Original articles

T. BRZOZOWSKI, D. DROZDOWICZ, J. MAJKA, S. J. KONTUREK

STUDIES ON GASTROPROTECTION INDUCED BY CAPSAICIN AND PAPAVERINE

Institute of Physiology, University Medical School, Kraków, Poland

Capsaicin and papaverine are potent vasorelaxants with strong gastroprotective activity against damage induced by absolute ethanol. This protection was originally attributed to the increase in gastric mucosal blood flow (GBF) but the possibility that NO mediates the protective and hyperemic effects of capsaicin and papaverine has been little studied. Using N-nitro-L-arginine (L-NNA), a selective blocker of NO synthase, and L-arginine as a substrate for NO, we investigated the role of NO in protective action of capsaicin and papaverine against ethanol-induced gastric damage and in GBF. Pretreatment with capsaicin (0.1—0.5 mg/kg i.g.) or papaverine (0.1—2 mg/kg i.g.) reduced dose-dependently the area of ethanol-induced lesions, the LD_{50} being 0.3 and 1 mg/kg, respectively. This protection was accompanied by a gradual increase in the GBF. Intravenous (i.v.) injection of L-NNA (1.2—5 mg/kg), which by itself caused only a small increase in ethanol lesions, reversed dose-dependently the protective and hyperemic effects of capsaicin and papaverine against ethanol-induced damage and attenuated the increase in GBF induced by each of these agents alone. This deleterious effect of L-NNA on the gastric mucosa and the GBF was fully antagonized by L-arginine (200 mg/kg i.v.) but not by D-arginine. L-arginine partly restored the decrease in GBF induced by L-NNA. Pretreatment with indomethacin (5 mg/kg i.p.), which suppressed the generation of PG by 85%, slightly enhaced the mucosal lesions induced by ethanol but failed to affect the fall in GBF induced by this irritant. Gastroprotective and hyperemic effects of capsaicin and papaverine were partly reversed by indomethacin suggesting that endogenous PG are also implicated in these effects. Addition of L-NNA to indomethacin completely eliminated both the protective and hyperemic effects of capsaicin and papaverine. We conclude that both NO and PG contribute to the gastroprotective and hyperemic effects of capsaicin and papaverine on the gastric mucosa.

INTRODUCTION

Capsaicin and papaverine are highly protective against acute gastric mucosal damage induced by absolute ethanol (1—4). Capsaicin, the pungent ingredient found in red pepper (5), represents a selective probe for the

stimulation of primary afferent neurons (6). Papaverine is a potent vasorelaxant showing gastroprotective action attributed to the stimulation of endogenous prostaglandins (PG) (7).

Afferent neurons are believed to participate in gastroprotection, since ablation of capsaicin-sensitive neurons leads to an aggravation of mucosal lesions induced by variety of ulcerogens (5, 8, 9). On the other hand, the stimulation of afferent neurons by intragastric administration of capsaicin was recently shown to induce the protection against gastric damage caused by absolute ethanol and aspirin (2, 10, 11) but the potential mediators and the mechanism of these effects have not been fully elucidated.

The local release of vasodilators such as epithelium derived relaxing factor (12), identified as nitric oxide (NO), is considered to play an essential role in the maintenance of mucosal integrity (13). The possibility that the protective action of capsaicin and papaverine is mediated by NO has not been fully explored, however, recent study by Peskar et al. (14) suggested that NO might be involved in gastroprotection induced by capsaicin. It is also unclear whether PG contribute to the gastroprotective activity of capsaicin and papaverine.

In this study, using N-nitro-L-arginine (L-NNA), a selective blocker of NO synthase, and indomethacin, a potent inhibitor of PG cyclooxygenase pathway, we attempted to determine the role of NO in both gastroprotective and circulatory effects of capsaicin and papaverine against lesions induced by absolute ethanol.

MATERIAL AND METHODS

Wistar rats of both sexes, weighing 180—220 g, were fasted 18 h in individual cages but had only free access to water before the study. Gastric lesions were induced by intragastric (i. g.) administration of 1.5 ml of 100% ethanol. Several series of experiments were carried out including; 1) control 100% ethanol (i. g.), 2) capsaicin (0.12—0.5 mg/kg i. g.) given 30 min. prior 100% ethanol, 3) papaverine (0.1—2 mg/kg i. g.) given 30 min prior 100% ethanol, 4) L-NNA (1.2—5 mg/kg i. v.) injected 15 min prior 100% ethanol, 5) L-NNA (1.2—5 mg/kg i. v.) injected 15 min prior to capsaicin (0.5 mg/kg i. g.) and then 30 min later followed by 100% ethanol, 6) L-NNA (1.2—5 mg/kg i. v.) injected 15 min prior to papaverine (1 mg/kg i. g.) and then followed 30 min later by 100% ethanol.

Since pretreatment with L-NNA abolished the gastroprotective activity of capsaicin and papaverine another series of experiments were performed using capsaicin (0.5 mg/kg) or papaverine (1 mg/kg) combined with L-NNA (5 mg/kg i. v.) alone or with L-arginine or D-arginine (200 mg/kg i. v.) followed 30 min later by 100% ethanol (1.5 ml). To assess the role of endogenous PG in gastroprotection by capsaicin and papaverine additional groups of rats were used and divided into the following treatment groups: 1) indomethacin (5 mg/kg i. p.) given 90 min prior 100% ethanol, 2) indomethacin (5 mg/kg i. p.) given 90 min prior capsaicin (0.5 mg/kg i. p.) then followed 30 min later by 100% ethanol, 3) indomethacin (5 mg/kg i. p.) given 90 min prior papaverine (1 mg/kg i. g.) then followed 30 min later by 100% ethanol, 4) L-NNA (5 mg/kg i. v.) injected 15 min prior to indomethacin and then 90 min later followed by the combination of

capsaicin (0.5 mg/kg i. g.) plus 100% ethanol, 5) L-NNA (5 mg/kg i. v.) injected 15 min prior indomethacin and then followed 90 min later by the combination of papaverine (1 mg/kg i. g.) plus 100% ethanol.

