Involvement of CGRP, Substance P and Blood Circulation in Aggravating Mechanism of Absolute Ethanol-Induced Antral Lesions by Capsaicin Treatment in Rats

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ABSTRACT—The effect of capsaicin-sensitive nerve degeneration (capsaicin-treatment) on the corpus and the antrum was investigated in the absolute ethanol-induced lesion model in rats. The gastric lesions in the antrum were significantly aggravated by the capsaicin-treatment, while those in the corpus were not affected. To clarify the different susceptibility between the antrum and the corpus, the effects on gastric mucosal blood flow (GMBF), mucus secretion and levels of calcitonin gene-related peptide (CGRP) or substance P (Sub P), were investigated by the hydrogen gas clearance method, histochemical methods and immunohistochemical methods, respectively. The GMBF in the antrum was significantly decreased by the capsaicin-treatment, but that in the corpus was not. Moreover, capsaicin-treatment increased the mucus secretion in the antrum, but not in the corpus. Capsaicin-treatment significantly decreased CGRP and Sub P-immunoreactive substances in the vascular smooth muscle in the antrum, but not in the corpus. On the 4th day after absolute ethanol, antral ulcers were observed. From the above results, it was suggested that capsaicin-treatment decreased the gastroprotective ability in the antrum to a greater extent than in the corpus and this may be caused by the decrease of GMBF through the decrease of CGRP- and Sub P-immunoreactive substances.

Keywords: Capsaicin-sensitive nerve, Blood flow, Mucus, Calcitonin gene-related peptide (CGRP)

Recently, capsaicin-sensitive nerve has been reported to play an important role in gastroprotection (1-4). The present authors also reported that the oral administration of capsaicin inhibited the formation of ethanol-induced gastric lesions and that, on the contrary, the protective effect of capsaicin was attenuated by capsaicin-sensitive nerve degeneration (capsaicin-treatment) (5).

Ethanol-induced gastric lesions were prominently observed in the corpus and rarely in the antrum. However, hyperemia or erosive lesions were often observed by capsaicin-treatment in the antral region. Esplugues and Whittle (6) also reported that morphine and capsaicin-treatment increased the antral damage induced by 25-100% ethanol. These findings suggest that capsaicin-sensitive nerve degeneration weakens the gastroprotective ability in the antral region to a greater extent than in the corpus.

Peripheral capsaicin-sensitive afferent nerve has been shown to release calcitonin gene-related peptide (CGRP) or substance P (Sub P) (7, 8). CGRP or Sub P is known to protect against gastric mucosal damages (9, 10).

In the present study, we investigated the effect of capsaicin-sensitive nerve degeneration on gastroprotective functions such as gastric mucosal blood flow and mucus secretion and on the level of CGRP and Sub P to clarify the differential effect on the corpus mucosa and the antral mucosa.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 220 to 250 g, were used after fasting for 24 hr, but allowed free access to drinking water, before the experiments. Ethanol was administered to the rats only in the study of lesion formation.

Lesion formation and evaluation

Gastric lesions were induced by the oral administration
of absolute ethanol (5 ml/kg). One hour after the treatment, the stomach was excised and cut along the greater curvature. The gastric lesions were grossly observed. Gastric lesions in the corpus were measured and expressed as the sum of the length of lesions (corpus lesion index, mm), and antral lesions were expressed as the product of the measured length and width (antral lesion index, mm²). Gastric lesions were induced in normal rats and capsaicin-treated rats.

Moreover, on the 4th day after ethanol treatment, gastric lesions were observed in the vehicle- or capsaicin-treated rats.

Measurement of gastric mucosal blood flow
Gastric mucosal blood flow was measured by the hydrogen gas clearance method in the vehicle- or capsaicin-treated rats. In brief, laparotomy was performed under urethane (1.2 g/kg, i.p.) anesthesia. Needle type electrodes (Unique Medical, Tokyo) were inserted from the serosa into the basal portion of the gastric mucosa and positioned at the submucosal border of the muscularis mucosae. The electrode was made of a platinum wire, and the sensitive portion was coated by platinum black. A reference electrode (Ag-AgCl) was placed on the hind paw with ECG cream. Measurement and computerized

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Fig. 1. Macroscopical findings of gastric lesions induced by oral administration of absolute ethanol (one hour after oral administration of absolute ethanol). Antral lesions were observed in a capsaicin-treated rat (top panel). On the 4th day after absolute ethanol, an antral ulcer, which penetrated to the muscularis mucosae, was observed (bottom panel).
analysis of gastric mucosal blood were performed with a digital UH meter (MHG-D1) (Unique Medical). Gastric mucosal blood flow was measured in both the antral region and the corpus region at the same time.

Histological examination

The stomach specimens in vehicle- or capsaicin-treated rats were fixed in buffered 10%-formalin solution. Tissue specimens were made by the regular method and stained by periodic acid Schiff (PAS)-alcan blue. The gastric mucus level was qualitatively graded into 5 classes according to the density of PAS-staining.

