Influence of N^G-nitro-L-arginine on non-adrenergic non-cholinergic relaxation in the guinea-pig gastric fundus

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1 The influence of N^G-nitro-L-Arginine (L-NNA) on non-adrenergic non-cholinergic (NANC) relaxations induced by electrical field stimulation was investigated in circular muscle strips of the guinea-pig gastric fundus.

2 In the presence of 10⁻⁶ M atropine and 4 × 10⁻⁶ M guanethidine, electrical field stimulation (40 V, 1 ms, 0.125–16 Hz) with 10 s trains at 5 min intervals induced short-lasting, frequency-dependent relaxations. Continuous stimulation, with cumulative increase of the stimulation frequency, induced sustained frequency-dependent relaxations. Both types of response were abolished by 3 × 10⁻⁶ M tetrodotoxin.

3 L-NNA (10⁻⁵ M and 10⁻⁴ M) concentration-dependently reduced both types of NANC response. Pre-incubation with 2 × 10⁻³ M L-arginine prevented the inhibitory action of 10⁻⁵ M L-NNA and partially antagonized that of 10⁻⁴ M L-NNA. D-arginine (2 × 10⁻³ M) did not protect against the inhibitory effect of L-NNA.

4 L-NNA did not consistently influence the basal tone of the tissues. L-Arginine and D-arginine likewise did not influence basal tone; they also had no influence on the electrically-induced NANC relaxations.

5 NO (10⁻⁶–10⁻⁴ M) induced short-lasting concentration-dependent relaxations, while vasoactive intestinal polypeptide (VIP, (10⁻⁹–10⁻⁷ M) induced more sustained relaxations, that developed at a slower rate. The NO- and VIP-induced relaxations were not influenced by 10⁻⁴ M L-NNA.

6 These results suggest that NO is involved in NANC neurotransmission of the guinea-pig gastric fundus; its contribution to sustained NANC relaxation in the guinea-pig gastric fundus is much more important than in the rat.

Keywords: Guinea-pig gastric fundus; NANC neurotransmission; N^G-nitro-L-arginine (L-NNA); NO; vasoactive intestinal polypeptide (VIP)

Introduction

Inhibitory non-adrenergic non-cholinergic (NANC) neurones are present throughout the gastrointestinal tract. Both purines and peptides, especially vasoactive intestinal polypeptide (VIP), have been proposed as possible mediators in different parts of the gut (McKirdy, 1988; Taylor & Bywater, 1989). Recent data suggest that nitric oxide (NO) is also an inhibitory NANC neurotransmitter. In several tissues such as the opossum lower oesophageal sphincter (Tøttrup *et al.*, 1991), the canine lower oesophageal sphincter and ileocolonic junction (Boeckxstaens *et al.*, 1990; De Man *et al.*, 1991) and the human ileum (Maggi *et al.*, 1991), NO synthesis inhibitors reduce electrically-induced NANC relaxations. Furthermore, the release of a vasorelaxant factor with the characteristics of a nitrovasodilator has been shown upon stimulation of the NANC nerves in the canine ileocolonic junction (Bult *et al.*, 1990), and NO synthase-like immunoreactivity has been observed in the myenteric plexus of the rat intestine (Bredt *et al.*, 1990). These data support the idea that NO has a more general transduction role in addition to endothelium-derived relaxation (Moncada *et al.*, 1991).

The NANC inhibitory neurones of the gastric fundus are involved in the receptive relaxation of the stomach during food intake (Abrahamsson, 1986). In the rat gastric fundus, NANC relaxation induced by prolonged electrical field stimulation is reduced in the presence of peptidases and VIP antiserum, but the initial component persists; this suggests that in addition to VIP, another, non-peptide, neurotransmitter is involved (De Beurme & Lefebvre, 1987; 1988). It was then shown that NANC relaxation induced by short train (10 s) field stimulation was greatly reduced by the NO synthesis inhibitors N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-L-arginine (L-NNA), especially at lower frequen-

cies of stimulation (Li & Rand, 1990; Boeckxstaens *et al.*, 1991). Both NO and VIP might thus be inhibitory NANC transmitters in the rat gastric fundus, whereby NO is released with short trains or low frequencies of stimulation, while VIP release is elicited by longer periods or higher frequencies of stimulation. NO and VIP might also be inhibitory transmitters in the guinea-pig trachea (Tucker *et al.*, 1990; Li & Rand, 1991). VIP has been proposed as the neural mediator of the NANC relaxation in the guinea-pig gastric fundus (Grider *et al.*, 1985; Grider & Makhoul, 1987; Grider, 1990); the NANC relaxation induced by field stimulation with 10 s trains at lower frequencies in this tissue was almost abolished in the presence of VIP antiserum (Grider *et al.*, 1985). Recently, it was shown that adaptive relaxation in response to increased gastric pressure in a guinea-pig isolated stomach model is antagonized by NO synthesis inhibitors, suggesting that NO is released from the inhibitory NANC neurones in the guinea-pig stomach (Desai *et al.*, 1991). We have now investigated the influence of L-NNA on NANC relaxation, induced by short train and prolonged field stimulation, in the guinea-pig gastric fundus. Our results suggest that both during short-lasting and continuous stimulation, NO is involved in the inhibitory NANC neurotransmission of the guinea-pig gastric fundus. A preliminary account of the results was given at the second international meeting on the biology of NO (Lefebvre *et al.*, 1991).

