

THE EFFECTS OF CALCITONIN ON STRESS-INDUCED GASTRIC ULCERS, GLUTATHIONE AND LIPID PEROXIDATION IN RATS*

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SUMMARY

It has been reported that cold-restraint stress significantly decreases hepatic and gastric glutathione and increases lipid peroxidation levels. In the present study, the effect of calcitonin on gastric and hepatic glutathione and lipid peroxidation levels after cold-restraint stress exposure was investigated in rats. Calcitonin pretreatment did not significantly influence the gastric glutathione and lipid peroxidation levels but significantly increased the hepatic glutathione and lipid peroxidation levels. Our results suggest that the anti-ulcerogenic effect of calcitonin is not related to changes in the gastric lipid peroxidation and glutathione levels. The effects of calcitonin on hepatic glutathione and lipid peroxidation must be further evaluated.

Key words: Calcitonin, Stress-ulcer, Glutathione, Lipid Peroxidation

INTRODUCTION

Growing evidence indicates that specific peptides act within the brain to influence the formation of stress-induced gastric lesions in rats (1). Most of these peptides have been shown to be distributed throughout the central nervous system with a high representation in brain areas known to influence gastric function. Interestingly, all peptides are also present in the gastrointestinal tract and distributed either in specific endocrine cells or peripheral nervous system.

Injection of bombesin, opioid peptides, neurotensin, corticotropin releasing factor and calcitonin into the cerebrospinal fluid prevented the development of gastric ulcers induced by cold-restraint stress (2-5). Salmon calcitonin is the most potent centrally acting peptide to prevent the development of gastric ulceration induced by cold-restraint stress (6).

Disturbance of gastric mucosal microcirculation (7-9), alteration in gastric secretion (10), abnormal gastric motility (11) and stimulation of gastric lipid

peroxidation (12) are mechanisms that have been reported to account for the stress induced mucosal lesions.

Glutathione is an important constituent of cellular protective mechanism against a number of noxious stimuli including O₂- derived free radicals (12,13). It was previously reported that hepatic and gastric glutathione levels decreased following cold-restraint stress (12,14,15).

The effect of intracerebroventricular injection of calcitonin on acid, pepsin, prostaglandin synthesis and gastrointestinal motility were studied as antiulcer mechanisms (13,16,17). Since its effect on oxidative stress has not yet been investigated, we planned to determine the effect of calcitonin on gastric and hepatic glutathione and lipid peroxidation levels after cold-restraint stress exposure in rat.

MATERIALS AND METHODS

Wistar rats weighing 170-220 g were used. The animals were not fed for 48 h, but were allowed free access to water. Rats were pretreated with 5 µg calcitonin (Sandoz) dissolved in saline at a concentration of 500 µg/ml or saline alone (n=12). Calcitonin or saline was injected into the lateral ventricle via a polyethylene cannula (PE50). The cannulas were implanted 2 mm posterior to the bregma, 2 mm lateral to the sagittal suture and 4 mm below the surface of the skull, under light ether anesthesia. Then, animals were kept in restraints cages at 4° C for 4h. 48h fasted animals were used as control (n = 11). Rats were killed with ether at the end of stress application. The proper position of the cannulas was checked at the autopsy.

The glandular stomach was examined macroscopically. Ulcer index was determined by measuring the longitudinal length of lesions and accepting 3 petechia as 1 mm. Gastric and hepatic glutathione and lipid peroxidation levels were determined as described previously (18). Glutathione levels were calculated using an extinction coefficient

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of $13600 \text{ M}^{-1} \text{ cm}^{-1}$. Lipid peroxide levels were expressed in terms of malondialdehyde (MDA) equivalents using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.

Data were expressed as "mean \pm S.E.M." Statistical analyses were carried out with the aid of an Apple Macintosh Plus computer using the program Statview 512+ and the data were analysed by means of Student's t-test.

RESULTS

Macroscopic lesions were observed in 58 % of the stomachs of saline pretreated animals after cold-restraint stress exposure (UI= $5.0 \pm 1.67 \text{ mm}$) (Fig. 1). Gastric and hepatic glutathione levels were significantly lower in cold-restraint, saline-pretreated group compared with values obtained from the control group ($p < 0.01$), whereas lipid peroxidation levels significantly increased ($p < 0.05$) (Figs. 2 and 3).

