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Effect of Malotilate on Ethanol-Induced Gastric Mucosal Damage in Capsaicin-Pretreated Rats

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Summary

We studied the role of afferent sensory neurons in malotilate-mediated gastric mucosal protection. Intact and capsaicin sensory-denervated rats were used in the experiments. Gross gastric mucosal injury was assessed and evaluated as a main criterion of the gastroprotective effect of the tested substances. Besides malotilate, methyl-prostaglandin E_2 was applied alone or in combination with malotilate to compare the effects and the mechanism of action of both substances. The results revealed that both malotilate as well as methyl-prostaglandin E_2 exerted a significant protective action on 96 % ethanol-induced gastric mucosal damage. However, there were no significant differences between intact and capsaicin-denervated rats. Only the use of 50 % ethanol as a milder mucosal irritating agent resulted in significant differences in both groups of animals. We propose that malotilate (like methyl-prostaglandin E_2) has a gastroprotective effect on ethanol-induced gastric mucosal injury. This effect is partly dependent on the sensory nervous system and the combination of both above substances has an additive effect.

Key words

Malotilate • Capsaicin • Methyl-prostaglandin E_2 • Ethanol • Gastroprotection

Introduction

Malotilate is known as a hepatoprotective agent with stimulatory effects on protein synthesis resulting from enhanced RNA synthesis (Wakasugi and Tomikawa 1985). Protein synthesis and some other mechanisms (e.g. increased liver blood supply) finally result in potentiated cellular regeneration (Igarashi 1980).

The gastric mucosal protective effect of malotilate has repeatedly been described in our laboratory (Mirossay *et al.* 1995, 1996a,b). The malotilate-induced gastroprotective effect seems to be multifactorial. Malotilate inhibits gastric acid secretion, mildly increases gastric mucus production and participates in different models of gastric mucosal damage. It significantly inhibits the formation of mucosal defects in indomethacin-induced, as well as in ethanol- and ischemia/reperfusion-induced gastric mucosal injury (Mirossay *et al.* 1995, 1996a). In spite of all information so far available, the mechanism of its action still remains obscure. The aim of the present paper was therefore to evaluate the role of sensory neurons in malotilate-induced gastroprotection. We thus employed capsaicin-denervated rats and ethanol as the injury inducing agent.

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Material and Methods

Male Wistar rats, weighing 200-250 g, were placed in cages with wire-net floors to prevent coprophagy. The animals were fasted for 24 h before the experimental procedure, but were allowed free access to water.

The rats were divided into two main groups comprising control (intact) animals and capsaicin-deafferented animals.

Degeneration of capsaicin-sensitive afferent nerves

To induce the degeneration of capsaicin-sensitive afferent nerve fibres, capsaicin pretreatment was performed by the method by Yonei *et al.* (1990). Capsaicin was dissolved in a vehicle consisting of 10 % ethanol, 10 % Tween 80 and 80 % saline. Rats received a total dose of 125 $mg.kg^{-1}$ capsaicin s.c. in the course of 2 days. The first dose consisted of 25 $mg.kg^{-1}$ of capsaicin in the morning, the second dose of 50 $mg.kg^{-1}$ in the afternoon on the first day. The third dose of 50 $mg.kg^{-1}$ was administered on the second day in the morning. Capsaicin was purchased from Fluka (Germany). Control animals received equal volumes of the vehicle. All injections were given under ether anesthesia. The rats were followed for 10 days after the pretreatment with capsaicin.To check the effectiveness of the denervation treatment, a drop of a 0.1 $mg.ml^{-1}$ solution of capsaicin in saline was instilled into the eye of each rat, and their protective wiping movements were counted. Vehicle-pretreated rats responded instantly with wiping movements while capsaicin-pretreated rats did not. The capsaicin-pretreated animals that showed any wiping movement were discarded from the study.

To counteract the respiratory impairment associated with capsaicin injection the rats were pretreated with terbutaline (0.1 $mg.kg^{-1}$, i.m.) and aminophylline (10 $mg.kg^{-1}$, i.m.) before capsaicin injection. Terbutaline was purchased from Sigma (Germany), aminophylline (Syntophyllin inj.) from Hoechst-Biotika (Slovakia).