One hour after 100% ethanol was introduced, animals were anesthetized with ether, the abdomen was opened and the stomach was exposed to measure the gastric mucosal blood flow (GBF). Then the stomachs were removed and opened along the greater curvature for macroscopic examination. The area of gastric lesions was measured planimetrically (Morphomat, Carl Zeiss, Germany).

Measurement of the gastric blood flow.

Gastric blood flow was measured in the gastric mucosa of rats without or with ethanol administration using H_2-gas clearance technique as described previously (15). Briefly, the double needle electrodes were inserted through the serosa into the oxyntic mucosa, one electrode was used for the local generation of H_2-gas and the other for the measurement of tissue H_2. With this method, the H_2 generated is carried away by the blood and the polarographic current detector gives the decreasing tissue H_2 content as the clearance curve, which is then used to calculate absolute flow rates (ml/min/100 g) in the tissue. For determination of GBF in the gastric mucosa not exposed to ethanol additional rats were divided into the following treatment groups (6—8 animals) including: 1) vehicle (control), 2) capsaicin (0.5 mg/kg) alone, 3) papaverine (1 mg/kg) alone, 4) capsaicin (0.5 mg/kg) followed 30 min later by L-NNA (5 mg/kg i. v.), 5) papaverine (1 mg/kg) followed 30 min later by L-NNA (5 mg/kg i. v.), 6) L-NNA (5 mg/kg i. v.) given 15 min prior to capsaicin (0.5 mg/kg) and 7) L-NNA (5 mg/kg i. v.) given 15 min prior to papaverine (1 mg/kg). 30 min after the last treatment dose, the animals were lightly anesthetized with ether, the abdomen was opened and the GBF was recorded in the oxyntic mucosa.

Capsaicin (8-methyl-N-vanillyl-6-nonenamide; Fluka, Buchs, Switzerland) was dissolved in vehicle consisting of 10% ethanol, 10% Tween 80 and 80% saline (vol/vol/vol) as described previously by Yonei et al (8) and given to the rats in a volume of 1 ml. Papaverine (6, 7-dimetoxy-1-veratrylisoquinoline; Sigma Co., St. Louis) was dissolved in 1 ml of 0.9% saline and prepared freshly before use. Control animals received 1 ml of vehicle (saline).

Results are presented as means ± SEM. Statistical evaluation was made by analysis of variance and, where appropriate, by the unpaired Student's t test, a P value of less then 0.05 being considered significant.

RESULTS

Effect of capsaicin and papaverine on the GBF and the area of lesions induced by 100% ethanol.

In vehicle-treated control rats, no lesions in the stomach were observed and the GBF averaged 47 ± 4 ml/min/100 g. Intragastric application of capsaicin (0.5 mg/kg) or papaverine (1 mg/kg) did not cause any mucosal lesions and resulted in a significant increase in the GBF by about 40% and 55%, respectively *(Table 1)*. Intravenous injection of L-NNA (5 mg/kg), which by itself also did not cause mucosal damage, resulted in a significant reduction in

Table 1. Effects of intragastric (i. g.) application of capsaicin (0.5 mg/kg) or papaverine (1 mg/kg) given alone or in the combination with L-NNA (5 mg/kg) injected i. v. 15 min before or 30 min after capsaicin or papaverine on the GBF in oxyntic mucosa. Results are mean ±SEM of 8 rats in each group. Asterisk indicates significant change as compared to the value obtained with the intact mucosa. Cross indicates significant decrease below the value obtained in the mucosa treated with capsaicin or papaverine alone.

Type of test	GBF (ml/min − 100 g)
Intact	47 ± 4
Capsaicin alone (0.5 mg/kg)	66 ± 8*
Papaverine alone (1 mg/kg)	73 ± 7*
L-NNA alone (5 mg/kg)	29 ± 3*
L-NNA + Capsaicin (15 min later)	37 ± 5*[+]
L-NNA + Papaverine (15 min later)	30 ± 2*[+]
Capsaicin + L-NNA (30 min later)	34 ± 4*[+]
Papaverine + L-NNA (30 min later)	38 ± 5*[+]

GBF by about 50% as compared to intact controls. Intravenous injection of L-NNA 15 min before i. g. administration of capsaicin or papaverine reduced significantly the increase in GBF induced by these agents by about 44% and 58%, respectively. When L-NNA was injected 30 min after the administration of capsaicin or papaverine, it also caused the reduction in GBF by about 50%.

Administration of 100% ethanol resulted in the appearance of gastric lesions in all animals tested and this was accompanied by a significant reduction in GBF. The mean lesion area in rats receiving ethanol was 78 ± 6 mm^2 and GBF was reduced by 56% comparing to vehicle-treated controls *(Fig. 1., Table 2)*. Capsaicin given i. g. in graded doses (0.12—0.5 mg/kg), reduced dose-dependently the mean lesion area, the dose reducing the lesion area by about 50% (ID$_{50}$) being 0.3 mg/kg *(Fig. 1)*. This reduction

Table 2. Effect of pretreatment with L-NNA (1.2—5 mg/kg i. v.) on the mean lesion area and GBF in rats receiving 100% ethanol. Results are mean ±SEM of 8 rats in each group. Asterisk indicates a significant decrease below the value obtained in intact mucosa not exposed to ethanol.

