Hiroshima Journal of Medical Sciences
Vol. 31, No. 1, March, 1982
HIJM 31-7

Effects of Active Amines on Development of Stress Ulcers: Review of Noradrenalin, 5-Hydroxytryptamine and Histamine Levels in Brain and Gastric Wall of Restrained and Cold Exposed Rats*)

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(Received December 22, 1981)

Key words: Stress ulcer, Active amines

ABSTRACT

Acute hemorrhagic gastroduodenal ulcers are frequently induced by various types of stress. With the purpose of studying the central nervous system factors and gastric mucosal local factors involved in the development of such ulcers, Wistar strain rats were subjected to restraint and cold exposure for 120 minutes, and the intragastric pH, and the noradrenarin, 5-hydroxytryptamine and histamine contents in the brain and gastric wall were measured over time, and the following results were obtained.

1. The intragastric pH decreased with time up to 120 minutes, but the difference with the pre-restraint value was not significant.

2. At 60 minutes after restraint when the ulcerogenic rate became markedly increased, of the amines in the brain, the noradrenalin content showed a significant decrease, while 5-hydroxytryptamine increased significantly, but the histamine value failed to show any significant change.

3. Of the amines in the gastric wall, the histamine level decreased significantly after 30 minutes, and the 5-hydroxytryptamine volue showed a significant decrease after 60 minutes.

On the basis of the above findings, it is assumed that the effects of gastric acid on the development of acute hemorrhagic gastroduodenal ulcers are smoll. However, it is considered that of the amines in the brain, the changes in values of noradrenalin and 5-hydroxytryptamine, and of these in the gastric wall, the changes in 5-hydroxytryptamine and histamine levels have important implications.

INTRODUCTION

Acute hemorrhagic gastroduodenal ulcers (referred to as acute ulcers here after) are induced by such stress fastors as severe burns, obstructive jaundice, renal failure, central nervous system disease such as stroke and head injury, surgery and episodes of infection, and are therefore also called stress ulcers[19] in a broad sense. According to Curtis and Thomas[6], the relationship between central nervous system diseases and acute ulcers was first pointed out by Schiff and Rokitansky in the mid-past of the 19th century, and as is well-known it was Cushing[7] who reported in 1932 on the close relationship between peridiencephalic and hemorrhagic gastric lesions. Further, in 1943 Selye[20] suggested the possibility of erosions and

*) 児玉 求，伊藤信昭，児玉 治，小川喜輝，加藤良隆，松山敏哉，吉田久信，江崎治夫：ストレス潰瘍発生におよぼす活性アミンの影響：寒冷拘束ラットにおける脳内および胃壁内ノルアドレナリン，5-ハイドロキシトリプタミン，ヒスタミンの検討

ulcerous lesions being formed on the gastroduodenal membrane as a result of various types of stress being applied to the autonomic nervous system and pituitary-adrenal system. Since then many reports have been published on the effects of central nervous system factors on the development of acute ulcers[11,17].

On the other hand, the developmental mechanism of acute ulcers localized in the gastric membrane has been discussed in many reports from the view point of balance between the attacking agents such as gastric acid and pepsin, and the defence fastors such as gastric mucus and gastric microcirculation. However, there appears to be few reports in which review has been made of both the central nervous system factors and localized gastric mucosal factors at the same time. Thus, with the objective of ascertaining the effects of both of these factors on the development of acute ulcers, Wistar rats were subjected to restraint and exposure to cold, and various experiments were performed using such active amines as noradrenalin(NA), 5-hydroxytryptamine(5-HT) and histamine(HA). As interesting findings were obtained, there will be presented.

METHODS

1) *Experimental animals and method of restraint and cold exposure*

The animals used were male Wistar strain rats, weighing 200～250 g. The rats were put into metal restraint cages after 24-hour-fasting, and placed in a cold room maintained at 4°C (refered to as cold restraint hereinafter) for 30, 60 and 120 minutes.

2) *Determination of intragastric pH*

The rats were decapitated after being subjectet to cold restraint, beld and stomach was resected. A single pole pH electrode (Toa Dempa Co. manufactured Model TSC-10A) was incerted into the stomach from the cardia to measure the pH.

3) *Observation of the state of ulcer development*

After measuring the intragastric pH, an incision was made along the larger curvature of the resected stomach, and after washing the insides with cold physiological saline, gross examination was made to determine whether or not there was any development of ulcers.

