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Influence of Amines in the Brain and Gastric Wall on Development of Stress Ulcers

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ABSTRACT

With a view to examining the influence of amines in the brain and gastric wall on the development of stress ulcers, the authors loaded rats with the stress of cold restraint after administration of the inhibitory agents of produce and metabolism of amines, and the following findings were obtained.

1. Noradrenalin contents in the brain were significantly reduced after stress. The reduction relatively induced vagotonia and developed ulcers. Accordingly, noradrenalin in the brain associates with the development of stress ulcers.
2. Serotonin contents in the brain were increased significantly after stress, unlike the changes of the noradrenalin contents. But serotonin in the brain was not associated with the development of stress ulcers.
3. Histamine and serotonin contents in the gastric wall were reduced significantly after stress and both amines were associated with the development of stress ulcers.
4. Either of histamine or serotonin in the gastric wall was not be able to be the main cause on the development of stress ulcers, and their association was almost the same degree.

It has long been reported that when a person fall intracerebral lesions or when a part of the brain of an animal is destroyed or stimulated, acute gastroduodnal ulcers often occur[4,6]. Thereafter, it has been believed now out of several researches that undoubtedly the central nervous system is associated with the development of acute stress ulcers[7,14,15].

Concerning the factors in the central nervous system, they are different from the local factors like gastric acid, mucus, active amines or gastric mucosal blood flow, and only a few factors like active amines, acetylcholine and a few kinds of polypeptide hormones, have so far reported[3,11,13]. Among these factors, particular attention has been paid to the relationship between the development of stress ulcers and active amines existing abundantly not only in the gastric wall but also in the brain[3,9,12,18]. The authors reported, using cold restraint rats, that the contents of noradrenalin (NA) and serotonin (5-HT) among amines in the brain, and the contents of histamine (HA) and 5-HT among amines in the gastric wall, were significantly changed[10]. In the present study, therefore, the authors increased and/or decreased the amine contents in the brain and gastric wall with the inhibitory agents of produce and metabolism of each amine and reviewed which amine of NA and 5-HT in the brain, or HA and 5-HT in the gastric wall was largely associated with the development of stress ulcers.

MATERIALS AND METHODS

Material: Male Wistar rats weighing appoximately 250g were classified into 7 groups.

a) Control group: Those subjected to no treatment.

b) Fusaric acid administered group: Those administered intraperitoneal injection of fusaric acid 100mg/kg at 3 hr prior to experiment.

c) Tetrabenazine administered group: Those administered intraperitoneal injection of tetrabenazine 20mg/kg at 30 min prior to experiment.

d) Tetrabenazine administered with vagotomy group: Those administered tetrabenazine by the above method after breeding for 3 weeks after which bilateral truncal vagotomy and pyloroplasty were performed.

e) Iproniazide administered group: Those administered intraperitoneal injection of iproniazide 40 mg/kg at 5 hr prior to experiment.

f) p-Chlorophenylalanine administered group: Those administered intraperitoneal injection of p-chlorophenylalanine (p-CPA) 100mg/kg/day for 3 days prior to the experiment.

g) Semicarbazide administered group: Those fed a $V.B_6$ free diet for 3 weeks after which subctaneous injections of semicarbazide were administered in dose of 50mg/kg × 2/day for 3 days prior to the experiment.

Method: Animals in all groups were fasted for 24 hr and were put into restraint cages and placed in a cold room maintained at 4°C 30, 60, 120 min.

(I) Observation of Ulcers

The animals were decapitated before and after stress loading, and the stomach was immediately extracted to observe the incidence of ulcers macroscopically.

(II) Collection of Specimens

The whole brain was immediately taken out from the decapitated rats, and it was cut into halves along the cerebral longitudinal fissure. For the determination of NA contents in the brain, the thalamus and hypothalamus in a cerebral hemisphere were used, while for the detrermination of 5-HT and HA contents in the brain, the whole brain in the other hemisphere was used.

The stomach was excised along the greater curvature. For the determination of HA contents in the gastric wall, the mucosal layer in the corpus was used, whereas for the determination of 5-HT contents in the gastric wall, the all layer of the antrum was used.

(III) Determination of Amine Contents

i) NA Content

After weighing the specimen, NA in tissue was extracted with alumina oxide[1], determination with high-performance liquid chromatography was made using o-phthalaldehyde (OPT)[17].

ii) 5-HT Content

After weighing the specimen, 5-HT in tissue was extracted with amberlite CG-50 resin. Subsequently under conc acidic condition it was reacted with OPT and intensity of fluorescense was measured by a spectrofluorophotometer[16].

iii) HA Content

After weighing the specimen, HA was extracted similarly with 5-HT, with amberlite CG-50 resin. Under conc alkaline condition it was reacted and determination was made with a spectrofluorometer[16].