Methods

Tissue preparation

Guinea-pigs of either sex (weight 320–670 g) were fasted for 24 h and were killed by a blow on the head and bleeding.

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The stomach was removed and after careful removal of the mucosa by sharp dissection, 2 circular muscle strips (length 2 cm) were prepared from the ventral part of the fundus. The strips were mounted under a load of 1 g in 6 ml (for the experiments with VIP) or 18 ml organ baths containing Krebs solution of the following composition (mM): NaCl 118.5, KCl 4.8, KH_2PO_4 1.2, MgSO_4 1.2, CaCl_2 1.9, NaHCO_3 25.0 and glucose 10.1. The Krebs solution always contained 10^{-6} M atropine and 4×10^{-6} M guanethidine; it was maintained at 37°C and bubbled with 95% O_2 :5% CO_2 . The guinea-pig gastric fundus is a spontaneous tone preparation; thus it is not necessary to raise tone in order to observe relaxant responses (Sahyoun *et al.*, 1982). Electrical stimulation at supramaximal voltage was applied by two parallel platinum electrodes with an S88 Grass stimulator and a constant current unit. Changes in length were recorded isotonicity with Bioscience T₃ transducers and registered on a Graphtec Linearcorder FWR 3701. For the experiments with VIP in the 6 ml organ baths, response were recorded auxotonically (Harvard heart – smooth muscle transducer) on a Graphtec Multicorder MC6625. The tissues were equilibrated for 1 h with rinsing every 15 min. Frequency-response curves to electrical field stimulation (40 V, 1 ms, 0.125–16 Hz) were obtained by stimulating the tissues either with 10 s trains at 5 min intervals (non-cumulative train stimulation) or continuously with stepwise increases of the frequency, once the response to a given frequency had become maximal (cumulative stimulation). At the end of each experiment a maximal relaxation was induced by administration of 10^{-4} M papaverine.

Protocols

To study the influence of L-NNA on the electrically-induced relaxations, and the modification of the effect of L-NNA by L- and D-arginine, four parallel tissues were studied, in which three non-cumulative or cumulative frequency-response curves (0.125–16 Hz) were obtained. Drugs were not added in the control strip; L-NNA (10^{-5} and 10^{-4} M) was added 10 min before the second and third curve respectively. L-Arginine (2×10^{-3} M) and D-arginine (2×10^{-3} M) were added 5 min before L-NNA. The influence of 10^{-4} M L-NNA was also studied on the relaxations induced by electrical stimulation at 0.5 and 4 Hz, both applied for 10 s and 3 min within the same strip; between the stimulus trains, an interval with rinsing was given to allow the tone of the strips to recover.

In an additional series of experiments, the influence of 3×10^{-6} M tetrodotoxin (TTX; incubated for 10 min), 2×10^{-3} M L-arginine and 2×10^{-3} M D-arginine (both incubated for 15 min) on non-cumulative and cumulative frequency-response curves was studied; the 2 types of stimulation were applied in different tissues. Also in these experiments, 3 frequency-response curves were constructed and TTX, L-arginine or D-arginine was added between the second and third curve.

In a last series, concentration-response curves were constructed for NO (10^{-6} – 10^{-4} M; non-cumulative administration) and VIP (10^{-9} – 10^{-7} M; cumulative administration) and repeated 10 min after adding 10^{-4} M L-NNA. In the control tissues, L-NNA was not added. The influence of NO was also studied when it was continuously administered to the tissue for 5 min; in view of the short half-life of NO, the amount yielding 10^{-5} M, when administered in bolus, was infused into the organ bath over a period of 10 s via a Braun infusion pump.