Induction of gastric mucosal defect

Gastric mucosal defects were induced by either 95 % or 50 % ethanol given to each animal intragastrically in a dose of 0.5 $ml.100\ g^{-1}$. The animals were killed 60 min after ethanol application by ether narcosis. Each stomach was removed immediately, opened along the greater curvature and all macroscopically visible lesions of the mucosa in the glandular part were recorded. The extent of lesions was measured, summed per stomach and expressed in millimeters.

Malotilate pretreatment

Malotilate was obtained from the Drug Research Institute, Modra (Slovakia) and suspended in 0.5 % methylcellulose. The groups of animals pretreated with malotilate received it orally in doses of 50, 100 and 200 $mg.kg^{-1}$ in a total volume of 0.5 $ml.kg^{-1}$ 15 min before ethanol application. Only 0.5 % methylcellulose was given to the control animals.

Methyl-prostaglandin E_2 pretreatment

Methyl-prostaglandin E_2 (methyl-PGE_2) from Sigma (Germany) was given orally in doses of 10, 30 and 100 $\mu g.kg^{-1}$. The parenteral ethanol solution was diluted to a final concentration of 5 % ethanol. The drug was given to the rats 15 min before the application of malotilate or vehicle (0.5 % methylcellulose). The total volume of administered solution was the same as in the case of malotilate. Control animals have received the same volume of 5 % ethanol.

Statistical analysis

Values are given as means ± S.E.M. The statistical significance of differences was determined by one-way analysis of variance (ANOVA) with contrasts. A probability level of 0.05 was considered to be significant.

Results

Methyl-PGE_2 in three different doses was tested in preliminary experiments. The effect of these three doses (10, 30 and 100 $\mu g.kg^{-1}$) is given in Figure 1. The lesions were induced by 96 % ethanol. The protection of gastric mucosa is dose-dependent and achieves about 30 % with 10 $\mu g.kg^{-1}$ ($p<0.05$) and about 70 % with 30 $\mu g.kg^{-1}$ ($p<0.001$). The highest dose of methyl-PGE_2 (100 $\mu g.kg^{-1}$) almost completely abolished gastric mucosal damage ($p<0.001$).

The dose-dependent effect of malotilate is shown in Figure 2. Gastric mucosal damage was induced by 96 % ethanol. Malotilate was administered in three different doses of 50, 100 or 200 $mg.kg^{-1}$ in 0.5 % methylcellulose. A significant gastroprotective effect was already provided by the first (the smallest) dose of malotilate ($p<0.001$). The next two doses exerted a higher protective effect, whereas practically total abolition of ethanol-induced injury was achieved with 200 $mg.kg^{-1}$ ($p<0.001$).

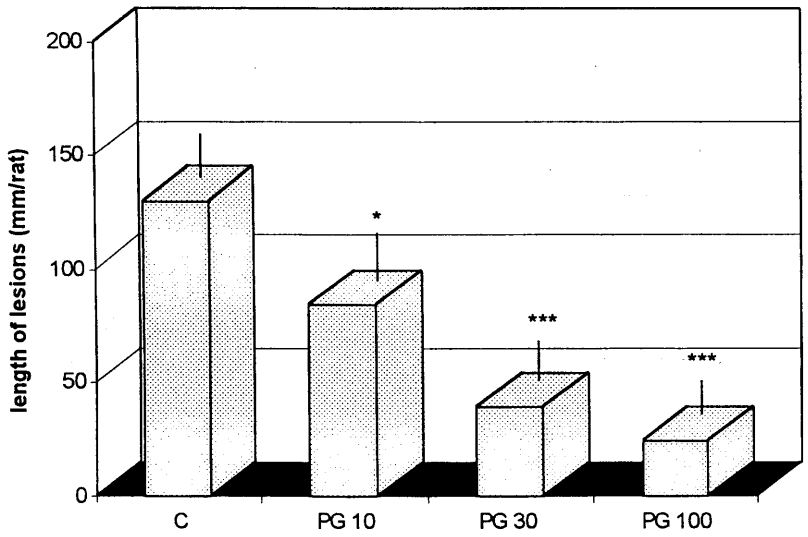

Fig. 1. *Effect of methyl-prostaglandin E_2 in doses of 10, 30 and 100 $\mu g.kg^{-1}$ (PG 10, PG 30, PG 100) on 96 % ethanol-induced gross gastric mucosal lesions. Results are expressed as means ± S.E.M. Significantly different from vehicle-pretreated (C) group: *p<0.05, ***p<0.001.*