RESULTS OF EXPERIMENT

1) Incidence of Ulcers

The incidence of ulcers in the control group was 33% after stress for 30 min, 75% for 60 min and 92% for 120 min, showing a marked increase after stress for 60 min (Table 1).

In fusaric acid and tetrabenazine administered groups, the incidence was 71% and 75%, respectively, after stress for 30 min, indicating

Table 1. Incidence of gastric ulcer on cold restrained rats

Cold restraint time	pre-test	30 min	60 min	120 min
Control	0%(0/12)	33%(4/12)	75%(9/12)	92%(11/12)
Fusaric acid administered	0 (0/ 7)	71 (5/ 7)	100 (7/ 7)	100 (7/ 7)
Tetrabenazine administered	0 (0/ 8)	75 (6/ 8)	100 (8/ 8)	100 (8/ 8)
Tetrabenazine administered with Vagotomy	0 (0/ 8)	0 (0/ 8)	25 (2/ 8)	25 (2/ 8)
Iproniazide administered	0 (0/ 8)	13 (1/ 8)	25 (2/ 8)	92 (11/12)
p-CPA administered	0 (0/ 8)	13 (1/ 8)	38 (3/ 8)	43 (3/ 7)
Semicarbazide administered	0 (0/ 8)	25 (2/ 8)	38 (3/ 8)	50 (4/ 8)

markedly high rates as compared with the control group in both groups.

In the tetrabenazine administered with vagotomy group, the incidence of ulcers was 25% even after stress for 120 min, showing a marked reduction in comparison with the control group.

In the iproniazide administered group, the incidence was 25% after stress for 60 min, and a marked reduction was noted similarly with the tetrabenazine administered with vagotomy group. But after stress for 120 min the rate rose up to 92%.

The incidence of ulcers in the p-CPA and semicarbazide administered groups was 38% after stress for 60 min in both groups, and the rates were 50% and 43%, respectively, even after 120 min, indicating marked reduction in both groups as compared with the control group.

2) Chages in Amine Contents in the Brain

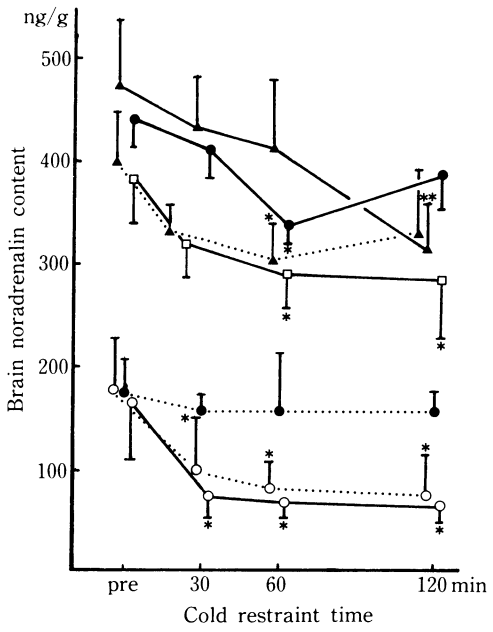

Fig. 1. Chronological changes of noradrenalin content in the brain on cold restrained rats. •—• Control (n=10), •···• Fusaric acid administered (n=6), ○—○ Tetrabenazine administered (n=6), ○···○ Tetrabenazine administered with vagotomy (n=6), ▲—▲ Iproniazide administered (n=6), ▲···▲ p-CPA administered (n=6), □—□ Semicarbazide administered (n=6)
*, ** Significance of the difference between means of the pre-test and restraint value within each study group. (M ± SD, *$p<0.05$, **$p<0.01$)

i) Changes in NA Content in the Brain

The NA content in the control group was 442.2 ± 26.4 ng/g (M ± SD) before stress, which was significantly decreased to 346.8 ± 28.0 ng/g after stress for 60 min ($p<0.05$, Fig. 1).

In the fusaric acid and tetrabenazine administered groups the NA contents were 176.4 ± 37.8 ng/g, 165.7 ± 50.6 ng/g before stress, and the contents were significantly decreased in both groups ($p<0.05$) as compared with the pre-stress value in the control. In the fusaric acid administered group the NA content remain low up to 120 min, while in the tetrabenazine administered group the NA content was significantly decreased to 75.8 ± 23.6 ng/g after stress for 30 min, in comparison with the pre-stress level and it was no change up to 120 min.

