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Interaction of nitric oxide and salivary gland epidermal growth factor in the modulation of rat gastric mucosal integrity

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1 The interaction between endogenous nitric oxide (NO) and factors from the rat submandibular salivary gland such as epidermal growth factor (EGF) on gastric mucosal integrity in the rat has been investigated.

2 Bolus administration of the NO synthase inhibitor, N^G nitro-L-arginine methyl ester (L-NAME; 6.25–50 mg kg^{-1}, i.v.) to animals treated intraluminally with 0.15 N HCl resulted in a significant increase in the area of mucosal haemorrhagic damage at doses 12.5 and 50 mg kg^{-1}. Concurrent administration of indomethacin (5 mg kg^{-1}, i.v.) resulted in a significant haemorrhagic mucosal damage in response to L-NAME (12.5–50 mg kg^{-1}). Administration of the highest dose of L-NAME resulted in an increase in histological damage to the rat gastric mucosa.

3 When compared to control animals, the extent of damage produced by L-NAME or L-NAME in combination with indomethacin was significantly exacerbated in rats which had been sialoadenectomized (SALX) by removal of the submandibular salivary glands. The mucosal damage in SALX rats was ameliorated by treatment with EGF (5 and 10 $\mu g\ kg^{-1}$, i.v.).

4 L-NAME administration resulted in a small reduction of gastric mucosal blood flow as assessed by laser Doppler flowmetry (LDF). The reduction in LDF by 25 and 50 mg kg^{-1} L-NAME was significantly greater in SALX rats than in rats with intact salivary glands. Pretreatment of SALX rats with indomethacin did not augment this large decrease in LDF suggesting that endogenous prostanoids do not interact with NO and salivary factors in regulating mucosal microcirculation.

5 Mucosal NO biosynthesis as assessed by [^{14}C]-citrulline formation was reduced in SALX rats when compared to control animals. Pretreatment of SALX animals with parenterally-administered EGF (10 $\mu g\ kg^{-1}$) was associated with an increase in [^{14}C]-citrulline formation in the gastric mucosa to levels observed in control SALX rats.

6 These data suggest that factors which originate from the salivary gland such as EGF interact with NO in the maintenance of mucosal integrity. The effects may be mediated at least in part by changes in gastric mucosal blood flow. Salivary glands and EGF may mediate these effects to some extent via changes in mucosal NO biosynthesis.

Keywords: Nitric oxide; salivary gland; epidermal growth factor; gastric mucosal integrity; gastric mucosal blood flow; N^G-nitro-L-arginine methyl ester; prostanoids

Introduction

The endothelium-derived vasodilator, nitric oxide, NO (Palmer *et al.*, 1987; Khan & Furchgott, 1987) plays a role in the regulation of the gastric microcirculation (Pique *et al.*, 1989; Whittle & Tepperman, 1991; Tepperman & Whittle, 1992). Furthermore, NO has been shown to interact with prostanoids and with sensory neuropeptides in the modulation of mucosal integrity (Whittle *et al.*, 1990; Tepperman & Whittle, 1991) and this may involve effects on both mucosal blood flow and the continuity of the microvasculature.

Epidermal growth factor (EGF) is a polypeptide originally isolated from the rodent submandibular salivary gland (Cohen, 1962). EGF administration has been shown to promote healing of duodenal ulcers and attenuate gastric mucosal damage in response to ethanol (Pilot *et al.*, 1979; Olsen *et al.*, 1984). Removal of the salivary glands in rats has been shown to increase the susceptibility of the gastric mucosa to ulcerogens and this damage could be reduced by EGF treatment (Skinner *et al.*, 1981; Olsen *et al.*, 1984). EGF has also been shown to contract isolated strips of vascular smooth muscle (Berk *et al.*, 1985; Muramatsu *et al.*, 1985) and to exert vasodilator actions on the splanchnic circulation (Gan *et al.*, 1987a,b). Therefore EGF may influence mucosal integrity via an action on the gastric microcirculation. Furthermore EGF has been shown to interact with sensory neuropeptides in the maintenance of gastric mucosal integrity (Evangelista *et al.*, 1991a,b). Since it is known that NO, prostanoids and neuropeptides from capsaicin-sensitive afferent fibres interact to influence gastric microcirculation and integrity, it is possible that similar interactions between NO and factors of salivary gland origin may also occur.