Intraventricular injection of $5 \mu\text{g}$ calcitonin significantly prevented gastric lesion formation induced by cold-restraint stress (UI= $0.61 \pm 0.33 \text{ mm}$) (Fig.1). Calcitonin did not significantly influence gastric glutathione and lipid peroxidation levels but both hepatic glutathione and lipid peroxidation levels significantly increased in calcitonin-pretreated animals ($p < 0.01$) (Figs. 2 and 3).

DISCUSSION

Salmon calcitonin is the most potent centrally acting peptide to inhibit the occurrence of gastroduodenal lesions induced by stress, TRH, aspirin and cysteamine (19,20). Specific receptors for salmon calcitonin have been characterized in rat and human brain (6). High biological activity of calcitonin and the presence of brain receptors indicate that calcitonin or calcitonin-like peptides may have relevant implications in the maintenance of gastroduodenal integrity (6).

Studies related to the distribution of ^{125}I salmon calcitonin after injection into lateral brain ventricles have shown that highest amounts of intact peptide are retained by the brain, mainly the hypothalamus and hind brain and to some extent by kidneys but not by the stomach (21). Microinjection studies confirmed that the lateral and ventromedial hypothalamus and the paraventricular nucleus were among the sites of the inhibitory action of calcitonin on gastric acid secretion (6). Although calcitonin was found to be homologous with CGRP which is a potent inhibitor of acid secretion, calcitonin is believed to have distinct anti-ulcer mechanism (16). The antiulcerogenic effect of calcitonin is not solely related to the potent inhibition of gastric acid secretion because when the peptide delivered into the ventromedial hypothalamus suppresses stress ulceration without altering acid

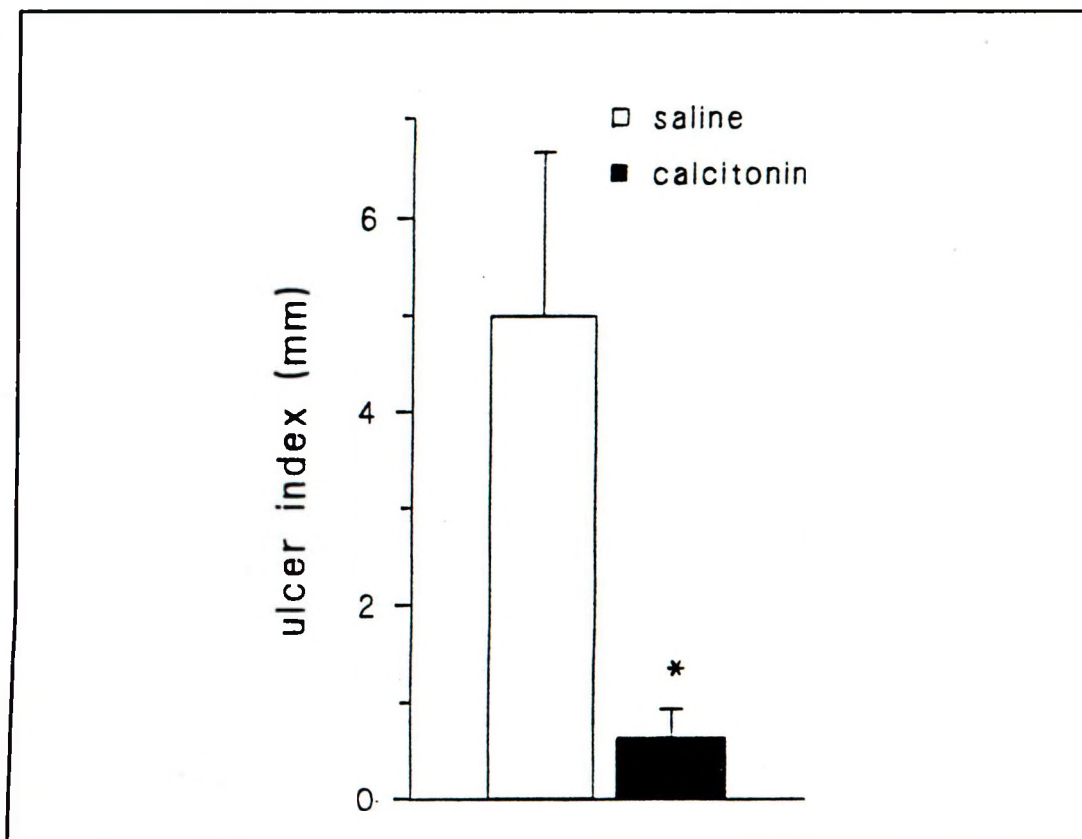


Fig. 1. Effect of calcitonin ($5 \mu\text{g}$, icv) on ulcer index induced by cold-restraint stress. *Significantly different than saline-pretreated group ($p < 0.05$).