In the tetrabenazine administered with vagotomy group, the changes of the NA content after stress were similar with in the tetrabenazine administered group.

The NA content in the iproniazide administered group before stress was 477.9 ± 69.8 ng/g, which was higher than the pre-stress level in the control group. After stress for 60 min the content was 423.7 ± 65.7 ng/g, which did not show a significant decrease in comparison with the pre-stress level, unlike the control. While after stress for 120 min when the incidence of ulcers rose markedly, the content was significantly decreased to 318.1 ± 51.7 ng/g as compared with the pre-stress level ($p<0.01$).

The NA content in the p-CPA and semicarbazide administered groups showed almost similar changes with those in the control group both before and after stress.

ii) Changes in 5-HT Content in the Brain

The 5-HT content in the control group was 230.6 ± 41.5 ng/g before stress, 331.8 ± 40.2 ng/g after stress for 60 min and 335.4 ± 26.7 ng/g for 120 min. Unlike the changes of the NA content, the 5-HT content was significantly increased as compared with the pre-stress level after stress for 60 min ($p<0.05$, Fig. 2).

The 5-HT contents in the tetrabenazine, tetrabenazine with vagotomy and p-CPA administered groups were 136.7 ± 25.2 ng/g, 138.2 ± 33.5 ng/g and 121.9 ± 35.5 ng/g before stress. The contents were all showing a significant decrease from the pre-stress level in the

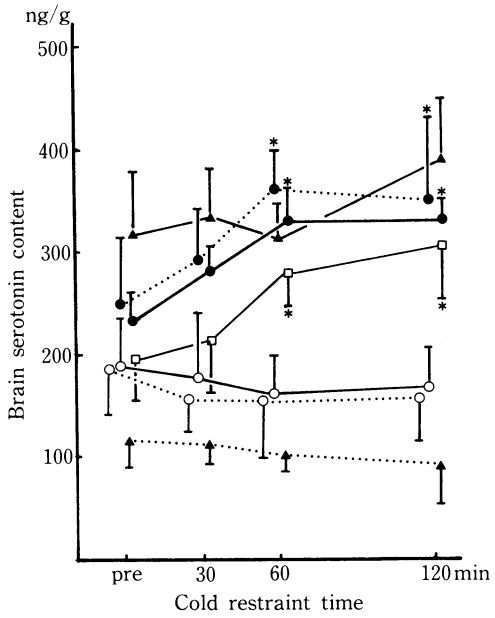

Fig. 2. Chronological changes of serotonin content in the brain on cold restrained rats. •—• Control (n=10), •···• Fusaric acid administered (n=6), ○—○ Tetrabenazine administered (n=6), ○···○ Tetrabenazine administered with vagotomy (n=6), ▲—▲ Iproniazide administered (n=6), ▲···▲ p-CPA administered (n=6), □—□ Semicarbazide administered (n=6)
*Significance of the difference between means of the pre-test and restraint value within each study group. (M ± SD, $p<0.05$)

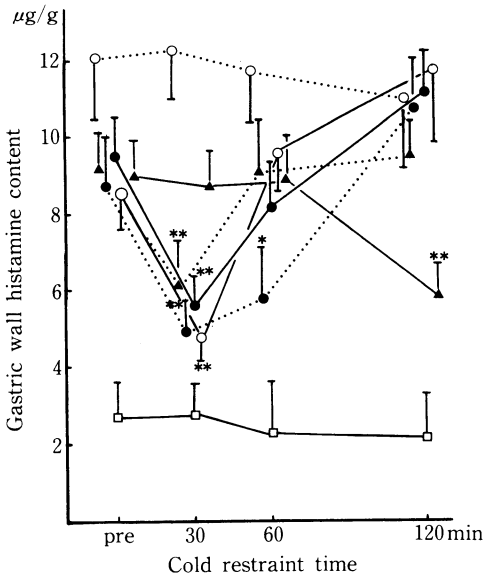

Fig. 3. Chronological changes of histamine content in gastric wall on cold restrained rats. •—• Control (n=10), •···• Fusaric acid administered (n=6), ○—○ Tetrabenazine administered (n=6), ○···○ Tetrabenazine administered with vagotomy (n=6), ▲—▲ Iproniazide administered (n=6), ▲···▲ p-CPA administered (n=6), □—□ Semicarbazide administered (n=6)
*, ** Significance of the difference between means of the pre-test and restrait value within each study group. (M ± SD, *$p<0.05$, **$p<0.01$)

control group ($p<0.01$). In all groups, the 5-HT contents were remained after stress up to 120 min without a significant change.