Type of test	Lesion area (mm^2)	GBF (ml/min − 100 g)
Intact	—	47 ± 4
100% Ethanol	78 ± 6	21 ± 4*
100% Ethanol + L-NNA (mg/kg) 1.2	75 ± 5	22 ± 3*
2.5	80 ± 4	20 ± 4*
5.0	81 ± 6	19 ± 2*

was followed by an increase in the GBF, that at a dose of 0.5 mg/kg of capsaicin amounted to about 85% of the value obtained in the vehicle-treated controls. Pretreatment with i. g. papaverine in a various doses (0.1—2.0 mg/kg) also resulted in a significant and dose-dependent reduction in the mean lesion area, the ID_{50} being about 1 mg/kg. *(Fig. 2)*. Papaverine given i. g. prior the administration of ethanol significantly increased the GBF, the significant increase to about 85% of the vehicle-treated control was achieved at a dose of 1 mg/kg of papaverine.
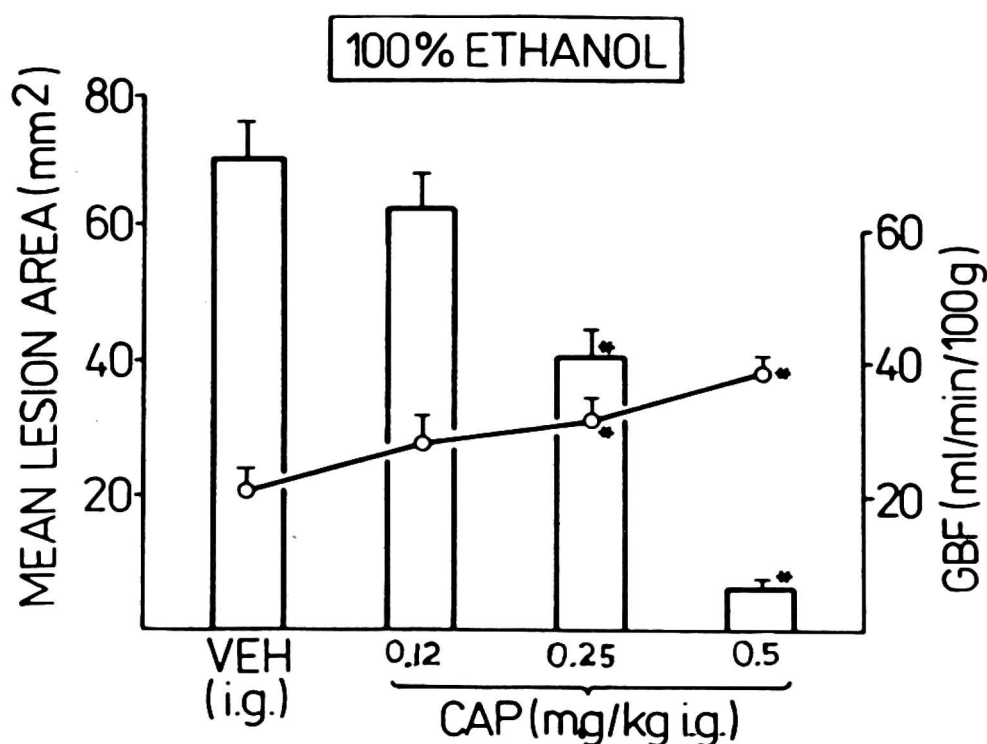

Fig. 1. Effect of various doses of capsaicin (0.12—0.5 mg/kg i. g) on the changes in GBF and mean lesion area of gastric lesions induced by 100% ethanol. Results are mean ±SEM of 8—10 rats in each group. Asterisk indicates significant decrease or increase below or above the value obtained with vehicle controls.

Effects of L-NNA (1.2—5.0 mg/kg) alone or in the combination with L-arginine (200 mg/kg) or D-arginine (200 mg/kg) on the changes in the GBF and gastroprotection induced by capsaicin and papaverine.

As shown in *Fig 3 and 4*, the pretreatment with a standard dose of capsaicin (0.5 mg/kg) or papaverine (1 mg/kg) reduced significantly the mean area of ethanol-induced lesions and increased markedly the GBF. Intravenous (i. v.) injection of L-NNA in various doses (1.2—5 mg/kg) failed to affect significantly the area of lesions and the fall in GBF induced by 100% ethanol alone *(Table 2)*. Such pretreatment with L-NNA resulted in a dose-dependent
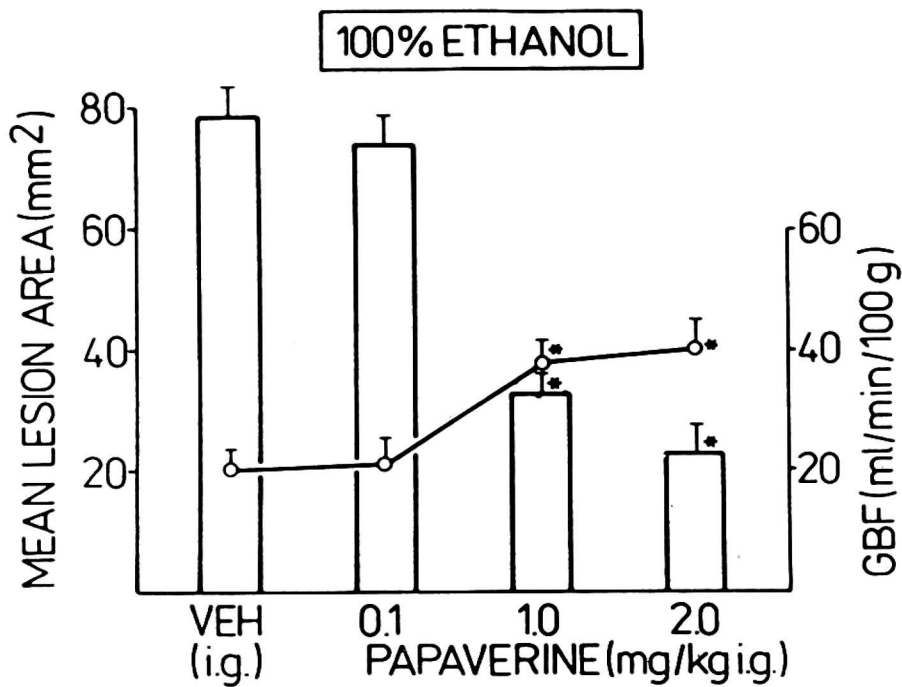
Fig. 2. Effect of various doses of papaverine (0.1—2.0 mg/kg i. g.) on the changes in GBF and mean lesion area of gastric lesions induced by 100% ethanol. Results are mean ±SEM of 8—10 rats in each group. Asterisk indicates significant decrease or increase below or above the value obtained with vehicle controls.
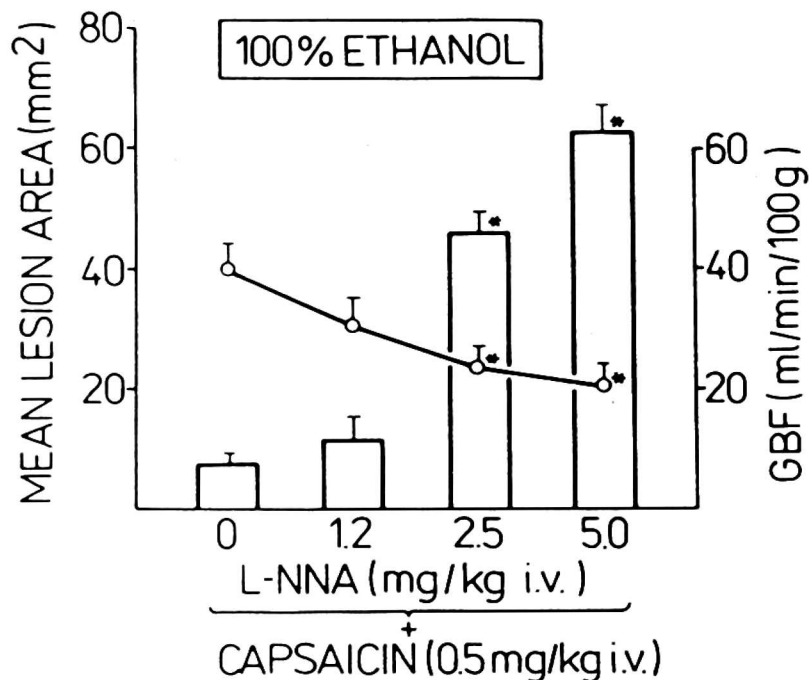

