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Short communication

Nitric oxide and sensory afferent neurones modulate the protective effects of low-dose endotoxin on rat gastric mucosal damage

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Abstract

Pretreatment (1 h) with low doses (5–40 μg/kg i.p.) of *Escherichia coli* endotoxin dose dependently reduced the gastric mucosal damage induced by a 10 min challenge with 1 ml ethanol (50% and 100%) in conscious rats. Treatment with the nitric oxide synthesis inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 5 and 10 mg/kg i.p.), significantly inhibited the protective effects of endotoxin (40 μg/kg i.p.). The actions of L-NAME were reversed by the prior administration of L-arginine (100 mg/kg i.p.). The protective effects of endotoxin were not influenced by pretreatment with dexamethasone (5 mg/kg s.c. twice) or indomethacin (5 mg/kg s.c.). However, ablation of sensory afferent neurones by capsaicin pretreatment (20, 30 and 50 mg/kg s.c.) abolished the mucosa protective effects of endotoxin (40 μg/kg). These findings suggest that the protection elicited by low doses of endotoxin against ethanol-induced mucosal damage involves synthesis of nitric oxide and activation of sensory neurones.

Keywords: Nitric oxide (NO); Endotoxin; Capsaicin; Gastric damage; Gastric defense; Stress

1. Introduction

Endotoxin can exert differential acute effects on the gastric mucosa depending on the dose administered. Thus, doses of endotoxin in the mg/kg range produce acute mucosal damage involving the release of different endogenous mediators harmful to the gastric mucosa such as thromboxane or platelet activating factor (Whittle and Esplugues, 1989). In contrast, administration of much lower doses of endotoxin (in the μg/kg range) protects the gastric mucosa against ulcerogenic stimuli such as stress, nonsteroid anti-inflammatory drugs and ethanol (Tsuji et al., 1993), but the mechanisms involved in these protective effects of endotoxin have not been explained. In previous studies, we have shown that low doses of endotoxin induce a rapid inhibition of gastric acid secretion by a process involving the synthesis of nitric oxide (NO) (Martínez-Cuesta et al., 1992). We have now investigated, by the use of the inhibitor of NO biosynthesis, N^G-nitro-L-arginine methyl ester (L-NAME), the role of endogenous nitric oxide in the protective effects of endotoxin against gastric mucosal damage induced by ethanol. Furthermore, the possible involvement of prostaglandins and sensory afferent neurones has also been assessed by the use of indomethacin to inhibit cyclo-oxygenase and capsaicin pretreatment to deplete sensory neuropeptides, respectively.

2. Materials and methods

Female Sprague-Dawley rats (200–250 g) were deprived of food but not water for 18–22 h prior to an experiment. Animals were injected i.p. with saline or endotoxin (5–40 μg/kg), 1 h before intragastric administration of 1 ml ethanol (50–100% v/v). Additional groups of rats were injected i.p. with either L-NAME (5 or 10 mg/kg), L-arginine (100 mg/kg) or saline immediately before endotoxin. In another group of experiments animals were treated with indomethacin

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(5 mg/kg s.c., 1 h before endotoxin) or dexamethasone (5 mg/kg s.c., 16 and 3 h before endotoxin).

In a further group of studies, adult rats were treated with increasing doses of capsaicin for 3 consecutive days (20, 30 and 50 mg/kg s.c.) to deplete sensory neuropeptides from primary afferent neurones (Esplugues and Whittle, 1990; Holzer, 1991). All capsaicin injections were performed under ether anaesthesia following pretreatment (i.m.) with salbutamol (0.1 mg/kg) and aminophylline (10 mg/kg), and the rats were used 12 days after the last dose of capsaicin. Control animals received a similar regimen of treatment with the capsaicin vehicle alone.

The rats were killed by cervical dislocation 10 min after ethanol administration. The stomachs were removed, opened along the greater curvature, pinned to a wax block, coded to avoid observer bias and photographed on colour transparency film. The area of mucosal damage was calculated via computerized planimetry and expressed as the percentage of the total gastric mucosa showing macroscopically visible damage (Esplugues and Whittle, 1990).

Escherichia coli endotoxin (serotype 0111:B4), L-NAME, and L-arginine were purchased from Sigma Chemical Co. Indomethacin (Inacid, Merck Sharp Dohme), dexamethasone (Fortecortin, Merck), salbutamol (Ventolin, Glaxo) and aminophylline (Eufilina, Elmu) were used as clinically available preparations. Capsaicin was obtained from Fluka Chemic and freshly prepared as a 50 mg/ml solution containing absolute ethanol, Tween 80 and isotonic saline (10:10:80 v/v/v). All other drugs were dissolved in saline immediately before use and administered in a volume of 1 ml/kg.

Results are expressed as means ± S.E.M. A one-way analysis of variance followed by the Bonferroni *t*-test was used for multiple comparison, and *P* values of less than 0.05 were taken as significant.

