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Effects of Stress Transmission Pathways on Acute Ulcerogenesis in Rats under Cold-Restraint Stress

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ABSTRACT

The effect of autonomic nerve and pituitary-adrenal pathways of stress transmission on stress-induced acute gastric ulceration was studied using splanchnicotomized (Sp), truncal vagotomized (TV) and splanchnicotomized plus vagotomized (Sp + TV) rats. Cold-restraint stress was loaded for 30, 60 and 120 min. With normal rats as a control group, the change in incidence of gastric mucosal lesions, contents of noradrenalin (NA), histamine (HA) and serotonin (5-HT) within gastric mucosa and levels of serum ACTH and 11-OHCS was compared. In the Sp group, there was no significant difference in the NA content before and after stress loading, but the HA content at 30 min after stress loading and the 5-HT content at 60 min were significantly decreased [5.0 ± 1.6 μg/g and 30.0 ± 0.6 μg/g, mean ± SD, respectively] as opposed to their respective contents before stress [8.6 ± 1.1 and 4.3 ± 1.1, respectively] [$p<0.01$ and $p<0.05$], as it did in the control group. The incidence of animals with gastric mucosal lesions amounted to a high rate of 88% at 120 min. On the other hand, in the TV and Sp + TV groups the HA and 5-HT contents underwent no significant decreases until 120 min after stress loading, and the incidences of lesions were suppressed to 13%. In the control group, the serum ACTH level at 30 min and the serum 11-OHCS level at 60 min were significantly increased as compared with their respective values before stress. The levels of serum ACTH and 11-OHCS in the Sp, TV and Sp + TV groups showed similar patterns of changes to those seen in the control group. The results indicate that the pituitary-adrenal pathway exerts a milder effect than the autonomic nerve pathway on acute ulcerogenesis induced by cold-restraint stress.

Key words: *Acute stress ulcer, Stress transmission patyways, Vasoactive amine, ACTH, 11-OHCS*

Emotional or physical stress easily causes acute stress ulcers with hemorrhagic and multiple lesions in the upper gastrointestinal tract. In the transmission of central impulses developed by stress between the cerebral cortex and the upper gastrointestinal tract via the hypothalamus, the following two pathways are presumed: a neurogenic pathway from the hypothalamus mediated by the autonomic nervous system, and a humoral pathway from the hypothalamus mediated by the anterior pituitary and adrenal system[5].

Stress transmitted to the stomach and duodenum produces alternations in the balance of aggressive and defensive factors[11], causes microcirculatory disturbance and induces the formation of acute ulcers. Mucosal microcirculatory disturbance is closely associated with the activation and release of intramucosallystored vasoactive amines such as noradrenalin (NA), histamine (HA), and serotonin (5-hydroxytryptamine; 5-HT). However, the relationship between the mechanisms of acute stress ulceration and the pathways of stress transmission remains obscure in many cases, with the exception of Cushing's ulcer in which an autonomic nerve pathway, especially the parasympathetic nerve pathway, has been proposed as playing an important role in intracranial diseases and gastroduodenal ulcerogenesis[1].

The aims of this study were to evaluate the effects of these two pathways of stress transmission on the development of cold-restraint induced ulcer in rats. In four groups of rats [normal; splanchnicotomy; vagotomy; splanchnicotomy plus vagotomy], we examined incidences of gastric mucosal lesions and changes of the NA, HA and 5-HT contents in the gastric mucosa under cold-restraint stress. In addition, the levels of serum adrenocorticotropic hormone (ACTH) and 11-hydrocorticosteriod (11-OHCS) were simultaneously determined to demonstrate whether the incidences of gastric mucosal lesions in four groups as described above were related to changes of these two hormones.

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MATERIALS AND METHODS

Male Wistar strain rats (weighing 250—300 g) kept in individual cages with a raised mesh bottom were used throughout the study.

Experimental Models:

The abdomen of anesthetized rats with 11 mg/kg body weight of pentobarbital sodium (Nembutal®, Dainabot Co.) administered intraperitoneally was incised and the following models of an autonomic neurectomy were prepared; (a) splanchnicotomized rats (Sp group) in which the splanchnic nerves were cut on both sides at anterior region of the celiac ganglion, together with cutting of the bilateral adrenal branches; (b) truncal vagotomized rats (TV group) in which the bilateral vagus nerve trunks were cut and a pyloroplasty was treated using the Heineke-Mikulicz method; and (c) splanchnicotomized plus vagotomized rats (Sp + TV group) in which splanchnicotomy and truncal vagotomy as described above were simultaneously performed with pyloroplasty. Thereafter, the laparotomy was closed by one layer of single interrupted stitches. After a three weeks breeding period in individual cages, the experiment of cold-restraint stress was carried out. In addition, normal rats without laparotomy bred under same conditions were used as control rats (control group).