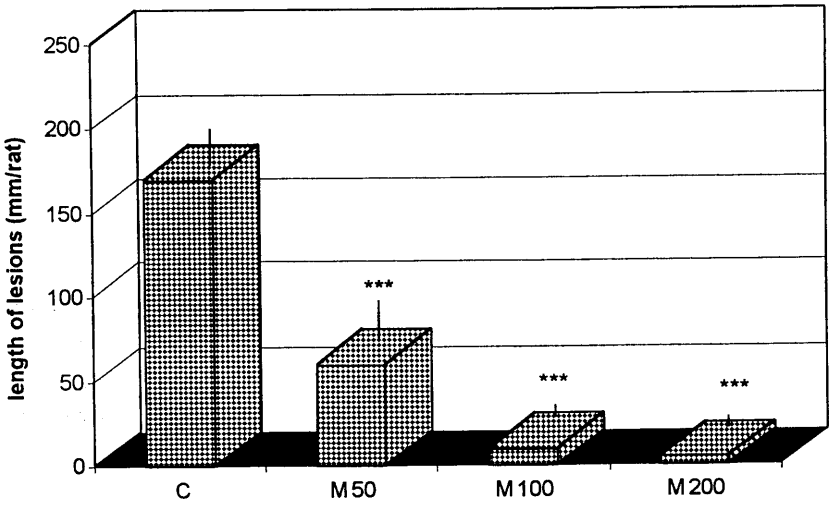

Fig. 2. *Effect of malotilate in doses of 50, 100 and 200 $mg.kg^{-1}$ (M 50, M 100, M 200) on 96 % ethanol-induced gross gastric mucosal lesions. Results are expressed as means ± S.E.M. Significantly different from vehicle-pretreated (C) group: *** p<0.001.*

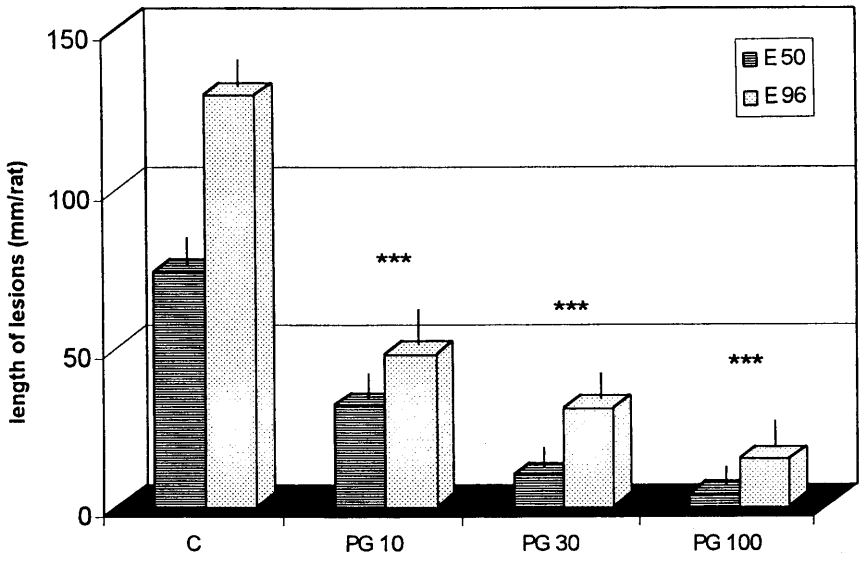

Fig. 3. Effect of methyl-prostaglandin E_2 in doses of 10, 30 and 100 $\mu g.kg^{-1}$ (PG 10, PG 30, PG 100) on 50 % and 96 % ethanol-induced gross gastric mucosal lesions. Results are expressed as mean ± S.E.M. Significantly different from vehicle-pretreated (C) group: ***p<0.001. The location of symbols in the center of two columns represents significant differences of both substances compared to the control groups.

To demonstrate the influence of sensory neurons on malotilate and methyl-PGE_2 gastric mucosal protection we applied the same substances under the same experimental conditions to capsaicin-induced

sensory denervated animals and compared them to control (intact) rats. First of all, we tested the dose-dependent effect of both methyl-PGE_2 and malotilate in capsaicin-denervated rats in the same doses used in the above experiments in normal animals. Ethanol as an irritating agent was used in the 96 % concentration. The results of these experiments are shown in Figures 3 and 4. The dose-dependent effect of both substances tested is evident. Methyl-PGE_2 as well as malotilate were significantly effective in all doses ($p<0.001$) and their effect in capsaicin-treated rats was very similar to normal (intact) rats.

