Participation of the Parasympathetic Nervous System in the Development of Activity-Stress Ulcers

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ABSTRACT: The present study investigates the effects of truncal vagotomy and drug treatment, comprising atropine methylbromide and chlorisondamine, on the development of activity-stress ulcers in rats. To induce gastric lesions, female rats were housed individually in activity-wheel cages and subjected to a food-restricted schedule of only 1 hr food availability per day. Bilateral truncal vagotomy significantly prevented gastric ulceration, while those rats with vagotomy showed more running activity than sham-operated rats. Daily treatment with either methylatropine (3 and 6 mg/kg, s.c.) or chlorisondamine (2 and 4 mg/kg, i.p.) also significantly decreased the severity of lesions without a significant reduction in running activity. This evidence suggests that the development of activity-stress ulcers is mainly due to the hyperactivity of the peripheral parasympathetic nervous system.

KEY WORDS: activity-stress ulcer, vagotomy, methylatropine, chlorisondamine

Introduction

Young rats which have been housed individually in activity-wheel cages manifest various physiological and behavioral changes when subjected to a restricted feeding schedule,[1] the most prominent effect being the appearance of several lesions in the glandular portion of the stomach. Since a remarkable increase in running activity plays an essential role in the development of such gastric ulceration, this gastric lesion has been designated "activity-stress ulcer."[2] Although psychological factors leading to an increase in running activity have been proposed,[1] studies on peripheral mechanisms which may participate in the development of activity-stress ulcers are limited.

It has been well documented that parasympathetic nervous activity participates in the manifestation of gastric ulceration. In animals, as well as humans, either a truncal vagotomy or the administration of anticholinergic drugs suppresses the gastric ulceration induced by a variety of experimental manipulations, such as; restraint,[3–5] water immersion,[6] and forced swimming.[7] However, there are contradictory reports concerning the effects of anticholinergic drugs on activity-stress ulcers. Paré[8] reported that atropine sulfate failed to prevent the development of activity-stress ulcers. Contrary to this, Houser et al.[9] indicated that methylscopolamine significantly decreased the incidence of gastric lesions. However, there is little information concerning whether or not a truncal vagotomy alters the gastric ulceration induced by the activity-

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wheel paradigm.

The present study was conducted to investigate the role of peripheral neural mechanisms in mediating the development of activity-stress ulcers in rats. For this, the effects of truncal vagotomy, anticholinergic drugs and ganglion blocking agents on gastric ulceration were determined. Running wheel activity and food intake were also measured.

Materials and Methods

Subjects

The animals used in the following experiments were female Long-Evans rats (160–180 g) obtained originally from the Charles River Company, Chicago, Illinois and bred in the Animal Center of the Ehime University School of Medicine. They were housed communally in conventional polycarbonate cages and had free access to food and water before the experiments. The temperature in the vivarium was maintained at 22 ± 2°C, and a 12 hr light-dark cycle was kept constant, with lights on at 07:00 and off at 19:00.

Apparatus

An activity-wheel cage (Yayoi Medical Co., Ltd., Tokyo) consisted of a running-wheel (11 cm wide and 33 cm in diameter) and an adjoining wire-mesh cage (40 × 15 × 15 cm) allowing access to food and water. Thus, the rat could move freely between the wheel and the cage. The number of wheel-revolutions was automatically recorded at 30-min intervals with the aid of a digital counting system.

Testing procedures

Each rat was placed in an activity-wheel cage with free access to food and water during the 3-day adaptation period. Restricted feeding began on the 4th day, with food being withdrawn from the cage at 22:00 and then made available for only 1 hr per day between 21:00 and 22:00, from the 5th day until termination of the experiments. Daily changes in running activity and the amount of food intake were determined.

Endoscopic observation

A time-dependent change in the mucosal surface of the stomach was observed by means of an ultra-thin fiberscope 2.7 mm in diameter (Model PF-27, Olympus Co., Japan). Under light ethyl ether anesthesia, the rat was held in the supine position on a wooden board and, after 6 ml of air had been infused into the stomach, the fiberscope was immediately inserted. Photographs were taken using an automatic exposure control unit in combination with a halogen light source (Model CLE-F, Olympus Co., Japan). When food residue or bile obstructed observation, the stomach was rinsed with 3 to 5 ml of warm saline before photographing. Endoscopic examination was performed repeatedly on days 3, 5, 7 and 10 after the restricted feeding had been started. Endoscopic examination was not performed on the vagotomized rats as these rats also received pyloroplasty. The details of these endoscopic techniques were described previously.[10,11]

Determination of severity of the lesions

After the experiment, the rats were sacrificed with diethylether. The stomach was resected immediately following the ligation of the duodenum and filled with 6 ml of saline. The ballooned stomach was then immersed in 10 per cent formaldehyde solution for 5 min, after which the lightly fixed specimen was cut along the greater curvature and the lesions inspected macroscopically. In this study, the severity of the lesions was expressed by an ulcer index.[12] Spot-like lesions of less than 0.5 mm in length were excluded from the data.