Induction of gastric mucosal lesions:

The animals were deprived of food to avoid any dietary influence but allowed free access to water for twenty-four hours before the beginning of the experiment. Animals were confined in metallic restraint cages using the technique of Senay et al[10], and exposed to a cold stress in a room cooled to 4°C for 30, 60 and 120 min. After stress loading, the animals were killed by decapitation immediately. The entire stomach was removed and opened along the greater curvature to observe the gastric mucosa macrographically using a magnifying glass. Animals with evidence of hemorrhage, erosion or ulcer in the gastric mucosa were designated positive cases of gastric mucosal lesions. The incidence of animals with lesions was calculated for each group.

Determination of HA and 5-HT contents in the gastric wall:

The mucosal layer of the posterior wall of the fundus was collected for determination of HA. The mucosal layer of the posterior wall of the antrum was collected for determination of 5-HT. The samples collected were immediately frozen and preserved at −80°C until measured.

The samples collected were homogenized after addition of a 0.4N perchloric acid. The homogenate was centrifuged (10000 rpm for 25 min). The supernatant was collected and absorbed in an Amberlite CG resin column (0.4 cm in diameter × 9 cm) adjusted to pH 6.5[12]. HA was allowed to react to the O-phthalaldehyde under an alkaline condition and 5-HT under an acidic condition. The intensity of fluorescense of HA and 5-HT fractions was determined by the fluorophotometry (RE 501 type, shimazu Co.) [Ex/Em= 360/440nm and Ex/Em=360/470nm, respectively].

Determination of NA content in the gastric wall:

The entire layers of the anterior fundic and antral walls were collected for determination of NA. They were homogenized after addition of a 5% trichloroacetic acid solution. The homogenate was cetrifuged (10000 rpm for 10 min). The supernatant was absorbed in an acidified alumina, and the NA fragment was eluted with a 1 N acetic acid solution. The eluate was lyophilized and dissolved in 100 μl of a sodium citrate solution at pH 5.28, and then the NA content was determined by high-performance liquid chromatography[13].

Determination of serum ACTH and 11-OHCS values:

Serum ACTH and 11-OHCS were determined before and after loading of cold-restraint stress. Animals were decapitated and blood samples were drawn from the neck for determination of serum ACTH and 11-OHCS values. The samples thus collected were centrifuged (3000 rpm for 10 min), and the serum was frozen and preserved at −80°C until measured. Serum ACTH value was dertemined by radioimmunoassay method (ACTH kit, Compagnie Oris Industrie S.A.) and serum 11-OHCS value by the sulfuric acid-induced fluorescent method[14].

Statistical analysis:

Data are presented as mean ± SD from 5 to 12 rats per group. The statistical significance of differences were evaluated using the Student's t-test. Probabilities of <0.05 were considered statistically significant.

RESULTS

Incidence of gastric mucosal lesions:

Table 1 shows the incidence of animals with gastric mucosal lesions at 30, 60 and 120 min after loading of cold-restraint stress. In the control group, the rate increased with time, i.e., 33% at 30 min, 75% at 60 min and 92% at 120 min. In the Sp group, similar increases were observed, i.e., 38% at 30 min, 75% at 60 min and 88% at 120 min. In the TV group, however, the rate remained only 13% at both 60 and 120 min. In the Sp + TV group, it was only 13% at 120 min. All the lesions in each group occurred in the fundus and consisted mainly of mucosal hemorrhage or multiple acute superficial ulcerations of Ul-l in grade.

Table 1. Incidence of gastric mucosal lesions in cold-restraint rats

Cold-restraint time	30 min	60 min	120 min
Control group (n=12)	33% (4/12)	75% (9/12)	92% (11/12)
Sp group (n=8)	38% (8/ 8)	75% (6/ 8)	88% (7/ 8)
TV group (n=8)	0% (0/ 8)	13% (1/ 8)	13% (1/ 8)
Sp + TV group (n=8)	0% (0/ 8)	0% (0/ 8)	13% (1/ 8)

Control group: normal rats without laparotomy, Sp group: Splanchnicotomized rats, TV group: Truncal vagotomized rats, Sp+TV group: Splanchnicotomized+Truncal vagotomized rats

