ROLE OF GASTRIC MOTILITY IN DEVELOPMENT OF STRESS-INDUCED GASTRIC LESIONS OF RATS

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Abstract—Gastric motility of stressed rats was studied to determine its role in producing stress-induced gastric lesions. Restraint and water immersion resulted in an increase in gastric motility which consisted of an increase in frequency and amplitude of contractions and a rise in gastric tone. This increase reached maximal levels 2 to 4 hr after stress, and persisted thereafter. Formation of gastric lesions was markedly accelerated after occurrence of the increased gastric motility. In contrast, restraint alone neither produced such a vigorous increase in gastric motility, nor were the gastric lesions severe. A continuous infusion of papaverine during restraint and water immersion inhibited increase in frequency and amplitude of gastric contractions and prevented formation of gastric lesions. It is concluded that increased gastric motility is closely associated with marked formation of gastric lesions under conditions of restraint and water immersion stress and is probably a main cause for their vigorous formation, although formation of lesions occurs to a small degree without involvement of gastric motility.

Restraint and water immersion stress produces a high incidence and severity of gastric lesions in rats (1, 2) and mice (3, 4). Exposure of rats to this stress induced an increase in gastric motility (5), and thus the increased gastric motility as well as the presence of gastric juice (6) and the disorder of gastric mucosal blood flow (7, 8) was postulated to play an important role in forming stress-induced gastric lesions (9, 10). However, there is apparently no documentation demonstrating a causal relationship between gastric motility changes and stress ulcer formation.

Gastric motility is increased by stress through vagal activity which becomes more prominent than sympatho-adrenal activity (11, 12). Therefore, gastric motility may vary in its increasing pattern depending on the stress since intensity and duration of stress affect the activation of vagal tone in a different manner.

The present study was designed to determine if gastric motility changes were associated with the formation of gastric lesions in stress and, if so, to determine whether this involvement was a main cause for the pathogenesis of stress-induced gastric lesions.

MATERIALS AND METHODS

Male Wistar rats weighing 240 to 280 g were deprived of food for 18 hr, but permitted water ad libitum.

Two procedures were used to induce stress gastric lesions. In the first procedure, animals were restrained in firmly fitted restraint cages and placed in a room of 22–23°C.
In the second procedure, animals were restrained in the same way and vertically immersed in water maintained at 25°C, to the level of the xyphoid process, according to the method of Takagi et al. (1).

Gastric motility was studied by measuring changes in volume of an internal balloon in the stomach. The animal was anesthetized with ether and the abdomen was opened. A rubber balloon (2.5 ml) attached to a polyethylene tube was passed into the glandular portion of the stomach through an incision of the forestomach and the tube was connected to a water manometer via a polyvinyl tube. Initial pressure of the balloon was set at the height of 8 cm of water. A catheter was placed in the abdominal cavity to infuse drugs i.p. in some experiments. Body movement was estimated by allowing the animal to press down a rubber balloon (3.0 ml) which was equipped on the floor of the restraint cage and connected to a water manometer. Changes in gastric motility and body movement were recorded on a kymographion. The position of the abdominal incision was closed with a suture and then covered with collodion. After recovery from the anesthetic, the animal was exposed to either stress procedure.

Gastric motility was evaluated for frequency and amplitude of contractions and also for gastric tone. Contractions greater than 0.2 ml in volume change were counted for 10 min at each measurement and frequency was expressed as contractions/min. The upper and lower ends of all contractions counted for this period were expressed as volume levels (ml) from the initial lowest baseline after stress, and a difference between volume levels of both ends was regarded as amplitude (ml). Gastric tone was considered as baseline in the recording of gastric motility and was also expressed as the mean volume level (ml) of the 10-min period.

A study was conducted to determine if there was any relationship between gastric motility changes and stress ulcer formation, in stress states. In the group of restraint and water immersion, the animals were sacrificed at 1 hr after stress or at 0, 3 or 18 hr after the observation that an increase in gastric motility reached maximal levels. The animals of the restraint group were sacrificed at corresponding times to those of the restraint and water immersion group. Evaluation of gastric motility in frequency, amplitude and tone was made for 10 min, just before each sacrifice.