Fig. 3. Effect of graded doses of L-NNA (1.2—5.0 mg/kg i. v.) on gastroprotection and GBF changes induced by capsaicin against ethanol injury. Results are mean ±SEM of 8—10 rats in each group. Asterisk indicates a significant decrease or increase below or above the value obtained in rats receiving the combination of capsaicin plus 100% ethanol.

reversion of gastroprotection induced by capsaicin or papaverine and in the reduction in GBF *(Figs 3 and 4)*. At the dose of 5 mg/kg of L-NNA, the area of lesions and GBF in rats treated with capsaicin or papaverine was not significantly different from that achieved with ethanol alone. *Figs 5 and 6* show that in rats treated with capsaicin or papaverine combined with L-NNA, the
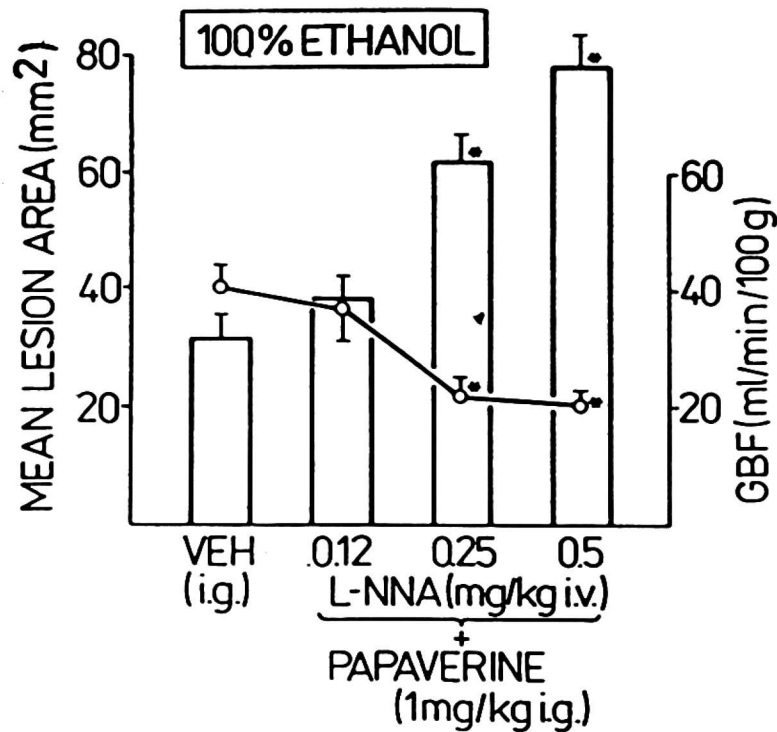
Fig. 4. Effects of graded doses of L-NNA (1.2—5.0 mg/kg i. v.) on gastroprotection and GBF changes induced by papaverine against injury caused by 100% ethanol. Results are mean ±SEM of 8—10 rats in each group. Asterisk indicates significant decrease or increase below or above the value recorded in vehicle controls.
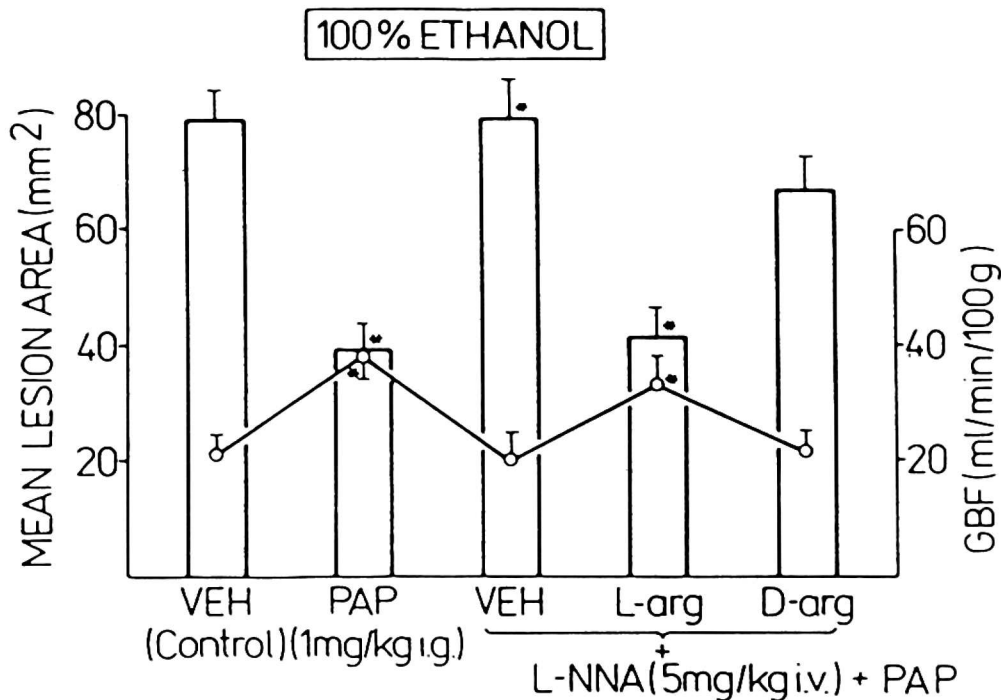

