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Modulation by opioids and by afferent sensory neurones of prostanoid protection of the rat gastric mucosa

J.V. Esplugues, [1,*] B.J.R. Whittle & *S. Moncada

Department of Pharmacology, University of Valencia, Valencia, Spain and *Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

1 Pretreatment with capsaicin, to deplete sensory neuropeptides from primary afferent neurones or the administration of morphine (9 mg kg^{-1}, i.v.), which can inhibit neuropeptide release, augmented gastric mucosal injury induced by a 5 min challenge with intragastric ethanol in the rat, as assessed by macroscopic and histological evaluation.

2 Morphine administration substantially attenuated the protective actions of the prostaglandin analogue 16,16 dimethyl prostaglandin E_2 (dm PGE_2; 0.5–20 $\mu g\ kg^{-1}$, p.o.) against ethanol-induced damage. This reduced degree of protection by dmPGE_2 was not however, the consequence of the enhanced level of damage.

3 These actions of morphine in reducing prostaglandin protection against mucosal injury were abolished by pretreatment (5 min) with naloxone (1 mg kg^{-1}, i.v.) or the peripherally acting opioid antagonist, N-methyl nalorphine (6 mg kg^{-1}, i.v.).

4 Capsaicin pretreatment (2 weeks before study), likewise attenuated the protective actions of dmPGE_2, although to a lesser degree than did morphine.

5 These findings, thus implicate the involvement of capsaicin- and opioid-sensitive afferent neurones in the processes by which exogenous prostanoids can protect the gastric mucosa from damage.

Keywords: Opioids; morphine, capsaicin; 16,16-dimethyl PGE_2; prostaglandins; sensory neurones; mucosal protection; gastric damage

Introduction

It has been proposed that the maintenance of gastric mucosal integrity is regulated by the interaction between local mediators such as sensory neuropeptides, nitric oxide (NO) and prostaglandins (Whittle *et al.*, 1990).

A role for sensory neuropeptides has been deduced from the findings that chronic systemic administration of capsaicin, which depletes sensory neuropeptides from primary afferent neurones, can substantially enhance gastric mucosal injury induced by a number of pro-ulcerogenic agents (Szolcsanyi & Bartho, 1981; Holzer & Sametz, 1986; Esplugues *et al.*, 1989). Under such conditions, the inhibitor of NO biosynthesis, N^G-monomethyl-L-arginine (L-NMMA), induces acute mucosal haemorrhage and erosion, indicating the involvement of NO in mucosal integrity and its interaction with sensory neuropeptides (Whittle *et al.*, 1990).

Opioids, that can act peripherally on sensory neurones (Ferreira & Nakamura, 1979; Bartho & Szolcsanyi, 1981; Smith & Buchan, 1984) and can inhibit neuropeptide release (Lembeck & Donnerer, 1985), can likewise augment mucosal damage (Esplugues *et al.*, 1989; Esplugues & Whittle, 1990; Whittle & Lopez-Belmonte, 1991; Holzer *et al.*, 1991). Conversely, acute intragastric capsaicin administration, which stimulates the neuronal release of sensory neuropeptides, protects the mucosa against damage (Holzer & Lippe, 1988; Holzer *et al.*, 1990). Furthermore, intra-arterial administration of calcitonin-gene related peptide (CGRP), the predominant sensory neuropeptide in the rat gastric mucosa (Green & Dockray, 1988), also reduces gastric injury (Lippe *et al.*, 1989; Whittle & Lopez-Belmonte, 1991).

The involvement of endogenous prostaglandins in the modulation of mucosal integrity is suggested by the enhanced susceptibility of the gastric mucosa to challenge following treatment with indomethacin and other cyclo-oxygenase inhibitors (Whittle, 1983; Whittle & Vane, 1987). In addition, it is well-established that naturally-occuring prostaglandins and their synthetic analogues can potently protect against most forms of experimental gastric damage, although their mechanisms of action are complex and not clearly defined (Robert, 1976; Whittle, 1976; Robert *et al.*, 1979; Guth *et al.*, 1984; Henagan *et al.*, 1984; Whittle & Steel, 1985; Whittle & Vane, 1987).

The acute mucosal damage induced by indomethacin is augmented in capsaicin-pretreated rats, suggesting interactions between endogenous prostanoids and sensory neuropeptides (Holzer & Sametz, 1986; Whittle *et al.*, 1990). In the present study, the interactions of these local mediators in the processes by which the gastric mucosa can resist challenge and injury, have been further explored. Thus, the dependence on endogenous sensory neuropeptides of a prostaglandin E_2 analogue (16, 16, dimethyl PGE_2) to exert its protective actions against ethanol challenge, has been investigated by use of opioids and capsaicin treatment to modulate their release in the rat.

