Transient Prevention of Ethanol-Induced Gastric Lesion by Capsaicin Due to Release of Endogenous Calcitonin Gene-Related Peptide in Rats

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ABSTRACT—Pre-exposure of the rat gastric mucosa to capsaicin reduced the mucosal lesion by 50% ethanol to 1/4. Treatment with an antagonist of calcitonin gene-related peptide (CGRP), CGRP (8 – 37), nullified the effect of capsaicin. During constant perfusion of the gastric lumen with physiological saline + pepstatin, the CGRP level was not increased by 50% ethanol, but it showed a peak (802.5 ± 145.7 pg/2 min) after 1.6 mM capsaicin. Four minutes after capsaicin, the CGRP level was kept at a high level and the gastric lesion was markedly reduced by re-exposure of the mucosa to 50% ethanol. At 20 – 30 min after capsaicin, the CGRP levels returned to the resting level and the reddened area by 50% ethanol was not reduced. It was concluded that capsaicin transiently prevented the mucosal lesion through CGRP release.

Keywords: Gastric lesion, Capsaicin, Calcitonin gene-related peptide

It is known that capsaicin acutely stimulates a group of afferent neurons with unmyelinated or thinly myelinated nerve fibers and release calcitonin gene-related peptide (CGRP), substance P (SP) and neurokinin A (NKA), which are contained in the spinal afferents (1). Although a neurotoxic dose of capsaicin does not cause damage by itself, capsaicin exacerbates formation of the mucosal lesion caused by injurious factors including ethanol. Capsaicin-induced ablation of afferent neurons compromises the ability of many factors and drugs to protect the gastric mucosa from injury, including prostaglandin E2 (1).

A number of reports indicated that short-term intragastric administration of capsaicin protects the rat and canine stomach from a wide spectrum of injurious factors, which include ethanol (2 – 5). CGRP was detected in the superfusion fluid of slices of the rat stomach (6) and in the venous effluent after intra-arterial administration of capsaicin (7). In the present experiments, CGRP that may be interstitially released was measured in the perfusate of the gastric lumen along the time course after intragastric application of capsaicin, and the interrelation between the time course of CGRP released by capsaicin and prevention of the ethanol-induced gastric lesion was examined.

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Male Sprague-Dawley strain rats (specific pathogen-free; Japan SLC, Inc., Hamamatsu), weighing 300 – 400 g, were starved for 18 h before the experiments, but had free access to water. The experiments were performed on animals anesthetized with urethane (1.25 g/kg, i.p.; Aldrich Chemical Co., Milwaukee, WI, USA). The experiments were carried out according to the Guidelines for the Treatment of Experimental Animals of Kitasato University School of Medicine.

The rat stomach was doubly cannulated with polyethylene cannulae, one from the esophageal side and one from the duodenal side (PE-280; Clay Adams, Parsippany, NJ, USA), as described previously (8). Physiological saline (37°C, pH 5.7) containing 10 μg/ml of pepstatin (pepstatin A; Peptide Institute, Inc., Osaka) was perfused at 2 ml/min, using a constant-rate pump (Model 11; Harvard Apparatus, Millis, MA, USA) connected to the esophageal cannula, and samples were collected from the duodenal cannula. Before collection of the first sample, the stomach was perfused with the above solution for more than 60 min to stabilize the stomach. CGRP in the sample tubes was determined with a CGRP EIA kit, which was provided by the Pharmacological Research Laboratories, Fujirebio, Inc., Tokyo. Methods of the extraction and EIA for CGRP were essentially similar to those for SP (9), and the recovery rate for exogenous CGRP was approximately 70%.

The exposure of the gastric mucosa to ethanol was per-
formed by switching the perfusion fluid from the physiological saline to 50% ethanol solution containing pepstatin, and 2 min later, the gastric fluid was again replaced by the physiological saline.

For measurement of the gastric lesion, the stomach was removed and opened by cutting along the greater curvature. The mucosa was gently wiped off and the stomach was placed between two glass plates (7.5 x 7.5 cm). Images of the stomach were captured with Adobe PhotoShop 2.5 J software, (Adobe Systems, Inc., Mountain View, CA, USA), and the reddened area was calculated using image analysis software (Ultimage, version 2.1.1; Grafter, Meudon-La-Foret, France) on a Macintosh computer.

The gastric mucosa was exposed for 2 min to 1.6 mM of capsaicin (Wako Pure Chemical Industries, Osaka) suspended in 0.25% gum arabic or to 0.25% gum arabic alone (a vehicle control) by replacing the physiological saline. Four minutes later, the mucosa was exposed to 50% ethanol solution for 2 min. A CGRP antagonist, CGRP (8–37) (Peptide Institute, Inc., Osaka) in the physiological saline was retrogradely infused into the gastric arteries through the splenic artery at the rate of 1 nmol/kg per min. Physiological saline was infused for the control experiments. Body temperature was maintained throughout at 37°C (Gb1 37) (Peptide Institute, Inc., Osaka) in the physiological saline. Four minutes later, the mucosa was exposed to 50% ethanol solution containing pepstatin, a CGRP antagonist, CGRP (8–37) into the trachea. Values are expressed as means ± S.E.M. Statistical analysis was made by ANOVA, and a P value <0.05 was regarded as significance.