Fig. 5. Effects of vehicle, papaverine (1 mg/kg i. g.) and L-NNA (5 mg/kg i. v.) with or without addition of L-arginine or D-arginine (200 mg/kg i. v.) on the area of ethanol induced gastric lesion and gastric blood flow. Results are mean ±SEM of 8—10 rats in each group. Asterisk indicates significant decrease or increase below or above the value obtained in vehicle controls.

addition of L-arginine (50 mg/kg) to i. v. injection of L-NNA almost completely reversed the deleterious effects of L-NNA on the mucosal lesions and GBF *(Figs 5 and 6)*. Addition of D-arginine failed to affect the L-NNA induced increase in mucosal damage and in the fall of GBF in rats treated with capsaicin or papaverine.
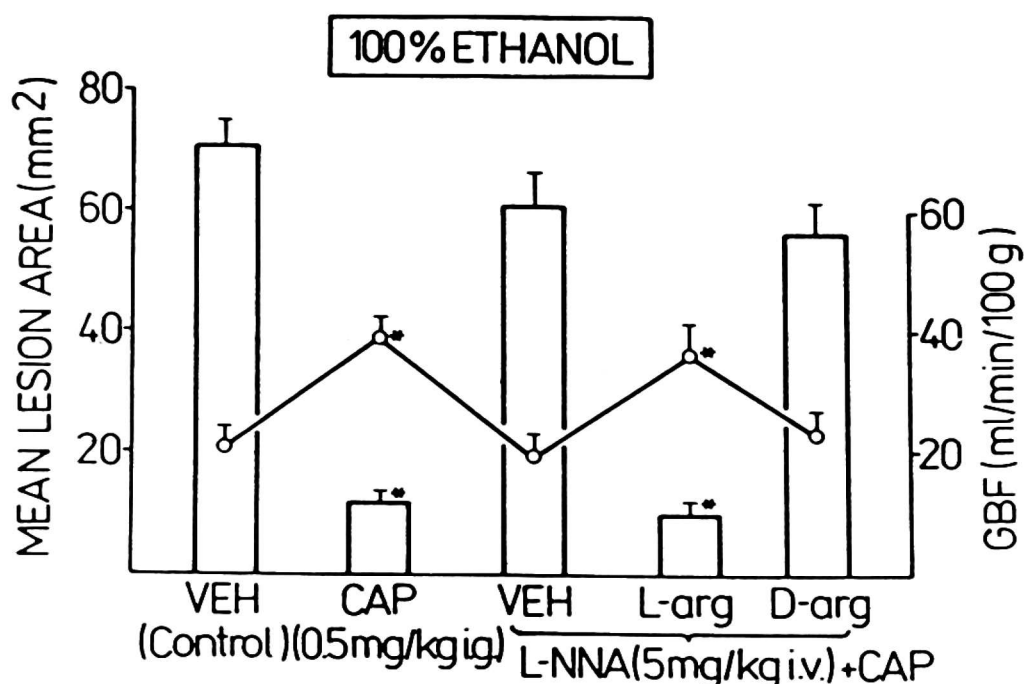
Fig. 6. Effects of vehicle, capsaicin (0.5 mg/kg i. g.) and L-NNA (5 mg/kg i. v.) with or without addition of L-arginine or D-arginine (200 mg/kg i. v.) on the area of ethanol induced gastric lesion and gastric blood flow. Results are mean ±SEM of 8—10 rats from 6 experiments. Asterisk indicates significant decrease or increase below or above the value recorded in vehicle controls.
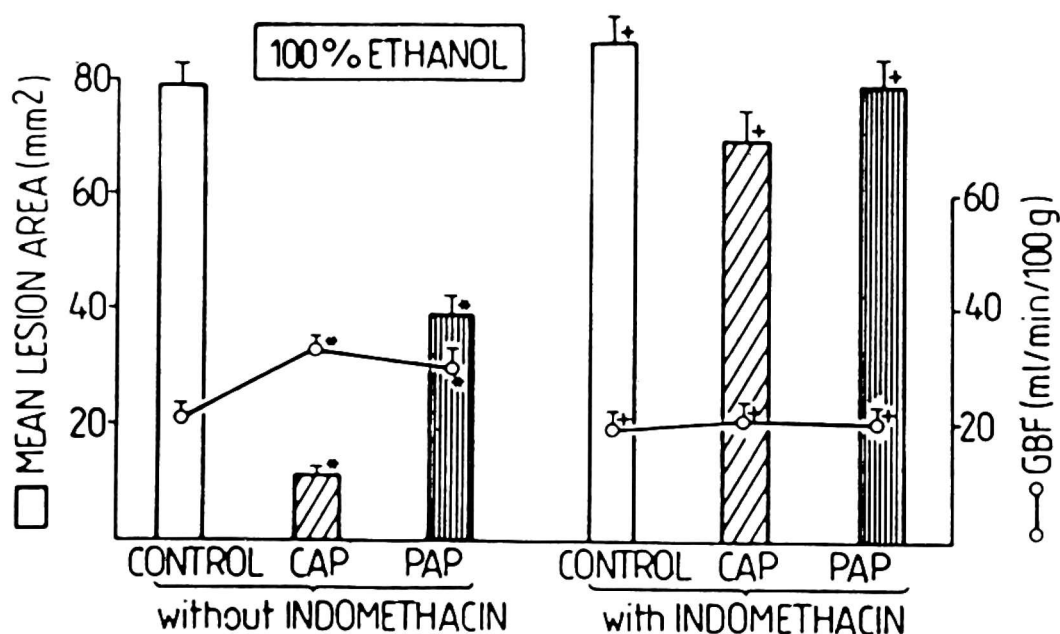