In the present study we have examined the possible interaction between endogenous NO, and EGF from the salivary gland. In order to examine these interactions we have investigated the pro-ulcerogenic actions of an inhibitor of NO formation, N^G-nitro-L-arginine methyl ester (L-NAME) in animals in which the submandibular salivary glands have been surgically extirpated.

Methods

Animal preparation

Male Sprague Dawley rats (200–250 g) were used in these studies. Sialoadenectomy (SALX) was performed under pentobarbitone anaesthesia (60 mg kg^{-1}, i.p.) by removal of the submandibular-sublingual salivary gland complexes after ligation of the ducts as previously described (Skinner & Tepperman, 1981; Skinner *et al.*, 1984). Sham-operated rats served as controls. Animals were used 2–3 weeks after surgery. Effective sialoadenectomy was confirmed by the observation of an increase in prandial drinking by approx-

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imately 200% compared to control animals (Epstein *et al.*, 1964). All experimental procedures were subsequently done on rats which were deprived of food but not water for 18–20 h before the experiments. For each experiment, rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.). All agents were administered intravenously via a catheter inserted into the tail vein.

Measurement of gastric mucosal damage

In anaesthetized rats, the stomach was exposed by a mid-line incision. After ligating the oesophagus and pylorus, 2 ml of acid saline (150 mN HCl in 150 mM NaCl) was instilled into the gastric lumen through the forestomach, followed by bolus intravenous administration of the agents under investigation. L-NAME was administered in a dose of 6.25–50 mg kg^{-1} via the tail vein. In some experiments indomethacin (5 mg kg^{-1}, i.v.) was given and 5 min later L-NAME was injected. Finally in some experiments epidermal growth factor (EGF) was injected intravenously (5 or 10 μg kg^{-1}) 15 min before administration of indomethacin and L-NAME. All injections were made in volumes of 0.5 ml or less. Thirty min after the last injection the animals were killed by cervical dislocation, the stomachs were excised and opened along the greater curvature, pinned to a wax block with the mucosal side up, immersed in neutral buffered formalin and then photographed on colour transparency film. The extent of macroscopically-visible damage was determined from these projected transparencies via computerized planimetry by the Sigma Scan programme (Mandel Scientific, Corte Madera, Calif., U.S.A.). The area of haemorrhagic mucosal damage was calculated as the % of the total gastric mucosal area.

A sample of gastric corpus (0.5 × 1.5 cm) was excised from either the dorsal or ventral aspect of the corpus mucosa, 0.5 cm below the limiting ridge, and was processed by routine techniques before being embedded in paraffin. Sections (4 μm) were stained with haematoxylin and eosin and examined under a light microscope. A 1 cm length of each histological section was assessed for epithelial cell damage (a score of 1 being assigned); glandular disruption, vasocongestion or oedema in the upper mucosa (a score of 2 being assigned); haemorrhagic damage in the mid to lower mucosa (a score of 3) and deep necrosis and ulceration (a score of 4). Each section was evaluated on a cumulative basis to give the histological damage index, the maximum score thus being 10. All determinations were performed in a randomized manner with both transparencies and histological sections coded to eliminate observer bias.