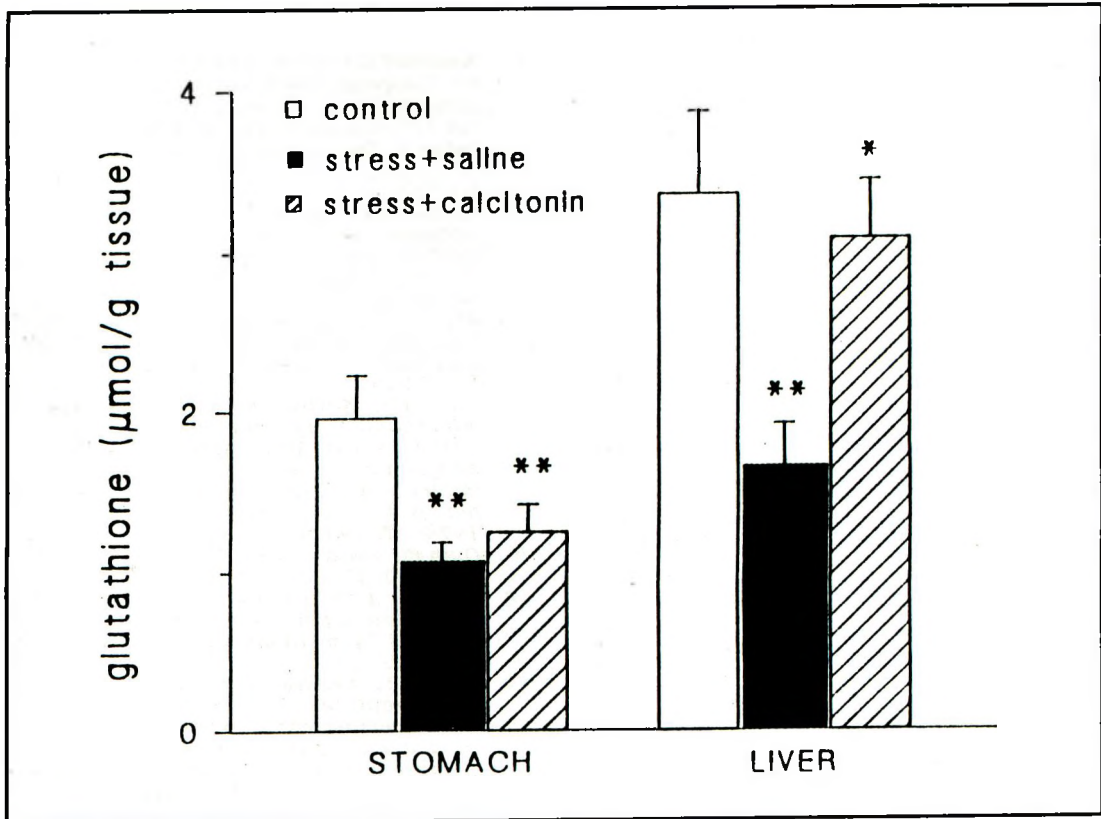


Fig. 2. Effect of calcitonin (5 μg , icv) on gastric and hepatic glutathione levels. * Significantly different than saline-pretreated group ($p < 0.01$). ** Significantly different than control ($p < 0.05$).