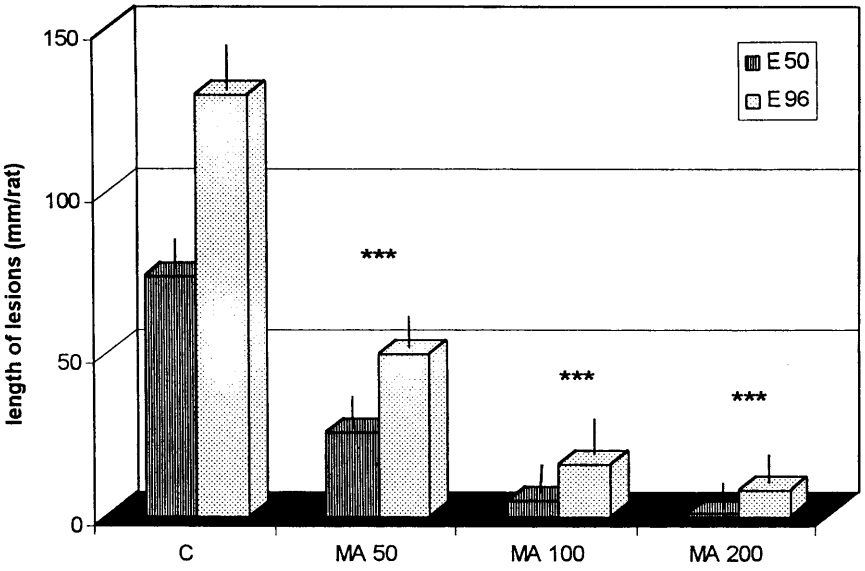

Fig. 4. *Effect of malotilate in doses of 50, 100 and 200 mg.kg^{-1} (M 50, M 100, M 200) on 50 % and 96 % ethanol-induced gross gastric mucosal lesions. Results are expressed as means ± S.E.M. Significantly different from vehicle-pretreated (C) group: *** $p<0.001$. The location of symbols in the center of two columns represents significant differences of both substances compared to the control groups.*

In further experiments, we also studied the effects of malotilate, methyl-PGE_2 and their cooperation in gastric mucosal protection. The doses of both substances were chosen according to their submaximal efficacy in order to observe their possible additive effects. Malotilate was given in a dose of 50 mg.kg^{-1} and methyl-PGE_2 in a dose of 10 μg.kg^{-1}. The concentration of 96 % ethanol was used for inducing gastric mucosal damage. The results of these experiments are presented in Figure 5. Both substances exerted a significant protective effect on gastric mucosa against ethanol-induced injury when given separately ($p<0.001$). Coadministration of malotilate and methyl-PGE_2 resulted in an additive gastroprotective effect which was significantly higher than when applied separately ($p<0.01$). Comparing the results of the group of non-treated rats to sensory denervated animals there are slight differences in the efficacy of individual substances as well as of their combination. In all cases the effects were significantly different from vehicle pretreated animals but they did not differ significantly when the effects of the same drugs were compared between non-pretreated and capsaicin-pretreated rats. Even the results in control animals did not differ in both experimental groups. The administration of highly concentrated ethanol is probably responsible for this lack of significant differences.

