Influence of Sialoadenectomy on Capsaicin-Sensitive “Gastric Defense Mechanism” in Rats

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ABSTRACT — Chemical ablation of sensory afferents produced by the treatment with the neurotoxin capsaicin worsened gastric ulcers induced by 50% ethanol in sham- and sialoadenectomized (SALX)-operated rats. Conversely, capsaicin worsened water immersion stress (WIS)-ulcers in SALX, but not those in sham-operated animals. The concomitant removal of two potent antisecretory factors, such as salivary gland containing epidermal growth factor and neuropeptides contained in the sensory afferents, is probably responsible for the enhanced vulnerability of the mucosa in WIS-ulcers.

Capsaicin is a selective sensory neurotoxin, which if given in high doses, destroys primary afferent neurons that abundantly innervate the stomach (1). Chemical ablation of these sensory nerves by systemic capsaicin pretreatment aggravates lesion formation induced by several chemical stimuli (2), while the stimulation of the same endings by intragastric administration of capsaicin protects the gastric mucosa (3). This capsaicin-sensitive “gastric defense mechanism” has been attributed to the presence of antiulcer and vasodilator peptides contained in these nerves (4).

Ablation of the salivary complex delays healing of experimental ulcers (5) and influences the gastric secretory response to a stimulus (6). The epidermal growth factor (EGF) contained in this complex has been held responsible for the control of these gastric functions (5).

The observation that these two systems could influence similar functions prompted us to investigate their relative influence on ulcers induced by water immersion stress (WIS) and 50% ethanol. These models differ in their method for ulcer genesis and are respectively dependent or not dependent on an enhanced gastric acid secretion.

Male albino rats of the Sprague-Dawley Nossan strain, weighing 180–210 g, were housed at constant room temperature (21 ± 1°C), relative humidity of 60% and a 12 hr light-dark cycle (light on 6:00 a.m.) and used for the experiments.

Under thiopental sodium anesthesia (50 mg/kg, i.p.), the rats were sialoadenectomized (SALX) by removal of the sublingual-submandibular gland complexes after the ducts were ligated. The effectiveness of this operation was confirmed by the dramatic increase in prandial drinking (200% as compared to controls). Sham-operated animals served as controls, they were anesthetized and their sublingual-submandibular gland complexes exposed but not excised. SALX-animals were used 2 weeks after the operation (7).

Desensitization of sensory neurons was obtained by capsaicin injection (50 + 100 mg/
kg, s.c., administered in two consecutive days); and in order to check the effectiveness of the treatment, one day before the experiments, a drop of a 0.33 mM solution of capsaicin was instilled into one eye of the rats and the wiping movements were counted. Capsaicin-pretreated rats that showed any wiping movements were excluded from the study (3). This dose of capsaicin caused a marked depletion of gastric calcitonin gene-related peptide-like immunoreactivity (6), which is considered as a marker for a subset of visceral primary afferent neurons which innervate the upper gastrointestinal tract (1).

One week after the last dose of capsaicin, the gastric ulcers were induced in 24-hr fasted rats by a submaximal ulcerogenic dose of ethanol (50% v/v) administered by gavage in a volume of 5 ml/kg or by WIS. In this latter method, the animals were individually placed in a plexiglass cylinder (40 cm height, 18 cm internal diameter) containing water (15 cm depth) maintained at 25 ± 1°C. The animals were autopsied 1 hr after the 50% ethanol and 3 hr after WIS, and their stomachs removed, opened along the greater curvature and examined for the presence of gastric lesions by an observer unaware of the treatments. The lengths of the gastric lesions were measured by means of a stereo-microscope (magnification ×10) to determine the lesion index. Lesions smaller than 1 mm were assigned a rating of 1, lesions measuring 1–2 mm were assigned a rating of 2 and lesions measuring more than 2 mm were given a rating according to their length in mm. The overall total was designated as the “lesion index” (3).

Statistical analysis was performed by analysis of variance followed by multiple comparison procedures. All data were expressed as the mean ± S.E.

Lengths of gastric lesions induced by 50% ethanol and WIS were unaffected by sialoadenectomy as compared to those in the sham-operated animals (Fig. 1).

Capsaicin pretreatment produced a significant aggravation of gastric lesions induced by 50% ethanol in both sham- and SALX-operated rats (Fig. 1, panel A). As shown in panel B of Fig. 1, capsaicin pretreatment aggravated WIS-ulcers in SALX-operated animals, but not in sham-rats. The 2 × 2 analysis of variance gave a significant interaction (F = 4.2; P < 0.05) for WIS-ulcers between capsaicin pretreatment and sialoadenectomy (Fig. 1, panel B).

Capsaicin pretreatment influenced ethanol-lesions but not WIS-ulcers in rats with intact salivary glands. Peripheral and central factors influencing the different pathogeneses of the two ulcerogens might explain these differences. In fact, ethanol, a known barrier break-
er, produced hemorrhagic lesions through local mechanisms: it rapidly penetrates the mucosa causing a complete cessation of blood flow in the interested area; this stasis is followed by deep hemorrhagic necrosis (8). It should be mentioned that acute capsaicin treatment reduced the lesions induced by ethanol (3), through an increase in gastric blood flow (9) produced by a local release of peptides (10). Conversely, formation of WIS-ulcers characteristically involves a central component such as brain glucose uptake that regulates a vagal mediated increase in gastric acid secretion a factor which play a major role in the mucosal damage induced by WIS (11). From the present results, it appears that afferent fibers participate more directly in the ulcers characterized by a local component.