Fig. 7. Effects of pretreatment with indomethacin (5 mg/kg i. p.) on the lesions area and GBF changes induced by ethanol alone or the combination of ethanol plus capsaicin (0.5 mg/kg i. g.) or papaverine (1 mg/kg i. g.). Results are mean ±SEM of 8—12 rats in each group. Asterisk indicates significant change below or above vehicle controls without indomethacin. Cross indicates significant decrease or increase below or above the value obtained in rats without the pretreatment with indomethacin.

Effect of pretreatment with indomethacin alone or in the combination with L-NNA on the GBF and gastroprotection induced by capsaicin and papaverine.

As shown in *Fig. 7,* the pretreatment with indomethacin given at a dose of 5 mg/kg (i. p.) 90 min before 100% ethanol caused a small but significant increase in the area of gastric lesions and a small but insignificant reduction in

the GBF below that recorded in animals receiving ethanol alone. Such pretreatment with indomethacin almost completely abolished the protective activity of capsaicin and papaverine and this action was associated with a significant attenuation of the GBF to the level similar to that obtained

Table 3. Effects of L-NNA (5 mg/kg i.v.) and indomethacin (Indo) (5 mg/kg i.p.) or their combination on the area of gastric lesions and changes in GBF induced by 100% ethanol in rats without or with pretreatment with capsaicin (CAP) (0.5 mg/kg i.g.) or papaverine (PAP) (1 mg/kg i.g.). Results are mean ±SEM of 8—10 rats from 6 experiments. Single asterisk indicates significant change as compared to the value obtained with 100% ethanol alone. Double asterisk indicates significant change as compared to the value recorded in rats treated with the combination of capsaicin or papaverine plus ethanol. Cross indicates significant change as compared to the value obtained in indomethacin pretreated rats receiving the combination of capsaicin or papaverine plus ethanol.

Test	Lesion area (mm^2)	GBF (ml/min/100 g)
100% ethanol (control)	78 ± 6	22 ± 3
CAP + 100% ethanol	$8 \pm 0.5^*$	$38 \pm 4^*$
PAP + 100% ethanol	$21 \pm 3^*$	$41 \pm 4^*$
L-NNA + CAP + 100% ethanol	$62 \pm 8^{**}$	$21 \pm 2^{**}$
Indo + 100% ethanol	$85 \pm 4^{**}$	$21 \pm 3^{**}$
L-NNA + PAP + 100% ethanol	$72 \pm 7^{**}$	$22 \pm 3^{**}$
Indo + CAP + 100% ethanol	$69 \pm 8^{**}$	$24 \pm 4^{**}$
Indo + PAP + 100% ethanol	$73 \pm 6^{**}$	$25 \pm 4^{**}$
L-NNA + Indo + CAP + 100% ethanol	$88 \pm 5^+$	$18 \pm 2^+$
L-NNA + Indo + PAP + 100% ethanol	$92 \pm 4^+$	$17 \pm 1^+$

with 100% ethanol (Fig. 7, Table 3). Addition of L-NNA to indomethacin resulted in further aggravation of the lesion area over that achieved with indomethacin alone and significantly diminished the blood flow changes below the value recorded in indomethacin treated rats (Table 3).

DISCUSSION

Our present study confirms previous findings (1—4) that capsaicin and papaverine protect the gastric mucosa against acute damage induced by ethanol and shows that this protection is accompanied by an increase in gastric mucosal blood flow. The current data demonstrate, that the selective blockade of NO synthase by L-NNA reversed, in part, the gastroprotective and hyperemic effects of capsaicin and papaverine and that these effects were completely eliminated by the combination of L-NNA and indomethacin,

indicating that both NO and PG are involved in the action of these compounds on the gastric mucosa.

Previous studies have shown that the stimulation of afferent sensory neurons by intragastric administration of capsaicin leads to the protection of gastric mucosa against the injury induced by various irritants and ulcerogens such as absolute ethanol (1, 2, 11), acidified aspirin (10) and pylorus ligation (5). This action was attributed to the release of vasoactive peptides such as CGRP from capsaicin-sensitive sensory nerve fibers (2, 16, 17). Furthermore, the stimulation of these nerves could result in an axon-reflex vasodilatation in the presence of sensory neurotransmitters as observed by others (17, 18) and confirmed in our present study. The local release of neuropeptides such as CGRP has also been implicated in the maintenance of mucosal integrity since the ablation of afferent neurons by the pretreatment with capsaicin enhanced gastric mucosal damage induced by various noxious agents (1, 5, 18, 20). Furthermore, exogenous CGRP resulted in gastric mucosal protection against ethanol damage and in the enhancement of the mucosal blood flow (18, 19).

Increased gastric blood flow is thought to be an important factor in gastric mucosal protection (21—23). Indeed, our present study demonstrates that intragastric application of capsaicin or papaverine caused a marked increase in GBF indicating that this increase might contribute to their protective activity. Whether endothelial factors such as endothelium-derived relaxing factor or nitric oxide (NO) mediate the protective action of capsaicin and papaverine remains unsettled. Whittle et al. (25) reported that the suppression of the NO biosynthesis by methyl-arginine (L-NMMA) greatly attenuated the mucosal blood flow, especially in capsaicin-pretreated rats, with depleted sensory neuropeptides from afferent neurons. An interaction between NO and sensory neurons have been proposed for the modulation of mucosal integrity. Peskar et al (14) demonstrated that L-NNA attenuated the protective action of capsaicin against ethanol damage, but the gastric blood flow was not measured in that study.

