Nitric oxide mediates the inhibition by interleukin-1 β of pentagastrin-stimulated rat gastric acid secretion

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Bolus injection of interleukin-1 β (2 $\mu\text{g kg}^{-1}$, i.v.) inhibited acid secretion induced by intravenous infusion of pentagastrin (8 $\mu\text{g kg}^{-1} \text{ h}^{-1}$) in the continuously perfused stomach of the anaesthetized rat. Administration of interleukin-1 β did not modify mean systemic arterial blood pressure. Pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME, 2–10 mg kg^{-1} , i.v.), but not dexamethasone (5 mg kg^{-1} , s.c. twice over 16 h), restored the acid secretory responses to pentagastrin. The actions of L-NAME were reversed by the prior administration of L-arginine (100 mg kg^{-1} , i.v.), but not by its enantiomer D-arginine (100 mg kg^{-1} , i.v.). L-NAME (5 mg kg^{-1} , i.v.) increased blood pressure but this was not the mechanism by which interleukin-induced acid inhibition was prevented, since similar systemic pressor responses induced by phenylephrine (10 $\mu\text{g kg}^{-1} \text{ min}^{-1}$, i.v.), had no such effect. These findings suggest that interleukin-induced inhibition of acid responses to pentagastrin involves synthesis of NO from L-arginine.

Keywords: Gastric acid; interleukin-1 β ; cytokines; endotoxin; NO; pentagastrin

Introduction Nitric oxide (NO) is formed from L-arginine by a calcium-dependent constitutive NO synthase in a variety of cell types, including the endothelium and neural tissue (Moncada *et al.*, 1991). The induction of a calcium-independent NO synthase is thought to play a key role in the vascular events and cellular injury triggered after several hours by endotoxin (Moncada *et al.*, 1991), while NO can also attenuate the acute vascular damage induced by endotoxin in the gastrointestinal mucosa (Hutcheson *et al.*, 1990). It is well established that endotoxin can stimulate the release of the various cytokines (Dinarello, 1988) which modulate several of the early responses to infection, as well as inducing the expression of a calcium-independent NO synthase in vascular and immune cells (see Moncada *et al.*, 1991). Furthermore, the cytokine interleukin-1 β (IL-1 β) has been shown to inhibit basal (Uehara *et al.*, 1989; Saperas *et al.*, 1990) and pentagastrin-stimulated (Wallace *et al.*, 1991) acid secretion in the rat. In the present study, we have now investigated by the use of the NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, Rees *et al.*, 1990), the role of endogenous NO in the inhibitory effects of IL-1 β on pentagastrin-stimulated gastric acid secretion.

Methods Wistar rats of either sex (180–250 g) were fasted for 24 h but allowed drinking water. Under urethane anaesthesia (1.5 g kg^{-1} , i.p.) the trachea was intubated, the jugular vein cannulated and, in some experiments, systemic arterial blood pressure measured from a carotid artery. The gastric lumen was perfused (0.9 ml min^{-1}) with isotonic saline and H⁺ output determined at 20 min intervals by automatic titration. IL-1 β (2 $\mu\text{g kg}^{-1}$, i.v.) was administered once acid output stimulated by the i.v. infusion of pentagastrin had remained constant for 120 min, with acid output being determined over the following 120 min and expressed as $\mu\text{Eq H}^+$ per 100 g body weight. Inhibition by IL-1 β of acid secretion and the effects of L-NAME, L- and D-arginine were calculated relative to values of acid output stimulated by pentagastrin in each animal before the administration of these compounds or vehicle. In further studies, phenylephrine was co-infused with pentagastrin for the duration of the experi-

ment at a dose (10 $\mu\text{g kg}^{-1} \text{ min}^{-1}$) that provided a similar increase in blood pressure to that produced by L-NAME (5 mg kg^{-1}).

Recombinant mouse IL-1 β (specific activity of 3.5×10^5 units mg^{-1}) was obtained from Genzyme Corporation (Cambridge, MA, U.S.A.). L-NAME, L-arginine, D-arginine and phenylephrine were from Sigma Chemical (St Louis, MO, U.S.A.). These and pentagastrin (Peptavlon, ICI, UK) and dexamethasone (Fortecortin, Merck, Germany) were dissolved in saline immediately before use and administered in a volume of 1 ml kg^{-1} . Data are expressed as mean $\pm$ s.e.mean. Comparisons between groups were made by Student's *t* test for unpaired data, except for blood pressure studies in which Student's *t* test for paired data was used. Differences were considered significant at $P < 0.05$.

