CONTRIBUTION OF SYMPATHO-ADRENAL SYSTEM TO THE GASTRIC MOVEMENT OF RATS SUBJECTED TO RESTRAINT AND WATER IMMERSION STRESS

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Abstract—Exposure of the rat to restraint and water immersion stress enhanced a characteristic gastric movement in a 2- to 4-hr latent period. The mechanism of producing such gastric movement was studied in connection with changes in the sympatho-adrenal activity. Acute bilateral adrenalectomy and reserpine pretreatment significantly hastened occurrence of the gastric movement and guanethidine pretreatment showed a similar tendency. Epinephrine content in the adrenals of the stressed group markedly decreased at the time of occurrence of the gastric movement. Urinary excretion of epinephrine and norepinephrine continued to increase for 3 hr and for 6 hr respectively, after the onset of stress, and both excretory rates declined thereafter. Epinephrine and norepinephrine were much more effective on depressing the stress-induced gastric movement than cortisone and ACTH. It was suggested that: 1) at the initial stage of stress, the adrenal function as well as the sympathetic nervous function was activated, and a large amount of epinephrine and norepinephrine was secreted, which brought about an inhibition of the gastric movement; 2) at the subsequent stage, the parasympathetic nervous function became more prominent than the sympato-adrenal one and initiated the gastric movement; and 3) contribution of corticosteroids and/or ACTH to the gastric movement was small, if any.

Restraint and water immersion stress developed the rat gastric movement which was characterized by an increase in the amplitude along with or without an elevation in the tone level and accompanied by a latent period of 2-4 hr (1). The formation of gastric lesions was markedly accelerated during the presence of this type of gastric movement (unpublished observations). The sympatho-adrenal activity is found to increase immediately after the onset of stress (2, 3) and to participate in many stress responses (4-6). The present work was undertaken to study the mechanism of the stress-induced gastric movement in connection with changes in the sympatho-adrenal activity.

MATERIALS AND METHODS

Male Wistar rats weighing 200-290 g were deprived of food overnight but permitted water ad libitum.

Recording of gastric movement and body movement under stress

Gastric movement of the rat under stress was recorded according to the method described previously (1). In short, the animal was lightly anesthetized with ether, the abdomen opened, and the stomach exteriorized. After an incision was made in the forestomach,
a rubber balloon (volume of about 2.5 ml) was passed into the stomach and placed at the glandular portion of the stomach to record gastric movement. The balloon was filled with water and connected to a water manometer through a polyethylene tube. Initial pressure of the balloon was kept at the height of 8 cm of water. A catheter was placed in the peritoneal cavity to administer drug solutions. A bilateral adrenalectomy using an anterior approach was performed at the same time in some experiments. Ten min after recovery from anesthesia, the animal was restrained in a stress cage which was equipped with a water-filled balloon (volume of about 3 ml) on the floor. Body movement was measured through a water manometer by letting the animal press down on the balloon in the stress cage. Both gastric movement and body movement were recorded on a kymographion. The animal was then immersed in a water bath of 25°C to the depth of the xiphoid process. Restraint alone was also employed as a comparative procedure when necessary.

Criteria for occurrence of gastric movement

As mentioned above, gastric movement which was characterized by an increase in the amplitude and mostly by an elevation in the tone level appeared in some latent period after the onset of stress. Occurrence of the increased gastric movement was expressed as the time when the tone level and amplitude reached the maximal response. In the cases where the tone elevation was not so manifest, the time of occurrence of the maximal amplitude was used for the measurement.

Criteria for inhibition of gastric movement

Inhibitory effect of drugs was examined during the presence of the increased gastric movement. Inhibition time of the gastric movement was measured as the period from beginning of drug-induced inhibition to recovery to the original level.