Surgical procedure

Vagotomy was performed 14 days before the rats were placed in activity cages. The operation was performed under pentobarbital anesthesia (40 mg/kg, i.p.). Each rat was placed on its dorsal surface and a small 2.0–2.5 cm midline incision made across the upper abdomen. After the esophago-gastric junction was exposed, the anterior and posterior vagal trunks were resected proximal to the stomach. Heineke-Mikulicz's pyloroplasty was also performed to prevent

Table 1. Effects of the Various Experimental Manipulations on Activity-Stress Ulcers in Rats

Treatment	No. of Animals	Percentage of Survivors				Ulcer Incidence (%)[b]				Ulcer Index[c]
		3	5	7	10 day[a]	3	5	7	10 day[a]	(Mean ± SE mm)
Sham-operation	9	100	100	89	33	—	—	—	100	13.9±2.6
Vagotomy	9	89	56	33[#]	22	—	—	—	33[##]	0.4±0.2**
Saline (s.c.)	10	100	50	10	0	50	80	90	100	26.7±4.5
Methylatropine (s.c.)										
3 mg/kg	10	100	80	20	0	0[#]	60	70	80	11.2±3.9*
6 mg/kg	9	67	44	0	0	44	67	78	78	6.8±3.2**
Saline (i.p.)	10	80	80	40	10	30	50	70	100	37.4±4.1
Chlorisondamine (i.p)										
2 mg/kg	10	80	70	30	0	20	30	100	—	16.8±2.5**
4 mg/kg	9	78	56	0	0	11	33	78	89	8.4±2.7**

a Day after the start of restricted feeding schedule.

b Incidence is expressed as a cumulative percentage of the rats with a gastric lesion per initial number of rats, based on periodical endoscopic observation.

c The lengths of the lesions were summed to calculate the ulcer index.

*: $p<0.05$ and **: $p<0.01$; significantly different from the corresponding control value (two-tailed Mann-Whitney U-test).

#: $p<0.05$ and ##: $p<0.01$; significantly different from the corresponding control value (two-tailed Fisher's exact probability test).

gastric stasis. The sham-operated animals underwent the same procedure as the vagotomized rats, but without resection of the vagal trunks.

Drug administration

Atropine methylbromide (3 and 6 mg/kg) and chlorisondamine (2 and 4 mg/kg) were dissolved in an isotonic saline vehicle. Atropine methylbromide was administered subcutaneously and chlorisondamine intraperitoneally. Both drugs were given in a volume of 0.1 ml per 100 g body weight. The administration of each drug was started from the first day of restricted feeding and rats were treated twice daily (08:00–09:00 and 16:00–17:00).

Statistical analysis

Statistical analysis was done by the two-tailed Mann-Whitney U-test comparing the treatment group with the corresponding control group. The incidence of gastric lesions and the percentage of survivors were analysed by Fisher's exact probability test.

Results

As shown in Table 1, truncal vagotomy significantly decreased the incidence of gastric lesions as compared to the sham-operated group ($p<0.01$), while all the other treatments failed to suppress the incidence of gastric lesions. However, the severity of the lesions was significantly suppressed in all the treatment groups, being vagotomy; U=0 ($p<0.01$), methylatropine 3 mg/kg; U=19 ($p<0.05$) and 6 mg/kg; U=7 ($p<0.01$) and chlorisondamine 2 mg/kg; U=7 ($p<0.01$) and 4 mg/kg; U=2 ($p<0.01$). Endoscopic appearance of the gastric mucosa following the experimental treatments is shown in Fig. 1.

Fig. 2 demonstrates the effects of the various treatments on running-wheel activity.