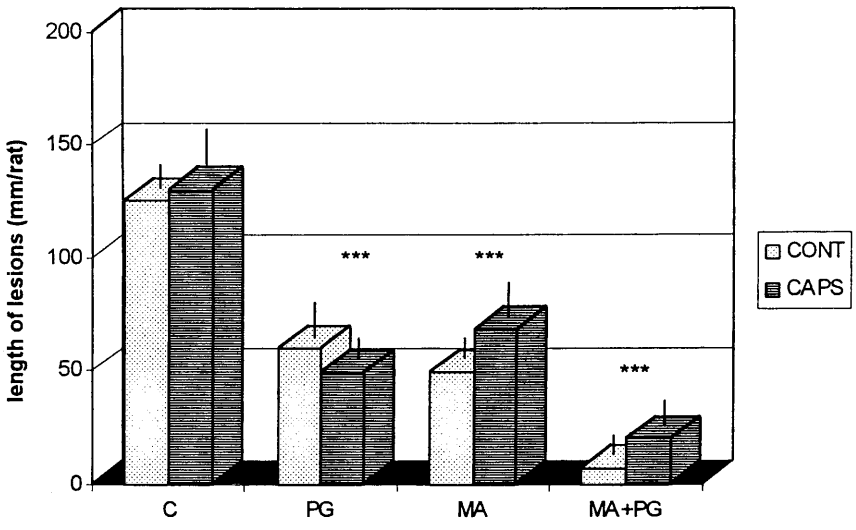

Fig. 5. *Effect of 50 mg.kg^{-1} malotilate (MA), 10 μg.kg^{-1} methyl-prostaglandin E_2 (PG) and their combination (MA+PG) on 96 % ethanol-induced gross gastric mucosal lesions in intact and capsaicin-induced sensory denervated rats. Results are expressed as means ± S.E.M. Significantly different from vehicle-pretreated (C) group: ***$p<0.001$. The location of symbols in the center of two columns represents significant differences of both substances compared to the control groups.*

For this reason, we have used in additional experiments another model of gastric injury, namely the model with 50 % ethanol. The results of the experiments with 50 % ethanol as the noxious stimulus are shown in Figures 3, 4 and 6. It is clear from the comparison of control groups of both capsaicin-pretreated and capsaicin non-treated rats that mucosal injury-induced by 50 % ethanol is much smaller than the effect of 96 % ethanol (Fig. 3 and 4). The difference between these groups is also significant ($p<0.001$). Methyl-PGE_2 as well as malotilate exerted a similar protective effect as in the model with 96 % ethanol, but the differences between control groups of capsaicin-pretreated and non-treated animals were significant for both substances given alone ($p<0.001$) (Fig. 6). The effect of the combination of both drugs was additive in capsaicin-pretreated rats and significant if compared with the effect of either substance applied alone ($p<0.01$). Significant additive effects were not found in intact animals because the effects of both drugs given alone (especially malotilate) almost completely abolished the gastrotoxic effect of 50 % ethanol.

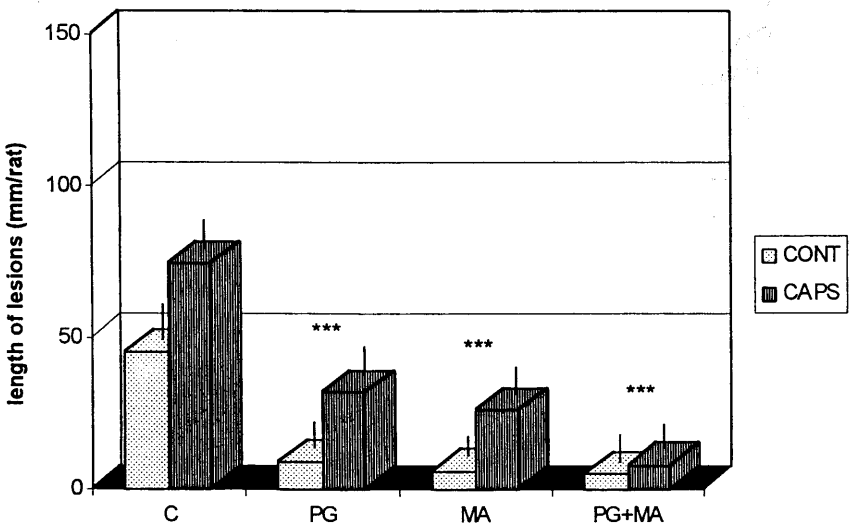

Fig. 6. *Effect of 50 mg.kg^{-1} malotilate (MA), 10 μg.kg^{-1} methyl-prostaglandin E_2 (PG) and their combination (MA+PG) on 50 % ethanol-induced gross gastric mucosal lesions in intact and capsaicin-induced sensory denervated rats. Results are expressed as means ± S.E.M. Significantly different from vehicle-pretreated (C) group: ***p<0.001. The location of symbols in the center of two columns represents significant differences of both substances compared to the control groups.*

Discussion

The effect of endogenous afferent sensory nervous system on the gastroprotective effect of malotilate was tested in intact (normal) and capsaicin-pretreated rats. The gastroprotective effect of malotilate has previously been established in several models of gastric mucosal injury, e. g. indomethacin- or ethanol-induced gastric mucosal lesions (Mirossay *et al.* 1995, 1996a,b). The protective effect of malotilate is quite evident, however, the mechanism of its action is not clear. Nevertheless, the effect of malotilate seems to be more pronounced in ethanol-induced gastric mucosal damage. For this reason, we only used ethanol-induced gastric lesions in these experiments.