4) *Collection of specimens*

After removal of the whole brain, it was split in half along the cerebral longitudinal fissure. The thalamus and hypothalamus of one half were used for determination of NA and 5-HT and HA were determined using the remaining hemisphere. Determination of the NA of the stomach was carried out by measuring all layers of the anterior wall of the gastric body, and 5-HT was determined by measuring all layers of the gastric antrum while HA was determined by making measurements of collecting sections of the mucous membrane of the posterior wall of the gastric body.

5) *Methods for determination of NA, 5-HT and HA*

i)Determination of NA

The specimens collected were deproteinized by adding a 10-fold volume of 5% trichloroacetic acid and 0.1 ml of 5% EDTA. This was subjected to high speed centrifugation at 3000×g for 10 minutes at room temperature and the supernatant was collected. NA was adsorbed on aluminium[2] and extracted by 1 ml of 1 N acetic acid. The extract was evaporation dried by lyophilization, dissolved with 100 μl of pH 5.8 citric acid buffer and determined by high performance liquid chromatography[23] (Fig. 1 and 2).

ii)Determination of 5-HT

The specimens were deproteinized by adding

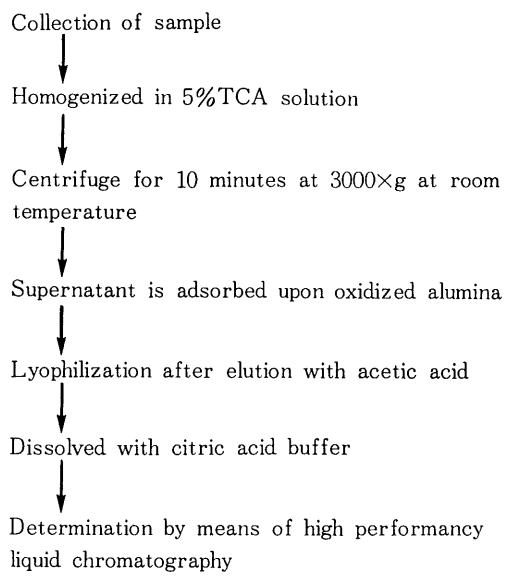


Fig. 1. Method of determination of noradrenalin levels in tissue

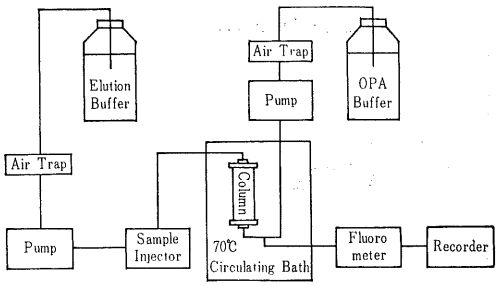


Fig. 2. Schematic illustration of noradrenalin analytical system using high performancy liquid chromatography

10-fold volume of 0.4 N perchloric acid and 0.5 ml of 5% EDTA. This was subjected to high speed centrifugation at 4°C and extracted with Amberlite CG-50 resin[22]. Determination by fluorescence was carried out using the o-phthalaldehyde (OPT) method[22] under acid conditions (Fig. 3).

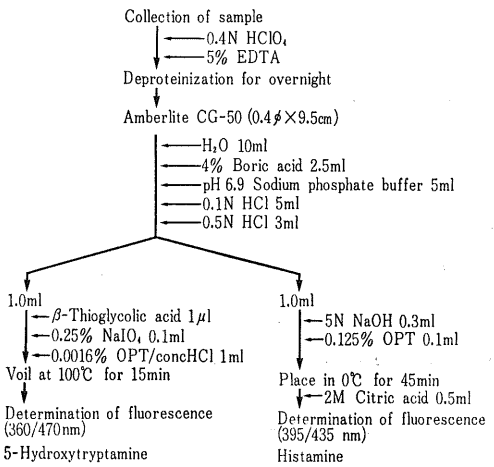


Fig. 3. Method of determination of 5-hydroxytryptamine and histamine levels in tissue

iii)Determination of HA

The same method as for 5-HT was used to extract HA from the specimens. Quantitation was performed by fluorescence determination using the OPT method[21] under alkaline conditions (Fig. 3).

