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Review article

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ACTIONS AND INTERACTIONS OF ENDOTHELINS, PROSTACYCLIN AND NITRIC OXIDE IN THE GASTRIC MUCOSA

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The modulation of the gastric mucosal microcirculation plays a key role in the maintenance of gastric integrity. Disruption of the balance between the local release of vasodilator and vasoconstrictor mediators could therefore be involved in the pathogenesis of mucosal injury. Thus, the endothelium derived peptide endothelin-1 (ET-1), induces mucosal injury following local administration. In contrast, the vasodilator prostanoids, prostacyclin and PGE_2 can protect against gastric damage, while inhibition of endogneous prostanoid formation by cyclo-oxygenase inhibitors augment mucosal damage, including that induced by ET-1. Sensory neuropeptides such as calcitonin gene-related peptide (CGRP) may also play a local protective role, since acute intragastric administration of capsaicin which stimulates neuropeptide release, protects against mucosal injury iduced by ET-1, as does local infusion of CGRP. Furthermore, chronic administration of capsaicin which deplete primary sensory neurones augments gastric damage induced by a number of ulcerogens including ET-1. Nitric oxide (NO) synthesized from L-arginine can regulate gastric mucosal blood flow, both under resting conditions and following stimulation of acid secretion. Inhibition of NO biosynthesis alone does not induce acute mucosal injury, yet extensive haemorrhagic damage results from concurrent inhibition of NO formation, cyclo-oxygenase inhibition and depletion of sensory neuropeptides. NO donors can protect against ulceration, although the unregulated release of high levels of NO can lead to mucosal injury. Thus, NO has a critical interactive role with other local protective mediators such as the prostanoids and sensory neuropeptides in the physiological regulation of mucosal integrity.

Key words: *endothelins, prostanoids, nitric oxide, sensory neuropeptides, gastric mucosal integrity*

INTRODUCTION

The regulation of gastric mucosal microcirculation plays a key role in the maintenance of gastric integrity. Thus, a balance between the local release of vasodilator and vasoconstrictor mediators regulates microvascular blood flow,

while a disruption in this balance could be involved in the pathogenesis of mucosal injury and peptic ulceration. In the present review, the actions, interactions and roles of the endothelium-derived vasoactive mediators, prostacyclin, endothelin-1 and nitric oxide in the gastric mucosa will be considered.

Prostacyclin

The labile cyclo-oxygenase product, prostacyclin (PGI_2), can be synthesized from the fatty acid precusor, arachidonic acid by endothelial cells (1). Its formation can be detected in gastric mucosal tissue using bioassay or radioimmunoassay techniques and is inhibited both *in vitro* and *ex vivo* by non-steroid anti-inflammatory drugs such as aspirin and indomethacin (2, 3).

Prostacyclin, like the other major prostanoid that is synthesized in the gastric mucosa, prostaglandin E_2 (PGE_2), has potent vasodilator properties in the gastric microcirculation, (4-7). Furthermore, like PGE_2, prostacyclin can inhibit gastric acid secretion in a number of experimental preparations (4, 5, 8, 9, 10, 11) and can stimulate the secretion of bicarbonate, a luminal protective factor (12). Such a profile of properties have been suggested to contribute to the protective actions of PGE_2 (3, 13) and may likewise underlie the protective properties of prostacyclin and its more-stable synthetic analogues against mucosal injury observed in a number of experimental models (4, 14, 15, 16).

The involvement of endogenous prostanoids in the modulation of mucosal integrity is suggested by the enhanced suceptibility of the gastric mucosa to challenge following cyclo-oxygenase inhibition by non-steroid antiinflammatory agents (17). It is of relevance to the endogenous role of these prostanoids that the haemorrhagic damage induced by aspirin or indomethacin is preceded by focal structural changes of the basement membrane of capillary and post-capillary endothelial cells, leading eventually to destruction of the microvessels (18, 19). Mucosal application of the topical irritant, ethanol also induces primary microvascular injury and prevention of endothelial damage and vascular stasis may be a major mechanism by which prostaglandins can protect against the subsequent necrotic damage (20, 21).

Nitric oxide

Endothelial cells also release the highly labile humoral vasodilator substance, originally known as endothelium-derived relaxing factor (EDRF), that mediates the vascular relaxation induced by agents such as acetylcholine (22, 23). It is now known that nitric oxide (NO), formed by endothelial cells from the amino acid L-arginine, accounts for the biological properties of EDRF (24—28).

The enzyme, NO synthase, generates NO from the terminal guanidino nitrogen atoms of the amino acid, L-arginine, through a process where molecular oxygen is also incorporated (29-31). The constitutively expressed NO synthase enzyme is calcium-, calmodulin- and NADPH-dependent, although a calcium-independent inducible isoform has been identified that can be expressed following incubation with endotoxin and various cytokines in phagocytic cells (32-35), in vascular tissue (36, 37) and in lung, liver and gut tissue (38, 39).