Assessment of gastric mucosal blood flow

In a separate group of experiments, the effects of L-NAME (6.25–50 mg kg^{-1}, i.v.) gastric mucosal blood flow in the presence or absence of indomethacin was assessed by laser Doppler flowmetry. Experiments were done on pentobarbitone-anaesthetized rats. The stomach was exposed by a mid-line incision. A small bore (8.5 mm o.d.) plastic cannula was then inserted via a small incision in the forestomach and tied in place, to allow free access to the gastric lumen. Gastric blood flow was recorded continuously with a laser Doppler blood flow monitor (Periflux 3; Perimed, Piscataway New Jersey, U.S.A.). A stainless steel laser optic probe (1.9 mm o.d.; Perimed) was inserted into the gastric lumen via the plastic cannula and was allowed to rest gently on the gastric corpus mucosa. Changes in laser Doppler flow (LDF) were assessed in response to intravenous bolus injection (0.5 ml kg^{-1}) of isotonic saline or the compound under investigation. Average LDF values in sham-operated and SALX rats were determined for a 3 min period prior to administration of L-NAME, indomethacin and/or EGF. Similarly the average LDF was calculated for a 3 min period, 15 min after injection of the agents where values of LDF had stabilized. Changes in LDF from pretreatment values were estimated with a software programme designed for use with the PeriFlux laser Doppler flowmeter (Perisoft; Perimed). The mean systemic arterial blood pressure (BP) was also measured from a cannula inserted into a carotid artery connected to a pressure transducer (Cobe CDX3, Lakewood, Colo., U.S.A.) and a chart researcher (Grass, model 79C).

Estimation of NO synthase activity

Experiments were done *in vitro* using segments of excised gastric mucosa. Gastric tissue was taken from pentobarbitone-anaesthetized sham-operated or SALX rats treated with saline or EGF (10 μg kg^{-1}, i.v.) as previously described. After treatment, rats were killed by cervial dislocation. The excised stomach was opened along the greater curvature. The mucosa was rinsed in ice-cold saline, scraped free of the underlying muscle with a blunt scalpel, weighed and placed in a preparative buffer consisting of 10 mM HEPES, 0.32 M sucrose, 0.1 mM EDTA, 1 mM dithiothreitol, 10 μg ml^{-1} soybean trypsin inhibitor, 10 μg ml^{-1} leupeptin, and 2 μg ml^{-1} aprotinin (pH 7.4). Samples were homogenized for 15 s in a Tekmar Ultra-Turrax homogenizer (Model SDT; Cincinnati, Ohio, U.S.A.) and then centrifuged at 10,000 *g* for 20 min at 4°C. NO synthase activity was estimated from the conversion of [^{14}C]-arginine to the NO co-product citrulline as described by Knowles *et al.* (1990). Briefly, the assay system (50 μl total volume, pH 7.2) contained 20 μl of broken cell supernatant and the following components (final concentrations): 30 mM potassium phosphate, 150 μM $CaCl_2$, 15 μM [^{14}C]-L-arginine (700,000 d.p.m. ml^{-1}), 0.7 mM NADPH, as well as 7 mM L-valine to inhibit any arginase. Incubations proceeded for 10 min at 37°C, after which time 1 ml of a 1:1 suspension of Dowex 50W in water was added to bind arginine. The resin was allowed to settle, and the supernatant was removed for estimation of the radiolabelled products by liquid scintillation counting. Product formation that was inhibited by removal of Ca^{2+} (1 mM EGTA in the assay system) or by 100 μM N^G monomethyl-L-arginine (L-NMMA) determined constitutive NO synthase activity (Knowles *et al.*, 1990).

Materials

L-NAME (Sigma Chemical, St. Louis, U.S.A.) was dissolved in isotonic saline immediately before use. Indomethacin (Sigma) was dissolved in 5% w/v Na_2CO_3 solution and diluted to 1.25% with distilled water. EGF (Receptor grade; Biomedical Technologies, Stoughton, Mass, U.S.A.) was dissolved in isotonic saline immediately before use. [^{14}C]-L-arginine monohydrochloride (319 mCi $mmol^{-1}$) was purchased from Amersham Canada, Oakville, Ontario. L-NMMA was purchased from Calbiochem (LaJolla, Calif., U.S.A.). All other components of the NO synthase assay were purchased from Sigma.

Statistical analysis

All data are expressed as the mean ± s.e.mean. Comparisons between SALX and sham-operated rats were made by Student's *t* test for unpaired data while comparisons within groups and between vehicle and indomethacin-treatment were made by analysis of variance (ANOVA) and Duncan's Multiple Range Test. *P* values of less than 0.05 were taken as significant.