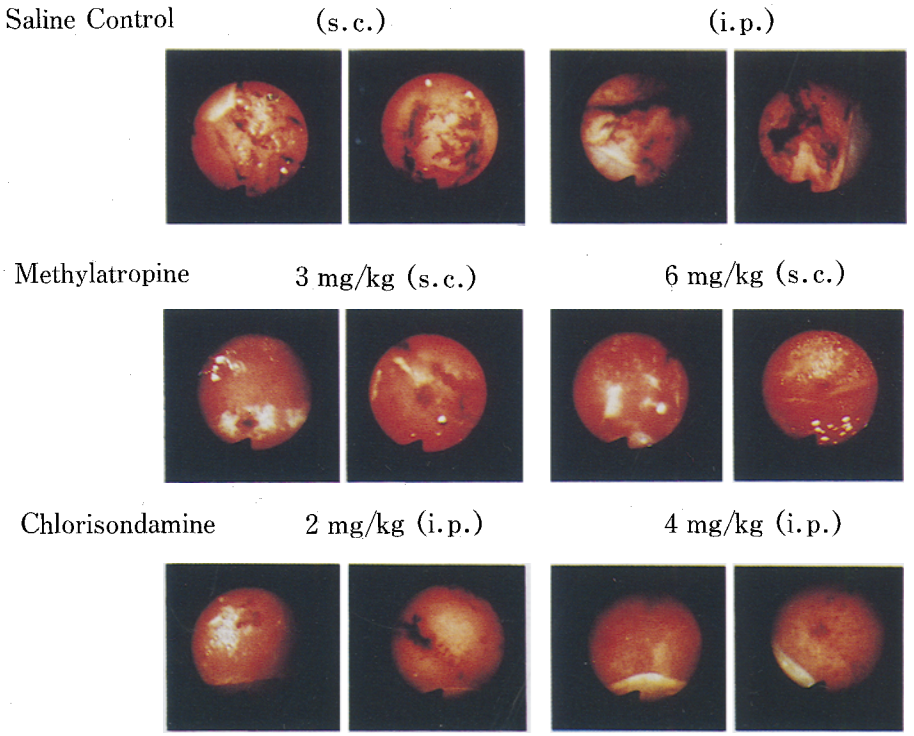

Fig. 1. Endoscopic appearance of the activity-stress ulcer in rats following the various experimental treatments. Note that the development of gastric lesions was suppressed by the treatment of methylatropine and chlorisondamine.

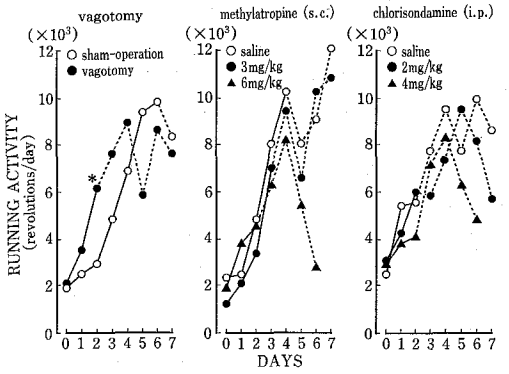

Fig. 2. Effect of the various experimental manipulations on running activity in rats. Day 0 indicates the control level when access to food was unrestricted. The dotted line indicates that the number of subjects decreased by death. *: $p<0.05$ and **: $p<0.01$; significantly different from the corresponding control value (two-tailed Mann-Whitney U-test).

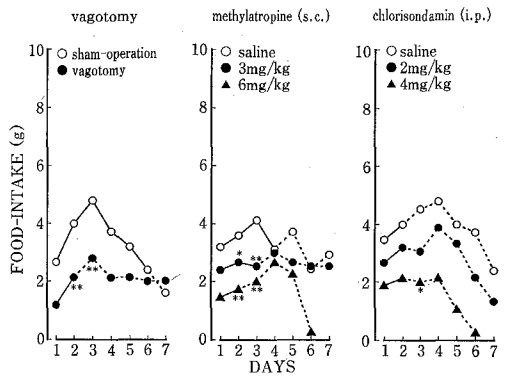

Fig. 3. Effect of the various experimental manipulations on food intake in rats. The dotted line indicates that the number of subjects decreased by death. *: $p<0.05$ and **: $p<0.01$; significantly different from the corresponding control value (two-tailed Mann-Whitney U-test).

Rats with truncal vagotomy revealed a significantly higher running activity than those of the corresponding sham-operated group, being U=14 at day 2 ($p<0.05$). The high doses of methylatropine and chlorisondamine tended to reduce running activity, although the differences were not statistically significant.