The formation of NO from L-arginine by a calcium-dependent constitutive NO synthase in rat gastric mucosal tissue has been directly demonstrated using a spectrophometeric technique (40). In a further study on the cellular distribution of NO synthase in the rat gastric mucosa, epithelial cells separated by elutriation demonstrated high levels of constitutive enzyme activity as determined by the conversion of radiolabelled L-arginine to the NO co-product, citrulline (41). These findings may reflect a non-vascular role for NO in the modulation of mucus or bicarbonate secretion from these cells, which, as in vascular tissue (42, 43) may involve activation of guanylate cyclase and elevation of cyclic GMP.

The formation of NO is selectively inhibited by L-arginine analogues such as N^G-monomethyl-L-arginine (L-NMMA) shown originally in *in vitro* studies on vascular tissue (29, 44). Studies in the rabbit, rat and guinea-pig *in vivo* demonstrated that L-NMMA increased systemic arterial blood pressure, an effect reversed by L-arginine but not the enantiomer, D-arginine, suggesting that endogenous NO biosynthesis from L-arginine modulates resting vascular tone *in vivo* (45-48). Local infusion of L-NMMA into the forearm also increased peripheral vascular tone in man (49), while studies in a number of species with other L-arginine analogues that inhibit NO biosynthesis, such as the more potent N^G-nitro-L-arginine methyl ester (L-NAME) have confirmed the importance of NO in the regulation of systemic arterial blood pressure (50).

Studies using hydrogen gas clearance in the rat have demonstrated that intravenous administration of L-NMMA dose-dependently reduced resting gastric mucosal blood flow (51). These effects were not shared by D-NMMA, while L-arginine but not D-arginine reversed these actions. Subsequently, using laser Doppler flowmetry, both L-NMMA and L-NAME have been shown to reduce resting mucosal blood flow (52).

Studies on the gastric mucosal hyperaemia induced by intravenous infusion of pentagastrin in the rat demonstrated that this response could be attenuated by concurrent infusion of L-NMMA or L-NAME (53). However, mucosal blood flow may be altered by changes in the rate of acid secretion. In a study to determine concurrent changes in acid output, pretreatment with a low dose of L-NMMA reduced the elevation of mucosal blood flow by 65% but had no significant effect on the plateau rates of acid secretion induced by pentagastrin,

thus indicating an effect on the microcirculation independent of secretory modulation (54). A higher dose of L-NMMA, which both reduced resting mucosal blood flow and abolished the hyperemic response, induced a small inhibition of pentagastrin-stimulated acid output. In contrast, administration of this dose of L-NMMA during stable rates of pentagastrin-stimulated acid output had no such effect on acid secretion, yet substantially reduced mucosal blood flow (54).

These findings therefore indicate that NO is a prime mediator of the blood flow changes associated with secretion, although inhibition of NO biosynthesis has no direct effect on the stimulation of acid secretion. Studies with other secretagogues as well as using isolated parietal cells will provide further information on the possible role of NO in such secretory events. Indeed, recent studies have demonstrated the involvement of NO in the process by which acute administration of endotoxin can inhibit acid secretion, but the mechanism is as yet, unknown (55).

INTERACTIONS OF PROSTANOIDS AND NO WITH SENSORY NEUROPEPTIDES

Vasodilator sensory neuropeptides, predominantly calcitonin-gene related peptide are stored in primary afferent neurones in the gastric mucosa (56) and their release is also considered to play an important role in the preservation of mucosal integrity. Thus, depletion of sensory neuropeptides by chronic administration of the pungent red pepper extract, capsaicin, which itself does not injure the gastric mucosa, greatly augments the damage induced by a number of ulcerogenic agents (57-61).

Recent studies have demonstrated that morphine administration, which is known to prevent neuropeptide release from sensory neurones, or capsaicin pretreatment, can attenuate the protective properties of PGE_2 and its 16, 16-dimethyl analogue against acute gastric challenge (62, 63). Such findings indicate an interaction between these protective mediators in the modulation of mucosal integrity. Furthermore, the mucosal injury induced by indomethacin is augmented in capsaicin-pretreated rats, again demonstrating such interactions between endogenous protective sensory neuropeptides and prostanoids (58, 64).

In studies on the interactions of these mediators with endogenous NO, administration of L-NMMA was demonstrated to induce acute gastric mucosal injury in rats pretreated with indomethacin, using doses of either agent that themselves did not provoke acute mucosal injury. Likewise, L-NMMA induced extensive haemorrhagic mucosal injury in rats chronically pretreated

with capsaicin. Furthermore, L-NMMA induced deep haemorrhagic necrosis involving virtually all of the mucosal area in rats pretreated concurrently with both indomethacin and capsaicin (64).

Such findings indicate a critical interaction between endogenous NO, sensory neuropeptides and prostanoids, all of which appear to subserve a modulator function in the regulation of gastric mucosal integrity. These mediators may not only exert local vasodilator actions on the microcirculation essential for adequate microvascular blood flow under physiological conditions, but may act to enhance or preserve endothelial cell function and continuity, especially under conditions of challenge.