Results

Gastric mucosal damage

In response to intraluminal instillation of 150 mM HCl there was no significant difference in the extent of haemorrhagic damage between SALX and sham-operated rats treated

parenterally with saline (Figure 1). L-NAME administration (12.5 and 50 mg kg^{-1}) resulted in a small but significant increase in the extent of haemorrhagic damage in sham-operated rats when compared to saline-treatment (Figure 1). In sialoadenectomized (SALX) rats the area of haemorrhagic damage in response to some doses of L-NAME (12.5–50 mg kg^{-1}) was significantly greater than that observed in the corresponding group of sham-operated control rats. By itself, indomethacin did not result in a significant increase in the area of mucosal damage in control or SALX rats when compared to similar groups of rats treated with vehicle. Indomethacin pretreatment was associated with a significant exacerbation of haemorrhagic damage in sham-operated rats given 12.5–25 mg kg^{-1} L-NAME. The area of haemorrhagic damage in SALX rats treated with indomethacin was signficantly greater than similarly treated sham-operated rats in response to all doses of L-NAME except 12.5 mg kg^{-1}. In indomethacin-treated SALX rats, only damage in response to 50 mg kg^{-1} L-NAME was greater than was observed in SALX control animals.

Pretreatment of SALX rats receiving the combination of indomethacin and L-NAME (50 mg kg^{-1}) with EGF (5 μg kg^{-1} or 10 μg kg^{-1}, i.v.) resulted in a significant dose-dependent decrease in mucosal haemorrhage damage from 12.7 ± 0.8% to 8.1 ± 0.7% and 6.5 ± 1.6% respectively (n = 5–7 for each group). In sham-operated rats treated with indomethacin and L-NAME (50 mg kg^{-1}), EGF (10 μg kg^{-1}) did not significantly reduce the extent of haemorrhagic damage (9.9 ± 0.5% vs 8.3 ± 1.4%; n = 5–7 each group).

Similarly the histological damage index, in response to individual administration of these agents, was greater in SALX rats than in control animals (Figure 2). In control rats the combination of indomethacin and L-NAME (50 mg kg^{-1}) resulted in a significant increase in the histological damage index compared to that observed when this dose of L-NAME was administered alone (Figure 2). There was a further and significant increase in both types of microscopic damage in SALX rats treated with the combination of L-NAME and indomethacin.

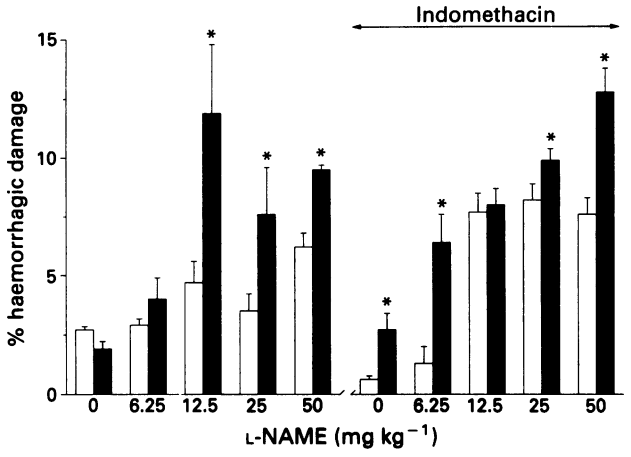

Figure 1 Percentage of the gastric mucosal area displaying haemorrhagic damage in response to intravenous injection of N^G-nitro-L-arginine methyl ester (L-NAME) alone or in combination with indomethacin (5 mg kg^{-1}, i.v.). Experiments were done using sialoadenectomized (SALX, solid columns) or sham-sialoadenectomized (open columns) rats. Results shown as mean ± s.e.mean. Asterisks (*) indicate significant differences between SALX and sham-operated animals receiving similar doses of L-NAME as determined by Student's t test for unpaired data within groups. L-NAME produced significant damage in sham-operated control animals (50 mg kg^{-1}) and sham-operated, indomethacin-treated animals (12.5–50 mg kg^{-1}) as determined by ANOVA and Duncan's multiple range test (n = 6–9 per group).