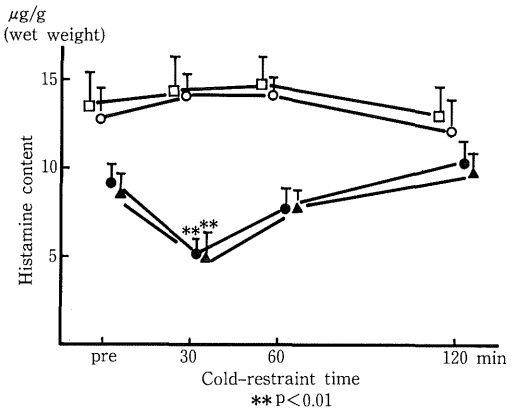

Fig. 1. Changes in the histamine (HA) contents of the fundic mucosa before and after loading of cold-restraint stress.
●—●: Control group (n=10), ▲—▲: Sp, group (n=6)
○—○: TV group (n=6), □—□: Sp+TV group (n=6)
Data represent mean ± SD in each group. Asterisks indicate statistically significant differences after stress loading in comparison with the pre-stress values.

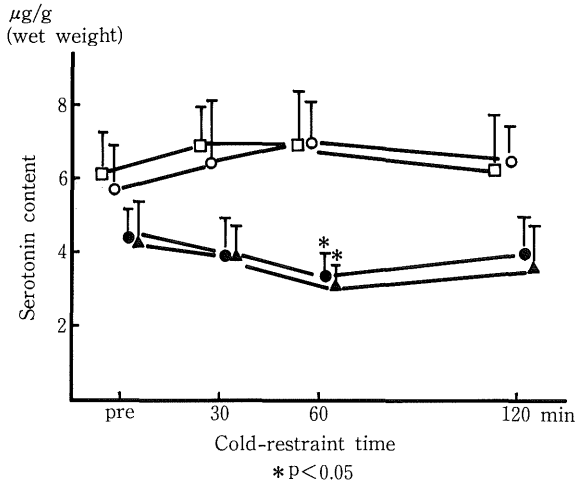

Fig. 2. Changes in the serotonin (5-TH) contents of antral mucosa before and after loading of cold-restraint stress.
For symbols, refer to Fig. 1.

Changes in vasoactive amine contents in the grastric wall:

Figure 1 shows the changes in the HA contents (wet weight) before and after loading of cold-restraint stress. The value before stress loading was 8.9 ± 1.2 μg/g in the control group, 8.6 ± 1.1 μg/g in the Sp group, 12.8 ± 1.8 μg/g in the TV group and 13.2 ± 2.1 μg/g in the Sp + TV group. The levels at 30 min after stress loading in the control and Sp groups were 5.2 ± 0.8 μg/g and 5.0 ± 1.6 μg/g respectively, which showed significant decreases in comparison with the pre-stress values in these groups ($p<0.05$). On the other hand, in the TV and Sp ± TV groups in which the incidences of gastric mucosal lesions were suppressed, no changes from the pre-stress values occurred until the passage of 60 min. At 120 min, however, the levels showed a slightly decreasing tendency.

Figure 2 shows the changes in the 5-HT contents (wet weight) before and after loading of cold-restraint stress. The pre-stress value was 4.5 ± 0.8 μg/g in the control group, 4.3 ± 1.1 μg/g in the Sp + group, 5.9 ± 1.1 μg/g in the TV group and 6.1 ± 2.1 μg/g in the Sp + TV group. In the control and Sp groups in which the incidences of gastric mucosal lesions were high, the levels at 60 min after stress loading were 3.4 ± 0.6 and 3.0 ± 0.6 μg/g respectively, showing significant decreases compared with the pre-stress values ($p<0.05$). In the TV and Sp + TV groups which demonstrated low rates of the formation of gastric mucosal lesions, the 5-HT values were 6.9 ± 1.3 and 7.0 ± 1.4 μg/g respectively at 60 min, indicating no significant differences compared with the pre-stress values. However, at 120 min after stress loading it tended to decrease slightly, as did the HA level.

Figure 3 shows the changes in the NA contents (wet weight) before and after loading of cold-restraint stress. The pre-stress value was 210.5 ± 22.6 ng/g in the control group, 167.9 ± 30.1 ng/g in the Sp group, 200.3 ± 29.3 ng/g in the TV group and 174.0 ± 38.4 ng/g in the Sp + TV group. However, the levels at 30, 60 and 120 min after stress loading showed no significant changes in all the groups compared with pre-stress values.