In the iproniazide administered group the pre-stress content was 323.1 ± 60.5 ng/g, showing a significant increase from the pre-stress level in the control ($p<0.05$). After stress the content was 390.3 ± 63.3 ng/g for 120 min, and no change was observed.

The 5-HT contents in the fusaric acid and semicarbazide administered groups were increased after stress for 60 min similarly with that in the control group ($p<0.05$).

3) Changes in Amine Contents in the Gastric Wall

i) Changes in HA Content in the Gastric Wall

The HA content in the control group was 8.9 ± 1.2 μg/g (M ± SD) before stress, which was decreased significantly to 5.2 ± 0.8 μg/g after stress for 30 min ($p<0.01$), gradually increasing thereafter up to 120 min (Fig. 3).

The pre-stress HA contents in the fusaric acid and tetrabenazine administered groups were 8.6 ± 1.6 μg/g and 8.0 ± 1.0 μg/g. After stress for 30 min, the contents were significantly reduced to 4.7 ± 1.0 μg/g and 4.9 ± 1.1 μg/g ($p<0.01$).

The HA content in the tetrabenazine administered with vagotomy group was 12.2 ± 1.6 μg/g before stress, not showing significant changes up to 120 min when the incidence of ulcers was not increased. In the iproniazide group, the pre-stress content was 8.8 ± 1.0 μg/g, which did not show significant changes until 60 min. However, the content was 5.7 ± 0.9 μg/g after stress for 120 min when the incidence of ulcers rose, indicating a significant decrease from the pre-stress level ($p<0.01$).

The HA content in the p-CPA administered group both before and after stress showed similar changes with those in the control, while the

HA content in the semicarbazide administered group was 2.6 ± 0.7 μg/g before stress, decreasing significantly as compared with the pre-stress level in the control group ($p<0.01$), while after stress no change was noted up to 120 min.

ii) Changes in 5-HT Content in the Gastric Wall

The 5-HT content in the control group was 4.5 ± 0.8 μg/g before stress (M ± SD), whereas it was significantly reduced to 3.4 ± 0.6 μg/g after stress for 60 min ($p<0.05$, Fig. 4).

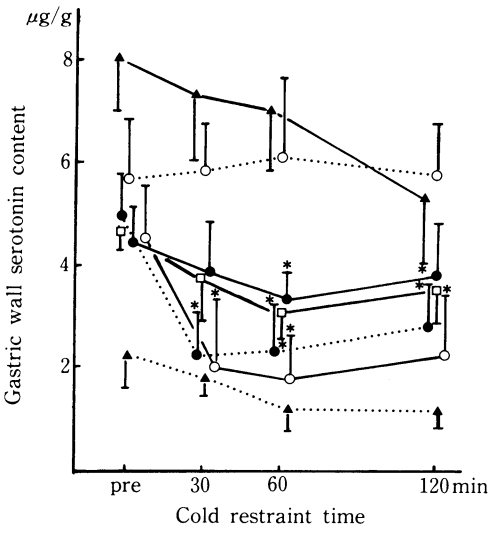

Fig. 4. Chronological changes of serotonin content in gastric wall on cold restrained rats. •—• Control (n=10), •···• Fusaric acid administered (n=6), ○—○ Tetrabenazine administered (n=6), ○···○ Tetrabenazine administered with vagotomy (n=6), ▲—▲ Iproniazide administered (n-6), ▲···▲ p-CPA administered (n=6), □—□ Semicarbazide administered (n=6)
*Significance of the difference between means of the pre-test and restraint value within each study group. (M ± SD, $p<0.05$)

The 5-HT contents in the fusaric acid and tetrabenazine administered groups were 5.0 ± 0.8 μg/g and 4.6 ± 1.1 μg/g before stress. After stress for 30 min when the incidence of ulcers was markedly increased, the contents were significantly reduced to 2.3 ± 0.9 μg/g and 2.0 ± 0.7 g/g as compared with the pre-stress value in each group ($p<0.05$).

In the tetrabenazine administered with vagotomy group, the 5-HT content was 5.7 ± 1.2 μg/g before stress, not showing significant changes up to 120 min similarly with changes in HA content. The pre-stress 5-HT content in the iproniazide group was 8.2 ± 1.2 μg/g and it did not show almost any change up to 60 min. However, after stress for 120 min when the incidence of ulcers rose, it was significantly reduced to 5.4 ± 1.4 μg/g ($p<0.05$).