The mucosal protective actions of a PGE_2 analogue against ethanol-induced injury do not however appear to depend on endogenous NO since they were not inhibited by N^G-nitro-L-arginine (65) although the protective actions of the anti-ulcer compounds, carbenoxolone and sucralfate was attenuated by this inhibitor of NO synthesis (65, 66). Moreover, other recent studies have shown that the protective actions against ethanol-induced injury of acute intraluminal instillation of capsaicin, which releases neuropeptides following initial stimulation of the sensory neurones (67, 68) are attenuated by N^G-nitro-L-arginine (66). This finding again implies interactions between neuropeptides and NO in the mechanisms subserving mucosal protection.

It is possible that neuropeptides originating form the afferent sensory neurones in the vicinity of the microvessels are involved in the regulation of release of the endothelium-derived mediators. Recent studies have shown that depletion of sensory neuropeptides greatly augments the fall in mucosal blood flow induced by L-NMMA and L-NAME (52). Furthermore, the acute increase in mucosal blood flow induced by sensory neuronal stimulation following instillation of capsaicin into the gastric lumen, is abolished by concurrent administration of L-NAME (69). This may reflect physiological interactions in the modulation of microvascular tone between NO and sensory neuropeptides, either directly on vascular smooth muscle or by the involvment of NO in the local vascular neuromodulator processes.

ENDOTHELINS

Vascular endothelial cells synthesize a 21-residue peptide known as endothelin-1 (ET-1) which exhibits vasoconstrictor actions both *in vitro* and *in vivo* (70, 71). Local intra-arterial infusion of picomole quantities of ET-1 has been demonstrated to induce substantial gastric mucosal vasocongestion and haemorrhagic injury in the rat (72), as shown in *Figure 1*. Furthermore, intravenous infusion of ET-1 has been shown to augment mucosal damage induced by intragastric instillation of ethanol or acid (73).

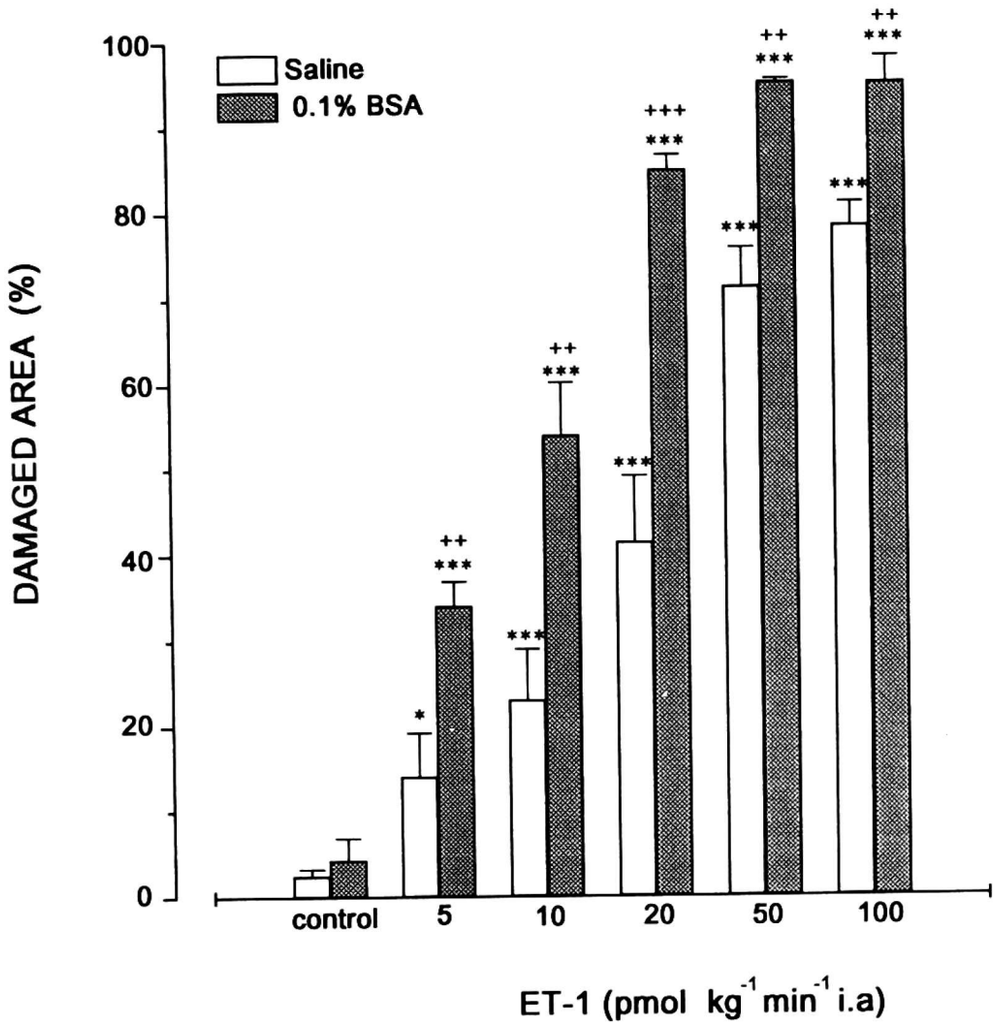

Fig. 1. Gastric mucosal injury induced by close-arterial infusion through the left gastric artery of endothelin-1 (ET-1; 5-100 pmol kg^{-1} min^{-1}) in isotonic saline or in 0.1% bovine serum albumin in the pentobarbitone-anaesthetised rat. Results, shown as the area of macroscopically assessed damage expressed as % total mucosal area, 20 min following termination of a 10 min infusion are mean ± s.e. mean of 4-8 experiments per group, where significant difference from control vehicle group is $P^* < 0.05$, $^{***}P < 0.001$, and significant difference between the two vehicles as $^{++}P < 0.01$, $^{+++}P < 0.001$.

