Influence of Immobilization Stress on Blood Pressure,
Plasma Renin Activity and Biosynthesis of Adrenocorticoid

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umerous reports indicate that the nervous system exercises over arterial pressure. The effects of mental stress on blood pressure and excretion of catecholamines in urine were studied in labile hypertensive patients, and the stress was reported to lead to changes in systolic and diastolic pressures which were significantly greater in hypertensives. On the other hand the nature of contribution of sympathetic nervous system to renal hypertension has become more clear, and the actions of angiotensin on the sympathetic nervous system appears to intensify its effects on the peripheral vascular system, and to stimulate release of catechols from adrenal medulla.

Our previous study on the EEG of the mesencephalic reticular formation and the measurement of arousal threshold by high frequent electrical stimulation in experimental renal hypertensive rabbits revealed that the mesencephalic reticular formation in ascending reticular activating system played an important role in regulation of the blood pressure. Furthermore, it was suggested that experimental renal hypertension seemed to be initiated by humoral mechanism, whereas its chronic phase may be accompanied by neural component.

In the present study experiments were carried out to elucidate the correlated changes of renin-angiotensin system and pituitary-adrenocortical system with elevation of blood pressure regulated from stress, i.e. immobilization stress.

Another purpose of this study was to investigate the influence of high frequent electrical stimulation to the brain on both systems.

MATERIALS AND METHODS

A total of one hundred and eighty eight New-Zealand white male rabbits, weighing 2.5-3.0 kg, were used throughout these experiments. All experiments were performed between 12:00 and 18:00.

For determination of the influence of immobilization stress upon plasma renin activity and adrenocortical activity a 6 hrs of immobilization, from 12:00 to 18:00, were applied at the rabbits by taping their four limbs to specially prepared metal mounts attached to a board. Blood samples were obtained by heart puncture at various time during the stress, and adrenal glands were removed from the decapitated carcass.

Adrenocortical biosynthetic activity was determined by measurement of in vitro transfer rates of $^{14}$C from $^{14}$C-1-acetate into corticosterone and 17-OHCS. Blood corticosterone concentration was determined by a modification of De Moor's technique and plasma renin activity was measured by bioassay.

For recording EEG activity and electrical stimulation, concentric bipolar electrodes were implanted stereotaxically by the atlas of Sawyer et al. into the several areas of brain; the mesencephalic reticular formation, mesencephalic central grey, centro-median nucleus, dorsomedial thalamus, anterior hypothalamic area, nucleus ventromedial of the hypothalamus, posterior hypothalamic area and so on. Experiments were performed after the recovery.

Key Words:
Stress, Renin, Corticosterone,
Adrenocortical biosynthetic activity,
Electrical stimulation of the brain

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TABLE 1  INFLUENCE OF IMMobilIZATION STRESS ON BLOOD PRESSURE, PLASMA RENIN ACTIVITY, PLASMA CORTICOSTERONE CONCENTRATION, BIOSYNTHETIC ACTIVITY OF ADRENOCORTICOID, PLASMA SODIUM CONCENTRATION AND PLASMA POTASSIUM CONCENTRATION