Gastric mucosal blood flow

In response to saline, LDF was not significantly changed in either SALX or sham-control rats (Figure 3). In sham-operated rats, L-NAME administration resulted in a small decrease in LDF. In SALX rats the decrease in LDF in response to L-NAME was significantly greater in response to 25 and 50 mg kg^{-1} L-NAME (Figure 3). There was no significant effect of indomethacin alone on LDF in sham-

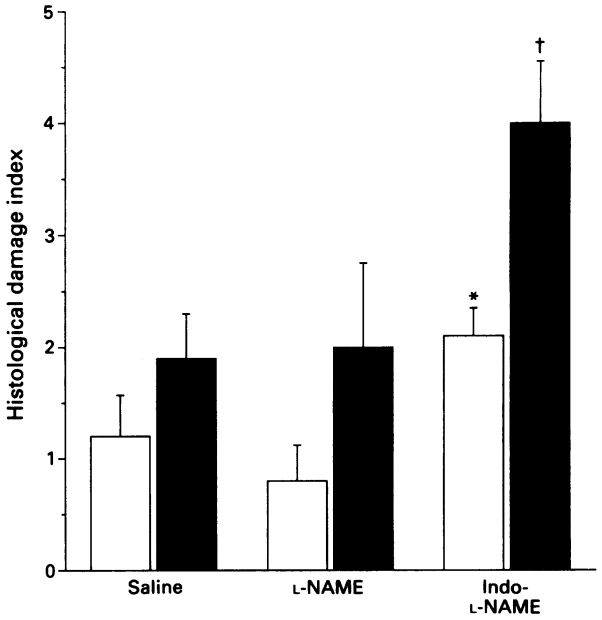

Figure 2 Histological damage index of the gastric mucosa of sialoadenectomized (SALX, solid columns) or sham sialoadenectomized (open columns) rats treated intravenously with saline, N^G-nitro-L-arginine methyl ester (L-NAME; 50 mg kg^{-1}) alone or indomethacin (5 mg kg^{-1}) in combination with L-NAME. Histological index was assessed as described in Methods. Results shown as mean ± s.e.mean. Asterisks (*) indicate significant increases over saline-treated sham-operated rats. Crosses (†) indicate significant differences between similarly treated sham-operated and SALX rates. Statistical significance was determined by ANOVA and Duncan's Multiple Range Test. (n = 5–7 per group).

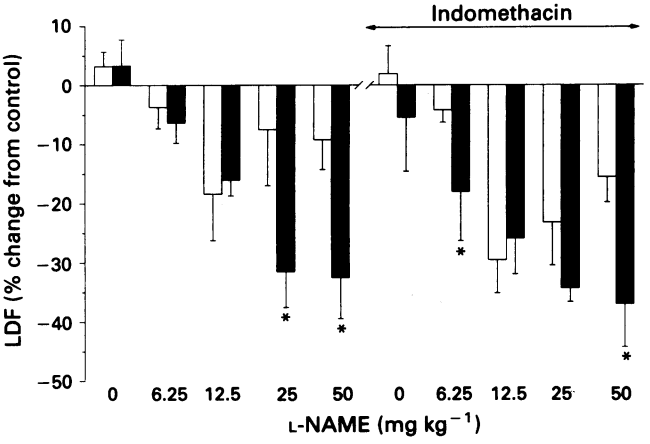

Figure 3 Laser Doppler flow (LDF) in sialoadenectomized (SALX, solid columns) or sham-operated (open columns) rats treated with N^G-nitro-L-arginine methyl ester (L-NAME, i.v.). LDF was calculated as a % change from basal LDF values. Results shown as mean ± s.e.mean. Asterisks (*) indicate significant differences between correspondingly treated sham-operated and SALX animals within groups as determined by Student's t test for unpaired data. (n = 5–7 per group).

operated or SALX rats. While indomethacin-treatment resulted in a further reduction in LDF in sham-operated rats in response to 25 and 50 mg kg^{-1} L-NAME, the decline in LDF was not greater in SALX rats when compared to SALX rats receiving vehicle in place of indomethacin.

