Implication of Sensory Neurons in the Diverse Mechanisms of Adaptive Cytoprotection in the Rat Stomach

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ABSTRACT—Adaptive cytoprotection is mediated by diverse mediators and mechanisms. We investigated the implication of capsaicin-sensitive afferent neurons in the adaptive cytoprotection in the rat stomach, taking special notice of nitric oxide, prostaglandins and luminal dilution. Sensory deafferentation abolished the protective effect of capsaicin against 0.6 N HCl-induced gastric injury but not the indomethacin-resistant or N\textsuperscript{G}-nitro-L-arginine-resistant adaptive cytoprotection conferred by 0.1 N NaOH. Nor did it attenuate the protection by 0.35 N HCl which accompanied luminal dilution. These findings suggest that certain mild irritants do not require sensory neurons to provide nitric oxide- and prostaglandins-mediated adaptive cytoprotection and, furthermore, that capsaicin-sensitive afferent neurons are not crucial, either, so long as there is a contribution of luminal dilution in the adaptive cytoprotection.

Keywords: Adaptive cytoprotection, Sensory neuron, Nitric oxide

As has been demonstrated, concurrent treatment with indomethacin, a potent cyclooxygenase inhibitor, and arginine analogs, biosynthesis inhibitors of nitric oxide (NO), in addition to the ablation of capsaicin-sensitive afferent neurons with neurotoxic amount of capsaicin resulted in severe gastric damage in rats, suggesting that prostaglandins (PG), NO and sensory neuropeptides may contribute to maintaining a favorable condition to counteract such aggressive factors as luminal acid, pepsin and bile (1). However, this study did not clarify the interrelations among those protective mediators in their production and effects. On the other hand, it has been reported that sensory neurons are not implicated in the adaptive cytoprotection because there was no influence of ablation of capsaicin-sensitive afferent neurons on the protection derived from the treatment with 0.2 M HCl and 20% ethanol against absolute ethanol-induced gastric lesion (2). We previously reported that adaptive cytoprotection involved various mechanisms, and which mechanism functioned was dependent on the concentration of mild irritants and on what kind of mild irritants were applied (3, 4). In this respect, the role of sensory neurons in the adaptive cytoprotection must be elucidated, taking notice of which mediator was mobilized and what mechanism works by mild irritants. We recently demonstrated that neither indomethacin nor N\textsuperscript{G}-nitro-L-arginine (L-NNA) affected the adaptive cytoprotection conferred by 0.1 N NaOH, but concurrent pretreatment abolished the protection (3). This supports the contention that indomethacin-resistant protection is NO-mediated and L-NNA-resistant protection is PG-mediated. In this study, we investigated the interactions of PG and NO generated in response to treatment with 0.1 N NaOH with sensory neurons by examining the effect of sensory deafferentation on indomethacin-resistant and L-NNA-resistant adaptive cytoprotection. We also investigated the influence of sensory deafferentation on the protective effects provided by 0.35 N HCl that are mediated by luminal dilution, the phenomenon that accumulated fluid in the gastric lumen dilutes necrotizing agents and results in the mucosal protection. From these studies, we tried to obtain further information about the participation of capsaicin-sensitive afferent neurons in the adaptive cytoprotection.

Fasted male Sprague-Dawley rats (Charles River Japan, Inc., Hino) were used. The animals weighed between 180 and 255 g at the time of the experiments. Rats were given 1 ml of 0.6 N HCl orally 30 min after administration of protective agents. Sixty minutes later, animals were sacrificed by cervical dislocation, and then their stomachs were quickly removed and fixed with 2% formalin. Macroscopical estimation of gastric lesions was
performed by measuring the major axis of each lesion in millimeters in the glandular portion and expressed as the sum of the lesion length in each stomach as the ulcer index (U.I.). Indomethacin (10 mg/kg) was injected subcutaneously 90 min before necrotizing agents. L-NNA was given orally 20 min before the treatment with protective agents. The dose of indomethacin was selected to show 93.4% inhibition of PGE2 content in the gastric mucosa, while the dose of L-NNA was selected to induce a marked increase of arterial blood pressure (unpublished data, Y. Hatakeyama et al.).

Chemical ablation of the sensory neurons was performed as described previously (5) with a slight modification. A total dose of 125 mg/kg capsaicin was injected subcutaneously over two days under pentobarbital anesthesia two weeks before the experiments. Rats were pretreated with terbutaline (0.1 mg/kg, s.c.) and aminophylline (10 mg/kg, i.p.) to avoid the respiratory impairment associated with capsaicin injection.

Drugs used in the present study were indomethacin, L-NNA, terbutaline, aminophylline (Sigma, St. Louis, MO, USA) and capsaicin (Nacalai Tesque, Kyoto). Indomethacin and L-NNA were suspended in 0.1% methylcellulose. Capsaicin was dissolved in a solution consisting of 10% ethanol, 10% Tween 80 (Tokyo Kasei, Tokyo) and 80% saline for subcutaneous injection (chemical ablation of sensory neurons) or suspended in 0.1% methylcellulose for oral administration. Agents administered subcutaneously or intraperitoneally were injected in a volume of 2 ml/kg or given in a volume of 5 ml/kg for oral administration. All mild irritants and capsaicin were administered orally in a volume of 1 ml.