Determination of epinephrine (Epi) and norepinephrine (Nor) content in various tissues and urine

Content of Epi and Nor in the adrenals, whole brain, glandular stomach and urine was determined at 1 hr after stress and at 0 or 3 hr after the time of occurrence of the increased gastric movement. At these times, the adrenals and stomach were excised under ether anesthesia, immediately frozen with liquid nitrogen and stored in a freezer (−15°C). Thereafter, the whole brain except the rhinencephalon was removed after being frozen in situ with liquid nitrogen, and stored at −15°C. Urine collection was carried out in another series of the experiment, where no operative procedure was performed. For the collection of urine, the orifice of a rubber pipette cap was attached to the external part of the genitals with an adhesive and the tip of the cap was connected with a sample bottle (about 20 ml) through a glass tube. This sample bottle contained 2–10 ml of 0.8 N perchloric acid and 0.2% sodium metabisulfite in advance and remained to be under atmospheric pressure via a fine polyethylene tube. At the time of urine collection, the urinary bladder of animals was exposed under anesthesia with sodium pentobarbital (50 mg/kg, i.p.) and urine in the bladder was collected and combined with the urine in the bottle. With minor modifications, the method of Shellenberger and Gordon (7) was used for the extraction of Epi and Nor.
from the tissues and urine and that of Anton and Sayre (8) for the determination of the respective amounts. Sham-operated or intact animals which served as control were always examined on the same experimental day as the stressed animals. The control animals were placed in an environment of 22°C and 60% humidity during the experimental period.

Statistical evaluation

All data were statistically analyzed using Student's t-test.

Drugs

Drugs used were as follows. ACTH (Acthar Injection, Armour), atropine methylbromide (Tokyo Kasei), cortisone acetate (Wako), l-epinephrine hydrochloride (Adrenalin Injection, Sankyo, for test of gastric movement), l-epinephrine bitartrate (Sigma, for Epi determination), guanethidine sulfate (Ismelin, CIBA), dl-norepinephrine hydrochloride (Noradrenalin Injection, Sankyo, for test of gastric movement), l-norepinephrine bitartrate (Wako, for Nor determination) and reserpine (Apoplon Injection, Daiichi). Drugs were dissolved or diluted in saline and solutions were i.p. injected in a volume of 1 ml/kg. Doses of catecholamines and reserpine were expressed in terms of the free base and those of guanethidine in terms of the salt.

RESULTS

Influence of reserpine pretreatment, guanethidine pretreatment and bilateral adrenalectomy on gastric movement of the rat subjected to restraint and water immersion stress

As shown in Fig. 1, gastric movement of the control group, characterized by an elevation in the tone level and an increase in the amplitude, appeared in 2 hr after the restraint and water immersion stress and persisted thereafter. On the contrary, this type of gastric movement never appeared under the stress of restraint alone. Typical recordings of the gastric movement influenced by reserpine pretreatment (5 hr before stress, 2.5 mg/kg), by guanethidine pretreatment (3 hr before stress, 30 mg/kg), and by bilateral adrenalectomy are illustrated in Fig. 1 and the time of occurrence of the increased gastric movement is given in Table 1. In the reserpine-treated group, the time of occurrence of the gastric movement was markedly shortened, that is, the tone level and the amplitude began to increase about 30 min after stress in most cases. In the guanethidine-treated group, the time of occurrence

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Dose (i.p.)</th>
<th>No of rats</th>
<th>Occurrence time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>11</td>
<td>147.8±11.1</td>
</tr>
<tr>
<td>Reserpine</td>
<td>2.5 mg/kg</td>
<td>9</td>
<td>84.3±10.2***</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td>11</td>
<td>127.1±8.9</td>
</tr>
<tr>
<td>Guanethidine</td>
<td>30 mg/kg</td>
<td>9</td>
<td>103.0±8.5</td>
</tr>
<tr>
<td>Sham-operated</td>
<td></td>
<td>8</td>
<td>136.2±14.0</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td></td>
<td>8</td>
<td>93.2±6.9*</td>
</tr>
</tbody>
</table>

*: P<0.05, ***: P<0.001.

Occurrence time is expressed as mean±S.E.
FIG. 1. Influence of reserpine (RSP), guanethidine (GNT) and bilateral adrenalectomy (ADX) on the gastric movement of the rat subjected to restraint and water immersion stress. BM: body movement, GM: gastric movement.

of the gastric movement was shortened in a similar manner, although not significantly. Elevation in the tone level, however, occurred in an earlier stage. In the adrenalectomized group, the gastric movement appeared significantly earlier and elevation in the tone level was observed 30 min after stress.