A special study was designed to observe whether prevention of gastric motility by papaverine gave rise to a protection against stress-induced gastric lesions. The animals underwent the operation requested to measure gastric motility. They were then exposed to restraint and water immersion, and simultaneously given an i.p. continuous infusion of papaverine in graded doses at intervals of 2 hr for 6 hr; successive doses were 10, 20, and 30 mg/kg/hr at intervals between 0 and 2 hr, 2 and 4 hr, and 4 and 6 hr after stress, respectively, and thus the total dose amounted to 120 mg/kg in the free base form. Papaverine hydrochloride was dissolved in saline and infused at a constant rate of 0.5 ml/hr with an infusion pump (Natsume, KN-201 D). Control animals were given saline in the same manner. Evaluation of gastric motility in frequency, amplitude and tone was made for 10 min just before 1st, 3rd and 5th hr after restraint and water immersion.
At the end of stress period, all animals were removed from the restraint cages and sacrificed under ether anesthesia. The stomachs were isolated and inflated with 10 ml of saline and placed in 0.5% formalin for 10 min, opened along the greater curvature, and examined for the presence of gastric erosions. Hemorrhagic areas with denuded epithelium greater than 1 mm in the largest dimension were considered to be erosions. The severity score of gastric erosions was calculated as the sum of the length of each erosion per stomach.

An attempt was made to investigate whether there was any action of papaverine on gastric secretion in the anesthetized rat. The animal was anesthetized with an i.p. injection of urethane at 1.2 g/kg and gastric secretion was measured according to the method of Lai (13). The stomach was perfused with saline at a rate of 1 ml/min and the perfusates were collected every 30 min. After basal collection of perfusates for 1 hr, either papaverine or saline was infused for 6 hr in the same doses as the above-mentioned experiment of gastric motility. Finally, histamine dihydrochloride was given i.v. in a dose of 1 mg/kg to observe the responsiveness of each preparation to gastric secretion. Gastric acid was titrated to pH 7.0 electrometrically with 0.01 N sodium hydroxide and titratable acid output was calculated as microequivalents/100 g of body weight/30 min.

Statistical analysis was carried out using Student's t-test.

RESULTS

The pattern of gastric motility in stressed rats

The pattern of gastric motility was measured in rats subjected either to restraint alone or to restraint and water immersion. Typical recordings of gastric motility and its evaluation in frequency, amplitude and tone are presented in Figs. 1 and 2, respectively. In restraint alone, an increase in gastric motility was not observed up to 6 hr but rather at 21 hr after stress. This type of gastric motility consisted of an increase in frequency and a moderate rise in amplitude, without a high gastric tone. On the other hand, in restraint and water immersion, gastric motility was increased vigorously and rapidly; a gradual rise in gastric

![Fig. 1. Pattern of gastric motility in stressed rats. Upper: recordings from a restrained rat; Lower: recordings from a restrained and water-immersed rat. Tracings are: BM, body movement; GM, gastric movement.](image-url)
tone appeared early, and an increase in frequency and amplitude of gastric contractions took place more than 1 hr after stress. These changes in gastric motility reached maximal levels 2 to 4 hr after stress and persisted thereafter. Thus, this type of gastric motility was characterized by the increased frequency and amplitude of contractions with the raised gastric tone.

Body movement was enhanced early in stress but reduced gradually with time of stress.

**Development of stress lesions in relation to gastric motility**

The development of gastric lesions was measured in connection with changes in the pattern of gastric motility after restraint and water immersion (Fig. 2). The time at which an increase in gastric motility reached maximal levels was 3.18 ± 0.34 hr (mean ± S.E.M.) after this stress. Formation of gastric lesions occurred to some extent 1 hr after stress without an appreciable increase in gastric motility, and then markedly increased from 0 to 3 hr after occurrence of the increased gastric motility. A comparative study was undertaken
in the group of restraint, and formation of gastric lesions was found to be slight throughout the restraint period.

**FIG. 3.** Effect of papaverine on the pattern of gastric motility during restraint and water immersion. Upper: recordings from a rat given saline; Lower: recordings from a rat given papaverine. Tracings are: BM, body movement; GM, gastric movement.

**FIG. 4.** Effect of papaverine on the pattern of gastric motility during restraint and water immersion. A; the upper and lower ends of gastric contractions (the top and bottom of columns) and gastric tone (dotted line), B; amplitude of gastric contractions, C; frequency of gastric contractions. Values are expressed as mean ± S.E.M. of 8 rats. a; p<0.05, b; p<0.01 when compared with the group given saline.
TABLE 1. Effect of papaverine on the development of gastric lesions during restraint and water immersion

| Infused drugs | No. of rats | Erosion severity (mm) Mean+ S.E.M. |
|---------------|-------------|-----------------------------------|
| Saline        | 8           | 17.2±3.8                           |
| Papaverine    | 8           | 6.9±2.0                            |

Fig. 5. Effect of papaverine on gastric secretion in the unstressed, anesthetized rat. Values are expressed as mean±S.E.M. *; p<0.05 when compared with the group given saline.