Methods

Induction and assessment of mucosal damage

Male Wistar rats (220–260 g body weight) were deprived of food, but not water, for 18–20 h before the experiment. One ml of ethanol (50% or 100% v/v in saline) or saline alone was administered orally by gavage, and the rats killed by cervical dislocation 5 min later. The stomachs were opened, pinned to a wax block immersed in neutral buffered formalin and photographed on colour transparency film.

The extent of macroscopically visible damage was subsequently determined from these slides in a randomized fashion via computerised planimetry. The area of mucosal damage was calculated as the % of the total gastric mucosal area showing macroscopically visible damage. The extent of macroscopic damage in both the corpus and antral regions

[1] Author for correspondence.

was also expressed as a % of the area of these individual regions.

Effects of 16, 16 dimethyl PGE_2

16, 16-dimethyl PGE_2 ($dmPGE_2$; 0.5, 1, 5 or 20 $\mu g\, kg^{-1}$), in doses chosen from previous studies (Whittle & Steel, 1985) or saline (1 ml kg^{-1}) was administered orally by gavage 10 min before challenge with intragastric ethanol. Macroscopic damage of the gastric mucosa was assessed as described above, 5 min after ethanol challenge.

Effects of morphine and opioid antagonists

Morphine (9 mg kg^{-1}) or saline (1 ml kg^{-1}) was injected into the tail vein 15 min before the intragastric challenge with ethanol. This dose of morphine has been shown in previous studies to induce a consistent near-maximal potentiation of ethanol-induced gastric damage in the rat (Esplugues & Whittle, 1990). Macroscopic mucosal damage was assessed 5 min after challenge.

In a further series of experiments, rats were pretreated with the opioid antagonists naloxone (1 mg kg^{-1}, i.v.) or N-methyl-nalorphine (6 mg kg^{-1}, i.v.) 5 min before the administration of morphine, in doses derived from previous studies in the rat (Esplugues & Whittle, 1990). Macroscopic damage was assessed 5 min after intragastric ethanol challenge.

Effects of pretreatment with capsaicin

Adult rats (190–220 g) were treated with capsaicin for three consecutive days (20, 30 and 50 mg kg^{-1}, s.c.). This regimen has been shown to lead to functional ablation of primary afferent neurones (see Holzer, 1988; 1991). All capsaicin injections were administered under halothane anaesthesia and, to counteract the respiratory impairment associated with the administration of capsaicin, the rats were pretreated with terbutaline (0.1 mg kg^{-1}, i.m.) and aminophylline (10 mg kg^{-1}, i.m.) prior to capsaicin injection. Control rats received a similar regimen with the capsaicin vehicle.

Two weeks after completion of the capsaicin treatment, $dmPGE_2$ (0.5, 1 or 20 $\mu g\, kg^{-1}$) or saline (1 ml kg^{-1}), was administered by gavage 10 min before intragastric ethanol challenge (50 or 100%, 1 ml) and the gastric damage was assessed 5 min later.

Histological assessment of mucosal damage

Two 1.5 × 0.5 cm segments of the corpus mucosa were excised from standardized areas of the stomach with tissue from both the dorsal and ventral aspects of the mid-corpus region being obtained 0.5 cm below the forestomach limiting ridge. Following processing by routine techniques and embedding in paraffin, the sections (4 μm) were stained with haematoxylin and eosin and examined under a light microscope.

The 1.5 cm length of each histological section was assessed for vasocongestion or haemorrhagic damage in the upper third of the mucosa (Type 1 damage); glandular disruption, vasocongestion or oedema in the mid mucosa (Type 2 damage); and haemorrhagic damage and necrosis in the lower mucosa (Type 3 damage). In this study, epithelial cell damage induced by ethanol and the various treatments was not separately assessed, since previous studies have shown it is affected by prostaglandin pretreatment only at high doses (Lacy & Ito, 1982; Whittle & Steel, 1985). All determinations were performed in a randomized manner with the histological sections coded to eliminate observer bias. The length of each section exhibiting each type of damage was expressed as a % of the total section length, and the mean value for the two sections of each corpus mucosa was calculated.