Figure 1 illustrates that the reddened area of the gastric lesion induced by 50% ethanol (20.8 ± 1.4% of the glandular stomach) was markedly reduced to 5.0 ± 0.7% (P<0.001) by previous exposure of the gastric mucosa to 1.6 mM capsaicin, and infusion of CGRP (8–37) into the gastric arteries completely prevented the reduction of the mucosal damage by capsaicin (18.9 ± 1.6%). This indicated that the preventive effect of capsaicin was due to release of CGRP from the sensory nerve endings in the gastric wall.

As shown in Fig. 2A, exposure of the gastric mucosa to 50% ethanol alone did not release CGRP into the gastric lumen. This showed a marked contrast to the release of SP, since the SP level was increased from the basal level (1.7 ± 0.23 pg/g/2 min) to 6.0 ± 1.6 pg/g/2 min during exposure to 50% ethanol for 2 min (Gb1 9). In contrast, 1.6 mM capsaicin released CGRP into the gastric lumen (Fig. 2B). The CGRP level in the gastric lumen showed a peak at the end of the 2 min-exposure (802.5 ± 145.7 pg/g/2 min), which was significantly higher (P<0.05) than the resting level (222.0 ± 30.5 pg/g/2 min) and declined slowly after changing the perfusion fluid to physiological saline. Four minutes after exposure to 1.6 mM capsaicin, the CGRP level in the gastric lumen was 482.5 ± 58.1 pg/g/2 min, which was still significantly higher than the resting level (Gb1 P<0.05). When the gastric mucosa was re-exposed to 50% ethanol for 2 min (Fig. 2B), the mucosal damage was markedly reduced from 16.7 ± 2.7% of the glandular stomach (vehicle + 50% ethanol) to 4.8 ± 2.0% (capsaicin + 50% ethanol) (P<0.05), as shown in the columns of Fig. 2).

This preventive effect of capsaicin was shown to be transient in the time course study. The CGRP levels in the gastric lumen 4 min after exposure to capsaicin was 470.0 ± 55.7 pg/g/2 min and suppressed the damaged area by 50% ethanol. The level was further decreased to 340.0 ± 46.2 pg/g/2 min (20 min after capsaicin) and was maintained at the lowered level for up to 50 min (356.7 ± 14.5 pg/g/2 min, n=3). Neither level was statistically different from the level before capsaicin (310.0 ± 37.9 pg/g/2 min). The damaged areas were 13.5 ± 5.1% (at 30 min, n=3) and 10.2 ± 5.3% (at 50 min, n=3) and almost resumed to the level after 50% ethanol alone (16.7 ± 2.7%), indicating that capsaicin prevented the mucosal damage only at the significantly higher levels of CGRP in the gastric lumen/mucosa.

Capsaicin is known to release not only CGRP, but also SP and NKA. SP alone exacerbates the mucosal damage induced by 50% ethanol (9, 10) by constricting the collecting venules of the gastric mucosa (11, 12). Pre-exposure of

![Fig. 1](image-url)
the gastric mucosa to 1.6 mM capsaicin increased the SP level in the gastric lumen (6.5 ± 1.5 pg/2 min), which is 130 times as low as the peak level of CGRP (9). Thus, although capsaicin released both CGRP and SP, the suppression of the gastric damage after the subsequent exposure to 50% ethanol was accounted for by a large amount of CGRP released by capsaicin in the gastric mucosa/lumen.

The preventive mechanism of capsaicin/CGRP on the gastric mucosal damage to high concentrations of ethanol was reported to be accounted for by hyperemia of the mucosal microcirculation (1). However, on the basis of intravital microscopic study of the microcirculation of the gastric mucosa (13), we have proposed that the gastric mucosal lesion by 30–50% ethanol is caused by congestion of the mucosal blood flow induced by the constriction of the collecting venules, which is induced by leukotriene C4 released from the mucosal type mast cells in the gastric mucosa (14), probably stimulated by SP released from the sensory nerves by 50% ethanol (11). The intravital microscopic observation revealed that mucosal application of capsaicin prevents the constriction of the collecting venules due to 50% ethanol (12, 15). The dilatation of the mucosal arterioles by capsaicin did not cause the prevention of the ethanol-induced gastric lesion, since 50% ethanol itself maximally dilated the mucosal arterioles (12). It was also observed that prostaglandin I2 generated in the gastric mucosa (8) accelerated the CGRP release from spinal afferents (16).

In conclusion, intragastric application of capsaicin prevented the 50% ethanol-induced gastric lesion by released CGRP, as evidenced by its increased levels in the gastric lumen, but its preventive effect was transient.

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