**Effect of papaverine on gastric motility and development of gastric lesions in the restrained and water-immersed rat**

Papaverine was continuously infused in the graded doses since, otherwise, this drug showed a refractoriness to its inhibitory action on gastric motility in preliminary studies. Papaverine antagonized an increase in frequency and amplitude of gastric contractions and moderately inhibited a rise in gastric tone (Figs. 3 and 4). Saline infusion did not affect the increasing pattern of gastric motility in stress. Treatment with papaverine significantly lowered the severity of gastric lesions as compared with that in the saline group (Table 1).

**Effect of papaverine on gastric secretion in the unstressed, anesthetized rat**

A comparison of the action on gastric secretion was made between saline and papaverine groups. Results are illustrated in Fig. 5. Papaverine produced a slight but significant increase in gastric secretion for four successive 30-minute periods in 30 min after infusion but this stimulatory action did not last until the end of infusion. There was no significant difference in secretory response to histamine between saline and papaverine groups at the end of infusion.
DISCUSSION

The present results show that restraint and water immersion, in 2 to 4 hr, caused a marked increase in frequency and amplitude of gastric contractions together with a rise in gastric tone, and produced a vigorous formation of gastric lesions predominantly after occurrence of this type of gastric motility. Thus, formation of gastric lesions after 1 hr of this stress remained low without an appreciable increase in gastric motility. On the other hand, restraint alone did not produce an increase in gastric motility up to 6 hr after stress and formation of gastric lesions was slight. These findings indicate that an increase in gastric motility such as that induced by restraint and water immersion is closely associated with marked formation of gastric lesions and is probably the main cause for their vigorous formation. However, formation of gastric lesions itself could occur to a small degree without involvement of gastric motility. In this respect, our view is not completely consistent with the suggestions of others (9, 10, 14) who postulated formation of stress-induced gastric lesions exclusively in terms of an increase in gastric motility.

Prevention of an increase in gastric motility is expected to produce an inhibition of gastric stress ulceration. Infusion of papaverine, a smooth muscle relaxant was given to confirm this possibility. Papaverine infusion during restraint and water immersion significantly inhibited an increase in frequency and amplitude of gastric contractions and concurrently provided protection against gastric stress ulceration. This finding may also support the concept of the correlation between gastric motility changes and formation of gastric lesions. However, actions of papaverine on gastric mucosal vasodilation and gastric secretion must be taken into account before concluding such causal relationship, as such would participate in the inhibitory effect of papaverine on formation of gastric lesions. According to Brodie and Hooke (15), there was no correlation between the effect of vasoactive drugs on stress-induced gastric hemorrhage and the degree of alteration of blood pressure, although a significant reduction in hemorrhage incidence was induced by certain vasodilators. However, there is no further evidence available to show whether vasodilatative action of drugs gives rise to an inhibition of stress-induced gastric lesions. Likewise, there are only few reports concerning actions of papaverine on gastric secretion. Brömster et al. (16) reported that oral administration of papaverine to humans did not alter the acid concentrations of gastric juice at doses of 200 to 800 mg, despite the fact that there were measurable serum concentrations of papaverine. An experiment done herein was to obtain definite observations of actions of papaverine on gastric secretion in anesthetized rats. Papaverine was found to cause a slight increase in gastric acid secretion. Accordingly it is unlikely that papaverine inhibits gastric stress ulceration by decreasing gastric secretion, although we cannot rule out the possibility that the gastric secretory pattern in stressed rats may be different from that in unstressed rats. It is most probable that papaverine prevented stress-induced gastric lesions by inhibiting the increase in gastric motility in restraint and water immersion.

Oral administration of a moderate dose of N-acetyl-L-glutamine aluminum complex (KW-110) caused a decrease in gastric motility and a prevention of stress ulceration in the
rat exposed to restraint and water immersion (17). KW-110 had no effect on the central and peripheral nervous system in tested doses (18), although KW-110 had an adhesive property to gastric mucosa (17). Inhibitory effect of the compound on the stress-induced gastric lesions would be due chiefly to a depressive action on the gastric motility. Therefore, this finding further supports the view that an increase in gastric motility is responsible for forming stress-induced gastric lesions.