Salivary glands exert an important influence on the gastric mucosal growth (5) and gastric acid secretion (6). Some studies have shown that SALX-rats showed a delayed healing of chronic gastric ulcers (5). Our results indicate that acutely induced ulcers are unaffected in SALX animals irrespective of the ulcerogen used.

Neuropeptides contained in the afferent fibers and EGF in the salivary complex seem to interact in the genesis of WIS-ulcers but not in the formation of ethanol lesions. The different pathogenetic mechanisms involved in the ulcerogenic action of ethanol and WIS might be again the cause of these different results.

Ethanol-lesions are unrelated to gastric acid secretion since they are insensitive to vagotomy and antisecretory compounds (12), while WIS-ulcers are directly dependent on increased acid secretion and are sensitive to the classical antisecretory drugs (13).

The observation that EGF antiserum increased the stimulated acid output (14) suggests that active parietal cells are subjected to a tonic inhibition by endogenous EGF. On the other hand, capsaicin sensitive fibers have been shown to influence vagal-stimulated but not basal gastric acid secretion (15). It has been reported that the concomitant absence of these two potent antisecretory systems containing EGF and sensory peptides resulted in an increased gastric acid secretion (6). Therefore, the removal of a tonic influence on stimulated gastric acid secretion can be responsible for the enhanced susceptibility to WIS-ulcers in capsaicin-treated animals with resected salivary glands.

Although further studies are needed to better understand the mechanisms involved, these findings show that a functional interaction exists between these two systems regulating the gastric mucosal protection in response to a gastric injury associated with hypersecretion.

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REFERENCES
1 Green, T. and Dockray, G.J.: Characterization of the peptidergic afferent innervation of the stomach in the rat, mouse and guinea-pig. Neuroscience 25, 181 – 193 (1988)
2 Evangelista, S., Maggi, C.A., Giuliani, S. and Meli, A.: Further studies on the role of the adrenals in the capsaicin-sensitive “gastric defense mechanism”. Int. J. Tissue React. 10, 253–255 (1988)
3 Holzer, P. and Lippe, I.Th.: Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. Neuroscience 27, 981–987 (1988)
4 Szolcsányi, J. and Barthó, L.: Impaired defense mechanism to peptic ulcer in the capsaicin-desensitized rat. In Adv. Physiol. Sc., Vol. 29, Gastrointestinal Defense Mechanisms, Edited by Mózsik, Gy., Hamnin, O. and Jávor, T., p. 39–51, Pergamon Press – Akadémiai Kiadó, Oxford and Budapest (1981)
5 Komurcek, S.I., Dembiski, A., Warzceha, Z., Brzozowski, T. and Gregory, H.: Role of epidermal growth factor in healing of chronic gastroduodenal ulcers in rats. Gastroenterology 94, 1300–1307 (1988)
6 Evangelista, S., Renzi, D., Guzzi, P. and Maggi, C.A.: Interactions between sialoadenectomy and capsaicin-sensitive afferent fibers on gastric acid secretion in rats. Life Sci. 48, PL37 – PL41 (1991)
7 Tepperman, B.L., Soper, B.D. and Morris, G.P.: Effect of sialoadenectomy on adaptive cytoprotect-
tion in the rat. Gastroenterology 97, 124–129 (1989)
8 Ohya, Y. and Guth, P.H.: Ethanol-induced gastric mucosal blood flow and vascular permeability changes in the rat. Dig. Dis. Sci. 33, 883–888 (1988)
9 Holzer, P., Livingston, E.H. and Guth, P.H.: Sensory neurons signal for an increase in rat gastric mucosal blood flow in the face of pending acid injury. Gastroenterology (1991) (in press)
10 Holzer, P., Peskar, B.M., Peskar, B.A. and Amann, R.: Release of calcitonin gene-related peptide induced by capsaicin in the vascularly perfused rat stomach. Neurosci. Lett. 108, 195–200 (1990)
11 Arai, I., Hirose, H., Muramatsu, M. and Aihara, H.: Effects of restraint and water-immersion stress and insulin on gastric acid secretion in rats. Physiol. Behav. 40, 357–361 (1987)
12 Foschi, D., Ferrante, F., Vari, L., Del Soldato, P. and Rovati, V.: Protection of gastric mucosa in rats. Differences between vagotomy, atropine and PGE2. Dig. Dis. Sci. 31, 289–296 (1986)
13 Kitagawa, H., Fujiwara, M. and Osumi, Y.: Effects of water-immersion stress on gastric secretion and mucosal blood flow in rats. Gastroenterology 77, 298–302 (1979)
14 Garner, A., Gregory, H., Hampston, S.E., Stainer, A.M., Willshire, I.R. and Young, J.A.: Epidermal growth factor: comparison of antisecretory and mitogenic activities. Digestion 46, Supp. 2, 254 (1990)
15 Raybould, H.E. and Taché, Y.: Capsaicin-sensitive vagal afferent fibers and stimulation of gastric acid secretion in anesthetized rats. Eur. J. Pharmacol. 167, 237–243 (1989)