Effect of epinephrine, norepinephrine, cortisone and ACTH on the rat gastric movement induced by restraint and water immersion stress

The effect of Epi, Nor, cortisone, ACTH and atropine methylbromide on the increased gastric movement under stress is shown in Fig. 2 and Table 2. Epi and Nor, in 0.1 mg/kg, completely depressed the gastric movement for 70–120 min. In contrast, 5–10 mg/kg of cortisone and 5–10 μg/kg of ACTH caused a weak depressive action with a complete depression of 20–50 min.
Effect of epinephrine, cortisone and ACTH on the rat gastric movement induced by restraint and water immersion stress. BM and GM: See Fig. 1. Doses of epinephrine and cortisone are expressed as mg%kg and those of ACTH as u/kg.

### Table 2. Inhibition time of the increased gastric movement

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (i.p.)</th>
<th>No of rats</th>
<th>Inhibition time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>15</td>
<td>6.9± 2.8</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.1 mg/kg</td>
<td>16</td>
<td>113.4± 7.4</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>0.1 mg/kg</td>
<td>16</td>
<td>75.8± 4.0</td>
</tr>
<tr>
<td>Cortisone</td>
<td>1 mg/kg</td>
<td>8</td>
<td>10.0± 2.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8</td>
<td>21.6± 7.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>39.8±12.1</td>
</tr>
<tr>
<td>ACTH</td>
<td>1 u/kg</td>
<td>8</td>
<td>10.5± 4.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>8</td>
<td>32.4±10.2</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>8</td>
<td>50.9±15.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>8</td>
<td>69.9±23.8</td>
</tr>
<tr>
<td>Atropine methylobromide</td>
<td>0.05 mg/kg</td>
<td>9</td>
<td>169.6±23.0</td>
</tr>
</tbody>
</table>

Inhibition time is expressed as mean ± S.E.

**Time course of changes in epinephrine and norepinephrine content in adrenals, glandular stomach, whole brain and urine of the rat during the stress period**

Epi and Nor content in tissues at 1 hr after stress, at the time of occurrence of the gastric movement (3.04±0.32 hr after stress) and at 3 hr after occurrence of the gastric movement (6.37±0.50 hr after stress) is shown in Fig. 3 (adrenals) and Fig. 4 (glandular stomach and whole brain). Epi content in the adrenals of the stressed group markedly decreased at the time of occurrence of the increased gastric movement in comparison with that of the control group and further decreased as the stress duration was prolonged.
FIG. 3. Adrenal catecholamine content. *: P<0.05, **: P<0.01 (significantly different from the respective control). Each time after stress or each point on the line is expressed as mean ± S.E. (n=6).

FIG. 4. Norepinephrine content of brain and stomach. Each time after stress or each point on the line is expressed as means ± S.E. (n = 6).

The other hand, there was not a significant decrease in the adrenal Nor content. Nor content in the glandular stomach and in the whole brain of the stressed group showed a tendency to increase and to decrease respectively, throughout the experimental period as compared with that of the control group. Urinary Epi and Nor content at 3, 6 and 21 hr after stress is illustrated in Fig. 5. In comparison with control values, Epi content of the stressed group rapidly increased for 3 hr after stress, while Nor content of the stressed group increased linearly for 6 hr after stress. The excretory rate of Epi and Nor in the stressed group became almost similar to that in the control group after 6 hr of stress.
DISCUSSION

Nervous control of gastrointestinal motility summarized so far is as follows (9-11). The digestive organs receive intrinsic auto-regulation by mural nerve cells and the extrinsic regulation by the autonomic nervous system. Sympathetic as well as parasympathetic nerves innervating the digestive organs include two kinds of neuron fibers, i.e. excitatory and inhibitory, and it has generally been accepted that, in normal states, the sympathetic nervous system mainly exerts an inhibitory action on the organ and the parasympathetic nervous system mainly does an excitatory one on it. In stressful states, however, the excitability of both nervous systems may change and a different motility from what is observed in normal states may occur.