The results of our study demonstrate that capsaicin induced both the increase in GBF and the protection against ethanol and that the administration of L-NNA, a selective inhibitor of NO synthase, eliminated these effects while the addition of L-arginine, a substrate of endogenous NO (12), restored both the mucosal protective and hyperemic activity of capsaicin. Such an supplementation of NO from L-arginine was found previously to enhance the endothelium-dependent vasodilatation in vitro (26, 27) suggesting that currently observed protective and vasoactive effect of L-arginine is highly specific. This notion is also supported by the fact that pretreatment with D-arginine under similar experimental conditions was completely ineffective.

Papaverine was originally shown as an agent capable of protecting the gastric mucosa against the damage by ethanol (3, 4) and stress (28, 29) and our results confirm these findings. In addition, we found for the first time that this protection is accompanied by a marked increase in GBF. The mechanism of this protection has not been extensively investigated, but Ligumsky et al (7) reported previously that papaverine stimulated the generation of PG from isolated rabbit gastric mucosal cells, suggesting that PG could contribute to its protective activity. Since in our present study, L-NNA greatly reduced the protection induced by papaverine, it is likely that the gastroprotective and hyperemic actions of this agent are mediated, at least in part, by NO. This notion was also supported by the fact that concurrent administration of L-arginine but not D-arginine antagonized the deleterious effects of L-NNA on papaverine-induced gastroprotection and the increase in blood flow. On the other hand, Kitagawa et al (30) reported that an increase in mucosal microcirculation induced by papaverine is not mediated by NO, because the inhibition of NO biosynthesis failed to affect the papaverine-induced vasodilation. In our *in vitro* study (unpublished observation) using rat fundic strips and aorta deprived of endothelium we also found that papaverine induced relaxation was not influenced by L-NNA, indicating that NO may not be responsible for the relaxing effect of this agent on smooth muscle. These data seem to be at variance with the results in the present study, especially regarding the involvement of NO in the increase in gastric blood flow by papaverine. Since there is an evidence that NO may be generated and released by nerve endings (6) and by the platelets (31) it is possible that papaverine, like capsaicin, stimulates the release of NO in the gastric mucosa from other sources than vascular endothelium, however, this hypothesis remains to be tested. It is also possible that papaverine like capsaicin (32) affects the function of platelets and this could contribute to its gastroprotective activity. It has recently been demonstrated that leukocyte adherence to the vascular endothelium is an important event in the pathogenesis of acute gastric lesions induced by number of ulcerogens including ethanol (33), aspirin (34), hemorrhagic shock (35) and ischemia reperfusion (35). Leukocytes might contribute to the ulceration by occluding microvessels, thereby reducing mucosal blood flow and by releasing various mediators such as leukotrienes or PAF that can induce damage to the gastric mucosa (37).

The most important finding of this study is the demonstration that the protective effect of capsaicin and papaverine was attenuated by pretreatment with indomethacin in a dose that was sufficient to cause almost complete inhibition of PG biosynthesis. Whittle and his coworkers (25) were the first who proposed that endogenous prostaglandis and NO interact on the gastric mucosa and play a crucial role in the maintenance of mucosal integrity. Our present data are in keeping with this hypothesis because hyperemic and

protective effect of papaverine and capsaicin were markedly reduced by indomethacin and the addition of L-NNA eliminated completely the protective activity of both agents. This observation indicates that both endogenous PG and NO released either by endothelium or by the afferent neurons contribute to the vasodilator and protective actions of capsaicin and papaverine. Our finding clearly disagrees with the recent report by Holzer et al. (11) showing that the protection of deep necrosis by capsaicin was not affected by indomethacin, that reduced *ex vivo* formation of PG by about 90%. This discrepancy could be explained by the difference in the experimental methods and conditions used in their (11) and our present study. In other studies, Uchida et al. (38) and Takeuchi et al. (39) demonstrated that the protection and the enhancement in the gastric motility and the blood flow caused by capsaicin was markedly reversed by indomethacin, suggesting a close relationship between the stimulation of primary afferent neurons and endogenous PG. They postulated (39) that afferent neurons are sensitized by endogenous PG and that this effect could contribute, at least in part, to protective activity of capsaicin. Thus, our present data indicate that close interaction between NO and PG systems contribute to the protective action of capsaicin and papaverine against ethanol damage.

REFERENCES

1. Holzer P, Sametz W. Gastric mucosal protection against ulcerogenic factors in the rat mediated by capsaicin-sensitive afferent neurons. Gastroenterology 1986; 91: 975—981.
2. Holzer P, Lippe IT. Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol induced damage of gastric mucosa. Neurosci 1988; 27: 981—987.
3. Hagel J, Renner H, Hirsch M, et al. Gastric cytoprotection by antacids and papaverine in rats. Hepato-Gastroenterol 1982; 29: 271—274.
4. Domschke W, Hagel J, Ruppin H, Kaduk B. Antacid protection of gastric mucosa. Klin Wochenschr 1986; 64: (Suppl VII), 28—31.
5. Szolcsanyi J, Bartho L. Impaired defence mechanism to peptic ulcer in the capsaicin-desensitized rat. In Gastrointestinal Defence Mechanisms, G. Mozsik, O. Hanuinen, T. Javor (eds.). Oxford and Budapest, Pergamon Press and Akademiai Kidao, 1981, pp. 39—51.
6. Buck SH, Burks TF. The neuropharmacology of capsaicin: review of some recent observations. Pharmacol Review 1986; 38: 179—226.
7. Ligumsky M, Rachmilewitz D, Zor V. Papaverine stimulation of prostaglandin E_2 production by cultured rabbit gastric mucosa. Gut 1979; 20: 882—885.
8. Yonei Y, Holzer P, Guth PH. Laparatomy-induced gastric protection against ethanol injury is mediated by capsaicin-sensitive sensory neurons. Gastroenterology 1990; 99: 3—9.
9. Esplugues JV, Whittle BJR, Mancada S. Local opioid-sensitive afferent sensory neurones in the modulation of gastric damage induced by PAF. Br J Pharmacol 1989; 97: 579—585.
10. Holzer P, Pabst MA, Lippe IT. Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the gastric mucosa. Gastroenterology 1989; 1425—1433.
11. Holzer P, Pabst MA, Lippe IT, et al. Afferent nerve-mediated protection against deep mucosal damage in the rat stomach. Gastroenterology 1990; 98: 838—848.

