

Cholecystokinin-8 Protects Gastric Mucosa against Ethanol-Induced Lesions in Rats (42806)

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Abstract. Subcutaneous administration of cholecystokinin-8 (CCK-8, 10-100 $\mu\text{g}/\text{kg}$) reduces in a dose-dependent manner gastric lesions induced by 96% ethanol in rats, and CCK-4, CCK-7, and the CCK-8 nonsulfated form (all up to 100 $\mu\text{g}/\text{kg}$ sc) were inactive. The presence of the entire molecule and sulfation of the tyrosine in position 2 are necessary for the mucosal protective properties of CCK-8 against 96% ethanol-induced gastric lesions. These effects are probably at least in part, due to a sulfhydryl-sensitive process. © 1988 Society for Experimental Biology and Medicine.

Subcutaneous administration of some peptides influences experimentally induced gastroduodenal ulcers (1-4). Among these, cholecystokinin-8 (CCK-8) has been found to protect the gastric mucosa against ethanol-induced gastric erosions (1). Recently it has been reported that CCK-8 gastroprotection was antagonized by the sulfhydryl (SH) blocker *N*-ethylmaleimide (2), suggesting a partial involvement of SH-groups in this gastric mucosal protective mechanisms. Since it has been reported that the anti-lesion properties of peripherally administered somatostatin are strictly related to the presence of cysteine, and amino acid containing SH groups, in its molecule, it appeared worthwhile to determine whether CCK-8 fragments and the CCK-8 nonsulfated form retain the anti-lesion properties of the entire molecule.

Materials and Methods. Male albino Sprague-Dawley rats of the Nossan strain, weighing 180-210 g, were housed at constant room temperature ($21 \pm 1^\circ\text{C}$) and relative humidity (60%) with a 12-hr light-dark cycle (light on 6:00 AM). Each experimental group, listed in Fig. 1, consisted of 8 rats and was chosen by means of a completely randomized schedule.

Induction of gastric lesions. After being housed in plastic cages with wire bottoms to minimize coprophagy, and fasting for 24 hr, the animals received the peptides subcutaneously (groups and treatments are listed in Fig. 1), and 30 min later 5 ml/kg 96% ethanol (v/v) by gavage. The rats were killed 1 hr after the ethanol challenge, and their stomachs were removed, opened along the greater

curvature, and examined for the presence of gastric lesions, whose degree was scored according to an arbitrary scale from 0 to 4 in relation to size, by an observer unaware of the treatment (1). The following peptides were tested: CCK-8 (Asp-Tyr(SO₃)-Met-Gly-Trp-Met-Asp-Phe-NH₂), its nonsulfated form (without SO₃ on Tyrosine in position 2), CCK-7 (Tyr-Met-Gly-Trp-Met-Asp-Phe-NH₂), and CCK-4 (Trp-Met-Asp-Phe-NH₂); they were purchased from Peninsula Labs, dissolved in saline, and administered in a volume of 5 ml/kg at doses which have been selected in a previous study (1).

Statistics. All data related to gastric lesions were analyzed by means of Smirnov's test for nonparametric samples (5).

Results and Discussion. CCK-4, CCK-7, and nonsulfated CCK-8 did not reduce the ethanol-induced gastric lesions, but CCK-8 appeared to be effective in a dose-dependent manner (Fig. 1). Ethanol-induced gastric lesions are unaffected by antisecretory compounds and alkalization (6) or vagotomy (7), and unlike in other experimentally induced gastric lesions, the increase of gastric acid secretion does not play a role in the formation of multifocal and superficial mucosal lesions induced by ethanol. This fact is substantiated by the observation that CCK-8 fragments and nonsulfated-CCK-8, which inhibited gastric acid secretion (8), were not effective against ethanol-induced gastric lesions. This supports the view that, unlike other peptides whose anti-lesion properties are related to their antisecretory activity (3,

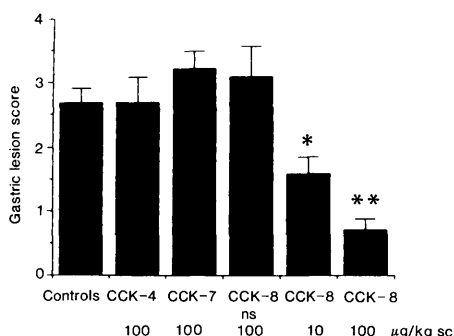


FIG. 1. Effect of cholecystokinin-4 (CCK-4), -7 (CCK-7), -8 nonsulfated form (CCK-8 ns) and -8 (CCK-8) on score of gastric lesions induced by 96% ethanol. Mean \pm SE of 8 rats for each group. * = $P < 0.05$ and ** = $P < 0.01$ as compared to controls.

9), CCK-8 reduced ethanol-induced gastric lesions by mechanism(s) unrelated to its inhibitory properties on gastric secretion.

Ethanol-induced gastric lesions are associated with a rapid decrease in the gastric mucosa content of SH-groups, and this effect can be antagonized by the administration of substances containing SH-groups (10). The presence of sulfhydryl groups appears to be responsible for mucosal defense (e.g., influence on membrane permeability, cell adhesion, and free radicals, see Ref. (11), and a SH-sensitive process can influence peptide gastric mucosal protection; in fact bombesin and CCK-8 (2)- and somatostatin (4)-gastroprotection were antagonized by SH-blockers, and somatostatin fragments without cysteine groups (4) were devoid of anti-lesion properties, and high doses of cysteine alone reduced ethanol-induced lesions (12).

In our studies, sulfation of tyrosine was necessary for CCK-8 to retain its antilesion activity, and it appears that SH-groups play

an important role in CCK-8 induced gastroprotection.

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Received February 4, 1988, P.S.E.B.M. 1988, Vol. 189.
Accepted July 15, 1988.