Results The intravenous infusion of pentagastrin (8 $\mu\text{g kg}^{-1} \text{ h}^{-1}$) induced a stable submaximal plateau rate of acid output, which in control rats ($n = 42$) remained between 9 and 11 $\mu\text{Eq H}^+ 100 \text{ g}^{-1} 20 \text{ min}^{-1}$ for the duration (240 min) of the experiments. As shown in Figure 1, IL-1 β administration (2 $\mu\text{g kg}^{-1}$, i.v., $n = 13$) following 2 h of acid stimulation substantially inhibited ($P < 0.001$) the subsequent acid response to pentagastrin. This near-maximal dose of IL-1 β , which was chosen from preliminary dose-response studies, did not produce any changes in systemic arterial blood pressure which remained between 95 and 105 mmHg ($n = 4$). Prior administration of dexamethasone (5 mg kg^{-1} s.c., 16 and 4 h before study) did not significantly modify the inhibitory effects of IL-1 β on gastric acid secretion ($80 \pm 6\%$ inhibition by IL-1 β , $n = 4$). Pretreatment (i.v.) with 2, 5 and 10 mg kg^{-1} of L-NAME ($n = 4, 7$ and 9 respectively) dose-dependently restored the secretory responses to pentagastrin in IL-treated rats (Figure 1). The reversal by L-NAME of the IL-1 β inhibition of acid output was prevented by the administration of L-arginine ($n = 6$, Figure 1), but not by its enantiomer D-arginine ($30 \pm 6\%$ inhibition by IL-1 β , $n = 14$). In control animals, pretreatment with L-NAME (5 mg kg^{-1}) did not significantly modify the secretory response to pentagastrin ($n = 11$), while L-arginine or D-arginine likewise had no significant effect ($n = 4$ for both).

The initial resting blood pressure was significantly

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Figure 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) (2, 5 and 10 mg kg⁻¹, i.v., ▨) on the inhibitory actions of interleukin-1β (IL-1β, 2 μg kg⁻¹, i.v., ▩) on gastric acid secretion induced by the intravenous infusion of pentagastrin (8 μg kg⁻¹ h⁻¹) in the anaesthetized rat. In animals treated with L-NAME (5 mg kg⁻¹) the administration (i.v.) of L-arginine (100 mg kg⁻¹, ■) restored the inhibitory effects of IL-1β on gastric acid secretion. Each column shows mean ± s.e. mean of at least 4 experiments, with % inhibition of acid secretion being calculated relative to values of acid production stimulated by pentagastrin in each animal during an equivalent period of time (120 min) before the administration of IL-1β, or vehicle (□).

Significant difference from the group receiving vehicle is shown as **P* < 0.05; ***P* < 0.01 and ****P* < 0.001; from the group receiving IL-1β only as †††*P* < 0.001, and from the group treated with the combination of IL-1β + L-NAME (5 mg kg⁻¹) as ##*P* < 0.01.

(*P* < 0.01) increased from 106 ± 11 to 155 ± 7 mmHg by L-NAME (5 mg kg⁻¹, i.v., *n* = 4). Subsequent administration of IL-1β (2 μg kg⁻¹) did not modify the hypertensive effects of L-NAME (143 ± 6 mmHg). In rats treated with IL-1β, the i.v. infusion of phenylephrine (10 μg kg⁻¹ min⁻¹, *n* = 4) produced a sustained increase in BP, from 104 ± 4 to 133 ± 3 mmHg (*P* < 0.01) but did not prevent the inhibition

by IL-1β of pentagastrin-stimulated acid output (79 ± 11% inhibition by IL-1β, *n* = 4).

Discussion The present results demonstrate that the NO-synthase inhibitor, L-NAME reverses the suppression by IL-1β of pentagastrin-stimulated gastric acid secretion. These effects of L-NAME were prevented by L-arginine but not by its enantiomer D-arginine, thus indicating the involvement of NO synthesis from L-arginine. However, the observation that administration of L-NAME in control, vehicle-treated animals did not affect the acid secretion stimulated by pentagastrin supports our previous studies using N^G-monomethyl-L-arginine and suggests that NO does not directly modulate H⁺ secretion under such conditions (Pique *et al.*, 1992). The restoration of the acid secretory responses by L-NAME is unlikely to be the consequence of its systemic vasopressor actions since phenylephrine, infused at a dose producing a similar vasopressor response as L-NAME, did not modify the acid-inhibitory effects of IL-1β. Furthermore, IL-1β did not produce any significant fall in blood pressure during the infusion of pentagastrin, which suggests an action on gastric acid output independent of any direct vascular changes induced by this cytokine.

It has been demonstrated that the induction of NO-synthase is responsible for the increased vascular synthesis of NO that occurs some 3–6 h after exposure to endotoxin and cytokines, and its expression is inhibited by glucocorticoids (Radomski *et al.*, 1990). It is therefore unlikely that such an inducible enzyme is involved in the inhibitory action of IL-1β on acid secretion since this rapidly occurs within 120 min and, moreover, it is not influenced by pretreatment with dexamethasone in a dose sufficient to prevent such induction (Moncada *et al.*, 1991). Thus, the present results suggest the involvement of an acute release or action of NO in the gastric inhibitory response to IL-1β, but the cellular origin of such NO is not known. If IL-1β inhibits pentagastrin-stimulation by preventing mucosal histamine release (Wallace *et al.*, 1991) this process may thus involve NO. In addition, NO may also influence the release of other endogenous mediators or be involved in the modulation of neuronal mechanisms evoked by IL-1β that could influence gastric acid secretion.

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