Endothelin-1-like immunoreactivity has been demonstrated in the rat gastric mucosa in both antral and corpus regions (74, 75). Much lower levels of immunoreactivity to endothelin-3 (ET-3), which differs from ET-1 by changes in six amino acids (71) has also been found in the rat stomach (74). ET-1 and ET-3 were found to be equipotent in inducing rat gastric haemorrhage following intravenous infusion, although *in vitro*, ET-1 was at least five fold more potent as a vasoconstrictor in the rat isolated stomach (76). ET-3 also has been shown to induce a small increase in canine gastric vascular resistance following close-arterial infusion (77). Local intra-arterial infusion of high doses of ET-3 induced vascular lesions in the rat gastric mucosa that involved the venules and capillaries and could potentiate the vascular injury induced by acid and ethanol (78). Furthermore, administration of an anti-ET-3 serum was found to reduce the extent of mucosal damage induced by intragastric ethanol,

suggesting that acute release of endogenous endothelins are involved in such mucosal injury (78).

It is likely that there is an interplay between endothelium-derived mediators with opposing vasoactive properties, within the gastric mucosal microcirculation following their release. Indeed, the cyclo-oxygenase inhibitor indomethacin, in doses sufficient to reduce prostacyclin and PGE_2 biosynthesis in the mucosa, substantially increased the haemorrhagic injury induced by local infusion and systemic administration of ET-1 (79, 80), as shown in *Figure 2.*

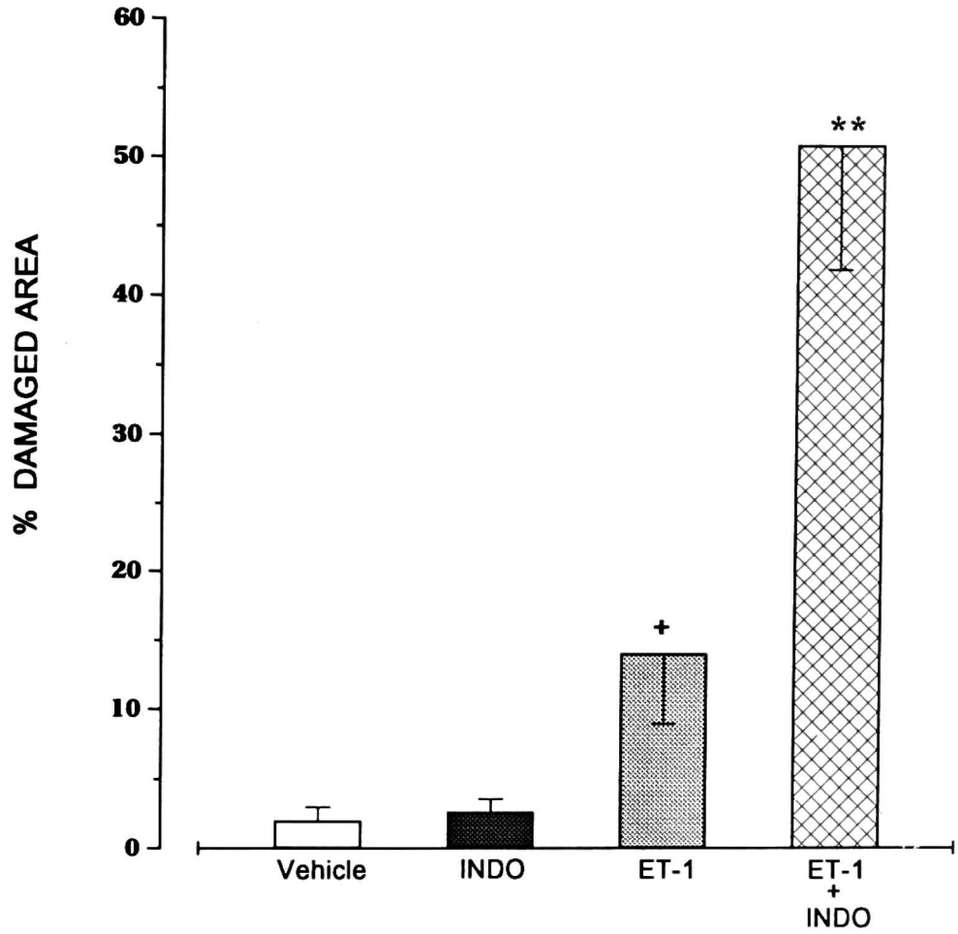

Fig. 2. Potentiation of rat gastric damage induced by close intra-arterial infusion of ET-1 (5 pmol kg^{-1} min^{-1} for 10 min) by pretreatment with indomethacin (5 mg kg^{-1} s.c. 1 hour before study). Results, shown as % of total mucosal area exhibiting macroscopic damage, assesed 20 min after infusion are the mean ± s.e. mean of 4-7 experiments in each group, where difference from the vehicle control is $^{+}P < 0.05$, and from ET-1 alone $^{**}P < 0.01$. Data are adapted from Whittle & Esplugues (99) and Whittle & Lopez-Belmonte (80).