Drugs

Morphine hydrochloride (MacFarlane Smith, Edinburgh, U.K.) naloxone hydrochloride (Endo Labs, New York, U.S.A.), 16, 16-dimethyl PGE_2 (stored in absolute ethanol, Cayman Chemicals, MI, U.S.A.), aminophylline (Sigma Chemical Co., Poole, Dorset), terbutaline sulphate (Astra, Kings Langley, U.K.) and N-methyl-nalorphine (synthesized in the Dept. of Medicinal Chemistry, Wellcome Research Labs.) were dissolved in isotonic saline immediately before use. Capsaicin (Fluka Chemic AG Buchi, Switzerland) was prepared in a 50 mg ml^{-1} solution containing absolute ethanol, Tween 80 and isotonic saline (10:10:80 v/v/v). Ethanol (analar; B.D.H. Poole, Dorset) was diluted with saline as appropriate.

Statistical analysis

All data are expressed as the mean ± s.e.mean. Comparisons between groups of parametric data were made by Student's *t* test for unpaired data. Comparisons between groups of non-parametric data (histological evaluation) were made by the Mann-Whitney U-test. *P* values of less than 0.05 were taken as significant.

Results

Effects of 16, 16-dimethyl PGE_2 on ethanol-induced mucosal damage

Intragastric challenge with 1 ml of ethanol (50 or 100%) for 5 min induced a concentration-dependent degree of macroscopically assessed mucosal injury. Intragastric administration of ethanol (100%) resulted in macroscopic damage involving 40 ± 6% (n = 17) of the total mucosal area (Figure 1). This damage was of a haemorrhagic nature and located both in the corpus and antral regions (Table 1). Likewise, intragastric challenge with 50% ethanol induced mucosal damage (Figure 2) that occurred in both corpus and antral

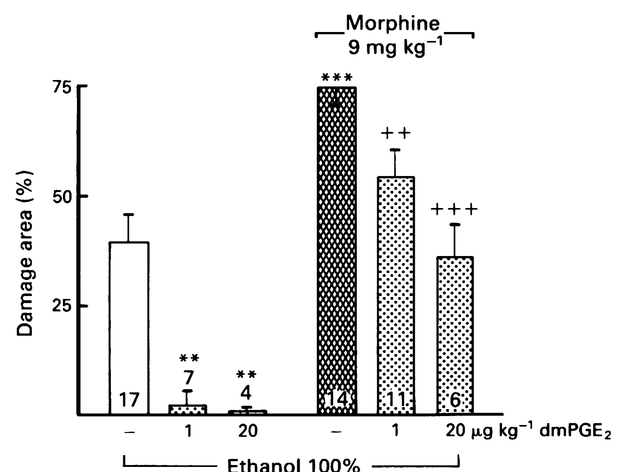

Figure 1 Effects of pretreatment with 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$, 1 and 20 $\mu g\, kg^{-1}$, p.o.) on the gastric mucosal damage induced by the intragastric administration of 100% ethanol (1 ml) alone, or in combination with morphine (9 mg kg^{-1}, i.v.). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, 5 min after challenge, are the mean with s.e.mean (vertical bars) of (n) experiments in each group. Significant difference from the control (ethanol-alone) group is given as $**P<0.01$ and $***P<0.001$, and from the corresponding ethanol and morphine group as ††$P<0.01$ and †††$P<0.001$. The inhibitory effects of $dmPGE_2$ (1 and 20 $\mu g\, kg^{-1}$) against ethanol-induced mucosal damage were significantly less ($P<0.001$ for both) in rats treated concurrently with morphine.

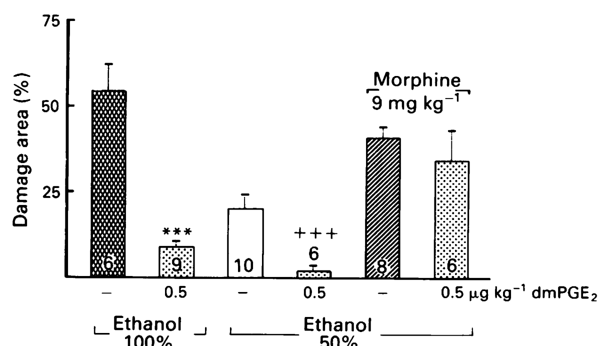

Figure 2 Effects of pretreatment with 16, 16-dimethyl prostaglandin E_2 ($dmPGE_2$, $0.5\,\mu g\,kg^{-1}$, p.o.) on the gastric mucosal damage induced by the intragastric administration of ethanol (50% or 100%, 1 ml) alone, or in combination with morphine ($9\,mg\,kg^{-1}$, i.v.). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, 5 min after challenge, are the mean with s.e.mean (vertical bars) of (n) experiments in each group. Significant difference from the respective control ethanol group is given as $^{***}P<0.001$; $\dagger\dagger\dagger P<0.001$. The inhibitory effects of $dmPGE_2$ against ethanol (50%)-induced damage were significantly less ($P<0.01$) in rats treated concurrently with morphine.

regions of the mucosa (Table 1). Gastric mucosal damage was not observed in any rat receiving only intragastric administration of 1 ml saline ($n = 5$).