RESULTS

1) *Variations in intragastric pH*

The intragastric pH failed to demonstrate any significant variations as compared against the pre-restraint value of 2.3±0.6 (mean value ± standerd deviation (S. D.)), i.e. 2.2±0.3 at 30 minutes after restraint, 2.0±0.4 at 60 minutes and 1.9±0.3 at 120 minutes (Fig. 4).

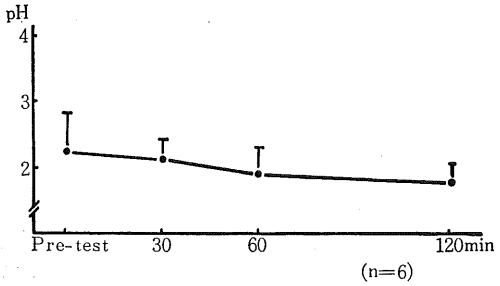


Fig. 4. Changes of intragastric pH in cold restraint rats

2) *Variation in frequency of ulcer development*

The ulcers were all found in the gastric body, and most were primarily erosions of Ul-I to II in grade. The frequency of development was 33% in the 30 minute group, 67% in the 60 minute group and 83% in the 120 minute group (Table 1).

Table 1. Incidence of gastric hemorrhage and erosion after cold restraint

Period of cold restraint	30 min.	60 min.	120 min.
Incidence	33% (2/6)	67% (4/6)	83% (5/6)

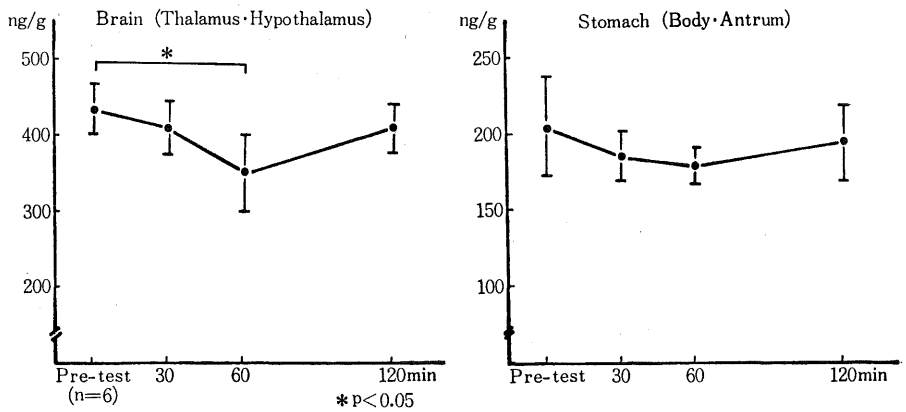
3) *Variations in amine contents in the brain and gastric wall*

i)Variation in NA content

The NA content in the brain showed a significant decrease from the pre-cold restraint value of 438.2±29.4 (mean ± S. D.) ng/g to a 60-minute post-cold restraint value of 351.8±53.3 ng/g ($p<0.01$). The pre-cold restraint NA value in the gastric wall was 205.8±37.5 ng/g as composed to the 30 and 60-minute post-cold restraint values of 186.7±17.5 and 179.6±9.3 respectively, and the differences were not significant (Fig. 5).

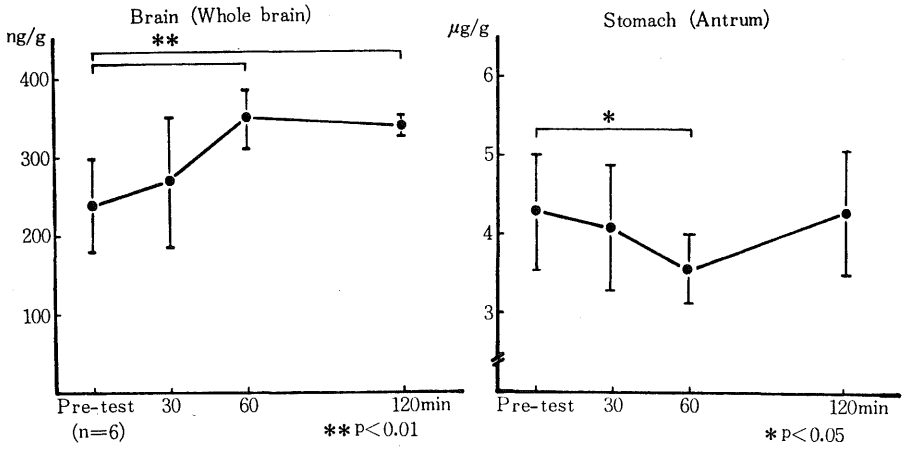
ii)Variation in 5-HT content

The pre-cold restraint content in the brain was 233.9±44.5 (mean ± S. D.) ng/g whereas the 60-minute post-cold restraint value showed a significant increase to 321.3±41.2 ng/g ($p<0.01$). The pre-cold restraint value in the gas-



