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Induction of rat gastric damage by the endothelium-derived peptide, endothelin

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The effect of the endogenous vasoconstrictor peptide, endothelin, on gastric mucosal integrity has been investigated in the rat. Local intra-arterial infusion of endothelin, in picomole doses, dose-dependently induced haemorrhagic and necrotic damage in the gastric mucosa. Such injury was not prevented by atropine, cimetidine, adrenoceptor antagonists, indomethacin, or the 5-lipoxygenase inhibitor BW A4C. These results suggest a potential pro-ulcerogenic role of endothelin in the pathogenesis of gastric damage and ulceration.

Introduction Endothelin, a recently described 21-residue peptide synthesized by vascular endothelial cells, exhibits vasoconstrictor actions both *in vitro* and *in vivo* (Yanagisawa *et al.*, 1988). Since an adequate blood flow in the gastric microcirculation is considered essential to maintain mucosal integrity, we have now investigated whether endothelin can induce gastric damage.

Methods Male Wistar rats (230–250 g) deprived of food but not water for 18–20 h, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), the stomach exposed by a mid-line incision and the left gastric artery cannulated (Esplugues & Whittle, 1988a). Systemic arterial blood pressure (BP) was measured from a cannula inserted into a carotid artery and connected to a pressure transducer and a chart recorder (Rikadenki, R-50).

Acid-saline (100 mM HCl, 50 mM NaCl; 2 ml) was instilled into the gastric lumen via a needle inserted through the forestomach, to ensure an acidic environment. Endothelin (Peninsula Labs, Inc., CA., USA, stored at −20°C) was prepared freshly in isotonic saline, and administered by close intra-arterial infusion (10 $\mu l\,min^{-1}$) for 10 min. Twenty minutes later, the stomachs were removed, opened along the greater curvature, pinned out in neutral buffered formalin and photographed. The extent of macroscopically-visible damage was determined in a randomized manner via computerized planimetry and calculated as the % of glandular mucosa exhibiting damage.

For histological assessment, samples were excised from standardized regions of the stomach and processed prior to embedding in paraffin. Sections (4 μm), stained with haematoxylin and eosin, were examined in a randomized manner under a light microscope, with a histological score being assigned according to previously defined criteria (Esplugues & Whittle, 1988a).

BW A4C (N-(3-phenoxycinnamyl)-acetohydroxamic acid) (Tateson *et al.*, 1988) was synthesized in the Wellcome Research Laboratories; cimetidine was obtained from SK&F Labs. and the other drugs from Sigma Chemical Company. Data are expressed as mean ± s.e.mean, with comparison between the groups being made by Student's t test for unpaired or paired data and by the Mann-Whitney U-test for the histological index, where $P < 0.05$ was taken as significant.

Results Local intra-arterial infusion of the vehicle, isotonic saline, induced minimal macroscopically-detectable damage of the gastric mucosa. Intra-arterial infusion of endothelin (10–250 ng kg^{-1} min^{-1}; 4–100 pmol kg^{-1} min^{-1}) however, induced a dose-dependent increase in macroscopically-assessed damage (Figure 1). Local infusion of endothelin (40 and 100 pmol kg^{-1} min^{-1}) also increased BP (by 7 ± 1 and 9 ± 1 mmHg, $n = 4$, $P < 0.05$ respectively; from a resting value of 107 ± 3 mmHg, $n = 12$). Intravenous infusion of endothelin (100 pmol kg^{-1} min^{-1} for 10 min) likewise increased BP (by 12 ± 4 mmHg, $n = 4$, $P < 0.05$), yet did not cause any detectable mucosal damage.

The macroscopic damage was apparent across the whole mucosal surface as areas of vasocongestion, haemorrhage and necrosis with sloughing of an opaque mucoid covering layer. On histological evaluation, epithelial disruption was noted, with diffuse microvascular congestion and haemorrhagic damage throughout the mucosa. With the higher dose of endothelin (100 pmol kg^{-1} min^{-1} locally), the control histological index was increased from 0.8 ± 0.2 to 20 ± 3 ($n = 4$, $P < 0.05$).