Intragastric pretreatment with $dmPGE_2$ (1 or $20\,\mu g\,kg^{-1}$) near-maximally inhibited ($P<0.01$ for both) the macroscopic damage induced by intragastric instillation of 100% ethanol (Figure 1). Lower doses of $dmPGE_2$ ($0.5\,\mu g\,kg^{-1}$) likewise caused significant inhibition ($85 \pm 5\%$, $n = 5$; $P<0.01$) of the mucosal damage induced by ethanol (100%), and near-maximally inhibited that induced by ethanol (50%), as shown in Figure 2. Inhibition of ethanol-induced macroscopic damage by $dmPGE_2$ ($0.5\,\mu g\,kg^{-1}$), was seen in both the corpus and antral regions of the mucosa (Table 1). Administration of $dmPGE_2$ ($20\,\mu g\,kg^{-1}$) alone did not cause any macroscopically detectable damage to the rat gastric mucosa ($n = 5$).

Effects of pretreatment with morphine

The area of damage induced by 100% ethanol was significantly ($P<0.001$) increased to $74 \pm 4\%$ ($n = 14$) of the gastric mucosa by pretreatment (15 min) with morphine ($9\,mg\,kg^{-1}$, i.v.), as shown in Figure 1. Pretreatment with this dose of morphine alone did not cause any macroscopically detectable damage to the rat gastric mucosa ($n = 5$).

Under such conditions of morphine pretreatment, administration of $dmPGE_2$ ($1\,\mu g\,kg^{-1}$, p.o.) did not substantially inhibit the degree of damage induced by ethanol challenge (Figure 1). Furthermore, following morphine administration, the reduction of ethanol-induced damage by $dmPGE_2$ ($20\,\mu g\,kg^{-1}$) was markedly attenuated (from $99 \pm 1\%$, $n = 7$ to $54 \pm 6\%$, $n = 6$, $P<0.001$), as shown in Figure 1.

Since the reduced ability of $dmPGE_2$ to inhibit the damage induced by 100% ethanol following pretreatment with morphine could have reflected the increased extent of mucosal damage, further studies were conducted with a lower ethanol concentration under similar conditions. Thus, pretreatment with morphine ($9\,mg\,kg^{-1}$, i.v.) augmented the area of damage induced by intragastric challenge with 50% ethanol to a level not significantly different from that observed with 100% ethanol alone (Figure 2). However, even under these conditions, $dmPGE_2$ ($0.5\,\mu g\,kg^{-1}$, p.o.) did not significantly inhibit damage caused by 50% ethanol following morphine pretreatment, although damage induced by intragastric challenge with 50% or 100% ethanol alone was reduced by

Table 1 Effect of morphine administration or capsaicin pretreatment on prostaglandin protection of different regions of the rat gastric mucosa

	Damage (% area)		
	Corpus	*Antrum*	*(n)*
Ethanol (100%)	31 ± 7	32 ± 7	(12)
+ $dmPGE_2$	$5 \pm 1^{**}$	$11 \pm 4^{*}$	(14)
Ethanol (50%)	19 ± 4	14 ± 5	(10)
+ $dmPGE_2$	$2 \pm 1^{**}$	$1 \pm 1^{**}$	(9)
Ethanol (50%)			
+ morphine	$35 \pm 4\dagger$	$37 \pm 6\dagger$	(11)
+ morphine + $dmPGE_2$	26 ± 6	32 ± 9	(9)
Ethanol (50%)			
+ capsaicin	$30 \pm 5\dagger$	$47 \pm 13\dagger$	(5)
+ capsaicin + $dmPGE_2$	$10 \pm 2^{*}$	21 ± 8	(5)

The data show mucosal damage in the antral and corpus regions of the rat stomach following a 5 min intragastric challenge with 50% or 100% ethanol (1 ml) and the effects of administration of 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$, $0.5\,\mu g\,kg^{-1}$, p.o.) morphine ($9\,mg\,kg^{-1}$, i.v.) or capsaicin pretreatment.