EGF administration (10 μg kg^{-1}, i.v.) resulted in a small but significant ($P<0.05$) increase in LDF above basal values in sham-operated (4.2 ± 0.3%, $n=6$) and SALX (3.1 ± 0.1%, $n=6$) rats. When EGF was administered to sham-operated or SALX rats treated concurrently with indomethacin and L-NAME (50 mg kg^{-1}), LDF values were not significantly different from zero (− 8.9 ± 7.6%, $n=4$ and 3.3 ± 11.5%, $n=4$ respectively).

Effects on systemic arterial blood pressure

Intravenous administration of L-NAME (6.25–50 mg kg^{-1}) induced a dose-dependent rise in systemic arterial blood pressure (Figure 4) above resting values (89 ± 6 mmHg; $n=25$). The hypertensive response was significantly augmented in SALX rats over the entire dose range of L-NAME examined. Indomethacin-treatment of sham-operated rats further increased the hypertensive response to L-NAME. In indomethacin-treated SALX rats, arterial pressure was elevated to the same degree regardless of the dose of L-NAME examined.

Mucosal NO synthase activity

NO synthase activity as estimated by [^{14}C]-citrulline formation was detected in the mucosa of sham-operated and SALX rats (Figure 5). The activity was significantly ($P<0.05$) in both groups by *in vitro* addition of 1 mM EGTA (− 92 ± 4%, sham-operated; − 87 ± 6%, SALX) or 100 μM L-NMMA (− 81 ± 8%, sham operated; − 83 ± 6%, SALX). Total NO formation was significantly less in SALX rats than in sham-operated animals (Figure 5). In rats pretreated with EGF (10 μg kg^{-1}, i.v.), NO synthase activity was not significantly affected in sham-operated rats (Figure 5). Furthermore, in SALX rats the amount of [^{14}C]-citrulline formed in response to EGF administration was not significantly different from that observed in sham-operated rats treated with saline.

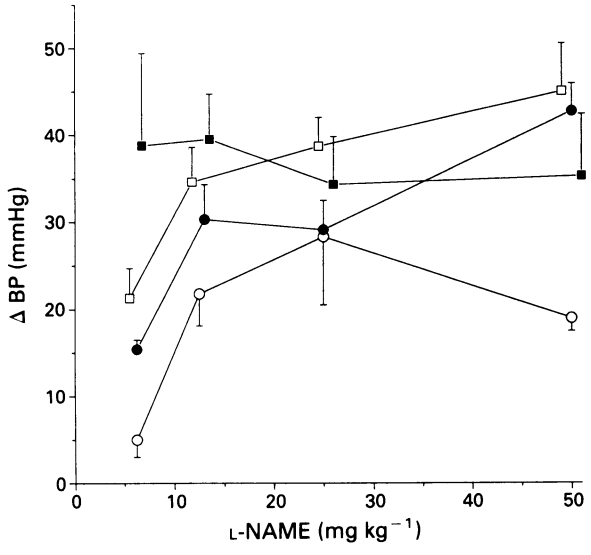

Figure 4 Effects of N^{G}-nitro-L-arginine methyl ester (L-NAME, 6.25–50 mg kg^{-1}, i.v) on changes (Δ) in systemic arterial blood pressure (BP) in sham-operated (○) and sialoadenectomized (□) control rats or after treatment with indomethacin (5 mg kg^{-1}, i.v.; ●, ■). *Results shown as mean ± s.e.mean. $n=4$–5 experiments for each group.