3. Results

Intragastric administration of 1 ml of 50% ethanol (10 min) resulted in macroscopically detectable damage to the rat gastric mucosa involving 11 ± 2% ($n = 26$) of the total mucosal area. Following i.p. pretreatment (1 h) with 5, 15, 30 and 40 μg/kg of endotoxin, the damage induced by 50% ethanol was decreased to 11 ± 3% ($n = 5$), 7 ± 3% ($n = 4$), 5 ± 1% ($n = 8$) and 3 ± 1% ($n = 26$, $P < 0.001$) of the total mucosa area respectively. Under our experimental conditions, administration of endotoxin (40 μg/kg i.p.) alone did not cause any macroscopic damage to the rat gastric mucosa ($n = 3$). As shown in Fig. 1, pretreatment with L-NAME (5 and 10 mg/kg i.p.) re-established the damaging effects of 50% ethanol on the gastric mucosa

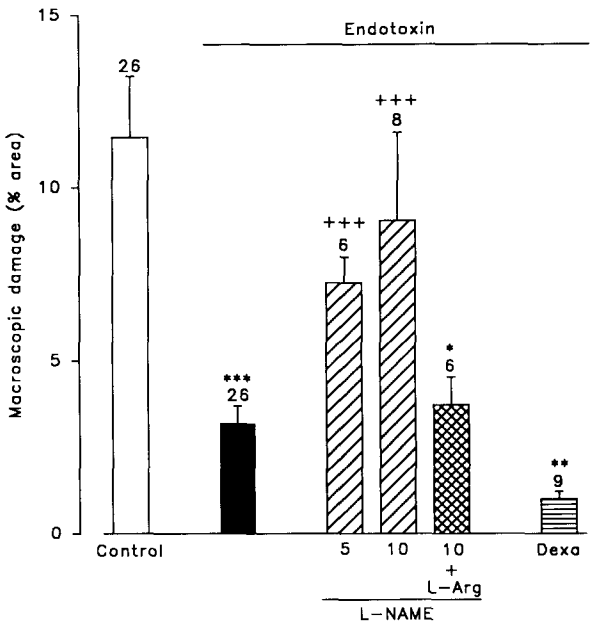

Fig. 1. Effect of N^G-nitro-L-arginine methyl ester (L-NAME, 5–10 mg/kg) on gastric mucosal protection induced by endotoxin (40 μg/kg i.p.) in rats challenged with intragastric 50% ethanol (1 ml). The administration of L-arginine (100 mg/kg i.p., L-Arg) restored endotoxin-induced gastric protection in animals treated with L-NAME. Pretreatment with dexamethasone (5 mg/kg s.c., 3 and 16 h before endotoxin, Dexa) did not modify the protective effects of endotoxin. Results, shown as the percentage of the total mucosal area that exhibited macroscopically assessed damage, are expressed as means ± S.E.M. of *n* (figures outside columns) experiments. Significant differences from the group receiving ethanol are shown as $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ and from the group receiving endotoxin are shown as $^{+++}P < 0.001$.

of animals treated with endotoxin (40 μg/kg i.p.). These effects of L-NAME were prevented by prior i.p. administration of L-arginine (100 mg/kg). Neither L-NAME (10 mg/kg) nor L-arginine (100 mg/kg) significantly modified the gastric mucosal damage induced by ethanol (50%), with values of 15 ± 2% and 11 ± 3% of the total mucosa respectively ($n = 5$ in both). Pretreatment with dexamethasone (5 mg/kg s.c., 16 and 3 h before endotoxin) did not reverse the mucosa protection elicited by endotoxin (40 μg/kg i.p.) on ethanol-induced mucosal damage (Fig. 1), nor did it significantly affect the damage induced by ethanol (50%) alone (12 ± 3, $n = 9$).

Treatment with indomethacin or pretreatment (12 days) with capsaicin increased the extent of mucosal damage induced by intragastric 50% ethanol (Fig. 2). Administration of endotoxin (40 μg/kg i.p.) significantly ($P < 0.01$) reduced this augmented gastric damage provoked by ethanol in indomethacin-treated rats. However, the same dose of endotoxin failed to influence the elevated mucosal damage elicited by ethanol in capsaicin-treated rats (Fig. 2). Since the reduced ability of endotoxin to inhibit damage in this latter