The effects of the various treatments on the amount of food intake are shown in Fig. 3. Vagotomy significantly suppressed the food intake, being U=7.5, and U=6 on days 2 and 3 respectively ($p<0.01$). The administration of methylatropine significantly decreased food intake in a dose-dependent manner: 3 mg/kg on day 2; U=20 ($p<0.05$) and day 3; U=15.5 ($p<0.01$); and 6 mg/kg on day 1; U=19 ($p<0.05$), day 2; U=12 ($p<0.01$) and day 3; U=4.5 ($p<0.01$). Chlorisondamine also caused a decrease in food intake as compared to the control group: 4 mg/kg on day 3; U=7.5 ($p<0.05$).

Discussion

The present findings, that either surgical or chemical vagotomy significantly suppressed the incidence and severity of activity-stress ulcers, clearly demonstrate that hyperactivity of the parasympathetic nervous system is mainly responsible for the development of gastric lesions in the activity-wheel situation. This view is in general accord with a previous report by Houser et al.[9] that methylscopolamine (0.06–0.25 mg/kg, i.p.) induced a suppressive effect on activity-stress ulcers. Since these manipulations do not exert a direct influence on the central nervous system, it seems most likely that the suppression of gastric ulceration is due to the inhibition of peripheral processes such as acid secretion, microcirculation, and motility. The suppressive effect of chlorisondamine, a long-lasting ganglion blocker, also supports this view.

With regard to acid secretion, Paré argued that gastric secretion is not essential to the formation of activity-stress ulcers, firstly because cimetidine failed to suppress the development of activity-stress ulcers,[13] and also because the activity-wheel paradigm results in a decrease in acid output rather than an increase.[14] He emphasized that lower running activity following the administration of atropine sulfate (2–8 mg/kg, s.c.) was a

critical factor in the suppression of gastric ulceration.[8] Among the variables which affect the incidence of activity-stress ulcer, indeed, the increase of running activity has been considered the most important because rats which did not show high revolutions in the running-wheel failed to develop gastric lesions.[1] In the present study, however, we did not find any significant difference in running activity between the methylatropine-treated and saline-treated rats. Interestingly, the rats which underwent truncal vagotomy showed a significantly elevated running activity during the early stage of the experimental period, although both the incidence and severity of ulcers were suppressed. Thus, it seems that an increase in running activity following chemical or surgical vagotomy is not critical. Moreover, because atropine sulfate as employed by Paré can penetrate into the brain, the distinctive effect of anticholinergic drugs on running activity may be accounted for by some central action of the drug.

Recently, studies concerning the relationship between gastric mucosal microcirculation and the autonomic nervous system have been accumulated, and several investigators have proposed the view that an impairment of gastric mucosal microcirculation may contribute to the development of stress ulcers.[15,16] Guth et al.[15] indicated that gastric mucosal vascular engorgement did occur prior to ulcer development in rats and suggested it to be a primary factor leading to a decrease in tissue resistance and permitting subsequent erosion by peptic acid secretion. They also demonstrated that both mucosal engorgement and restraint stress ulcers were blocked by surgical vagotomy.[4] Moreover, Dai et al.[5] observed that atropine treatment improved the mucosal vascular engorgement of the rat stomach exposed to stress. Further support comes from histochemical study showing that vagus nerves directly innervate the capillaries and arterioles in the gastric mucosa, and that vagotomy causes degeneration of the nerve endings which innervated these vessels.[17] These pieces of evidence suggest the possibility that parasympathetic nerve activity modulates the formation of stress ulcers through the impairment of gastric microcirculation.

All the experimental treatments employed in the present study decreased the amount of food intake during the restricted feeding period, and these treated-animals showed a short survival time as compared to the control group (Table 1). Thus, it appears that a decrease in food intake facilitates a malnutritional state leading to death and does not correlate directly to the suppression of activity-stress ulcers.

In this study, we periodically determined the pathology of the gastric mucosa using an endoscopic technique. We frequently observed that the development of gastric lesions was accompanied by a regurgitation of bile, the stomach often being covered with bubbled bile before being washed with saline, especially in rats which manifested severe lesions. Because bile acts as an aggravating factor on the process of gastric ulceration,[3] it is conceivable that the regurgitation of bile may play a role in the pathogenesis of the activity-stress ulcer. It is also possible that vagus hyperactivity causes a disorder of gastroduodenal motility[6,18] leading to the regurgitation of bile.

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