Capsaicin-Sensitive Afferent Neurons in Adaptive Responses of the Rat Stomach Induced by a Mild Irritant

Jiro Matsumoto, Koji Ueshima, Koji Takeuchi* and Susumu Okabe

Department of Applied Pharmacology, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607, Japan

Received September 13, 1990 Accepted November 21, 1990

ABSTRACT — Exposure of rat stomach to 1 M NaCl reduced the transmucosal potential difference (PD) followed by an increase of luminal pH and gastric mucosal blood flow (GMBF). Desensitization of capsaicin-sensitive afferent neurons significantly mitigated the increase in GMBF without affecting PD and pH responses. Mucosal application of capsaicin increased GMBF with no effect on PD and pH. The findings suggest that capsaicin-sensitive afferent neurons may be involved in the regulatory mechanism of GMBF responses induced by a mild irritant.

Recent studies have shown that capsaicin-sensitive afferent neurons may play roles in the defensive mechanism of the gastric mucosa against noxious stimuli (1–3). Desensitization of these neurons worsens various types of gastric lesions (1), while intragastric capsaicin protected the mucosa against damage induced by aspirin or ethanol (2, 3). On the other hand, mild irritants induced adaptive cytoprotection in the stomach by enhancing the self-defensive mechanism such as an increase of gastric mucosal blood flow (GMBF) and an inhibition of acid secretion (4–6). However, the relation of capsaicin-sensitive afferent neurons to these adaptive responses remains undetermined. In the present study, we examined whether functional ablation of these neurons affects adaptive responses of the stomach induced by 1 M NaCl as a mild irritant.

Male Sprague Dawley rats (230–300 g), kept in individual cages with wide mesh bottoms, were deprived of food but allowed free access to tap water for 18 hr prior to the experiment. Under anesthetized conditions induced by urethane (Tokyo Kasei, 1.25 g/kg, i.p.), transmucosal potential difference (PD), luminal pH, GMBF and mean arterial blood pressure (MBP) were measured simultaneously using a Lucite chamber. Briefly, the stomach was exposed through a midline incision and mounted on the chamber as described in a previous paper (7). Under these conditions, the mucosa was perfused at a flow rate of 1 ml/min with saline (154 mM NaCl, 37°C). The pH of gastric effluent was measured using a pH glass electrode of the flow type (Horiba Model 6901-25T), while PD was determined using two agar bridges, one positioned in the chamber and the other in the abdominal cavity. Changes in PD were continuously monitored on a two channel recorder (Nippon Densi Kagaku, Model U-228) simultaneously with those of pH. GMBF was determined using laser Doppler flowmetry (Advance, Model ALF-2100) and by softly touching the probe (1 mm in diameter) on the surface of the corpus mucosa, while MBP was monitored via the femoral artery by a blood pressure transducer.

* To whom correspondence should be addressed.
and amplifier system (San-ei, Type 45277; Type 1829). After all parameters had stabilized, the perfusion was discontinued, the luminal solution was removed, and then the mucosa was exposed for 10 min to 2 ml of 1 M NaCl or capsaicin (Wako, 6 mg/ml) suspended in 1% carboxymethylcellulose (CMC). After application, the mucosa was rinsed with saline, another 2 ml of saline was instilled, and the perfusion was resumed. The pH monitoring was interrupted for 10 min while the mucosa was exposed to the above agents, whereas other parameters were continuously monitored during a 2-hr test period. Indomethacin (Sigma, 5 mg/kg), suspended in Tween-saline, was given s.c. 30 min before exposure to 1 M NaCl. Ablation of afferent neurons was performed by s.c. injection of capsaicin once daily for 3 consecutive days (20, 30, 50 mg/kg) 2 weeks prior to the experiment (8). All capsaicin injections were performed under ether anesthesia, and the rats were pretreated with terbutaline (Bricanyl®, Fujisawa; 0.1 mg/kg, i.m.) and aminophylline (Neophylin®, Eisai; 10 mg/kg, i.m.) for counteracting the respiratory impairment associated with capsaicin injection. The effectiveness of the treatment was tested by examining the protective wiping movements of the eye (9). Data are presented as the mean ± S.E. from 4 to 8 rats per group. Statistical analysis was performed using a two-tailed Dunnett's multiple comparison test or Student's t-test, and values of P < 0.05 were regarded as significant.

Under the present conditions, all parameters had stabilized about 30 min after operation and remained unchanged during a 2-hr test period. Instillation of saline into the chamber changed neither PD, pH, GMBF nor MBP, while CMC application caused a small elevation of PD without affecting the other parameters (Table 1 and Fig. 1). On the other hand, exposure of the mucosa to 1 M NaCl caused a marked reduction of PD from -31.4 ± 2.1 mV to -16.9 ± 1.5 mV, followed by a significant increase of luminal pH (3.55 ± 0.10 to 5.87 ± 0.17) and GMBF (11.5 ± 1.1 ml/min/100 g to the maximal values of 15.8 ± 1.6 ml/min/100 g) without significant change in MBP (Fig. 1A). The pH remained elevated