12. Palmer RMJ, Ferriga AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524—526.

13. Mac Naughton WK, Cirino G, Wallace JL. Endothelium-derived relaxing factor (nitric oxide) has protective actions in the stomach. Life Sci 1989; 45: 1869—1876.

14. Peskar BM, Respondek M, Muller KM, Peskar BA. A role for nitric oxide in capsaicin-induced gastroprotection. Eur J Pharmacol 1991; 198: 113—114.

15. Konturek SJ, Brzozowski T, Drozdowicz D, et al. Nocloprost, a unique prostaglandin E$_2$ analog with local gastroprotective and ulcer-healing activity. Eur J Pharmacol 1991; 195: 347—357.

16. Holzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neurosci 1988; 24: 739—768.

17. Holzer P, Peskar BM, Peskar BA, Amann R. Release of calcitonin gene-related peptide induced by capsaicin in the vasculary perfused rat stomach. Neurosci Lett 1990; 180: 195—200.

18. Lippe IT, Pabst MA, Holzer P. Intragastric capsaicin enhances rat gastric elimination and mucosal blood flow by afferent nerve stimulation. Br J Pharmacol 1989; 96: 91—100.

19. Holzer P, Guth PH. Neuropeptide control of rat gastric mucosal blood flow. Circulation Res 1991; 68: 100—105.

20. Evangelista S, Maggi CA, Meli A. Evidence for a role of adrenals in the capsaicin-sensitive "gastric defense mechanism" in rats. Proc Soc Exp Biol Med 1986; 182: 568—569.

21. Guth PH, Leung FW. Physiology of gastric circulation. In Physiology of Gastrointestinal Tract, Johnson LR (ed). New York, Raven Press 1987, pp. 1031—1053.

22. Holm L. Gastric mucosal blood flow and mucosal protection. J Clin Gastroenterol 1989; 10 (Suppl 1): 114—119.

23. Cheung LY. Gastric mucosal blood flow: its measurement and importance in mucosal defense mechanism. J Surg Res 1984; 36: 282—288.

24. Konturek P, Brzozowski T, Konturek SJ, Dembiński A. Role of epidermal growth factor, prostaglandin and sulfhydrys in stress-induced gastric lesions. Gastroenterology 1990; 99: 1607—1615.

25. Whittle BJR, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. Br J Pharmacol 1990; 99: 607—611.

26. Kitagawa H, Takeda F, Kohei H. Gastric mucosal protective actions of endothelium-derived relaxing factor. Scand J Gastroenterol 1989; 24 (Suppl 162): 131—133.

27. Palmer RMJ, Rees DD, Ashton DS, Moncada S. L-arginine is the physiological precursor for the formation of nitric oxide in endothelium-dependent relaxation. Biochem Biophys Res Commun 1988; 153: 1251—1256.

28. Garrick T, Buack S, Bass P. Gastric motility is a major factor in cold restraint-induced lesion formation in rats. Am J Physiol 1986; 250: G191—G199.

29. Ohno T, Okabe S. Gastric surface epithelial cell damage induced by restraint and water-immersion stress in rats. Protective effects of 16,16-dimethyl prostaglandin E$_2$. J Clin Gastroenterol 1988; 10 (Suppl 1): S46—S52.

30. Kitagawa H, Takeda F, Kohei H. Effect of endothelium-derived relaxing factor on the gastric lesions induced by HCl in rats. J Pharm Exp Ther 1990; 253: 1133—1137.

31. Moncada S, Radomski N. M., Palmer R. M. Endothelium-derived relaxing factor. Identification as nitric oxide and role in the control of vascular tone and platelet function. Biochem Pharmacol 1988; 37: 2495—2501.

32. Hagoboam CM, Wallace JL. Inhibition of platelet aggregation by capsaicin. An effect unrelated to actions on sensory afferent neurons. Eur J Pharmacol 1991; 202: 129—131.

33. Kvietys PR, Twohig B, Danzell J, Special RD. Ethanol-induced injury to the rat gastric mucosa. Role of neutrophils and xanthine oxidase-derived radicals. Gastroenterology 1990; 98: 909—920.

34. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. Am P J Physiol 1990; 259: G 462—G467.

35. Smith SML, Holm-Rutili L, Perry MA et al. Role of neutrophils in haemorrhagic shock-induced gastric mucosal injury in the rat. Gastroenterology 1987; 93: 466—471.

36. Parks DA, Granger DN. Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. Am J Physiol 1983; 245: G285—289.

37. Konturek SJ, Brzozowski T, Drozdowicz D, Garlicki J, Beck G. Role of leukotrienes and platelet activating factor in acute gastric mucosal lesions in rats. Eur J Pharmacol 1989; 164: 285—292.

38. Uchida M, Yano S, Watanabe K. The role of capsaicin-sensitive afferent nerves in protective effect of capsaicin against absolute ethanol induced lesions in rats. Jpn J Pharmacol 1991; 55: 279—282.

39. Takeuchi K, Niida H, Matsumoto J, Ueshina K, Okabe S. Gastric motility changes in capsaicin-induced cytoprotection in the rat stomach. Jpn J Pharmacol 1991; 55: 147—155.

Author's address: Prof. S. Konturek, Institute of Physiology, University Medical School, 31-531 Kraków, ul. Grzegórzecka 16, Poland.