Increased gastric motility in stress is initiated mainly through vagal tone (14) which would be more prominent not only by a declining sympatho-adrenal function but also by an augmenting vagal activity (12). Thus, each stress procedure would differ in the increasing pattern of gastric motility; the more intense the severity of stress, the shorter the period needed for initiating the increased gastric motility. An increase in gastric motility may be ulcerogenic through 'mechanical rubbing' of gastric mucosa, because most of linear gastric lesions were observed after occurrence of the increased gastric motility. For these reasons, each stress procedure would vary in ulcerogenic property, especially those associated with potency in initiating an increase in gastric motility.

REFERENCES

1) TAKAGI, K., KASUYA, Y. AND WATANABE, K.: Studies on the drugs for peptic ulcer. A reliable method for producing stress ulcer in rats. Chem. Pharm. Bull. 12, 465-472 (1964)
2) TAKAGI, K. AND OKABE, S.: The effects of drugs on the production and recovery processes of the stress ulcer. Japan. J. Pharmacol. 18, 9-18 (1968)
3) PFEIFFER, C.J.: Cold immersion, restraint stress: an ulcer model in the mouse for rapid, massive screening. Peptic Ulcer, Edited by PFEIFFER, C.J., p. 84-91, J.B. Lippincott Company, Philadelphia and Toronto (1971)
4) YANO, S. AND HARADA, M.: A method for the production of stress erosion in the mouse stomach and related pharmacological studies. Japan. J. Pharmacol. 23, 57-64 (1973)
5) WATANABE, K.: Some pharmacological factors involved in formation and prevention of stress ulcer in rats. Chem. Pharm. Bull. 14, 101-107 (1966)
6) GUTH, P.H. AND KOZBUR, X.: Microcirculatory and mast cell changes in restraint stress. Role of gastric acid. Am. J. dig. Dis. 14, 113-117 (1969)
7) GUTH, P.H. AND HALL, P.: Microcirculatory and mast cell changes in restraint-induced gastric ulcer. Gastroenterology 50, 562-570 (1966)
8) HASE, T. AND MOSS, B.J.: Microvascular changes of gastric mucosa in the development of stress ulcer in rats. Gastroenterology 65, 224-234 (1973)
9) GOLDMAN, H. AND ROSEFF, C.B.: Pathogenesis of acute gastric stress ulcers. Am. J. Pathol. 52, 227-243 (1968)
10) DAI, S. AND OGLE, C.W.: Gastric ulcers induced by acid accumulation and by stress in pylorus-occluded rats. Europ. J. Pharmacol. 26, 15-21 (1974)
11) HÜRNBERG, G., KLEIN, H.J. AND EDER, M.: The gastric glands of the rat after stimulation with pentagastrin, Histalog, and in acute stress. A comparative fine structural study. Acta Hepato-Gastroenterol. 19, 388-394 (1972)
12) YANO, S., AKAHANE, M. AND HARADA, M.: Contribution of sympatho-adrenal system to the gastric movement of rats subjected to restraint and water immersion stress. Japan. J. Pharmacol. 27, 635-643 (1977)
13) LAI, K.S.: Studies on gastrin. I. A method of biological assay of gastrin. Gut 5, 327-333 (1964)
14) CHIO, C.H., OGLE, C.W. AND DAI, S.: Acute gastric ulcer formation in response to electrical vagal stimulation in rats. Europ. J. Pharmacol. 35, 215-219 (1976)
15) BRODIE, D.A. AND HOOKE, K.F.: The effect of vasoactive agents on stress-induced gastric hemorrhage in the rat. Digestion 4, 193-204 (1971)
16) BRÖMSTER, D., LINDGREN, E., ROSENN, A. AND THEORELL, M.: Effect of papaverine on gastric
emptying and gastric secretion in man. *Acta radiol. Diagn., Stockholm* 8, 354–364 (1969)

17) **Harada, M. and Yano, S.**: Inhibitory effect of N-acetyl-L-glutamine aluminum complex (KW-110) and related compounds on gastric erosion and motility in stressed animals. *Pharmacoenergetics* 8, 1–6 (1974)

18) **Tanaka, H., Shuto, K., Ishii, S., Orima, H. and Takahira, H.**: Antiulcerogenic actions and other pharmacological properties of N-acetyl-L-glutamine aluminum complex (KW-110). *Folia pharmacol. japon.* 68, 602–617 (1972) (Abs. in English)