The 5-HT content in the p-CPA administered group was 2.4 ± 0.7 μg/g before stress and it was significantly reduced in comparison with the pre-stress level in the control ($p<0.05$). After stress, however, there was no change up to 120 min. The 5-HT content in the semicarbazide administered group before and after stress showed almost similar changes with those in the control group.

DISCUSSION

It was found when the relation between the changes in the NA content in the brain and the development of stress ulcers was examined that in the control group the NA content was significantly decreased after stress for 60 min when the incidence of ulcers was increased. It was found that when after reducing the NA content in the brain with fusaric acid or tetrabenazine stress was loaded, the incidence of ulcers was markedly increased. It was further found, however, that even when the NA content in the brain was reduced and stress was loaded, if vagotomy was made in advance, the incidence of ulcers was clearly inhibited. Further, if the decrease in the NA content in the brain at stress was inhibited with iproniazide, the incidence of ulcers was inhibited to the similar degree as when vagotomy was performed.

Fujiwara et al[9] noted in their experiment using the precursor of amine that the increase and decrease in the NA content in the brain was associated with the development of stress ulcers. Further, these researchers reported on the association of NA in the brain for the development of ulcers that it was due to the inhibition of the parasympathicotonia in the gastric wall by the sympathicotonia causing the release of NA in the brain. Ban[2] clarified that a part of the sympathetic nerve fiber from the cerebral limbic system and hypothalamus reached the dorsal nucleus of vagus existing in the bulb, pointing out that the high-level center in the sympathetic nervous system directly inhibited

the low-level center in the parasympathetic nervous system.

It was postulated from these findings that NA in the brain was associated largely in the development of stress ulcers. As its mechanism, it was presumed that the tension of the sympathetic nerve due to the release of NA in the brain inhibited the occurrence of the tension of the parasympathetic nerve, although it was not clear whether it took place in the brain or in the gastric wall. It was also presumed that the decrease in the NA content in the brain due to the duration of stress relatively brought about the tension of the parasympathetic nerve, causing stress ulcers to form.

Our subsequent discussion refers to the relationship between the 5-HT content in the brain and development of ulcers. It was observed in the control group that the 5-HT content in the brain was increased significantly after stress when the incidence of ulcers was increased. But, even when the 5-HT content in the brain did not show changes after stress, due to administration of tetrabenazine, the incidence of ulcers was markedly increased. Even in a state where the 5-HT content in the brain before and after stress was increased with iproniazide or in a state where the 5-HT content was decreased with p-CPA, incidence of ulcers was greatly reduced. In other words, a definite tendency between the changes in the 5-HT content in the brain and the incidence of ulcers was not noted in the experiment performed by the authors.

Fujiwara et al[9] observed the changes in amine contents in the brain on water restraint rats, using precursors of NA and 5-HT, reporting that it was important that the changes in NA content was more important on the development of stress rather than 5-HT content in the brain. It has been considered that 5-HT fibers are governing organs in the pelvis, but their relationship with the upper digestive organs is not so close as NA fibers are.

It is presumed from these findings that the 5-HT content in the brain changes significantly at the loading of stress, but that the influence of 5-HT in the brain on the development of stress ulcers is not much.

On the other hand, the influence of amines in the gastric wall for the development of ulcers was examined. Fuchs et al[8] pointed out that the increase in the secretion of gastric juice due to release of HA in the gastric wall was the main cause of stress ulcers. However, Doteuchi[5] reported that stress ulcers were caused by the resisting deminish of the gastric mucosal endothelium, originating from the release of 5-HT due to stress.

According to the results obtained by the authors, both HA and 5-HT contents were significantly decreased in the control group in which the incidence of ulcers was high. In the semicarbazide administered group, on the other hand, in which the changes in HA content alone were inhibited, as well as in the p-CPA administered group in which the changes in 5-HT content alone were inhibited, the incidence of ulcers was a little higher, a compared with those in the tetrabenazine administered with vagotomy group, but as compared with those in the control group, it was markedly reduced. When the incidences of ulcers in both groups were compared, however, both were 38% after stress for 30 min and 43%, 50%, respectively, even for 120 min, indicating almost no difference between both groups.

It is presumed from these findings that both HA and 5-HT in the gastric wall are associated for the development of stress ulcers, and that either of them may not constitute the main cause, giving the influence of similar degree.

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