Drugs

The following drugs were used: atropine sulphate (Boehringer Ingelheim, Germany), D-arginine hydrochloride (Sigma, St. Louis, MO, U.S.A.), L-arginine hydrochloride (Sigma), bacitracin (Sigma), bovine serum albumin (Sigma),

guanethidine sulphate (Ciba-Geigy, Groot-Bijgaarden, Belgium), N^o-nitro-L-Arginine (Sigma), tetrodotoxin (TTX; Janssen Pharmaceutica, Beerse, Belgium) and vasoactive intestinal polypeptide (VIP; CRB, Northwich, U.K.). A saturated NO solution was prepared as described by Kelm & Schrader (1988) from NO gas (l'Air Liquide, Belgium). Drugs were dissolved and diluted with distilled water. Atropine, guanethidine, bacitracin and bovine serum albumin were added to the Krebs solution reservoir. Stock solutions of 10^{-3} M TTX and 10^{-3} M VIP were kept frozen at -20°C and dilutions were made the day of the experiment.

Statistical analysis

All relaxant responses are expressed as a percentage of the papaverine-induced relaxation. Results are given as means $\pm$ s.e.mean. Results were compared by the Wilcoxon signed-ranks test, *P* values of less than 0.05 being considered statistically significant.

Results

Stimulation with 10 s trains induced frequency-dependent relaxations (Figures 1, 2). Upon stimulation, tone very quickly declined; at the end of the 10 s stimulus train, a biphasic recovery of tone was evident in some tissues, an initial rapid phase being followed by a second slower phase (Figure 1). In other tissues, recovery was monophasic, with tone rising at a slower rate than it had declined (Figure 2). For calculations, the amplitude of the first phase of relaxation was taken into account. TTX (3×10^{-6} M) nearly abolished the relaxation induced by train stimulation at all frequencies tested. The relaxation induced by stimulation at 16 Hz was abolished in 3 strips and reduced from 47 to 10% in a fourth strip, while the response was well maintained in control tissues.

A representative experiment showing the influence of L-NNA on NANC relaxations induced by train stimulation is given in Figure 1, while the mean results are given in Figure 3. In the control tissues, the NANC response increased somewhat from the first to the second curve but then remained stable. L-NNA concentration-dependently reduced the electrically-induced relaxations. The administration of L-NNA did not consistently increase the basal tension of the strips. Administration of 2×10^{-3} M L-arginine prevented the inhibitory action of 10^{-5} M L-NNA; as in the control tissues, the responses increased from the first to the second frequency-response curve. L-Arginine partially antagonized the inhibitory effect of 10^{-4} M L-NNA. The inhibitory effect of L-NNA in the presence of 2×10^{-3} M D-arginine was similar to that in the absence of D-arginine, although the concentration-dependency was less clear. Neither L-arginine nor D-arginine influenced the NANC relaxations induced by train stimulation *per se* ($n = 4$ for each compound), nor did they influence basal tone.

Continuous stimulation with cumulative increases of the stimulation frequency induced sustained relaxations (Figure 4). The maximal relaxation was reached at 8 Hz as some contraction occurred when the stimulation frequency was increased to 16 Hz. The maximal relaxation, ranging between 60 to 90% of the papaverine-induced relaxation, was more pronounced than the maximal effect induced by 10 s train stimulation (40 to 50%). As with the trains, this type of relaxant response was nearly abolished by 3×10^{-6} M TTX: the relaxation obtained at a stimulation frequency of 8 Hz was reduced from $80 \pm 2\%$ to $5 \pm 1\%$ ($n = 4$). L-NNA reduced the sustained relaxations in a concentration-dependent manner (Figures 4, 5). The relaxation induced by stimulation at 8 Hz was reduced from $61 \pm 11\%$ to $33 \pm 10\%$ and $14 \pm 5\%$ in the presence of 10^{-5} and 10^{-4} M L-NNA respectively. In the presence of the higher concentration of L-NNA, some contraction started to occur at a stimulation

Figure 1 Original recording of a circular muscle strip of the guinea-pig gastric fundus, showing the isotonic responses to electrical stimulation (40 V, 1 ms) with 10 s trains (0.125–16 Hz) at 5 min intervals in the absence (a) and in the presence of 10^{-5} M (b) and 10^{-4} M (c) N^G -nitro-L-arginine (L-NNA). L-NNA was added 10 min before stimulation. Between (b) and (c), the sensitivity of the recorder was increased by a factor of 2.