<table>
<thead>
<tr>
<th></th>
<th>Blood pressure (mmHg)</th>
<th>Plasma renin activity (ng/ml)</th>
<th>Plasma corticosterone concentration (µg/dl)</th>
<th>¹⁴C incorporated CS (dpm)</th>
<th>¹⁴C incorporated 17-OHCS (dpm)</th>
<th>Plasma sodium concentration (mEq/l)</th>
<th>Plasma potassium concentration (mEq/l)</th>
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<tbody>
<tr>
<td>Control</td>
<td>60.0 ± 6.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9 ± 4.7</td>
<td>3.48 ± 1.35</td>
<td>860.6 ± 5.6</td>
<td>197.0 ± 5.6</td>
<td>132.3 ± 6.6</td>
<td>4.58 ± 0.64</td>
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<td>15 min</td>
<td>118.0 ± 7.3&lt;sup&gt;***&lt;/sup&gt;</td>
<td>24.7 ± 13.1&lt;sup&gt;***&lt;/sup&gt;</td>
<td>11.41 ± 3.30&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1052.8 ± 17.2**</td>
<td>218.8 ± 5.9&lt;sup&gt;***&lt;/sup&gt;</td>
<td>136.6 ± 7.8</td>
<td>3.81 ± 0.67&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 h</td>
<td>91.7 ± 14.4&lt;sup&gt;***&lt;/sup&gt;</td>
<td>19.3 ± 11.8&lt;sup&gt;*&lt;/sup&gt;</td>
<td>11.85 ± 3.16&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1149.0 ± 32.2&lt;sup&gt;***&lt;/sup&gt;</td>
<td>236.2 ± 3.6&lt;sup&gt;***&lt;/sup&gt;</td>
<td>136.1 ± 7.7</td>
<td>3.99 ± 0.56&lt;sup&gt;***&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 h</td>
<td>74.3 ± 13.7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>19.8 ± 6.4&lt;sup&gt;**&lt;/sup&gt;</td>
<td>9.64 ± 4.53&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1215.2 ± 37.7&lt;sup&gt;***&lt;/sup&gt;</td>
<td>239.4 ± 2.4&lt;sup&gt;***&lt;/sup&gt;</td>
<td>135.9 ± 3.9</td>
<td>4.15 ± 0.58&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 h</td>
<td>51.3 ± 3.0</td>
<td>13.3 ± 3.7</td>
<td>9.51 ± 4.20&lt;sup&gt;**&lt;/sup&gt;</td>
<td>139.9 ± 6.0</td>
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<tr>
<td>5 h</td>
<td>51.7 ± 6.6</td>
<td></td>
<td></td>
<td>1416.9 ± 31.4&lt;sup&gt;***&lt;/sup&gt;</td>
<td>288.4 ± 12.8&lt;sup&gt;***&lt;/sup&gt;</td>
<td></td>
<td></td>
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<tr>
<td>6 h</td>
<td>58.0 ± 9.5</td>
<td>9.5 ± 4.2</td>
<td>8.46 ± 3.29&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1322.0 ± 49.1&lt;sup&gt;***&lt;/sup&gt;</td>
<td>270.8 ± 12.7&lt;sup&gt;***&lt;/sup&gt;</td>
<td>138.0 ± 7.8</td>
<td>4.36 ± 0.43</td>
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</table>

<sup>a</sup>: Mean ± standard error,  <sup>b</sup>: Numbers of animals,  <sup>c</sup>: Mean dpm in ¹⁴C-1-corticosterone (CS) and ¹⁴C-1-17 hydroxycorticosterone (17-OHCS) per mg adrenal protein

<sup>*</sup>: p 0.05 vs. control,  <sup>**</sup>: p 0.01 vs. control,  <sup>***</sup>: p 0.001 vs. control
period of more than 20 days.

Recording of EEG were conducted before and during the application of immobilization stress and simultaneously blood pressure were measured. Effects of electrical stimulation on plasma renin activity, plasma corticosterone levels and adrenocortical biosynthetic activity were determined on the samples obtained immediately after the stimulation as described above.

Stimulation consisting of monophasic square wave pulses of 0.5 msec duration, 300 cps and 250–280 μA was delivered unilaterally for 20 min, 0.5 min on and 4.5 min off for the measurement of the changes in plasma renin activity. Stimulation of 0.1 msec duration, 100 cps and 250–280 μA was delivered unilaterally for 60 min, 5 sec on and 55 sec off for the measurement of the changes in plasma corticosterone levels and adrenocortical biosynthetic activity.

RESULTS

1. Effects of Immobilization Stress on the Blood Pressure, Plasma Renin Activity, Plasma Corticosterone Concentration and Biosynthesis of Adrenocorticoid.