Furthermore, interactions of ET-1 with other endogenous mediators such as sensory neuropeptides is also apparent. Thus, chronic administration of capsaicin, or administration of morphine to deplete and prevent neuropeptide release greatly augments damage induced by ET-1 (80). Moreover, concurrent local infusion of CGRP reduced the mucosal disruption induced by ET-1 (80). The haemorrhagic mucosal damage induced by local infusion of other vasoactive mediators such as platelet activating factor (PAF), which involves micro-

vascular injury, is likewise augmented by capsaicin pretreatment or morphine administration (60), as are the deleterious actions on mucosal blood flow (81) suggesting a local interaction of these vasoactive mediators with sensory neuropeptides within the mucosal microcirculation.

PROTECTION AND INJURY BY NO DONORS

The release of NO from nitrovasodilator agents either following metabolic transformation in the vasculature as with glyceryl trinitrate (GTN) and isoamyl nitrite, or spontaneously as with nitroprusside, is responsible for their ability to activate guanylate cyclase, elevate cyclic GMP and to relax vascular smooth muscle (42, 82-84).

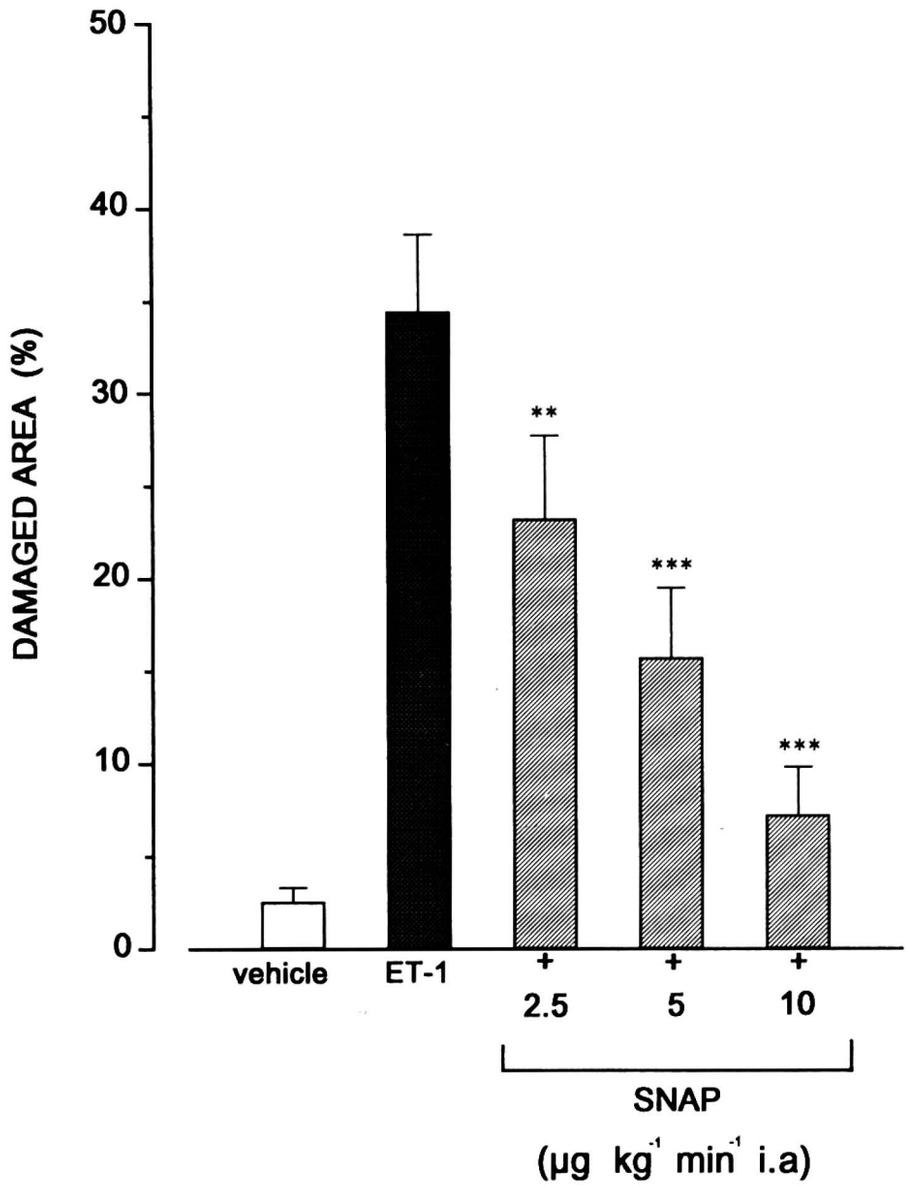

Fig. 3. Effects of concurrent close-arterial infusion of the NO donor, S-nitroso-N-acetyl-penicillamine (SNAP; 2.5-10 μg kg^{-1} min^{-1} for 15 min) on the rat gastric mucosal damage induced by ET-1 (5 pmol kg^{-1} min^{-1} for 15 min). Results, shown as the area of macroscopically assessed damage expressed as % of total mucosal area, are mean ± s.e. mean of 4-8 experiments per group, where significant difference from ET-1 group alone is shown as $^{**}P < 0.01$, $^{***}P < 0.001$. Data are adapted from Lopez-Belmonte et al (89).

Following intragastric application, these nitrovasodilators reduced the acute haemorrhagic mucosal injury induced by topical irritants and by intravenous infusion of ET-1 (85, 86). The nitrosothiol, S-nitroso-N-acetyl-penicillamine (SNAP), which spontaneously liberates NO (82) has also been demonstrated to protect against acute microvascular injury in the stomach and small intestine induced by PAF or endotoxin following intravenous administration (87, 88).

The protective actions of locally infused SNAP on the mucosal damage by close-arterial infusion of ET-1 has also been investigated (89). Local intra-arterial infusion of ET-1 induced dose-dependent mucosal damage, when assessed macroscopically 20 min later (*Figure 1*). This damage, which consisted of areas of mucosal vasocongestion and haemorrhage (72, 80), was significantly reduced by concurrent local intra-arterial infusion of SNAP, as shown in *Figure 3*.

By contrast, local infusion of higher doses of SNAP (20-40 μg kg^{-1} min^{-1}) did not significantly reduce the mucosal damage induced by ET-1. Furthermore, local infusion of these doses of SNAP for 15 min, itself induced haemorrhagic injury to the mucosa as shown in *Fig. 4*. At the higher local dose