Results, shown as the % of the mucosal area of the antrum or of the corpus that exhibited macroscopic damage, are the mean $\pm$ s.e.mean of (n) experiments in each group. Significant difference between the ethanol-challenged groups treated with vehicle and those treated with $dmPGE_2$ are shown as $^{*}P<0.05$, $^{**}P<0.01$. The significant increase in ethanol (50%)-induced damage by morphine or capsaicin treatment in both regions is shown as $\dagger P<0.05$.

85% and 98% respectively ($P<0.001$ for both) as shown in Figure 2. This ablation of the protective actions of $dmPGE_2$ ($0.5\,\mu g\,kg^{-1}$) by morphine pretreatment was observed in both the corpus and antral regions of the mucosa (Table 1).

In further studies, 5 min pretreatment (i.v.) with either the opioid antagonist naloxone ($1\,mg\,kg^{-1}$), which acts on both central and peripheral opioid receptors, or N-methyl nalorphine ($6\,mg\,kg^{-1}$), a peripherally acting quaternary opioid receptor antagonist, restored the protective effects of $dmPGE_2$ ($0.5\,\mu g\,kg^{-1}$) as shown in Figure 3. Neither naloxone nor N-methyl nalorphine significantly affected the area of damage induced by 50% ethanol alone ($n = 4$ for each, data not shown).

Effects of pretreatment with capsaicin

Capsaicin pretreatment, 2 weeks before the study, significantly ($P<0.01$) increased the area of macroscopic damage induced by the intragastric administration of 50 or 100% ethanol (Table 1 and Figure 4 respectively). Gastric damage was not observed in capsaicin-pretreated animals receiving a similar intragastric volume (1 ml) of saline ($n = 4$). The increase in ethanol-induced mucosal damage by capsaicin pretreatment was seen in both the corpus and antral regions (Table 1).

Pretreatment (10 min) with $dmPGE_2$ ($1\,\mu g\,kg^{-1}$, p.o.) near-maximally inhibited the macroscopically assessed mucosal damage induced by 100% ethanol in control vehicle-pretreated rats, as shown in Figure 4. However, in capsaicin-pretreated rats, the effects of $dmPGE_2$ ($1\,\mu g\,kg^{-1}$) were significantly ($P<0.001$) attenuated (Figure 4). Furthermore, under these conditions, even high doses of $dmPGE_2$ ($20\,\mu g\,kg^{-1}$) failed to abolish this macroscopic damage (Figure 4).

Further studies were conducted to ensure that the reduced protection by $dmPGE_2$ in capsaicin-pretreated rats was not simply a reflection of the higher degree of mucosal damage induced by ethanol challenge. Thus, the level of damage induced by intragastric 50% ethanol in capsaicin-pretreated rats was not significantly different from that induced by 100% ethanol in vehicle-pretreated animals (Figure 5).

Administration of $dmPGE_2$ (0.5 µg kg^{-1}) near-maximally inhibited the mucosal damage induced by challenge with 100% ethanol alone, whereas in capsaicin-pretreated rats, there was a significant attenuation of the protective actions of $dmPGE_2$ against challenge with 50% ethanol (Figure 5), which was observed in both corpus and antral mucosal regions (Table 1).

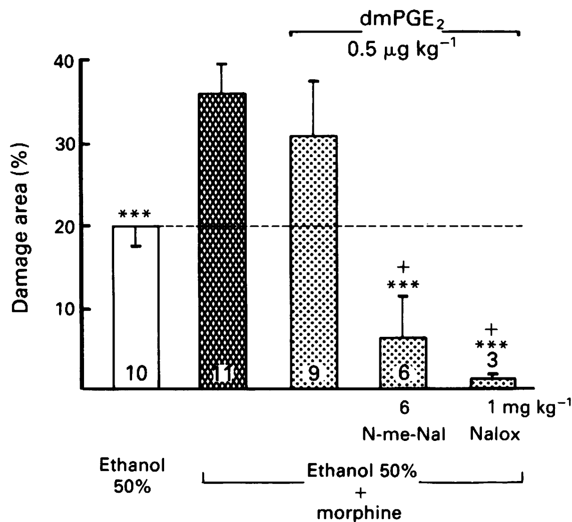

Figure 3 Pretreatment with the opioid antagonist naloxone (1 mg kg^{-1}, i.v.) and the peripherally acting antagonist, N-methyl nalorphine (N-me-Nal 6 mg kg^{-1}, i.v.) restores the protective effect of 16, 16-dimethyl prostaglandin E_2 ($dmPGE_2$, 0.5 µg kg^{-1}, p.o.) against gastric mucosal damage induced by 50% ethanol (1 ml, p.o.) in rats treated with morphine (9 mg kg^{-1}, i.v.). Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage 5 min after challenge, are the mean with s.e.mean (vertical bars) of (*n*) experiments in each group. Significant difference from the control ethanol-morphine group is given as $^{***}P<0.001$, and from the ethanol, morphine and $dmPGE_2$ group as $†P<0.001$.