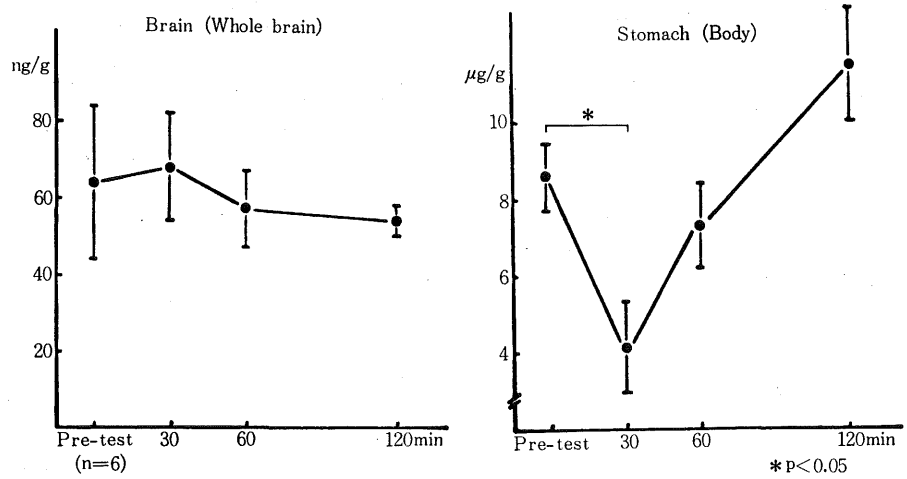
* Significance of the difference between means of the pre-test and restraint value

Fig. 5. Changes of noradrenalin levels in brain and stomach in cold restraint rats



* Significance of the difference between means of the pre-test and restraint value

Fig. 6. Changes of 5-hydroxytryptamine levels in cold restraint rats



* Significance of the difference between means of the pre-test and restraint value

Fig. 7. Changes of histamine levels in cold restraint rats

tric wall was 4.3±0.78 μg/g as composed to the 60-minute post-cold restraint value of 3.5± 0.52, which was a significant decrease ($p<0.05$) (Fig. 6).

iii)Variation in HA content

The pre-cold restraint content in the brain was 64.2±19.4 (mean ± S.D.) ng/g whereas the 30, 60 and 120-minute post-cold restraint values were 68.5±14.1, 57.2±10.5 and 53.9± 3.6 ng/g respectively, which are not significant differences. However, the pre-cold restraint value in the gastric wall was 8.4±1.1 μg/g as compared to the 30-minute post-cold restraint value of 4.1±1.5, which is a significant decrease ($p<0.05$) (Fig. 7).

DISCUSSION

Referring to the reports of Schiff and Rokitansky[6] which suggest the possibility of association between autonomal nerve disturvance and peptic ulcers, and the fact that gastroduodenal ulcers tend to develop at high frequency after brain surgery, Cushing[7] proposed on the basis of studies in 1932 the term of neurogenic ulcer for gastroduodenal ulcer which developed after experimental stimulation of the parasympathetic nervous center in the hypothalamus. Subsequently, French[11] and Porter[17] et al. pointed out the presence of two autonomic nerve pathways, one linking the anterior portion of the hypothalamus and vagus nerve system, and the other the posterior portion of the hypothalamus and sympathetic nervous system, and thus stated their opinions that not only the vagus nerves, but also the sympathetic nervous system was involved in the development of acute ulcers. Thus, it is presumed that in the development of such acute ulcers, not only are the various local factors in the gastric mucosa which is the site for ulcer genesis involved, but the central nervous system also has an important effect upon the gastric mucosa.