Summarised data on the effects of immobilization stress were shown in Table I. Fifteen minutes after the beginning of immobilization stress, there was a remarkable elevation in blood pressure and a significant increase in plasma renin levels. These changes were also observed at 2 hours. But during the next 4 hours until the releasing of immobilization, the changes were not so significant.

On the other hand immediate increases observed after the beginning of immobilization in the plasma corticosterone levels and the adrenocortical biosynthetic activity lasted for 6 hours throughout the immobilization period. The changes of adrenal biosynthetic activity approximately coincided with those of plasma corticosterone concentrations.

Plasma levels of sodium and potassium before and during the immobilization stress were measured at the same time. Plasma potassium concentrations were decreased after the beginning of stress, while the changes were restored after that. Plasma sodium concentrations were increased compared to that before immobilization, though being not significant.

2. Changes of EEG Activity during Immobilization Stress.

The dominant activity of the mesencephalic reticular formation tended to consist of regular waves at 4–8/sec under the nonstressful and arousal condition. When the rabbits were exposed to immobilization stress, the following types of electrical activity were recognized in the mesencephalic reticular formation and the mesencephalic central grey; (i) slow waves at 2–4/sec increased, (ii) low-amplitude waves at 8–13/sec decreased. Almost no remarkable changes of EEG activity were observed in the pontine reticular formation, centromedian nucleus and dorsomedial thalamus under the immobilization stress.

3. Effects of Electrical Stimulation of the Brain on Plasma Renin Levels.

The effects of electrical stimulation on plasma renin levels were investigated on the non-immobilized rabbits and a tendency to be increased compared to the pre-stimulation levels were showed. The most remarkable increases in plasma renin levels (mean increase 9.0 ng/ml or more) were observed after the stimulation of the mesencephalic reticular formation, the mesencephalic central grey and the posterior hypothalamic area.

4. Effects of Electrical Stimulation of the Brain on Plasma Corticosterone Levels and Adrenocortical Biosynthetic Activity.

As shown in Table II, under the non-immobilized conditions, stimulation of the posterior hypothalamic area and the hypothalamic ventromedial nucleus induced the most remarkable increase of corticosterone concentration and the transfer rates of 14C into corticosterone and 17-OHCS in the adrenal homogenates.

On the contrary stimulation to the septal area and nucleus accumbens induced the decrease of them.

Under the immobilized condition the same stimulation into nucleus ventro-medialis and medial preoptic area induced more remarkable increase of adrenocortical biosynthetic activity than under the non-immobilized condition.

DISCUSSION

These experiments indicated that activation of the renin-angiotensin system and the pituitary adrenocortical system immediately after the beginning of the immobilization stress participated in elevation of arterial pressure. In addition sympathetic system may probably play an important role in high raised arterial pressure and in increasing plasma renin activity under the stress.
<table>
<thead>
<tr>
<th>Sites of stimulation</th>
<th>% changes in CS</th>
<th>$^{14}C$ Incorporated</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>CS (dpm)</td>
<td>17-OHCS (dpm)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>100 (10)</td>
<td>875 ± 25 (10)</td>
<td>208 ± 12 (10)</td>
<td></td>
</tr>
<tr>
<td>Central grey</td>
<td>139 ± 12 (5)</td>
<td>1022 ± 25 (5)</td>
<td>243 ± 8 (5)</td>
<td></td>
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<tr>
<td>Mesencephalic reticular formation</td>
<td>183 ± 22 (5)</td>
<td>1059 ± 27 (5)</td>
<td>271 ± 7 (5)</td>
<td></td>
</tr>
<tr>
<td>Anterior hypothalamic area</td>
<td>131 ± 9 (4)</td>
<td>1242 ± 35 (4)</td>
<td>262 ± 7 (4)</td>
<td></td>
</tr>
<tr>
<td>N. ventromedialis hypothalami</td>
<td>206 ± 14 (10)</td>
<td>1305 ± 30 (5)</td>
<td>285 ± 10 (5)</td>
<td></td>
</tr>
<tr>
<td>Posterior hypothalamic area</td>
<td>175 ± 12 (7)</td>
<td>1215 ± 39 (7)</td>
<td>270 ± 9 (7)</td>
<td></td>
</tr>
<tr>
<td>Medial preoptic area</td>
<td>132 ± 8 (5)</td>
<td>1290 ± 33 (5)</td>
<td>272 ± 7 (5)</td>
<td></td>
</tr>
<tr>
<td>Hippocampus (CA 3)</td>
<td>166 ± 9 (10)</td>
<td>1015 ± 26 (10)</td>
<td>237 ± 9 (10)</td>
<td></td>
</tr>
<tr>
<td>Amygdala (boso-med)</td>
<td>135 ± 8 (5)</td>
<td>1050 ± 17 (5)</td>
<td>291 ± 9 (5)</td>
<td></td>
</tr>
<tr>
<td>Amygdala (boso-lat.)</td>
<td>140 ± 10 (5)</td>
<td>1172 ± 22 (5)</td>
<td>320 ± 11 (5)</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean dpm in $^{14}$C-1-corticosterone (CS) and $^{14}$C-1.17 hydroxycorticosterone (17-OHCS) per mg adrenal protein. N ± standard deviation.