The degree of gastric damage induced by a submaximal dose of endothelin (40 pmol kg^{-1} min^{-1}) was not significantly reduced by pretreatment (15 min) with atropine (1.5 mg kg^{-1} i.v.), by the α-

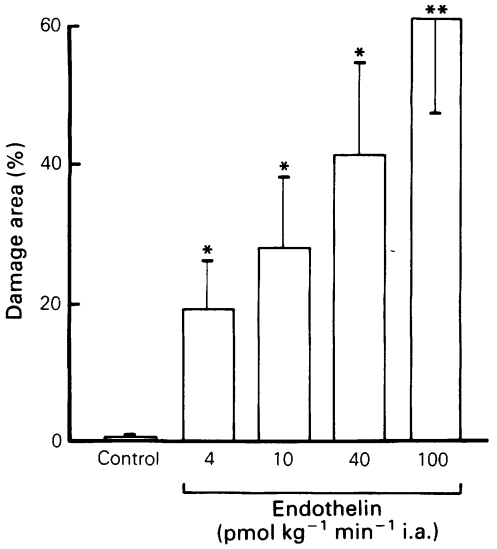

Figure 1 Gastric damage in the rat in the presence of intraluminal acid (100 mM HCl, 50 mM NaCl), induced by a 10 min local intra-arterial infusion of endothelin (4–100 pmol kg^{-1} min^{-1}). Results, expressed as the % of the total mucosal area that exhibited macroscopically-assessed damage, are the mean of 4 experiments in each group; vertical lines show s.e.mean. Statistical significance from the control (saline-infused) group is shown as * $P < 0.05$, ** $P < 0.01$.

or β-adrenoceptor antagonists phentolamine (10 mg kg^{-1}, i.v.) and propanolol (1 mg kg^{-1}, i.v.), by an anti-secretory dose of cimetidine (50 mg kg^{-1}, i.v.), by the cyclo-oxygenase inhibitor, indomethacin (5 mg kg^{-1}, s.c., a dose that itself did not induce mucosal damage) or by BW A4C (20 mg kg^{-1}, orally), a 5-lipoxygenase inhibitor ($n = 3$ or 4 per group, $P > 0.05$ for each compared to those receiving endothelin alone).

Discussion These results indicate that local intra-arterial infusion of low doses of endothelin induced extensive damage to the rat gastric mucosa, and it is therefore one of the most potent endogenous ulcerogens described. Although the subsequent release of pro-ulcerogenic mediators cannot yet be entirely excluded, the mechanism by which endothelin provokes such gastric damage is likely to involve vasoconstriction in the mucosal microcirculation. Thus, previous studies have demonstrated that the endogenous vasoconstrictor thromboxane A_2 (TXA_2), generated locally from blood-borne platelets, potentiated gastric ulceration in the dog (Whittle *et al.*, 1981). Furthermore, local administration of a vasoconstrictor thromboxane mimetic induced haemorrhagic damage to the rat gastric mucosa (Esplugues & Whittle, 1988b).

Under pathological conditions, the endogenous release of platelet-derived TXA_2 or the endothelium-derived endothelin may be involved in initiating or promoting gastric mucosal damage. Furthermore, a local balance between endothelin and endothelium-derived relaxing factors or prostacyclin, which exerts vasodilator and protective actions in the gastric mucosa, may be of physiological importance in regulating microvascular blood flow and hence mucosal integrity. Thus, inhibition of prostacyclin formation by non-steroid anti-inflammatory agents or vascular disease may lead to an imbalance in such regulation, leading to endothelin-induced vascular spasm and ischaemia in the gastric microcirculation, events that could induce or predispose to mucosal damage.

The processes that regulate endothelin production from endothelial cells are not yet clear, although adrenaline stimulates the formation of the precursor *in vitro* (Yanagisawa *et al.*, 1988). Enhanced endothelin formation in the mucosal microcirculation, perhaps as a consequence of the release of catecholamines *in vivo* during stress conditions, may therefore contribute to any associated gastric damage. Indeed, since disturbances to microvascular endothelium or ischaemia are likely to be prime events leading to mucosal disruption, local inappropriate formation and release of vasoconstrictor mediators such as endothelin may be intimately involved with the pathogenesis of gastric ulceration.

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References

ESPLUGUES, J.V. & WHITTLE, B.J.R. (1988a). Gastric mucosal damage induced by local intra-arterial administration of Paf in the rat. *Br. J. Pharmacol.*, **93**, 222–228.

ESPLUGUES, J.V. & WHITTLE, B.J.R. (1988b). Close-arterial administration of the thromboxane mimetic U-46619 induces damage to the rat gastric mucosa. *Prostaglandins*, **35**, 137–148.

TATESON, J.E., RANDALL, R.W., REYNOLDS, C.H., JACKSON, W.P., BHATTACHERJEE, P., SALMON, J.A. & GARLAND, L.G. (1988). Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids:

Biochemical assessment *in vitro* and *ex vivo*. *Br. J. Pharmacol.*, **94**, 528–539.

WHITTLE, B.J.R., KAUFFMAN, G.L. & MONCADA, S. (1981). Vasoconstriction with thromboxane A_2 induces ulceration of the gastric mucosa. *Nature*, **292**, 472–474.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332**, 411–415.

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