Changes of serum ACTH and 11-OHCS values:

Figure 4 shows the changes in the serum ACTH levels before and after loading of cold-restrant stress. In the control group, the pre-stress value was 98.9 ± 60.6 pg/ml which increased to 399.0 ± 118.1 pg/ml at 30 min, to 567.0 ± 147.7 pg/ml at 60 min, and to 525.0 ± 109.1 pg/ml at 120 min. Thus, the ACTH levels increased significantly at and after 30 min ($p<0.01$). In the Sp, TV and Sp + TV groups, the pre-stress values were 61.6 ± 13.6, 131.7 ± 81.6 and 56.2 ± 38.1 pg/ml respec-

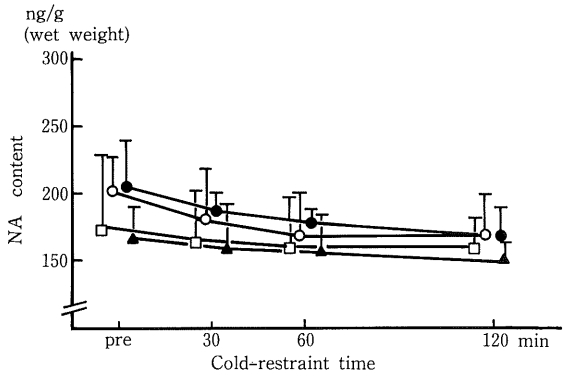

Fig. 3. Changes in the noradrenalin (NA) contents in the whole layers of the fundus and antrum before and after loading of cold-restraint stress.
For symbols, refer to Fig. 1.

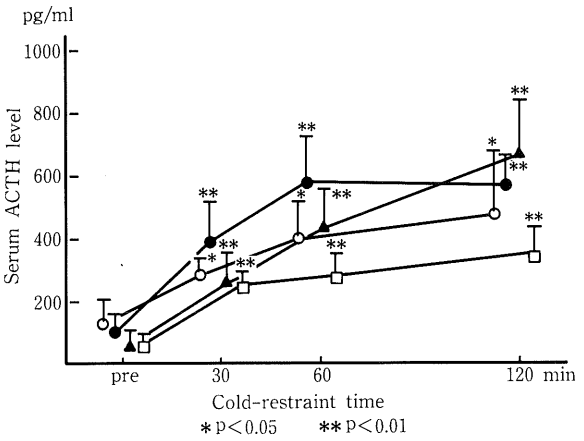

Fig. 4. Changes in the serum ACTH values before and after loading of cold-restraint stress.
Each group consists of 5 animals. For symbols, refer to Fig. 1.

tively, showing no significant differences from the pre-stress value in the control group. The levels at 30 min after stress loading were 235.0 ± 110.7 pg/ml in the Sp group ($p<0.05$), and 244.0 ± 27.3 pg/ml in the Sp + TV group ($p<0.01$), and these were significantly high compared with the pre-stress values in these groups. Furthermore, the levels at 60 and 120 min after stress loading increased significantly, indicating patterns of changes similar to that in the control group.

Figure 5 shows the changes in the serum 11-OHCS levels before and after loading of cold-restraint stress. The pre-stress value in the control group was 35.2 ± 8.4 μg/dl, which significantly increased to 53.6 ± 6.3 μg/dl at 60 minutes and to 57.7 ± 10.1 μg/dl at 120 min ($p<0.05$). On the other hand, the pre-stress levels in the Sp, TV and Sp + TV groups were 24.2 ± 7.2, 35.5 ± 12.7 and 23.1 ± 7.3 μg/dl respectively, indicating no differences from the corresponding value in the control group. The levels at 60 min after stress loading were 60.6 ± 9.7 μg/dl in the Sp group ($p<0.01$),

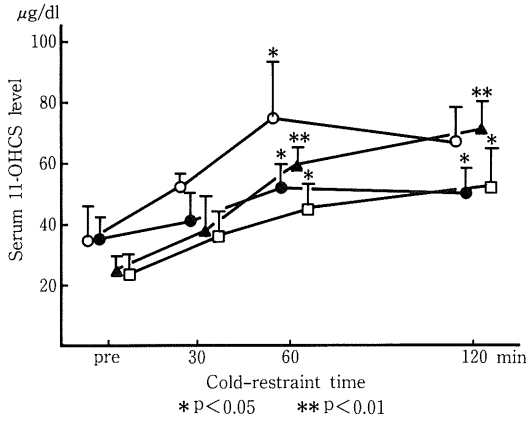

Fig. 5. Changes in the serum 11-OHCS values before and after loading of cold-restraint stress.
Each group consists of 5 animals. For symbols, refer to Fig. 1.