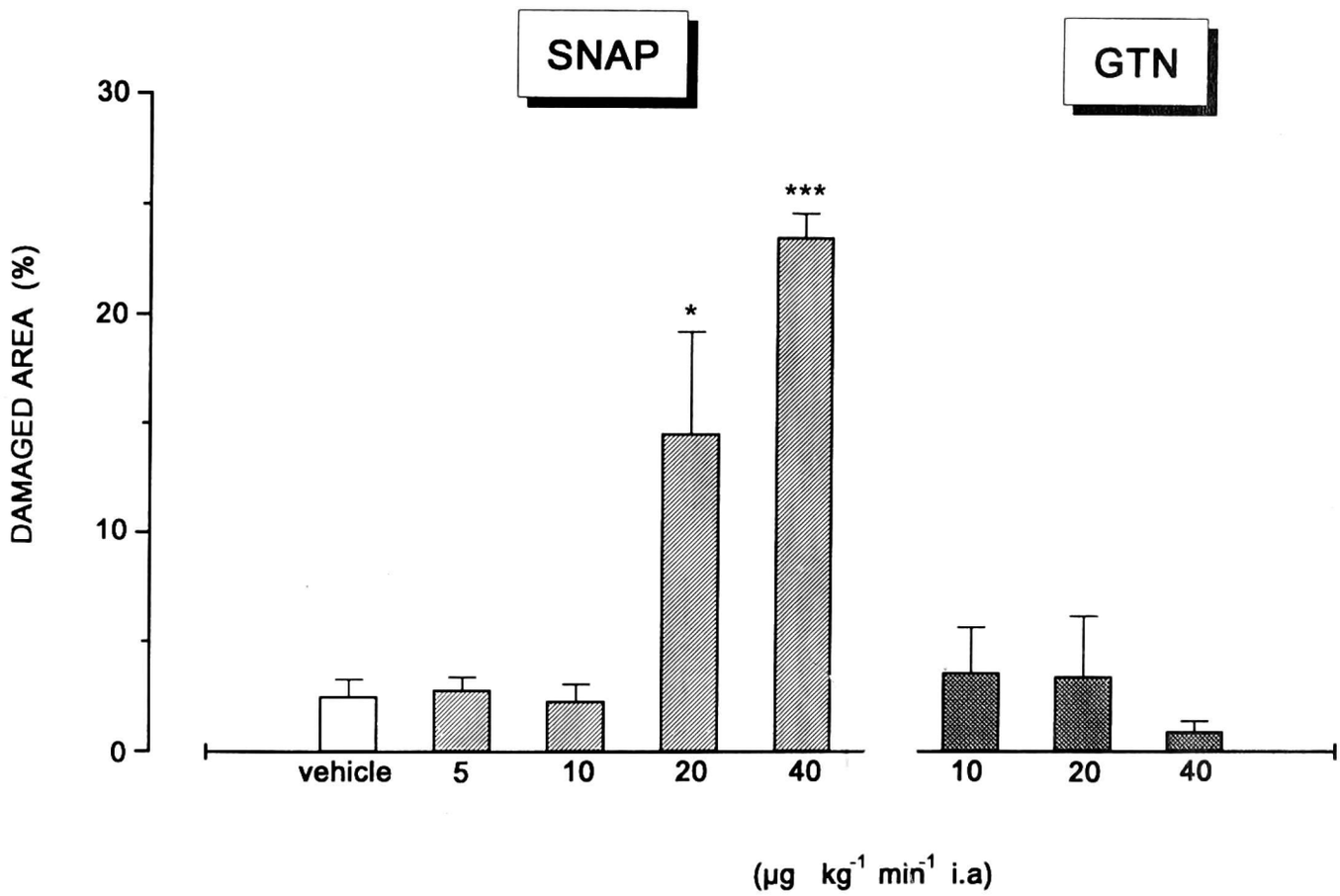

Fig. 4. Induction of rat gastric mucosal damage by close-arterial infusion of S-nitroso-N-acetyl-penicillamine (SNAP; 5-40 μg kg^{-1} min^{-1} for 15 min) but not by glyceryl trinitrate (GTN 10-40 μg kg^{-1} min^{-1} i.a.). Results, shown as the area of macroscopically assessed injury, expressed as % total mucosal area, are mean ± s.e. mean of at least 5 experiments in each group, where significant difference for control (saline infusion) is given as $^{*}P < 0.05$, $^{***}P < 0.001$. Data are adapted from Lopez-Belmonte et al (89).

of SNAP, a fall in systemic arterial blood pressure was observed (*Fig. 5*), indicating its escape into the systemic circulation. This fall in arterial blood pressure, like the injurious action on the gastric mucosa, was dependent on NO release since incubation of this thermodynamically and photosensitive NO donor for 48 hours at 37°C in ambient light to deplete its NO content, abolished both of these actions (*Fig. 5*). Local infusion of glyceryl trinitrate, which requires metabolic transformation, protects against ET-1 induced injury (89) but in contrast to SNAP, does not itself cause any damage (*Fig. 4*).

Local infusion of nitroprusside, which like SNAP also spontaneously releases NO, likewise induced gastric mucosal necrosis and reduced blood pressure (*Figure 5*). However, the degree of damage induced by nitroprusside was greater than that induced by a similar dose of SNAP, yet the fall in BP was less, indicating a dissociation between these events. Infusion of ferricyanide, in which the nitroso moiety is replaced by a further cyano group did not induce gastric mucosal damage (*Figure 5*), indicating the involvement of NO release in the cytotoxicity of nitroprusside.