Therefore, the authors conducted a study on the relationship of development of acute ulcers and active amines, particularly NA, 5-HT and HA which are present in the brain and gastric wall and are reportedly associated with the autonomic nerve. The NA content per tissue weight in the brain was highly concentrated in the nerve stem region which is densely populated with adrenergic nerve cells, but was most highly concentrated in the hypothalamus which is the terminal nervous region where NA serves as a neurotransmissible substance[3]. Thus, the authors studied the variation in NA content in the hypothalamus which is the autonomous nerve center. Reports on reviews made of intracranial NA content under stress, indicate that the NA content is decreased by the application of stress which in turn, many feel, causes tension of the vagus nerve, thus inducing ulcer of the gastric mucosa[12,16]. The experimental results obtained by the authors also indicate that the NA content of the hypothalamus showed a significant decrease at 60 minutes after subjecting to cold restraint, which is when there was an increase in the rate of ulcers development. However, it was not possible to reach a conclusion from the results of this experiment on whether the decrease in NA content in the brain causes vagus dominance which leads to ulcerogenesis, or whether it acts to maintain homeostasis as a general adaptation syndrome as pointed by Selye, and it is considered further study including dissection of the sympathetic and/or vagus nerves will be necessary.

On the other hand, the serotonergic nerve cells converge around the raphe nucleus of the brain stem, and the distribution of concentration approximates that of the intraserebral NA[9]. However, as the method of 5-HT determination used by the authors did not permit selective measurement of the hypothalamus alone, determination was made of the cerebral hemisphere including the hypothalamus. There are conflicting reports on experimental results of pre- and post-stress variation of intracerebral 5-HT, some claiming there is change[10,18] while others say there is not[4]. Thus, it appears the opinions are not consistent. The findings of the experiment performed by the authors showed that contrary to the results of the NA content, there was a significant increase of the 5-HT value. The 5-HT in the brain has an inhibitory effect upon excitation of the central nervous system, and thus is said to counter the effects of intracerebral NA. Therefore, it is assumed that the increase in 5-HT in the brain following exposure to stress occurred to counter the release of NA which caused activation and excitation of the central nervous system. Further, direct and indirect actions of 5-HT upon the center of the body temperature regulatig mechanism in the hypothalamus have been observed. In view

of this, the increase of intracerebral 5-HT may be interpreted as action by 5-HT to maintain the body temperature of the rats exposed to the cold of 4°C. However, as it is reported that 5-hydroxyindoracetic acid(5-HIAA), a metabolite of 5-HT, is increased in the brain as a result of stress restraint[10], it is considered reasonable to assume that 5-HT metabolism is activated by restraint, which resulted in the increase of 5-HT in the cerebrum. Further, as reported by Yuwiller[24], it is presumed that metabolism of 5-HT activated in the brain, stimulates the hypothalamo-pituitary-adrenal system, which causes acute gastric ulcers under colt restraint conditions.

On the other hand, a definite histochemical method to detect HA in the brain has not yet been established, and as a result it is extremely difficult to identify histaminergic nerve fibers, but during recent years attention is being focussed upon HA as a neurotransmissible substance within the living body. The distribution of HA within the brain showes it to be highly concentrated in the hypothalamus as in the case of NA and 5-HT, followed by the mesencephalon and telencephalon[1]. However, the HA content per total brain weight is about 1/5～1/10 that of NA or 5-HT. As the method of determination employed by the authors did not permit the measurement of HA present in the hypothalamus only, there was no alternative but to use the cerebral hemisphere as in the case of 5-HT. Review of reports on the variation of HA content in the brain undur stress revealed that in most, such changes could not be observed[16]. The experiments performed by the authors also failed to demonstrate a significant difference between the pre- and post-cold restraint values of HA. Although there is much in the physiological action of intracerebral HA which has not yet been elucidated, when considering the fact that the center of body temperature is primarily activated by HA and 5-HT as explained above, it is considered there is little possibility that HA is directly involved in the mechanism responsible for genesis of acute ulcers.

In view of the experimental results obtained by the authors, it was suggested that the effects of active amines in the brain on the development of gastric ulcers were significantly correlated to the vatiations in NA and 5-HT contents rather than in the variation of HA values.

In considering the effects of gastric acid secretion in the development of gastric ulcers, there is an old saying which goes, "No acid, no ulcer", but in experiments on stress ulcers, there have been reports which claim there is hypersecretion[15] of acid resulting in high acidity while there are others which state the acid level is low[5], and thus, it seems there is yet no consolidated opinion. The authors' experiment showed that there was a tendency for a higher level of gastric acid as the rate of gastric ulcer development increased following cold restraint, but no significant correlation could be demonstrated between the two. However, as the 'back diffusion theory' advanced by Davenport[8] et al. cannot be neglected when considering the variation of gastric pH as the mechanism for ulcerogenesis, it is felt that no conclusive statements can be drawn from our experiment.