77.4 ± 16.3 μg/dl in the TV group ($p<0.05$) and 46.5 ± 7.2 μg/dl in the Sp + TV group ($p<0.05$), thus indicating significantly high levels compared with the pre-stress values in these groups. A similar tendency was observed in the levels at 120 min after stress loading.

DISCUSSION

In the gastric wall of living organisms vasoactive amines such as NA, HA and 5-HT are distributed in abundance, but they are specific in localization and density. NA, which exists in high concentration at the terminals of the sympathetic nerve, is almost evenly distributed in the gastric wall. NA is considered to have the same distribution and density in the fundus and antrum[9]. In humans, monkeys and dogs, HA is contained only in mucosal and submucosal mast cells of the gastric wall. However, in rats and mice, it also exists in mucosal enterochromaffin-like cells, and in especially high concentration in the fundus[4,8]. 5-HT is stored mainly in enterochromaffin cells and is contained in high concentration in the antral mucosa[6]. Accordingly, for determination of amines within the gastric wall in this experiment, the authors chose as tissue samples for NA the whole layer of the gastric wall except at the fore-stomach; for HA the fundic mucosa where it exists in high density; and for 5-HT the antral mucosa.

The HA content in the control group was significantly decreased at 30 min after stress loading, compared with the pre-stress level. The 5-HT content was significantly decreased at 60 min, and the incidence of gastric mucosal lesions was increased. The decrease in HA and 5-HT within the gastric wall is believed to have resulted from the release of these amines from storing cells after stress loading, causing microcirculatory disturbance in the mucosa and inducing stress ulcers. Transmission of

cold stress, which was sensed by the cerebrocortical and limbic system and mediated by the sympathetic nerve, the parasympathetic nerve and the pituitary-adrenal pathways, is associated with the release of these amines in the gastric wall.

The HA contents at 30 min after stress loading were significantly decreased in the Sp group but not in the TV and Sp + TV groups. Likewise, the 5-HT contents in the TV and Sp + TV groups underwent no significant changes at 60 min after stress loading. The release of HA and 5-HT in the gastric wall in the TV and Sp + TV groups was inhibited by vagotomy, and the incidences of gastric mucosal lesions were lower than that in the Sp group.

The NA contents before stress loading were low in the Sp and Sp + TV groups compared with that in the control group. But in each group, stress loading resulted in no significant changes in the NA. In the Sp and Sp + TV groups, the release of NA in the gastric wall was inhibited by splanchnicotomy. In the TV group, on the other hand, the sympathetic nerve pathway was still preserved as in the control group, and it was presumed that the release of NA from the gastric wall was accelerated under cold-restraint stress. However, although the incidence of gastric mucosal lesions in the TV group was markedly inhibited after stress loading compared with those in the control and Sp groups, the NA content within the gastric wall showed no significant changes.

It may be concluded, therefore, that the release of HA and 5-HT from storing cells in the gastric wall by activation of the vagus nerve system is more closely related to the effects of vasoactive amines on the ulcerogenetic mechanism under cold-restraint stress than the release of NA evoked by the sympathetic nerve system.

HA and 5-HT within the gastric wall are released not only by neurogenic factors but also by humoral factors-including steroid hormones[3]. The gradual decreases in HA and 5-HT within the gastric wall at 120 min after stress loading in the TV and Sp + TV groups may indicate that the pituitary-adrenal pathway exerted a larger influence than the autonomic nervous system. Although the reaction of the pituitary-adrenal system to stress could be confirmed immediately after loading of cold-restraint stress, its direct on the gastric mucosa might be presumed to appear from 120 min after stress loading. The time lag of reactions between the autonomic nerve and pituitary-adrenal pathways has been similarly described in studies of gastric acid secretion in monkeys under insulin stimulation or degranulation of gastric mucosal mast cells in rats under restraint stress[2,7]. Regardless of the type of autonomic neurotomy, the levels of serum ACTH and 11-OHCS followed the same pattern of changes, and the incidence of gastric mucosal lesions at 120 min after stress loading was markedly inhibited to 13% in the TV and Sp + TV groups, unlike in the control and Sp groups.

This led to the conclusion that the direct effect of the pituitary-adrenal system on the gastric mucosa in association with the mechanism of acute ulcerogenesis in cold-restraint rats appeared from 120 min after stress loading, but its effect was milder than that of the autonomic nerve pathway, especially the vagus nerve system.

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