Numbers in parenthesis: number of animals

All the stimulation induced the significant ($p < 0.001$) changes.

It was found that there were marked increases in catecholamine excretion during the period of immobilization and increase in plasma renin levels in exercising and pain-stress rats were inhibited by administration of ganglionic blocking agents. However, evidence for a neurogenic mechanism to regulate the arterial pressure has been entirely equivocal.

In our previous study intravenous injection of angiotensin II increased blood pressure and stimulated the EEG activity of the mesencephalic reticular formation but 15 minutes after the injection it was inactivated even though the blood pressure was still higher. Baust et al. reported that intravenous injection of adrenaline and vasopressin induced cortical EEG activation simultaneously with the beginning of rise in the blood pressure and after that the sleep patterns always reappeared when the blood pressure was still elevated.

In this experiment the changes of EEG activity of the mesencephalic reticular formation and the mesencephalic central grey after the beginning of immobilization stress were recognized that slow waves at 2–4/sec increased and low amplitude waves at 8–13/sec decreased while the blood pressure was still elevated. These observations suggest that the major homeostatic mechanisms mediated by the activity of the mesencephalic reticular formation and the mesencephalic central grey seem to regulate the arterial pressure.

Since the central nervous system controls the endocrine secretion and the hormonal regulation of vascular tone is extremely powerful, a great deal of efforts has been expended on discovering the effects of electrical stimulation of different areas of the brain through implanted electrodes. Goldfien et al. studying adrenal medullary and adrenal cortical response to electrical stimulation of the diencephalon in anesthetized dogs, observed that catecholamines were increased with stimulation of the dorsomedial and posterior regions of the hypothalamus.
The involvement of the mesencephalic reticular formation and the mesencephalic central grey was also suggested by the stimulation experiments; that is, the mesencephalic reticular formation, the mesencephalic central grey and the posterior hypothalamic area seemed to be more effective on the release of renin than on the release of corticosterone, while the other areas in the brain such as the hippocampus, the amygdala and the anterior hypothalamic area were not so effective on the release of renin.

REFERENCES

Discussion:
Chairman: KOICHI OGINO Kyoto Univ.
Dr. OGINO: In this report, the following problems were discussed.
(1) What is stress to the rabbit in this study?
(2) Hasn't the bloodletting influence in this experiment on renin activity in the rabbit's plasma?
(3) Isn't it indispensable for the rabbits to be quantitatively and qualitatively analyzed the change in plasma or urinary steroids under such mechanical stress as tight-fastening?
(4) Does the increase in plasma renin activity surely play the primary causative role on pressor effect of the rabbit in this study?
Concerning the problem (1), following discussions were made.