| Treatment                         | No. of rats | PD (−mV) before | PD (−mV) after* | pH before | pH after† | MBP (mmHg) before | MBP (mmHg) after# |
|----------------------------------|-------------|-----------------|-----------------|-----------|-----------|-------------------|-------------------|
| Saline                           | 6           | 32.3 ± 1.5      | 32.3 ± 1.9      | 3.33 ± 0.15 | 3.41 ± 0.13 | 75.6 ± 2.7        | 77.0 ± 3.3        |
| 1 M NaCl                          | 8           | 31.4 ± 2.1      | 16.9 ± 1.5*     | 3.55 ± 0.10 | 5.87 ± 0.17* | 72.6 ± 2.2        | 75.6 ± 2.6        |
| Indomethacin plus 1 M NaCl       | 8           | 32.6 ± 1.1      | 19.2 ± 0.6*     | 3.70 ± 0.06 | 5.16 ± 0.15* | 77.4 ± 4.3        | 77.8 ± 4.2        |
| Capsaicin-pretreatment plus 1 M NaCl | 8         | 32.3 ± 1.3      | 16.8 ± 0.6*     | 3.62 ± 0.13 | 5.98 ± 0.09* | 71.3 ± 2.1        | 73.8 ± 1.9        |
| CMC                              | 7           | 33.7 ± 1.7      | 35.7 ± 1.3      | 3.80 ± 0.10 | 3.85 ± 0.11 | 65.6 ± 4.9        | 68.7 ± 5.0        |
| Capsaicin (6 mg/ml)               | 7           | 33.3 ± 1.1      | 36.6 ± 1.1      | 3.73 ± 0.08 | 3.89 ± 0.06 | 71.3 ± 3.7        | 78.7 ± 5.6        |
| Capsaicin-pretreatment plus Capsaicin | 4         | 33.0 ± 1.7      | 33.9 ± 2.0      | 3.91 ± 0.13 | 3.89 ± 0.10 | 70.8 ± 5.7        | 66.0 ± 4.2        |

Data are presented as the mean ± S.E. from 4–8 rats. *: values at 10 min after reperfusion of saline; †: values at 10 min after application of 1 M NaCl or capsaicin (6 mg/ml). Statistically significant difference at P < 0.05: *, from the corresponding values observed before the treatment in each group; †‡, from the corresponding values in normal rats treated with 1 M NaCl.
for 1 hr, whereas the increase of GMBF persisted only for about 20 min. Pretreatment with indomethacin (5 mg/kg, s.c.) significantly attenuated both the pH and GMBF responses induced by 1 M NaCl without affecting the PD. In contrast, functional ablation of capsaicin-sensitive afferent neurons significantly mitigated the increase of GMBF seen after exposure to 1 M NaCl, despite that the pH and PD responses remained unaltered in these animals. Similar to 1 M NaCl, mucosal application of capsaicin (6 mg/ml) caused a sig-

Fig. 1. Effects of mucosal application of 1 M NaCl [A] and capsaicin [B] on gastric mucosal blood flow (GMBF) in rats. The stomach was exposed for 10 min to 2 ml of 1 M NaCl or capsaicin (6 mg/ml). Indomethacin (5 mg/kg) was given subcutaneously 30 min before the exposure. “Capsaicin-pretreatment” means ablation of primary afferent neurons. Data are expressed as the percentage (%) for the initial values and represent the mean ± S.E. of values determined every 2 or 10 min from 4–8 rats. Statistically significant difference at P < 0.05: *, from the control (group treated with saline [A] or 1% CMC [B]); **, from the group treated with 1 M NaCl [A] or capsaicin (6 mg/ml) [B] alone.
significant increase of GMBF but had no effect on both PD and pH (Table 1 and Fig. 1B). At this concentration, GMBF was increased from 12.2 ± 0.7 ml/min/100 g to the maximal values of 19.7 ± 1.9 ml/min/100 g and remained elevated for more than 1 hr. Although capsaicin action on GMBF was accompanied by a slight increase of MBP, this effect was not statistically significant and persisted for only 20 min. The increased GMBF responses caused by capsaicin at 6 mg/ml were completely blocked by desensitization of capsaicin-sensitive afferent neurons. In all of the above experiments, the gastric mucosa showed no macroscopic damage and bleeding.

The gastric mucosa is kept intact by multiple protective mechanisms, despite its exposure to acid and other chemical hazards. An increase of GMBF seen after exposure to mild irritants is one of those protective mechanisms and may be important in the self-defensive ability of the stomach under adverse conditions (6). The present study showed that capsaicin-sensitive afferent neurons may be involved in the regulatory process of such GMBF responses induced by mild irritants.

First, we confirmed in this study that exposure of the gastric mucosa to 1 M NaCl produced a reduction of PD followed by an increase of luminal pH and GMBF (4-6, 10). These responses may be partly mediated by endogenous PGs, inasmuch as an inhibitor of cyclooxygenase or phospholipase A₂ could significantly attenuate the increase of pH and GMBF seen after exposure to mild irritants (4–6). In fact, a number of studies showed that the PG biosynthetic activity in the gastric mucosa was increased several fold greater by application of mild irritants (6, 11). It is assumed that irritation of the surface cells may activate phospholipase A₂, releases membrane phospholipids, and increases PGs in the gastric mucosa.