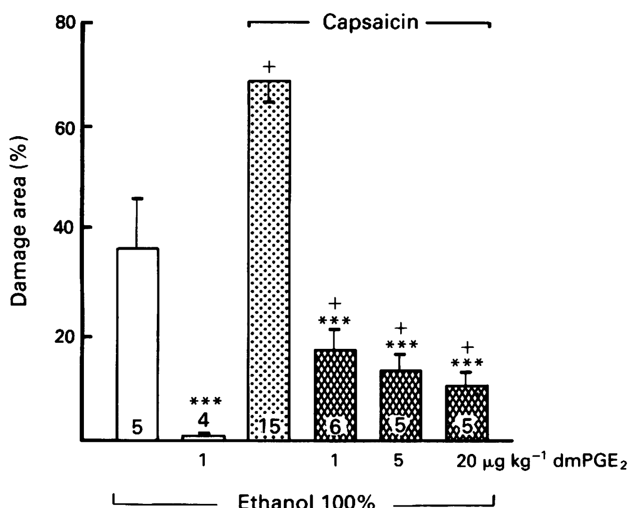

Figure 4 Effects of 16, 16-dimethyl prostaglandin E_2 ($dmPGE_2$ 1, 5 and 20 µg kg^{-1}, p.o.) on the gastric mucosal damage induced by the intragastric administration of 100% ethanol (1 ml), in control or capsaicin-pretreated rats. Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, 5 min after challenge, are the mean with s.e.mean (vertical bars) of (*n*) experiments in each group. Significant difference from the control (ethanol-alone) group is given as $^{***}P<0.001$ and from the corresponding ethanol or ethanol-capsaicin group as $†P<0.01$.

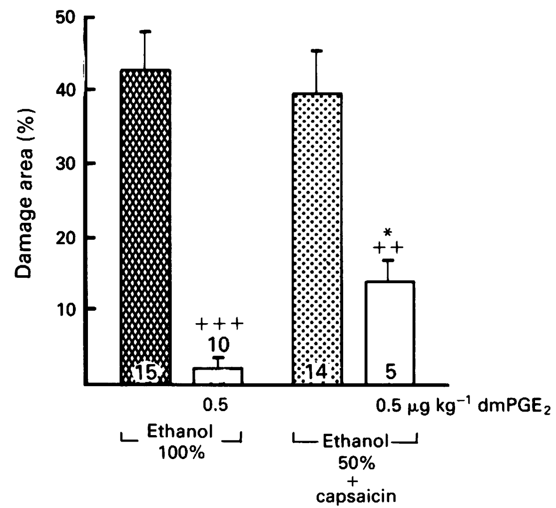

Figure 5 Effects of pretreatment with 16, 16-dimethyl prostaglandin E_2 ($dmPGE_2$, 0.5 µg kg^{-1}, p.o.) on the gastric mucosal damage induced by the intragastric administration of 100% ethanol (1 ml) alone, or 50% ethanol in capsaicin pretreated rats. Results, shown as the % of the total mucosal area that exhibited macroscopically assessed damage, 5 min after challenge, are the mean with s.e.mean (vertical bars) of (*n*) experiments in each group. Significant difference from the respective control ethanol-challenge group is given $††P<0.01$ or $†††P<0.001$, and between prostaglandin treated groups as $^{*}P<0.01$.

Histological assessment of gastric damage

Intragastric administration of 100% ethanol (1 ml) caused damage to the upper (Type 1 damage) and, mid (Type 2 damage) regions of the mucosa, accompanied by Type 3 damage (characterized as deep haemorrhagic damage and necrosis), as shown in Table 2. Administration of $dmPGE_2$ (0.5 µg kg^{-1}) reduced the level of histological damage after challenge with 100% ethanol to values similar to those of the non-challenged control group receiving intragastric saline (Table 2).

Rats treated with morphine (9 mg kg^{-1}) or pretreated with capsaicin prior to challenge with 50% ethanol showed levels of histologically assessed damage similar to that following challenge with 100% ethanol alone (Table 2). Pretreatment with either morphine or capsaicin alone did not significantly modify the extent of Type 1, 2 or 3 damage compared to vehicle control groups in the absence of ethanol challenge (Table 2).