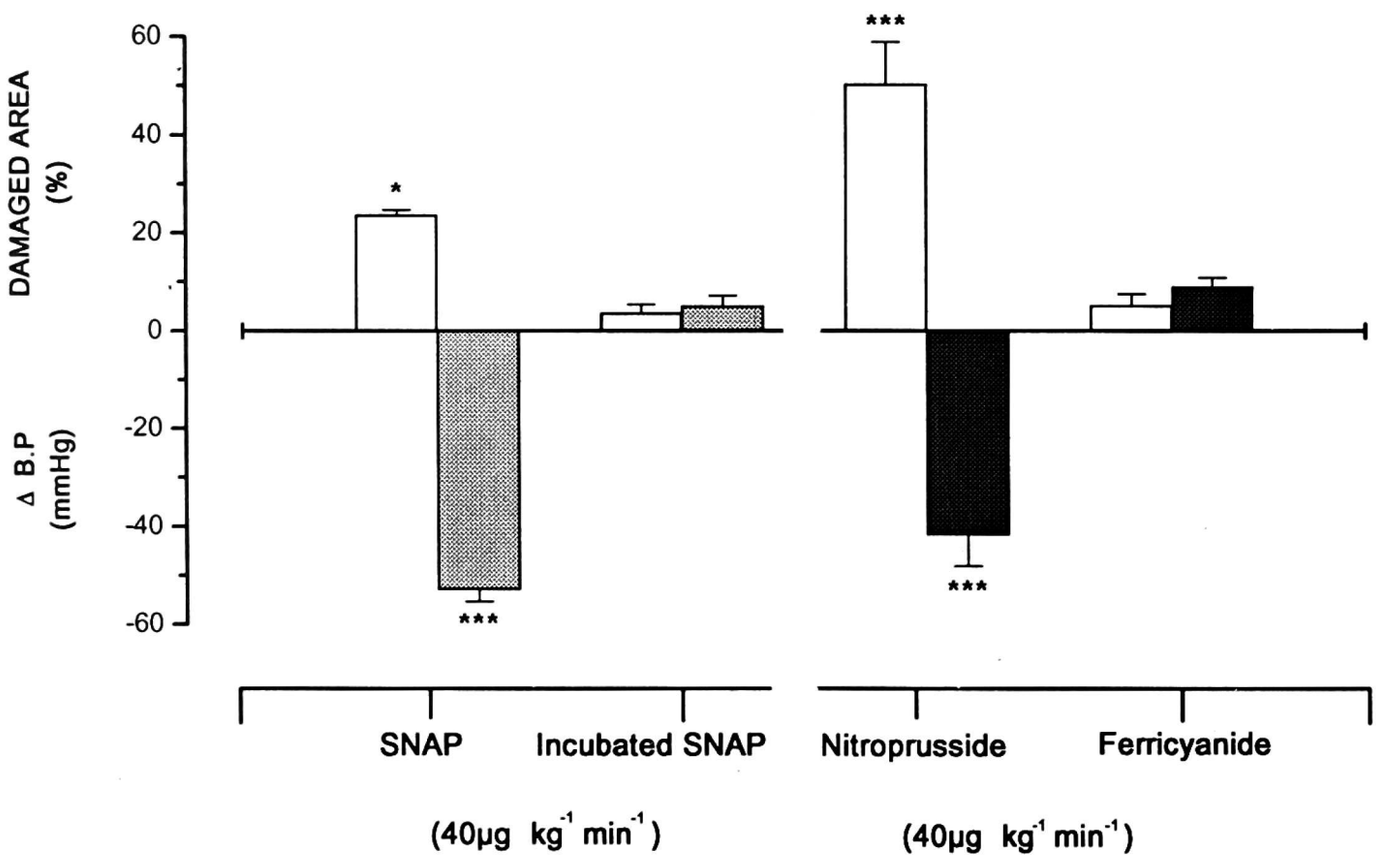

Fig. 5. Effect of nitroprusside or ferricynanide, S-nitroso-N-acetyl-penicillamine (SNAP) or SNAP incubated for 48h at 37°C in ambient light, on the induction of rat gastric mucosal injury and fall in systemic arterial blood pressure (BP) following close-arterial infusion (40μg kg^{-1} min^{-1} for 15 min for each). Results, shown as area of macroscopically assessed mucosal injury as % of total mucosal area and change in BP (ΔmmHg), are mean ± s.e. mean of at least 5 experiments in each group, where significant difference for control is given as $*P < 0.05$, $***P<0.0001$. Data are adapted form Lopez-Belmonte et al (89).

CONCLUSIONS

These findings indicate that local administration of the nitrosothiol, SNAP, in low doses can protect the gastric mucosa from damage induced by intraarterial infusion of ET-1. Likewise, local intra-arterial infusion of GTN protected against such ET-1 induced damage (89). Such actions are likely to reflect vascular interactions between the vasoconstrictor peptide and the locally generated NO in the mucosal microcirculation.

The mucosal injury associated with the spontaneous and unregulated release of NO from higher doses of SNAP, as well as from nitroprusside may indicate cytotoxic actions of high levels of NO on the microvascular endothelium. Indeed, the excessive production of NO by an inducible NO synthase in endothelial cells is considered to underlie the reduction in viability of these cells in culture following exposure over a 48h period to endotoxin and the cytokine, interferon-α (91), while induction of NO synthesis is also considered to be involved in damage to adenocarcinoma cells (90). In addition, the substantial synthesis of NO by the immunologically induced NO synthase in macrophages accounts for the cytotoxic actions against tumour cells (32, 33, 92).

The induction of NO synthase has also been implicated in the cardiovascular crisis and collapse seen over several hours in both animals and man in endotoxaemia (93, 94). Furthermore, the microvascular permeability changes, an index of endothelial injury, seen in the rat small and large intestine four to six hours after endotoxin administration is correlated with the induction of a calcium-independent NO synthase over this period (95).