Secondly, the present study showed that the mechanism of adaptive responses caused by mild irritants involve capsaicin-sensitive afferent neurons in addition to endogenous PGs. However, the neuronal involvement was observed only in the GMBF responses but not in the pH and PD changes, suggesting that the adaptive response is mediated by multi-pathways in different ways depending upon the function. This is based on the following findings: (a) desensitization of these afferent neurons significantly attenuated the increase of GMBF induced by 1 M NaCl without affecting PD and pH responses, and (b) mucosal application of capsaicin, a selective probe for sensory neuronal mechanisms (1–3), produced a marked increase of GMBF without any effect on PD and pH. Certainly, this increase of GMBF induced by capsaicin was completely blocked by ablation of capsaicin-sensitive afferent neurons. We found a slight elevation of MBP after application of capsaicin at 6 mg/ml. However, it is unlikely that the increased GMBF responses induced by capsaicin may be accounted for by MBP changes, because this agent at much lower concentrations (0.1–0.6 mg/ml) significantly increased GMBF without being accompanied by such MBP changes (J. Matsumoto et al., unpublished). Thus, a mild irritant, similar to capsaicin, may stimulate the nerve endings of these afferent neurons in the mucosa, resulting in an increase of GMBF. As evidenced in this study, the GMBF response induced by 1 M NaCl was partially mitigated by indomethacin. Moreover, it was reported that capsaicin effect on GMBF was significantly inhibited by indomethacin (12). It may be assumed that endogenous PGs released in the stomach exposed to mild irritants increase GMBF by acting on the vasculature and also by sensitizing these afferent neurons to stimulation by the irritant, although it remains undefined how PGs and these neurons interact in this process and what is the site of this interaction.

Taken together, the present study showed that capsaicin-sensitive afferent neurons are involved in the mechanism of GMBF responses induced by a mild irritant in the stomach. Irritation of the mucosa may be sensed by chemosensitive afferent nerves, which in turn signal various cells to alter their functions such as GMBF. Further study are required to
clarify the neurotransmitter related to the GMBF responses induced by a mild irritant and mediated by capsaicin-sensitive afferent neurons.

REFERENCES

1 Holzer, P. and Sametz, W.: Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. Gastroenterology 91, 975 – 981 (1986)

2 Holzer, P. and Lippe, I. Th.: Stimulation of different nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. Neuroscience 27, 981 – 987 (1988)

3 Holzer, P., Pabst, M. A. and Lippe, I. Th.: Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa. Gastroenterology 96, 1425 – 1433 (1989)

4 Nobuhara, Y. and Takeuchi, K.: Possible role of endogenous prostaglandins in alkaline response in rat gastric mucosa damaged by hypertonic NaCl. Dig. Dis. Sci. 29, 1142 – 1147 (1984)

5 Takeuchi, K., Yamakuni, H., Nobuhara, Y. and Okabe, S.: Functional and morphological alterations in the rat stomach following exposure to hypertonic NaCl solution. Japan. J. Pharmacol. 42, 549 – 560 (1986)

6 Takeuchi, K.: Adaptive functional alterations involved in self-defensive mechanisms of gastric mucosa and their regulations by endogenous prostaglandins. In Advances in Pharmaceutical Sciences. Edited by Honda, Y., No. 3, p. 114 – 133, The Research Foundation for Pharmaceutical Sciences, Tokyo (1987)

7 Takeuchi, K., Ishihara, Y., Okada, M., Niida, H. and Okabe, S.: A continuous monitoring of mucosal integrity and secretory activity in rat stomach: A preparation using a Lucite chamber. Japan. J. Pharmacol. 49, 235 – 244 (1989)

8 Espplugues, J. V. and Whittle, B. J. R.: Morphine potentiation of ethanol-induced gastric mucosal damage in the rat. Gastroenterology 90, 82 – 89 (1990)

9 Yonei, Y., Holzer, P. and Guth, P. H.: Laparotomy-induced gastric protection against ethanol injury is mediated by capsaicin-sensitive sensory neurons. Gastroenterology 99, 3 – 9 (1990)

10 Svanes, K., Varhaug, J. E., Dzienis, H. and Gronbech, J. E.: Gastric mucosal blood flow related to acute mucosal damage. Scand. J. Gastroenterol. 19, 62 – 66 (1984)

11 Robert, A., Nezamis, J. E., Lancaster, C., Davis, J. P., Field, S. O. and Hanchar, A. J.: Mild irritant prevent gastric necrosis through “adaptive cytoprotection” mediated by prostaglandins. Am. J. Physiol. 245, G113 – G121 (1983)

12 Takeuchi, K., Matsumoto, J., Ueshima, K. and Okabe, S.: Gastric motility changes in cytoprotective action of N-ethylmaleimide and capsaicin in the rat stomach. In Mechanism of Gastric Mucosal Protection. Edited by Kawai, K. and Szabo, S., Elsevier Press, Amsterdam (1990) (in press)