The Role of Capsaicin-Sensitive Afferent Nerves in Protective Effect of Capsaicin against Absolute Ethanol-Induced Gastric Lesions in Rats

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ABSTRACT—The role of capsaicin-sensitive afferent nerves in gastroprotection by capsaicin was investigated in the absolute ethanol-induced gastric lesion model in rats. Capsaicin (0.1 and 0.5 mg/kg, p.o.) inhibited the lesion formation dose-dependently. The protective effect of capsaicin was attenuated by indomethacin-pretreatment and disappeared in capsaicin-sensitive nerve degenerated rats. Capsaicin did not induce the distension of gastric mucosal folds. These results suggested that stimulation of capsaicin-sensitive afferent nerves by capsaicin would enhance the prostaglandin formation, leading to an inhibition of gastric lesions.

Capsaicin, the pungent component of red peppers of the genus Capsicum, has become widely used as a pharmacological tool to assess the involvement of sensory neurons in biological functions. Capsaicin-sensitive nerve degeneration by capsaicin pretreatment was reported to augment gastric damage induced by ethanol (1). On the other hand, Holzer and Lippe (2) reported that capsaicin (3.2-640 μM), administered with 25% ethanol, inhibited the development of hemorrhagic gastric lesions in a dose-dependent manner. Thus, the present study was performed to investigate whether capsaicin inhibits the lesion formation by absolute ethanol in normal rats and capsaicin-sensitive nerve degenerated rats by capsaicin and whether indomethacin affects the protective effect of capsaicin. Moreover, influences of capsaicin on gastric mucosal folds were also investigated, as distention of mucosal folds was reported to be gastroprotective against ethanol-induced gastric lesions (3).

Sprague-Dawley male rats weighing from 220-240 g (7 weeks) were used in the present study after 24 hr fasting. Gastric lesions were induced by the administration of absolute ethanol (0.5 ml/100 g). One hour later, gastric lesions were observed macroscopically. The length of gastric lesion was measured, and the sum of the length was expressed as the lesion index. Capsaicin (0.1 and 0.5 mg/kg) (Wako Pure Chemical) dissolved in 5% ethanol solution was administered orally 15 min before absolute ethanol. Indomethacin (10 mg/kg, s.c.) (Sigma) was administered 1 hr before capsaicin.

To degenerate the capsaicin-sensitive afferent nerves, capsaicin pretreatment was performed by the method by Yonei et al. (4). Capsaicin was dissolved in vehicle consisting of 10% ethanol, 10% Tween 80 (Nacalai Tesque) 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 for the experiment 10 days after the pretreatment with capsaicin. In order
to check the effectiveness of the treatment, a drop of 0.01% solution of capsaicin in saline was instilled into one eye of the rats, and their protective wiping movements were counted. The capsaicin-treated animals that showed any wiping movement were excluded from the study. Influences of capsaicin on gastric mucosal folds were studied according to the method reported by Mersereau et al. (3) with slight modification. Five conscious rats were gavaged with 0.25 ml of a 0.7 mg/100 ml aqueous solution of methyl violet. One hour later, they were killed by cervical dislocation and 10 ml of 5% formalin solution was administered orally. Ten minutes after formalin fixation, the stomach was excised and cut along the greater curvature. The lengths of methyl violet bounded mucosal folds were measured and the sum of the lengths was expressed as the length of mucosal folds. Capsaicin was administered 15 min before methyl violet. Statistical analysis was performed by Dunnett’s multiple comparison test.

The administration of absolute ethanol resulted in macroscopically detectable damage to the rat gastric mucosa and the lesion index was 51.5 ± 8.2 mm (n = 6). Pretreatment with capsaicin significantly inhibited the lesion formation by absolute ethanol in a dose-dependent manner, and the lesion indexes at the doses of 0.1 and 0.5 mg/kg were 18.4 ± 4.2 (n = 5, P < 0.01) and 1.8 ± 0.8 mm (n = 5, P < 0.01), respectively. On the other hand, indomethacin pretreatment did not show significant aggravating effect on ethanol-induced gastric lesions (Fig. 1). This result was in accord with the report by Yonei et al. (4). However, indomethacin pretreatment attenuated the inhibitory effect of capsaicin (0.5 mg/kg) (Fig. 1). In the capsaicin-sensitive nerve degenerated rats, the gastric lesions were aggravated, although not significantly, and the protective effect of capsaicin was not observed (Fig. 2). The lengths of methyl violet bounded mucosal folds in the control group and the capsaicin-administered group were 85.8 ± 6.7 (n = 5) and 87.2 ± 18.6 mm (n = 5), respectively, and there was no significant difference between the two groups.

The present results that capsaicin induces gastroprotection in the capsaicin-sensitive nerve intact rats coincided with the report by Holzer and Lippe (2), although the experimental conditions including the lesion model were different from the present study. Our findings provide clear evidence that the protective effect of capsaicin is due to the stimulation of capsaicin-sensitive afferent neurons.

In the rat stomach, capsaicin-sensitive afferent nerves have been localized histochemically to the mucosa and submucosa and associated with blood vessels (5). Capsaicin has been reported to increase gastric mucosal blood flow (6). Immunohistochemistry has shown that capsaicin-sensitive afferent neurons innervating the rat stomach contain substance P (5, 7) and calcitonin gene-related peptide (5, 8). However, subcutaneous administration of substance P and calcitonin gene-related peptide did not protect the stomach against ethanol-induced mucosal damage (9). Moreover, both peptides do not definitely enhance blood flow in the gastric mucosa (10), although they enhance the blood flow in a
variety of vascular beds (11). These findings are, however, still inconclusive with respect to what effect these peptides could exert when they are released locally within the gastric mucosa.

In the present study, pretreatment with indomethacin reduced the protective effect of capsaicin against absolute ethanol-induced gastric lesion. Indomethacin has been known to inhibit prostaglandin (PG) formation by inhibiting the cyclo-oxygenase. On the other hand, PGs, such as PGE1, PGE2 and PGI2, have been known to increase the gastric mucosal blood flow (12). Therefore, it was suggested that stimulation of capsaicin-sensitive afferent nerves enhanced the PG formation, which in turn increased the gastric mucosal blood flow, and led to an inhibition of gastric mucosal lesions induced by absolute ethanol. Holzer et al. (13), however, reported that the protective action of capsaicin against ethanol remained unchanged by indomethacin. Therefore, to clarify this mechanism, further experiments are needed. On the other hand, the distention of gastric mucosal folds was not induced by capsaicin, and thus, such a mechanism was not responsible for the gastroprotective effect of capsaicin.

Fig. 2. Effect of capsaicin on gastric lesions induced by absolute ethanol in capsaicin-sensitive afferent nerve degenerated rats. Cap 0.5 mg/kg: capsaicin, 0.5 mg/kg, p.o. Afferent nerve degenerated: capsaicin-sensitive afferent nerve was degenerated by capsaicin. *: Significant difference from the control (P < 0.05). **: Significant difference from the capsaicin group (P < 0.01). Bars represent the mean ± S.E. of 5 rats.

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