It is feasible that local high concentrations of NO, generated from SNAP, may also form tissue destructive species such as the peroxynitrite and hydroxyl moieties (96) and hence bring about endothelial injury in the microvasculature, leading to mucosal necrosis and ulceration. However, it is also clear that the physiological formation of NO by the constitutive enzyme has importance in protecting against the initial detrimental cardiovascular changes and the intestinal vascular injury seen in the acute phases of endotoxin shock (97, 98).

It is apparent therefore, that NO may be involved in both physiological and pathological events in the gastrointestinal mucosa. Thus, endogenous NO plays an important role in the modulation of mucosal blood flow. Furthermore, NO has a key interactive role with other local protective mediators such as the prostanoids and sensory neuropeptides in the physiological regulation of mucosal integrity, and inhibition of its formation can provoke gastric tissue damage especially under conditions where the synthesis or release of these other mediators is compromised (64). Such interactions between these protective mediators, as well as interactions with pro-ulcerogenic mediators such as ET-1, will need to be considered when evaluating mechanisms

underlying peptic ulceration. However, an excess, unregulated, liberation of NO has also ulcerogenic potential. The factors that regulate the synthesis and release of endogenous NO by both the constitutive enzyme, likely to be involved in physiological processes, and by the inducible enzyme that may underlie certain pathological events, remain to be clarified. It is evident therefore that knowledge of these processes and how they interact with the release of other protective mediators, as well as with the release of injurious mediators, will be of significant importance to the understanding of the pathogenesis of ulcer disease.

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