Next, consideration will be given to the active amines in the gastric wall and ulcerogenesis. The gastric wall of living bodies including rats has an abundance of active amines such as NA, and 5-HT and HA. The peripheral NA is found primarily in the terminal region of the sympathetic nerve, and is released by stimulation of that nerve. 5-HT is present in enterochromaffin(EC) cells which are found sporadically in the gastric mucosa, while HA is contained prymarily in mast cells, but in rats it is also present in EC-like cells. Further, it has been confirmed histologically that both 5-HT and HA are released primarily by stimulation of the vagus nerve.

Reports on study of the variation of NA in the gastric wall at the time of stress also indicate that the value decreases after subjected to stress as noted by the authors, and it is well known that the NA released causes contraction of the capillaries in the mucosa because of its α-action, thus inducing an ischemic condition. However, with regards to its ulcerogenic action, there are opinions which claim that when exogenously administered, it has inhibiting action towards development of ulcer[12,14], and it appears there is considerable dissociation between the endogenous actions of NA including the problem of pharmacological dose. Another ulcerogenic factor is hypoxia of the gastric mucosa due to gastric microcirculation disturbance.

Also as are views that NA opens the A-V shunt which is assumed to be located in the lower layer of the mucosa, it is considered the NA in the gastric wall released by stress will by α-action create an ischemic state on the superficial aspect of the mucosa which together with the opening of the A-V shunt will cause a greater degree of hypoxia and result in the creation of a pre-ulcerous state.

Next, there are conflicting reports on the variations of 5-HT and HA in the gastric wall with stress, some claiming they both decrease with stress[12,16], while another says they increase[13]. This is based on assumption, but such discrepancy may be due to differences in the type of experimental animals used or method of stress application. However, whatever the cause may be, it is a fact that there is variation with stress. The authors' experimental results indicate that there are significant decreases in 5-HT and HA in the gastric wall after exposure to stress. Generally, the physiological activity of 5-HT consists of contraction of the intestinal tube and promotion of vascular permeability. In the rat, it is said the 5-HT activity is greater than HA in the latter. Similarly, the physiological activity of HA is said to cause dilation of the microvasculature and promote gastric secretion. In consideration of these physiological activities, it is felt the variations in 5-HT and HA values have great effects upon the ulcerogenic mechanism. In the development of acute ulcers, not only the effects of these active amines but also gastric movement, gastrin, digestive tract hormones and other factors such as mucous substances, naturally, cannot be neglected, but it is considered that 5-HT and HA in the gastric wall are released as a result of exposure to stress, and these two active amines act synergisically, causing microcirculatory disturbance and various other ulcerogenic actions.

The active amines variations of NA, 5-HT and HA contents in cold restraint rats presented different patterns for those in the brain and those in the gastric wall, but it was not possible to draw conclusions on the relationship of ulcerogenesis and variations in the brain and gastric wall contents of the respective amines. However, following exposure to cold restraint findings suggestive of significant correlations between ulcerogenesis and variations in intracerebral NA and 5-HT contents, and the 5-HT and HA contents in the gastric wall were observed. Therefore, it is felt that in the elucidation of the mechanism for acute ulcerogenesis, it is necessary to study not only the amine effects upon the local gastric mucosa, but also not to neglect the effects of the intracerebral amines, and the variations of amine contents in the brain and in the gastric wall must be determined at the same time.

CONCLUSION

Wistar strain male rats (body weight 200~250 g) were subjected to cold restraint, and the intragastric pH and the NA, 5-HT and HA contents in the brain and gastric wall were determined over time. The following results were obtained.

1. It is presumed that there is little gastric acid effect on acute ulcer development.
2. In the development of gastric ulcers, it is assumed that of the intracerebral amines, variations in NA and 5-HT contents are important.
3. Of the amines in the gastric wall, it is presumed that variations in 5-HT and HA contents are involved in ulcerogenesis.
4. In the elucidation of the developmental mechanism of acute ulcers, it is considered necessary to determine the amines in the brain and gastric wall at the same time.

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