The results are expressed as means±S.E. of 6–8 rats per group. Statistical analyses were performed by one way analysis of variance (ANOVA), and the statistical significance of the differences among groups was determined by Dunnett's multiple comparison test. Student's t-test was utilized for analyses between two groups. Values of P<0.05 were regarded as significant.

Capsaicin (20 mg/kg) administered orally reduced gastric mucosal lesions caused by 0.6 N HCl (84.9±8.2 vs 16.8±4.1 mm), and this protection was abolished by the functional ablation of the sensory neurons (83.6±9.6 vs 79.3±12.0 mm). On the contrary, L-NNA-resistant protection conferred by 0.1 N NaOH was not abolished by sensory deafferentation, but indomethacin-resistant protection was slightly attenuated by capsaicin (Fig. 1). The extent of mucosal protection was 65.3% in normal rats, 74.6% in capsaicin pretreated rats, 47.3% in indomethacin and capsaicin treated rats and 83.6% in L-NNA and capsaicin treated rats. Similarly, sensory denervation did not affect the protection afforded by an acid mild irritant (Fig. 2). The extent of protection by 0.2 N and 0.35 N HCl was 66.7% and 89.8% in normal rats and 66.6% and
82.3% in capsaicin pretreated rats, respectively.

The present findings demonstrate that sodium hydroxide does not require capsaicin-sensitive afferent neurons to provide NO-based and PG-based adaptive cytoprotection, nor does hydrochloride require those neurons to provide adaptive cytoprotection, at least that caused by luminal dilution.

Our previous finding (3) supports the contention that indomethacin-resistant adaptive cytoprotection conferred by 0.1 N NaOH is NO-based because it is abolished by an additional pretreatment with L-NNA and restored by L-arginine. The present finding that indomethacin-resistant adaptive cytoprotection is still obvious in capsaicin-pretreated rats supports the proposal that mild irritants do not require sensory neurons to provide NO-based adaptive cytoprotection. It should not be concluded, however, that mild irritants do not stimulate the sensory neurons - CGRP (calcitonin gene-related peptide) - NO pathway or that sensory neurons are not involved in the adaptive cytoprotection, as it is plausible that those agents induce NO generation via both sensory neuron-dependent and independent ways. The present finding that indomethacin partially counteracted the protective effect of 0.1 N NaOH in capsaicin-pretreated rats could have reflected the existence of a component of NO produced through the stimulation of sensory neurons. Further studies are thereby needed to elucidate the contribution of sensory neurons to the protection afforded by 0.1 N NaOH. However, the present results confirm that mild irritants are capable of inducing NO-based adaptive cytoprotection without capsaicin-sensitive afferent neurons, indicating that certain mild irritants can enhance NO generation in ways different from capsaicin. It remains unclear where the source of NO, which is independent on sensory neurons in its generation, is and whether there is a difference in the physiological role between sensory neuron-dependent and independent NO.

Whether PG interact with sensory neurons is still controversial. Several reports have implied the interaction between sensory neurons and PG (6–8), whereas another report has denied the contribution of prostanoid formation to the afferent nerve-mediated gastric mucosal protection (9). The protection afforded by capsaicin against ethanol-induced gastric lesion has been demonstrated to be sensitive to the pretreatment with both arginine analogs and indomethacin (10). If it is the case that one regulates the production of the other, both arginine analogs and indomethacin would then abolish the protection as Brzozowski et al. (10) observed. In the current study, however, L-NNA hardly affected and indomethacin only partially reversed the protective effect of 0.1 N NaOH, indicating the existence of PG- and NO-systems mobilized by mild irritants that do not interact with sensory neurons.

Mild irritants such as 1 M NaCl and 0.2 N HCl have been reported to increase gastric mucosal blood flow, which is abolished by both indomethacin and capsaicin-pretreatment (11). As evidenced in this study, sensory deafferentation was ineffective on the adaptive cytoprotection provided by 0.2 N HCl. Considering that the
hyperemic response is thought to be the most likely mechanism for gastric mucosal protection, these findings raise the possibility that there is no correlation between hyperemic responses and the adaptive cytoprotection induced by mild irritants. However, the possibility also remains that protection is provided by diverse mechanisms and that some mechanisms other than hyperemic response compensate the deleterious effects caused by indomethacin or sensory deafferentation, the result being no influence of sensory deafferentation on protective actions. It could be possible that adaptive cytoprotection involves mechanisms that are both sensitive and insensitive to indomethacin or capsaicin pretreatment. Actually, we have recently shown that 0.35 N HCl increases gastric fluid in the lumen, which is never reduced by sensory deafferentation (4). This also explains why ablation of the sensory neurons had little or no effect on the protective action of acid mild irritant.

In summary, certain mild irritants do not require sensory neurons to provide the adaptive cytoprotection induced by NO, PG and luminal dilution, indicating that there is a lack of interaction of NO and PG mobilized by certain mild irritants with capsaicin-sensitive afferent neurons. Further studies are however needed to confirm whether 0.1 N NaOH induces NO generation through capsaicin-sensitive afferent neurons since indomethacin partially counteracted the protective effect of 0.1 N NaOH in capsaicin-treated rats.

REFERENCES