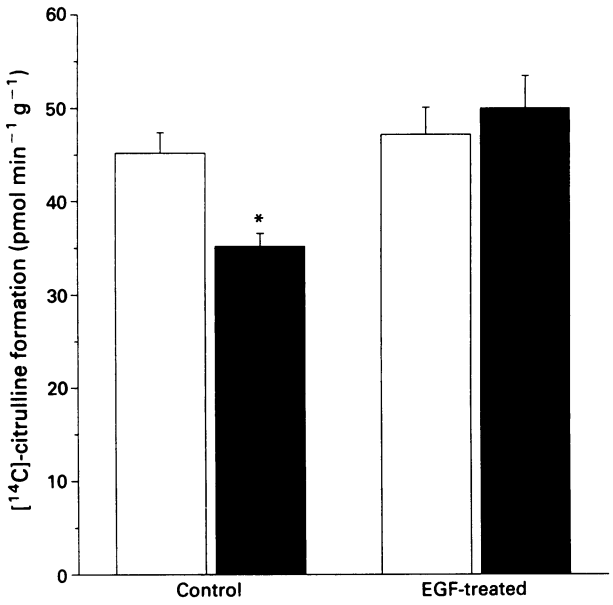

Figure 5 Nitric oxide synthase activity of rat gastric mucosa as assessed by [^{14}C]-citrulline formation. Experiments were done in sialoadenectomized (SALX, solid columns) or sham-operated (open columns) rats treated parenterally with saline (control) or epidermal growth factor, (EGF, 10 μg kg^{-1}). Results shown as mean ± s.e. mean. Asterisks (*) indicate significant differences between sham-operated and SALX control animals as determined by Student's *t* test for unpaired data. [^{14}C]-citrulline formation in EGF-treated SALX rats was significantly ($P<0.05$) greater than the levels determined in SALX control animals as determined by Student's *t* test for unpaired data. ($n=6$–7 per group).

Discussion

The present study demonstrates that in control rats, the inhibitor of NO synthase activity N^{G} nitro-L-arginine methyl ester, L-NAME, by itself resulted in a small degree of macroscopically or histologically detectable gastric mucosal damage. This finding is similar to what has been previously reported by Whittle *et al.* (1990) and Whittle & Tepperman (1991) using the NO synthase inhibitor N^{G} monomethyl-L-arginine (L-NMMA). However a key finding in the present study was that under conditions of sialoadenectomy (SALX) both macroscopic and microscopic damage induced by administration of L-NAME were significantly enhanced. Furthermore the present data also demonstrated that this augmentation in damage could be reversed by pretreatment with EGF. These data suggest that some factors from the salivary gland, possibly EGF, interacts with NO to influence gastric mucosal integrity.

The role of the salivary glands in gastric mucosal integrity has been examined previously. Removal of the salivary glands in rats has been shown to increase the susceptibility of the gastric mucosa to a number of ulcerogens including cysteamine, bile salts and ethanol (Skinner & Tepperman, 1981; Olsen *et al.*, 1984). This increased susceptibility to damage could be reversed by administration of exogenous EGF. In animals with intact salivary glands, EGF has been shown to exert a protective influence on the gastric mucosa (Pilot *et al.*, 1979; Konturek *et al.*, 1981) and levels of EGF in saliva have been shown to increase if the mucosa is damaged (Gysin *et al.*, 1988). The results of these studies suggest that endogenous EGF, released from the salivary gland, plays a role in maintaining mucosal integrity.

The mechanism by which salivary factors such as EGF affect mucosal integrity is uncertain. Konturek and colleagues (1981; 1988) have speculated that the protective

action of EGF may be due to a mitogenic effect while Sarosiek *et al.* (1988) have demonstrated that salivary EGF maintains the gastric mucus coat. In addition, a role for salivary EGF in the gastric microvasculature has been suggested. EGF can influence splanchnic blood flow (Gan *et al.*, 1987a,b) and recently in a preliminary study EGF has been shown to increase gastric mucosal blood flow (Hui *et al.*, 1991). In addition Namiki & Akatsuka (1990) have demonstrated that the vasoactive effect of EGF on rat aorta depends on intact endothelial layer. In the present study, the reduction in LDF to high doses of L-NAME (>12.5 mg kg^{-1}) was augmented in SALX rats. Furthermore, parenterally administered EGF resulted in an increase in mucosal blood flow as assessed by LDF and EGF treatment reversed the LDF fall in response to L-NAME in SALX rats. These data suggest that a factor from the salivary glands, possibly EGF, interacts with NO to modulate mucosal blood flow and hence tissue integrity.