Following pretreatment with morphine (9 mg kg^{-1}), dm-PGE_2 (0.5 µg kg^{-1}) failed to reduce significantly the degree of Type 1, Type 2 and Type 3 damage induced by 50% ethanol (Table 2). Likewise, in capsaicin-pretreated rats, the protective effects of $dmPGE_2$ (0.5 µg kg^{-1}) against Type 1, 2 and 3 damage induced by 50% ethanol were substantially less than its actions against the mucosal damage induced by 100% ethanol alone (Table 2).

Discussion

The present study demonstrates that morphine administration or capsaicin pretreatment, which alone do not induce detectable damage to the gastric mucosa, potentiated the mucosal injury following a 5 min challenge with intragastric ethanol, confirming previous studies (Esplugues & Whittle, 1990). Since capsaicin administration depletes sensory neuropeptides from afferent sensory neurones (Holzer, 1988; 1991), while morphine can affect capsaicin-sensitive neurones and inhibit neuropeptide release (Bartho & Szolcsanyi, 1981; Lembeck & Donnerer, 1985; Mantelli *et al.*, 1989) these

Table 2 Histological assessment of the effect of morphine administration or capsaicin-pretreatment on prostaglandin protection of the rat gastric mucosa

	Histological damage type (% total length)			
	1	*2*	*3*	*(n)*
Control	3 ± 2	0	0	(4)
Morphine	2 ± 3	0	0	(6)
Capsaicin	5 ± 3	0	0	(4)
Ethanol (100%)	85 ± 7	47 ± 7	18 ± 8	(7)
Ethanol (100%) + $dmPGE_2$	9 ± 3***	3 ± 3**	0***	(8)
Ethanol (50%) + morphine	79 ± 9	55 ± 6	21 ± 3	(15)
Ethanol (50%) + morphine + $dmPGE_2$	66 ± 5	47 ± 4	19 ± 3	(6)
Ethanol (50%) + capsaicin	89 ± 5	60 ± 5	26 ± 8	(14)
Ethanol (50%) + capsaicin + $dmPGE_2$	43 ± 7†	23 ± 4†	6 ± 2††	(5)

The influence of morphine (9 mg kg^{-1}, i.v.) or capsaicin pretreatment on the histologically assessed protective effects of 16, 16-dimethyl prostaglandin E_2 ($dmPGE_2$; 0.5 μg kg^{-1}, p.o.) on gastric mucosal damage induced by a 5 min intragastric challenge with ethanol (50 or 100%) is shown.

The data are expressed as the length of section exhibiting damage of varying degrees, Type 1 (vasocongestion or haemorrhagic damage in the upper mucosa), Type 2 (glandular disruption and haemorrhagic damage in the mid mucosa) and Type 3 (deeper haemorrhage and necrosis), expressed as % of total section length. Results are given as mean ± s.e.mean of (*n*) values, where statistically significant difference from the ethanol 100% group is shown as $^{**}P<0.01$, $^{***}P<0.001$ and difference from the 50% ethanol with capsaicin as †$P<0.05$, ††$P<0.05$.

findings support the concept that such endogenous sensory neuropeptides play an important role in the regulation of mucosal protection against challenge (Szolcsanyi & Bartho, 1981; Holzer & Sametz, 1986; Esplugues *et al.*, 1989). Furthermore, the present findings demonstrate that the protection of the gastric mucosa by the prostaglandin analogue 16,16-dimethyl PGE_2 ($dmPGE_2$) is also modulated by capsaicin or morphine administration, implicating a role for endogenous sensory neuropeptides in its mechanism of action. In other studies, morphine has likewise been shown to attenuate the mucosal protection elicited by PGE_2 (Esplugues & Whittle, 1991).

The enhanced mucosal injury induced by morphine and capsaicin pretreatment, observed macroscopically, in both antral and corpus regions, was characterized histologically as an increase in vasocongestion, haemorrhage and glandular disruption. With such techniques, it was demonstrated by varying the concentration of the intragastric ethanol, that the attenuation by capsaicin and morphine of the protective actions of $dmPGE_2$ was not a result of the enhanced degree of damage. These actions of morphine were abolished by the opioid μ receptor antagonist naloxone and by the peripherally acting N-methyl nalorphine, consistent with an action of morphine on peripheral sensory neurones (Ferreira & Nakamura, 1979; Smith & Buchan, 1984; Esplugues & Whittle, 1990). However, although both morphine and capsaicin treatment augmented mucosal damage to a comparable degree, morphine was more effective than capsaicin pretreatment in attenuating the mucosal protective actions of $dmPGE_2$. This may reflect the ability of morphine to affect additional processes other than those that are capsaicin-sensitive. Thin unmyelinated C-fibres are primarily susceptible to the actions of capsaicin pretreatment in adult rats leading to neuropeptide depletion (Holzer, 1988; 1991), while morphine can not only prevent neuropeptide release from such neurones, but can also modulate the excitability of other sensory neurones such as A-delta afferents (Sastry, 1978). Additional effects of morphine through actions on opioid receptors located on other tissues or cells cannot, however, be excluded.