Questions: What significance has the tight-fastening as stress to the rabbit? (Dr. E. Ueda)
Did you consider the struggle of the animal during
the fastening to be a stress? (Dr. J. Fujii) Why didn't you prefer another stimulation, e.g. painful stimuli in this study? (Dr. Ogino) How many milliliter of blood was collected from the heart? (Dr. J. Fujii) Does't venesection also act as stress to the rabbit? (Dr. E. Ueda).

Speaker. Dr. TSUKIYAMA: answered as follows.

Heart puncture in the control period was made from 2 days before the experiment on the rabbits tamed with same procedure by daily repeat of holding in the examiner's arm and, blood collection was restricted within 30 seconds in time and 4 cc in volume per a puncture, totally within 20 ml per an experiment. Therefore, the speaker considers the influence of heart puncture to be cancelled out in the case of bloodletting under the tight fastening on the fixing table. Fixing on fastening was seemed to have more stable effect as stress to the rabbit than painful stimulus e.g. by the injection of formalin solution.

Concerning the problem (2), discussion were made as follows. Condition during the blood collection is very important in discussing the change in PRA of animals (Dr. E. Ueda). Although the values of PRA determined with Pickens' method is reliable in discussing the increase of activity, they are disputable in referring to the decrease in activity, especially the decrease with the elapse of time, because the consumption of renin substrate or the half life of renin in the blood must be considered. (Dr. K. Tanaka).

Question: What is the change in PRA of rabbits after continuous bloodletting under the condition without any stress? (Dr. F. Fujii).

Speaker Dr. Tsukiyama replied as follows. Blood collection in fastening was seemed to be the considerable stress to the rabbits judging from the increase in PRA of these blood. PRA in the control period, if determined on the blood collected with such procedure, is thought to be unreliable.

As to problem (3), discussion were made as follows. It is indispensalbe, from the following reasons, to investigate the qualitative changes of steroid pattern in discussing the alteration of steroids under the stress, especially in such experiment as the speaker's one. 1) In the rabbit, actual features of 17 OHCS is not always clarified. 2) Recently, specific steroids were reported in sera of rabbits under stress. 3) Incorporation rate of isotope acetate, systematically administrated, into steroids is too low to be hardly determined. (Dr. R. Takeda).

Question: Which is preceded between the increase in PRA and the increase in plasma glucocorticoids on loading the stress? (Dr. K. Yamamoto).

Speaker Dr. Tsukiyama answered as follows. Regarding the problem of question, I have not enough evidences to reply to Dr. Yamamoto. Increase in PRA by loading the stress of fastening is recovered to the control level 2 hours after the beginning of stress, while, plasma concentration of corticosteroids kept to the higher level throughout the stress. Thus, speaker should like to take notice to the difference between response-curves of two substances.

Discussion on the problem (4).

Question: Are you convinced the elevation of blood pressure under the stress in your experiment would be caused by the increase in PRA? We have observed that the abrupt elevation of blood pressure was caused by the injection of iron particle centering around the brain stem, though the procedure differs from the speaker's one. In our case, the increase in catecholamine and the sympathetic stimulation was thought to be the primary cause of pressor effect and then, the increase in PRA was the secondary phenomena. (Dr. K. Tanaka)

Answer from the speaker. Various mechanism are presumed on the increase in PRA. As mentioned in the consideration, the increase of PRA in our present study is thought to be largely ascribed to the sympathetic nervous system.

Question: Which change of blood pressure were caused by chronic central stimulation to the rabbit? Were there observed the difference in blood pressure responsiveness between the normal and Goldblatt type rabbit?

Answer: We have observed previously the change of blood pressure in chronic electrical stimulation with high frequency of various areas in the brain. Pressor effect was induced by daily electrically stimulating for 2 months the reticular formation and the central gray matter of midbrain, and high blood pressure was kept up for a while after the stop of stimulation. And it is interesting in the present study that the changes of EEG under the fastening stress are remarkable in the abovementioned area and the marked increase of PRA is induced by the stimulation of the same area.