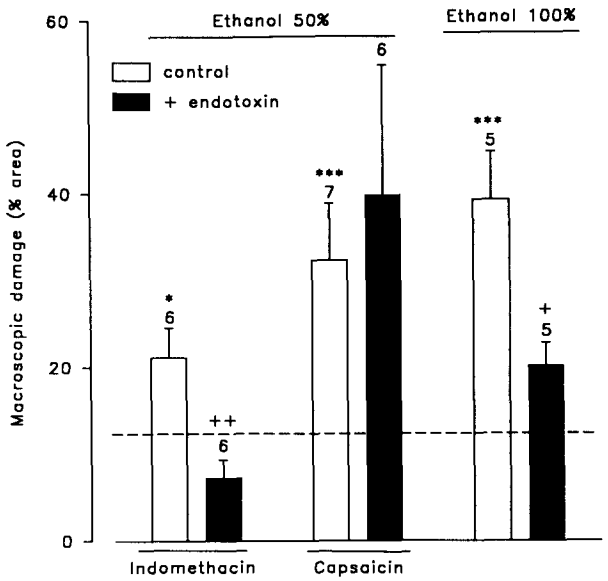

Fig. 2. Effect of indomethacin (5 mg/kg s.c.) and capsaicin pretreatment (2 weeks earlier) on gastric mucosal damage induced by intragastric challenge with 50% ethanol (1 ml). The administration of endotoxin (40 μg/kg i.p.) significantly reduced gastric damage in indomethacin-treated rats without influencing that of capsaicin-treated animals. In animals challenged with 100% ethanol (1 ml), endotoxin (40 μg/kg i.p.) protected against gastric mucosal damage alone. Results, shown as the percentage of the total mucosal area that exhibited macroscopically assessed damage, are expressed as means ± S.E.M. of n (figures outside columns) experiments. The dotted line represents the level of gastric damage in vehicle-treated rats receiving 1 ml of 50% ethanol and significant differences from this group are shown as $^{*}P < 0.05$ and $^{***}P < 0.001$. Significant difference from the respective control group is shown as $^{+}P < 0.05$ and $^{++}P < 0.01$.

group could reflect the greatly increased extent of mucosal damage, further studies were also conducted following intragastric challenge with a higher ethanol concentration. Thus, treatment with 100% ethanol alone induced a level of damage that was not significantly different from that observed with 50% ethanol following pretreatment with capsaicin. However, endotoxin (40 μg/kg i.p.) substantially reduced the gastric mucosal damage induced by ethanol (100%) alone (Fig. 2).

4. Discussion

It has been previously shown that low doses of endotoxin, a substance long considered as pro-ulcerogenic, acutely protect the gastric mucosa against different mucosa-damaging agents when administered at small doses (Tsuji et al., 1993). The present study confirms this protective effect of low doses of endotoxin in a model of ethanol-induced mucosal damage. Furthermore, the finding that pretreatment with L-NAME reversed the effects of endotoxin, an action prevented by L-arginine, suggests a role for endogenous NO in such a protective response. Endogenous nitric oxide has been implicated in the control of gastric functions, in particular NO plays an important role in the modulation of gastric microcirculation (Piqué et al., 1989). Furthermore, NO participates in the gastroprotection elicited by endogenous peptides such as cholecystokinin (Stroff et al., 1994), or drugs such as sucralfate (Konturek et al., 1992) or antacids (Lambrecht et al., 1993). The systemic vasopressor actions of L-NAME do not seem responsible for the increase of gastric mucosal damage occurring in endotoxin-treated rats, since in control rats, administration of L-NAME did not significantly enhance mucosal damage induced by 50% ethanol. Furthermore, the doses of endotoxin used in our experiments do not produce any significant fall in blood pressure (Martínez-Cuesta et al., 1994; Barrachina et al., 1995).

It is well known that the inducible NO-synthase is responsible for the increase in synthesis of NO that occurs some 3–6 h after exposure to endotoxin, and its expression is inhibited by glucocorticoids (Whittle, 1994). It is therefore unlikely that such an inducible isoenzyme could be involved in the protection elicited by endotoxin against mucosal damage since ethanol was administered only 60 min after endotoxin and, in addition, this protective response was not influenced by dexamethasone in doses sufficient to prevent its induction in gastrointestinal tissue (Whittle, 1994).

In previous studies we have shown that similar low doses of endotoxin inhibit gastric acid secretion in vivo by acute activation of a neuronal pathway involving the endogenous release of NO (Martínez-Cuesta et al., 1994; Barrachina et al., 1995), and an analogous mechanism could explain the origin of the NO involved in the mucosa protective actions of endotoxin. Although further experiments will be needed to confirm this concept, there is already evidence for a role of endogenous NO in gastric mucosa protection following central vagal stimulation (Király et al., 1993). The presence of acid is of little importance in the mucosa damaging effects of ethanol (Morris and Wallace, 1981) and therefore inhibition of acid production cannot explain the protective effects of endotoxin in this present model. In addition, in the current study the effects of endotoxin were not influenced by pretreatment with indomethacin, thus indicating a protective action of endotoxin independent of the biosynthesis of endogenous prostaglandins. However, ablation of sensory afferent neurones with capsaicin abolished the protective effects of endotoxin. This finding reinforces the idea of a neural process that involves NO in these protective actions of endotoxin. Likewise, it supports the proposed interaction between endogenous NO and sensory neuropeptides, released from primary afferent

neurones, in the modulation of gastric mucosa integrity (Whittle et al., 1990).

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