site of action from the ventricular wall etc. We are now investigating on the intracerebral distribution of these noradrenergic inhibitory mechanisms. 2) As to the interrelationship between central and peripheral nervous mechanisms, I suppose that an insufficiency in the central inhibitory mechanism possibly causes hyperfunction of the peripheral sympathetic nervous system, because we have ever observed an increase in activity of Ach-esterase and G-6-P dehydrogenase in sympathetic ganglia of SHR, and a positive correlation of them with the level of blood pressure in the F2 generation obtained by crosses between SHR and normotensive rats. 3) An increase in metabolites of DOPA or dopamine was demonstrated in the urinary specimen of SHR. I do not know any report concerning definite knowledge on the central receptor mechanism as yet. However, the centrogeneric decrease in blood pressure induced by alpha-methyl-norepinephrine as well as by norepinephrine might indicate that functional character of the central alpha-adrenergic receptor is different from that of the peripheral.

Dr. KEIJI NAKAMURA (Japan Roche): 1) Different from your result, Hauvel (1970) observed only a temporary depressor response to intraventricular injection of 6-hydroxy-dopamine (6-OHDA) in normotensive rats, and also I could not observe any definite change in blood pressure following direct administration of 6-OHDA into the medulla, pons, hypothalamus or other regions in the brain. 2) The depressor effect of combined treatment with L-DOPA and peripheral decarboxylase inhibitor (PDI), I think, would be caused not by norepinephrine (NE) originating from L-DOPA but rather by the augmented
dopamine (DA) affecting on noradrenergic neurones in the vasomotor areas, since we have observed that the combined injection revealed a significant increase in DA-level in various brain portions formerly rich in NE but no remarkable change in NE-content. 3) I also imagine that the central catecholamine metabolism in rats would be different by the type of experimental hypertension. For example, we observed that the combined injection of L-DOPA and Ro 4-4602 elicited an antihypertensive effect and concomitant DA increase in various portions of the brain, which was more remarkable in DOPA-salt-hypertensive rats than in renal hypertensive animals brought about by constriction of the renal artery.

Dr. YAMORI: Your observation is very interesting that the centrogenic depressor reaction to DOPA is different in magnitude between DOCA and renal hypertensions. You might think that DOCA-induced depressor reaction is caused by dopamine, but I suppose that norepinephrine plays an important role in the mechanism of centrogenic depression because an increase in blood pressure is observed by inhibition of intracerebral dopamine-beta-hydroxylase. It is not certain, however, that the decrease in norepinephrine is directly related to the elevation of blood pressure. Possibility remains that central dopaminergic neurones could take a part in the blood pressure elevation. The depressor effect of intraventricular administration of dopamine mostly varies among those of various sorts of catecholamines as shown by us and Kroneberg (Professor of Pharmacology, Frankfurt).

Dr. HIROFUMI SOKABE (Toho Univ., Tokyo): 1) What kind of angiotensin did you use for intraventricular injection in the rat? 2) How do you explain the fact that pressor effect of angiotensin injected into the cerebral ventricle was enhanced in the renal hypertensive rats?

Dr. YAMORI: 1) I used Hypertensin (CIBA) dissolved in 0.9% saline. 2) We presume two kinds of mechanisms for the central action of angiotensin; first, stimulation of the central noradrenergic pressor system, and second, competition with the central noradrenergic inhibitory system. However, the latter seems more probable because the centrally applied angiotensin causes a weak pressor effect in rats depleted of brain norepinephrine by 6-hydroxydopamine or in SHR, while it produces a prominent increase in blood pressure in rats stimulated of central alpha-receptor by clonidine.

Dr. YOSHIHIRO KANEKO (Tokyo Univ., Tokyo): I felt rather difficult to draw conclusions from plenty of results the speaker presented. What are the unequivocal abnormalities you found in the metabolism of neurotransmitters in the brain of spontaneously hypertensive, DOCA-hypertensive and renal hypertensive rats?

Dr. YAMORI: According to our observations one moth after the development of hypertension, turnover of norepinephrine in the brain stem was delayed in SHR and DOCA-hypertensive rats, but not significantly changed in rats with renal hypertension caused by renal infarction. But the turnover rate in the brain stem was reduced in the three types of hypertensive animals compared with that in the fore-brain.

Therefore, noradrenergic pressor mechanism might exist in the fore-brain.