The maintenance of mucosal integrity depends critically on the status of the microcirculation. Reduction in microvascular perfusion can lead to development of mucosal damage (Whittle, 1977). Removal of an endogenous vasodilator such as NO can lead to reductions in blood flow. Whittle and colleagues (Whittle *et al.*, 1990; Whittle & Tepperman 1991) have demonstrated that an inhibitor of NO biosynthesis such as L-NMMA reduced gastric mucosal blood flow. In the present study we have observed that by itself L-NAME at doses of 12.5–50 mg kg^{-1} did not consistently reduce mucosal blood flow. In a previous study Tepperman & Whittle (1992) have demonstrated that doses of 6.25 and 12.5 mg kg^{-1} L-NAME significantly reduced blood flow. The reasons for these differences are uncertain although we have used a different laser Doppler flowmeter and flow probe to estimate blood flow than was used in the study cited above. However Lippe & Holzer (1992) have demonstrated that the effect of NO synthesis inhibition on mucosal blood flow depends critically on the level of systemic arterial blood pressure. Blockade of NO formation lowered blood flow only if blood pressure was above a critical value. Thus the differences between studies may be due to the resting mean arterial pressures in the anaesthetized rats used in these studies.

The present study also confirms that L-NAME induced a dose-dependent increase in systemic blood pressure. The hypertensive action of all doses of L-NAME examined here were augmented in SALX rats. Since gastric microvascular changes in SALX rats were only seen at the higher doses of L-NAME, this suggests the interactions between endogenous NO and salivary gland factors on the gastric mucosa are not exclusively the result of changes in systemic arterial blood pressure.

Indomethacin pretreatment significantly augmented macroscopic mucosal damage in sham-operated rats in response to 12.5 and 25 mg kg^{-1} doses of L-NAME. These responses are similar to those observed by Whittle *et al.* (1990), Tepperman & Whittle (1992) and Whittle & Tepperman (1991) in which indomethacin enhanced mucosal injury in rats treated with L-NMMA. Furthermore in these animals, mucosal blood was further reduced in response to the highest dose of L-NAME. These present findings suggest that under the present conditions, endogenous prostanoids interact to some extent with NO to influence mucosal integrity by a modulation of mucosal blood flow, although direct effects on the microvascular endothelium cannot be excluded. In contrast, in indomethacin-treated SALX rats, neither damage nor LDF values were significantly different from control SALX animals suggesting that endogenous prostanoids do not interact with salivary gland factors and NO on the maintenance of mucosal integrity and vascular perfusion.

In the present study, using radiolabelled citrulline formation as an index of enzyme activity, NO synthase was found to be present in homogenates of rat gastric mucosa. This confirms findings by Whittle *et al.* (1991) using spectrophotometric determinations of NO synthase activity. In the present study, we have observed that SALX was associated with a reduction in NO synthesis. Furthermore, EGF pretreatment resulted in a restoration of mucosal NO biosynthesis to levels observed in sham sialoadenectomized rats. These data could suggest that SALX exacerbates mucosal damage in part by a further reduction in endogenous NO production and thereby influences mucosal blood flow. The mechanism(s) whereby SALX and salivary gland factors such as EGF affect mucosal NO synthase activity is currently under investigation in this laboratory.

Our data are comparable to results produced by Evangelista *et al.* (1991a,b). In those studies, sialoadenectomy was shown to interact with capsaicin-sensitive afferent fibres to augment water immersion stress-induced ulcers in rats. Previous studies have shown that sensory afferent nerves interact with NO in the maintenance of mucosal blood flow and integrity. Thus the present investigation confirms and extends these previous studies by the demonstration that factors from the salivary gland such as EGF may also interact with NO in the maintenance of gastric mucosal integrity. The interaction appears to be mediated, at least in part, by an action on the gastric microvasculature.

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