Functions of the sympathetic nervous system and of the adrenals in the body are activated by various stress procedures such as cold, restraint, forced exercise, etc. (2, 3, 6). On the other hand, it has been suggested that functions of the parasympathetic nervous system are also activated, which event contributes to the gastric motility under stress. Using the rat subjected to restraint and water immersion stress, Watanabe (12) observed increased gastric movement and suggested that stress influenced gastric functions through an activation of the vagus nerve. Hübner et al. (4) studied the time course of changes in the gastric microcirculation in the restrained rat. They observed that smooth muscles of gastric submucosal arterioles were constricted during the whole stress period and that the stomach was relaxed at the time of 3 hr after stress and was contracted later. They called the early stage a sympathico-adrenergic phase and the late stage a counter regulatory cholinergic phase. Hase et al. (13) observed a decrease in blood glucose in pylorus-ligated rats 2 hr after rotation stress, discussing that this change in blood glucose would enhance gastric secretion via the
vagus nerve, which favored the stress ulceration. These results definitely point out that stress activates sympato-adrenal and/or parasympathetic functions, but knowledge which concerns the interaction of both functions and its mechanisms has so far been lacked.

In the present experiment, reserpine pretreatment, guanethidine pretreatment and bilateral adrenalectomy all shortened the time of occurrence of the increased gastric movement under stress. The former two pretreatments like the latter are considered to reduce sympato-adrenal activity, as suggested elsewhere (14, 15), though both the drugs differ from each other in depleting action on catecholamines in the brain and adrenals. These facts indicate that a decrease in the sympato-adrenal tone induces earlier occurrence of the increased gastric movement. Catchpole (16), summarizing a number of references which involved the decrease in gastric movement caused by laparotomy and ileus, concluded that the symptoms could be ameliorated by depressing the sympathetic tone and that guanethidine was clinically the most suitable drug for this purpose.

The inhibitory action of Epi and Nor on the gastric movement under stress was markedly potent compared with that of cortisone and ACTH. Based on the dosage of these drugs used here, it is considered that drugs which are related to the sympato-adrenal system play a more important role in depressing the gastric movement than adrenocortical hormones. Therefore, the same situation would occur among actions of Epi, Nor and adrenocortical hormones which are intrinsically released during stress.

Epi and Nor content in various tissues and urine was measured in order to study the time course of changes in the sympato-adrenal activity under stress. Among these, Epi and Nor content excreted in urine is considered to be a favorable indicator for its activity (17). In the stressed group, Epi content in the adrenals decreased to 40% of control level at the time of occurrence of the increased gastric movement, while Nor content in the adrenals decreased slightly. Excretion of Epi in urine rapidly increased up to the time of occurrence of the gastric movement and declined thereafter. On the other hand, excretion of Nor continued linearly to increase for about 6 hr beyond the time of occurrence of the gastric movement after stress. Urinary Epi is considered to originate in the adrenals, and urinary Nor in the sympathetic nerve endings and adrenals. Accordingly, it is assumed that the adrenal function activated by stress begins to decline in activity at the time of occurrence of the gastric motility, whereas the sympathetic nervous function maintains its raised activity until 3 hr later. Kaartinen (18) reported that the turnover rate of Nor in the rat's stomach and duodenum increased under restraint and electroshock stress. Djahanguiri et al. (2) observed that there was not a definite change in Nor content in the glandular stomach under restraint stress but an increase in its turnover rate. In the present study, no significant change in Nor content in the glandular stomach was observed, either, between unstressed-control and stressed groups.

It is quite possible that gastric motility is stimulated not only by a declining sympato-adrenal function, but also by an augmenting vagal activity after stress. This hypothesis is supported by the fact that there still remains relatively high activity of the sympato-adrenal function at the time of occurrence of the increased gastric motility. To study the mecha-
nisms in detail, it has to be clarified exactly how the level of vagal activity varies during stress.

In conclusion, the activation of the sympatho-adrenal function may prevent the increase in gastric movement at the initial stage of restraint and water immersion stress, while the activation of parasympathetic nervous function which becomes more prominent as the sympatho-adrenal activity gradually declines may be responsible for occurrence and persistence of the increased gastric movement at the subsequent stage.

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REFERENCES

6) Buckley, J.P.: J. Pharmacol. Sci. 61, 1175 (1972)