Immunohistochemical examination

After rapid dissection of the antrum and the corpus of vehicle- or capsaicin-treated rats, the specimens were frozen in liquid nitrogen. Sections of 8-μm-thickness were cut on a cryostat. After fixation with acetone, CGRP- and Sub P-immunoreactive substances were stained according to the avidin-biotin peroxidase complex method (ABC) by using selective anti- sera of CGRP and Sub P, respectively (Cambridge Research Biochemicals, Cambridge, England). CGRP- or Sub P-immunoreactive substances were visualized with an image analyzer and qualitatively graded into 3 classes according to the amount of immunoreactive substances.

Denervation of the capsaicin-sensitive afferent nerves

To degenerate the capsaicin-sensitive afferent nerves, capsaicin pretreatment was performed according to the method of Yonei et al. (11). Capsaicin was dissolved in the vehicle consisting of 10% ethanol, 10% Tween 80 and 80% saline (vol./vol./vol.). Rats received a total dose of 125 mg/kg capsaicin, s.c. over 2 days, with 25 mg/kg in the morning and 50 mg/kg in the afternoon on the first day and 50 mg/kg once on the second day. The rats were used 10 days after the pretreatment with capsaicin. To check the effectiveness of the degeneration treatment, a drop of 0.01% solution of capsaicin in saline was instilled into either eye of the rats, and their protective wiping movements were observed. The capsaicin-treated rats that showed any wiping movement were excluded from the present study.

Data analysis

Data were expressed as the mean±S.E. Statistical analysis of the data was performed by Dunnett’s multiple comparison test and the Wilcoxon rank sum test.

RESULTS

Gastric lesions

None of the gastric lesions were observed in both the corpus and the antrum on the 10th or 14th days after capsaicin-treatment.

By oral administration of ethanol, in the vehicle-treated rats, most of the gastric lesions were induced in the corpus, while in the capsaicin-treated rats, gastric lesions were induced in both the corpus and the antrum (Fig. 1). As for the corpus lesion index, no significant difference was observed between the vehicle-treated and capsaicin-treated groups (n=5 or 6) (Fig. 2). However, a significant difference in the antral lesion index was observed between the vehicle-treated and capsaicin-treated groups (P < 0.05) (n=5 or 6) (Fig. 2).

On the 4th day after absolute ethanol, antral ulcers, which histologically penetrated to the muscularis mucosae, were observed in the capsaicin-treated group (Fig. 1), and the antral lesion index and incidence were 21.6±7.5 mm² and 5/5, respectively. However, in the vehicle-treated group, no antral lesions were not observed (n=4).

Gastric mucosal blood flow

In the vehicle-treated rats, the values of gastric mucosal blood flow in the corpus region and the antral region were...
86.3±10.6 (n=5) and 96.4±4.4 (n=5), respectively (Fig. 3). In the capsaicin-treated rats, the value of gastric mucosal blood flow at the antral region was significantly lower than that in the vehicle-treated rats (P < 0.01), while there was no significant difference in the value at the corpus region between vehicle-treated rats and capsaicin-treated rats (Fig. 3).

**Histological examination**

PAS-positive substance in the gastric pit was significantly increased by the capsaicin-treatment (Fig. 4 and Table 1). On the contrary, in the corpus, it was not affected by the capsaicin-treatment.

**Immunohistochemical examination**

CGRP-immunoreactive substance was observed around the vascular smooth muscle in the corpus and the antrum (Fig. 5). However, the density was higher in the antrum than in the corpus. By the capsaicin-treatment, CGRP-immunoreactive substance was significantly decreased in the antrum, but not in the corpus (Table 2).

**Fig. 3.** Effect of capsaicin-sensitive afferent nerve degeneration on gastric mucosal blood flow. In the antral region, a significant decrease of gastric mucosal blood flow was observed by capsaicin-sensitive afferent nerve degeneration, but not in the corpus region. The data represent the mean ± S.E. of 5 rats. **:** Significant decrease from the vehicle-treated group (P < 0.01).

![Graph showing gastric mucosal blood flow](image)

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<th>Grade</th>
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N.S. P < 0.01

Grades indicate the condition of mucus secretion, and the greater the number, the more prominent the mucus secretion. Values represent the number of animals. N.S.: Not significantly different from the vehicle-treated group. P < 0.01: Significant difference from the vehicle-treated group.

**Fig. 4.** Effect of capsaicin-sensitive afferent nerve degeneration on gastric mucus secretion. PAS-positive substances were markedly increased in the antral region in a capsaicin-sensitive afferent nerve degenerated rat (right panel) as compared with that in a vehicle-treated rat (left panel). (PAS alcian blue staining, ×40).
On the other hand, Sub P-immunoreactive substance was also observed in the vascular smooth muscle in both the corpus and the antrum. Sub P-immunoreactive substance was also markedly decreased by the capsaicin-treatment in the antrum, but not in the corpus (Table 2).