The processes involved in the interactions between prostanoids and sensory neurones in the gastric mucosa are not clear. However, it is known that PGE_2 potently stimulates afferent vagal C-fibres in the lungs after a single systemic exposure in the cat (Coleridge *et al.*, 1976; 1978). In rat skin, capsaicin-pretreatment reduces the oedema induced by intracutaneous injection of PGE_1, (Arvier *et al.*, 1979), while morphine inhibits the acute hyperalgesic actions of PGE_2 on cutaneous sensory neurones (Ferreira & Nakamura, 1979). Furthermore, a long lasting hyperalgesia action in the rat paw is induced by multiple intraplantar injections of PGE_2 or prostacyclin which reflects actions on sensory nerves (Nakamura-Craig & Smith, 1989). PGE_2 can also activate a capsaicin-sensitive reflex micturition in the rat (Maggi *et al.*, 1988). Since capsaicin-sensitive vagal afferent fibres are known to influence gastric function (Raybould & Tache, 1989; Esplugues *et al.*, 1990; Thiefin *et al.*, 1990), it is pertinent that earlier studies have demonstrated that vagotomy could abolish the protective actions of $dmPGE_2$ against ethanol-induced challenge (Henagan *et al.*, 1984).

The mechanisms underlying the protective actions of prostanoids in the stomach are complex, but local vascular actions are strongly implicated (Robert, 1981; Whittle & Vane, 1987). While direct vasodilator actions on the gastric mucosa by PGE_2, prostacyclin and their analogues may play a role (Whittle & Vane, 1987), prostanoids have also been shown to prevent stasis in the microcirculation following challenge by ethanol (Guth *et al.*, 1984; Pihan *et al.*, 1986), perhaps by altering cellular interactions within the microvasculature and preventing endothelial cell damage. Capsaicin-sensitive sensory neurones are located in close association with the submucosal vessels that regulate mucosal blood flow (Sharkey *et al.*, 1984; Ekblad *et al.*, 1985; Sternini *et al.*, 1987; Su *et al.*, 1987; Green & Dockray, 1988) while functional ablation of such neurones with capsaicin or morphine augments the detrimental microvascular changes following intravascular challenge (Pique *et al.*, 1990). Furthermore, sensory neuropeptide release induced by capsaicin, or close arterial infusion of CGRP, which protect against mucosal injury (Holzer & Lippe, 1988; Lippe & Holzer, 1989; Whittle & Lopez-Belmonte, 1991), also can elevate mucosal blood flow (Holzer *et al.*, 1991; Holzer & Guth, 1991) although it is possible that sensory neuropeptides can exert a direct action on the endothelium, preventing microvascular injury in the mucosa. Although not abolished, the protective actions of even high doses of $dmPGE_2$ were substantially attenuated by morphine or capsaicin treatment, and it is therefore feasible that sensory neuronal activation and neuropeptide release contribute to the vascular actions of prostanoids involved in the mucosal protective processes.

Endogenous prostanoids do not appear to be directly involved in the protective actions of endogenous sensory

neuropeptides since cyclo-oxygenase inhibition by indomethacin or aspirin did not modulate the protection following acute intragastric capsaicin administration (Holzer & Sametz, 1986; Holzer *et al.*, 1990). However, recent studies suggest that an inhibitor of NO biosynthesis can reduce the protective actions of such acute capsaicin application (Peskar *et al.*, 1991), which is consistent with the actions of L-NMMA in attenuating the cardiovascular actions of CGRP (Whittle, 1990). Furthermore, the reduction in resting gastric mucosal blood flow induced by L-NMMA (Pique *et al.*, 1989; 1992) or N^G-nitro-L-arginine methyl ester is substantially enhanced by capsaicin pretreatment, indicating an interactive role of endogenous neuropeptides and NO in the mucosal microcirculation (Whittle & Tepperman, 1991; Tepperman & Whittle, 1992). The present findings that gastric mucosal protection by an E-type prostaglandin can be modulated by sensory neuronal mechanisms thus further re-inforce the concept of interactions between sensory neuropeptides and prostanoids, which together with NO are involved in the regulation of mucosal integrity (Whittle *et al.*, 1990).

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