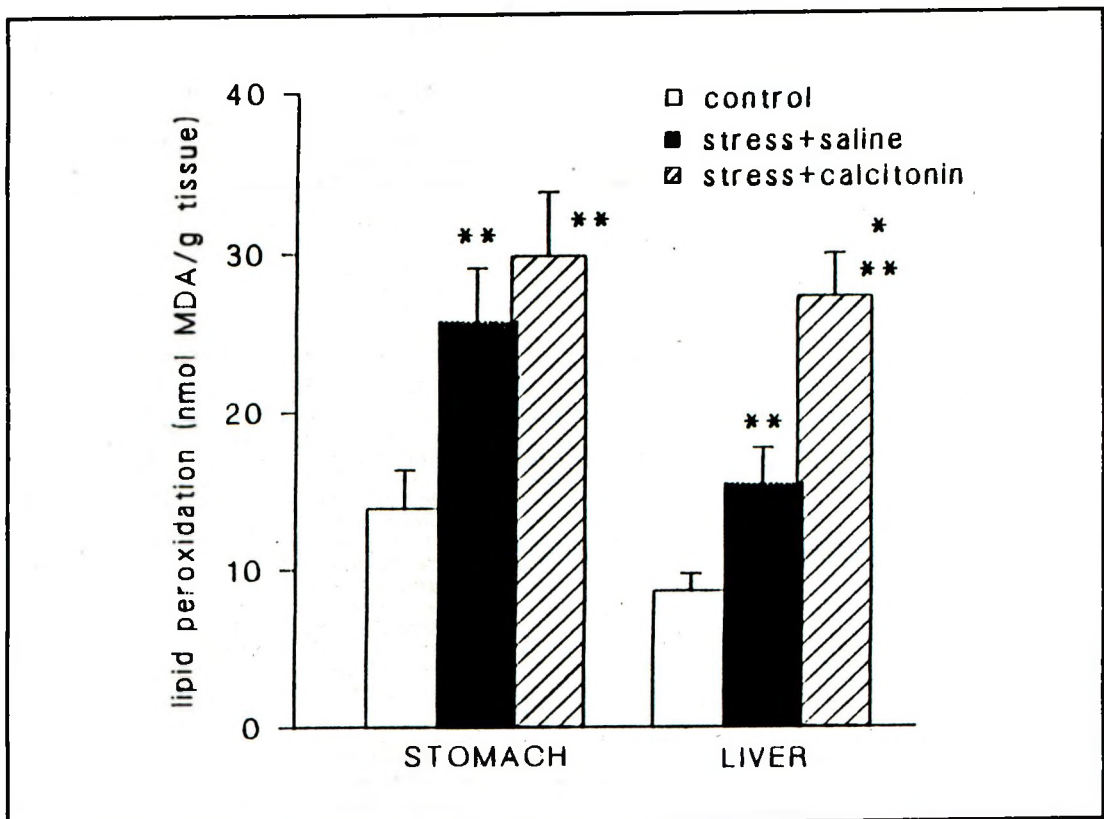


Fig. 3. Effects of calcitonin (5 μg , icv) on gastric and hepatic lipid peroxidation. * Significantly different than saline-pretreated group ($p < 0.01$). ** Significantly different than control ($p < 0.01$).

secretion (16). The antiulcerogenic effect of calcitonin microinjected into lateral hypothalamus was dose dependent within the doses ranging from 1-100 ng and specific since CGRP had no effect even at 1.1 µg dose (16).

Icv injection of calcitonin at antiulcer doses was reported to suppress pepsin secretion (6), inhibit gastric emptying of liquid meal and alter gastrointestinal motility (17). Prostaglandin generation in the gastric mucosa was not modified by CSF injection of calcitonin and this peptide inhibited gastric ulcer formation in the presence of aspirin (20).

Under hypothermic restraint stress a marked increase in blood viscosity with a mirror-image decrease in gastric mucosal blood flow has been detected (8). Partial arterial occlusion of a segment of cat small intestine followed by reperfusion caused histologically observable damage to the tissue and increased intestinal vascular permeability. Intravenous administration of superoxide dismutase (SOD) or oral administration of allopurinol (both the inhibitors of the enzyme xanthine oxidase which leads free radical formation in the ischemic tissue) to the animals before removal of the arterial occlusion offered protection against damage. Allopurinol is also a powerful hydroxyl radical scavenger and inhibitor of lipid peroxidation (22). Glutathione is an important constituent of cellular protective mechanisms against O₂- derived free radicals (12,13). It has been reported that cold-restraint stress significantly decreases hepatic and gastric glutathione and increases lipid-peroxidation levels (12).

In the present study, we have shown that calcitonin prevented stress-induced ulcer development but did not alter gastric glutathione and lipid peroxidation levels which are thought to be alternative mechanisms for ulcerogenesis in this model (12). Therefore, gastric glutathione and lipid peroxidation system may not play any role in the antiulcer effect of calcitonin.

On the other hand, peripheral injection of eel-calcitonin was shown to increase calcium content of rat liver cells and this increase in calcium was found to be associated with the activation of hepatic glycogenolysis and gluconeogenesis (23). In this study, it has been shown that icv injection of calcitonin returned the hepatic glutathione levels to control levels. In addition, lipid peroxidation levels were also further increased. The physiological cause and consequence of these effects needs to be further investigated.

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