DISCUSSION

Capsaicin-sensitive afferent nerve has been considered to be responsible for gastric mucosal protection (1–4). The present authors previously reported a significant aggravation of antral ulcers induced by 2-deoxy-D-glucose, aspirin and ammonia in capsaicin-treated rats and suggested that capsaicin-sensitive nerve degeneration modifies the gastroprotective ability in the antral mucosa to a greater extent than in the corpus mucosa (12). In the present study, a significant aggravation of antral gastric lesions induced by absolute ethanol was observed by the capsaicin-treatment. Moreover, gastric mucosal blood flow in the antrum was significantly decreased, and CGRP- and Sub P-immunoreactive substances were also significantly decreased. The increase of gastric mucosal blood flow has
Table 2. Effect of capsaicin-sensitive afferent nerve degeneration on the calcitonin gene-related peptide (CGRP) and substance P (Sub P) levels in rats

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<th>Grade</th>
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Grades indicate the levels of CGRP- or Sub P-immunoreactive substance, and the greater the number, the more prominent the levels of CGRP- or Sub P-immunoreactive substance. Values represent the number of animals. N.S.: Not significantly different from the vehicle-treated group. P < 0.01: Significant difference from the vehicle-treated group.

been suggested as a mechanism for the gastroprotection by capsaicin (13, 14). On the 4th day after absolute ethanol treatment, antral ulcer was observed. These findings suggest that capsaicin-sensitive afferent nerves play a more important gastroprotective role in the antrum than in the corpus.

Many factors have been reported to play an important role in gastroprotective functions such as gastric mucosal blood flow, gastric mucus secretion and alkaline secretion. Among them, gastric mucosal blood flow appears to play a major role in the gastroprotection through supplying nutrients and oxygen (15) and to counteract the disruption of the gastric mucosal barrier and acid back-diffusion (16, 17). In this study, the decrease of gastric mucosal blood flow was observed in the antral region by the capsaicin-treatment. Therefore, the decrease of gastric mucosal blood flow by capsaicin-treatment may be the cause of the aggravation of antral gastric lesions induced by absolute ethanol.

On the other hand, mucus secretion also plays an important role in the gastric mucosal protection. Ross et al. (18) reported that a pH gradient developed across the mucus layer on the corpus mucosa of the rat stomach. The present authors (19) and others (20) also reported the existence of a pH gradient across the mucus. In the present study, the increase of gastric mucus was observed in the antral mucosa by the capsaicin-treatment. The increase of the mucus may counteract the back-diffusion of acid. However, the antral lesions induced by ethanol were aggravated by capsaicin-treatment. To maintain the pH gradient across the mucus, the alkaline secretion has an important role. Most of the alkaline secretion is supported by the blood. Takeuchi et al. (21) reported the decrease of alkaline secretion in capsaicin-treated rats, although the experimental specimens were from the duodenum of anesthetized rats. Therefore, the decrease of alkaline secretion by the reduced gastric mucosal blood flow might induce the aggravation of gastric lesions. To confirm this speculation, elucidation of the effect of capsaicin-treatment on the gastric mucosal pH gradient in the antrum will be necessary.

The stomach receives a peptidergic afferent innervation mainly from splanchnic nerves (22-24). The stimulation of peripheral endings of capsaicin-sensitive nerves in the stomach has proven to evoke the release of vasodilatory peptides such as CGRP or Sub P (25, 26), whose administration protected against gastric mucosal damage such as ethanol-induced (9, 14) or indomethacin-induced lesions (10). In the present study, capsaicin treatment caused a marked decrease of CGRP- and Sub P-immunoreactive substance in the antral vascular smooth muscle, but not in the corpus vascular smooth muscle. CGRP has been suggested to protect against mucosal damage by its vasodilatory property (8, 9, 24). These results were supported by the present finding that antral gastric mucosal blood flow was decreased by capsaicin-treatment.

As to gastric acid secretion, we previously reported that it was enhanced by capsaicin-treatment in rats (12). Therefore, the increase of gastric acid secretion may have aggravating actions on gastric lesions induced by ethanol in capsaicin-treated rats.

In general, gastric erosive lesions are clearly separated from gastric ulcer by histological criteria, and it is known that erosive lesions do not develop into ulcers. Therefore, it would be very interesting to clarify this situation. The decrease of CGRP- or Sub P-immunoreactive substance by capsaicin-treatment may be one of the reasons for the ulceration, but to clarify these points, further experiments are needed.

As to the corpus lesions, there was no significant difference between vehicle-treated rats and capsaicin-treated rats. This may be explained by the findings that gastric mucosal blood flow and CGRP- or Sub P-immunoreactive substance in the corpus were not affected by the capsaicin-treatment.

In conclusion, capsaicin-treatment weakens the antral gastroprotection to a greater extent than does the corpus gastroprotection, presumably by the decrease of gastric mucosal blood flow through the decrease of CGRP- or Sub P-immunoreactive substance.
Effect of Capsaicin on Gastroprotection

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