To determine the possible mechanism of action of malotilate, we performed experiments targeted to different pathophysiological pathways involved in gastric mucosal protection. We observed a significant inhibitory effect on the secreted volume and acidity of gastric juice (Mirossay *et al.* 1995). This inhibition positively correlated with the protection against indomethacin-induced gastric mucosal damage. The next step in our experimental procedure was to evaluate the effect of endogenous prostaglandin production on the effectiveness of malotilate. We suppressed this production with non-ulcerogenic doses of indomethacin (5 mg.kg^{-1}), but there was no evidence that malotilate acts through this pathway (Mirossay *et al.* 1996b). In additional experiments presented in this paper, the oral application of methyl-PGE_2 had an additive effect if given with malotilate, both substances being given in submaximal effective doses. We concluded from these previous results that the mechanism of malotilate action should partially depend on the inhibition of gastric acid secretion. At the same time, its effect is independent of the endogenous production of prostaglandins. This was concluded on the basis of their suppression, which was not associated with any change in malotilate efficacy, and their additive effect with malotilate when given orally.

When the effectiveness of malotilate in different gastric ulcer models is compared, it should be stressed that it was more effective in ethanol-induced gastric lesions. Therefore, malotilate should be considered as a

directly acting gastroprotective agent providing protection independent of gastric acid secretion, a property ascribed to prostaglandins (Robert *et al.* 1984). If these two types of agents act independently as we have proposed above, we should look for some other mechanism of malotilate action.

Furthermore, evidence has been presented that capsaicin-sensitive primary sensory neurons participate in the physiological control of gastric mucosal protection from experimental injury (Holzer *et al.* 1991a,b).

These neurons exert their effect in acute gastroprotection by liberating vasodilator substances and mediate the gastroprotective effect of various agents. Gastric protection provided, for example, by honey is solely dependent on the presence of intact sensory neurons, whereas sucralfate-induced gastroprotection is mediated by sensory neurons and prostaglandin system (Al-Swayeh and Ali 1998). Besides this mediating effect, afferent neurons constitute an emergency system that is called into operation when the gastric mucosa is endangered by acid and other noxious chemicals. Peptides released from the peripheral endings of sensory neurons are using nitric oxide as their common messenger responsible for vasodilation. This mechanism limits injury to the surface of the mucosa and creates favorable conditions for rapid restitution and healing of the damaged mucosa (Holzer 1998). This has been also demonstrated in the present paper (see below).

We have compared intact and sensory capsaicin-denervated rats in our experiments. Both malotilate and methyl-PGE_2 decreased the injury induced by 96 % ethanol in a dose-dependent manner. However, there was no significant difference between intact and capsaicin-denervated rats as had been observed by other researchers (Holzer *et al.* 1991). We have found that 96 % ethanol is a highly noxious agent which is responsible for this lack of difference between normal and sensory denervated rats. Significantly different results in groups treated with the same substance were obtained only after using a milder irritant, namely 50 % ethanol. Distinct lesser injury in saline-pretreated animals as well as a higher effect of both methyl-PGE_2 or malotilate were observed in groups of intact versus sensory denervated rats. However, there was no significant difference in gastric injury between these two groups when treated with a combination of the tested substances. It is possible that the protective function of sensory nerves does not depend on vagal efferent or sympathetic neurons, or involves prostaglandins. It has previously been shown that the protective effect of prostacyclin on gastric mucosa is retained after systemic or topical capsaicin desensitization (Abdel-Salam *et al.* 1997). This was also demonstrated for methyl-PGE_2 in experiments in the present paper. Very similar results were obtained for malotilate.

In conclusion, we propose that, as in normally innervated gastric mucosa, the protective effects of both malotilate and prostaglandin in capsaicin-denervated animals i) are only partly dependent on the appropriate sensory nerve supply, ii) their protective effects are additive, iii) they depend on the concentration and type of the noxious stimulus, and iv) malotilate acts by similar mechanism(s) as prostaglandins but probably use other receptors or transduction pathways leading to the protective effect on the rat gastric mucosa.

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