Figure 2 Original recording of a circular muscle strip of the guinea-pig gastric fundus, showing the isotonic responses to electrical stimulation (ES, 40 V, 1 ms) with 10 s trains at 5 min intervals and to NO.

frequency of 8 Hz. L-Arginine (2×10^{-3} M) prevented the inhibitory effect of 10^{-5} M L-NNA, although the latter still induced some non-significant inhibition at the lower stimulation frequencies. D-Arginine did not influence the effect of L-NNA. When incubated alone, L-arginine ($n = 7$) and D-arginine ($n = 4$) did not change the electrically-induced sustained relaxations. Also, when sustained stimulation (3 min) at a given frequency was performed in isolation and not as part of a cumulative frequency-response curve (experiments with stimulation at 0.5 and 4 Hz), the elicited relaxation was

antagonized by 10^{-4} M L-NNA. The relaxation induced by stimulation for 3 min at a frequency of 0.5 Hz was reduced from $30 \pm 6\%$ to $2 \pm 1\%$ ($n = 6$, $P < 0.05$), while that at 4 Hz was reduced from $49 \pm 9\%$ to $6 \pm 3\%$ ($n = 6$, $P < 0.05$). When a 10 s train was used for stimulation the values were $13 \pm 3\%$ and $1 \pm 1\%$ ($n = 6$, $P < 0.05$) for 0.5 Hz and $25 \pm 5\%$ and $7 \pm 1\%$ ($n = 6$, $P < 0.05$) for 4 Hz.

NO, administered in bolus, induced short-lasting concentration-dependent relaxations, similar to the NANC responses obtained with short train stimulation (Figure 2). The

maximal relaxation was about 60% of that induced by papaverine. The NO-induced relaxations were not influenced by 10⁻⁴ M L-NNA (Figure 6). VIP induced a more sustained relaxation of the tissues, resembling the picture obtained by continuous stimulation. Sustained relaxation was, however, also obtained by infusing NO into the organ bath ($n = 4$). The relaxant effect by the highest concentration of VIP used (10⁻⁷ M) was only 33% ($n = 14$). The VIP-induced relaxations were not influenced by 10⁻⁴ M L-NNA. The results with VIP were obtained in the absence of bovine serum albumin and bacitracin, as an additional series showed that the presence of 50 mg l⁻¹ bovine serum albumin, to prevent adhesion of VIP to the glassware, and 30 mg l⁻¹ bacitracin, to prevent VIP metabolism by peptidases, in the Krebs solution did not significantly increase the relaxant effect of VIP.

Discussion

N^G-nitro-L-arginine (L-NNA) has been shown to be a potent inhibitor of NO biosynthesis (Ishii *et al.*, 1990; Moore *et al.*,

Figure 4 Original recording of a circular muscle strip of the guinea-pig gastric fundus, showing the responses to continuous electrical stimulation (40 V, 1 ms) at increasing frequency in the absence (a) and in the presence of 10⁻⁵ M (b) and 10⁻⁴ M (c) N^G-nitro-L-arginine (L-NNA). L-NNA was added 10 min before stimulation. Between (b) and (c) the sensitivity of the recorder was increased by a factor of 2.

Figure 3 Relaxation responses to electrical stimulation (40 V, 1 ms) with 10 s trains at 5 min intervals. (a) In control tissues, 3 frequency-response curves were constructed without addition of drugs (x, 1st curve; O, 2nd curve; Δ, 3rd curve). (b) Responses in the absence (x) and in the presence of 10⁻⁵ M (O) and 10⁻⁴ M (Δ) N^G-nitro-L-arginine (L-NNA). (c) As in (b) but tissues were incubated with 2 × 10⁻³ M L-arginine before L-NNA was administered. (d) As in (b) but tissues were incubated with 2 × 10⁻³ M D-arginine before L-NNA was administered. Mean of $n = 5$ to 6; vertical bars show s.e.mean. * $P < 0.05$: significantly different from the control values (signed-ranks test).

Figure 5 Responses to continuous electrical stimulation (40 V, 1 ms) at increasing frequency. (a) In control tissues, 3 frequency-response curves were constructed without addition of drugs (×, 1st curve; ○, 2nd curve; ▲, 3rd curve). (b) Responses in the absence (×) and in the presence of 10⁻⁵M (○) and 10⁻⁴M (▲) N^G-nitro-L-arginine (L-NNA). (c) As in (b) but tissues were incubated with 2 × 10⁻³M L-arginine before L-NNA was administered. (d) As in (b) but tissues were incubated with 2 × 10⁻³M D-arginine before L-NNA was administered. Mean of *n* = 5 (c, d) or 6 (a, b); vertical bars show s.e.mean. **P* < 0.05 (one tailed in c and d because *n* = 5); significantly different from the control values (signed-ranks test).

1990; Mülsch & Busse, 1990). In the present study, L-NNA concentration-dependently reduced NANC relaxations in the guinea-pig gastric fundus, induced by short train and continuous stimulation. Both types of response were blocked by TTX, illustrating their neurogenic nature. The tendency to contraction at 16 Hz, occurring at the end of a cumulative frequency-response curve might be due to acetylcholine overcoming the atropine-mediated blockade of the muscarinic receptors in a very relaxed tissue or to release of a non-cholinergic contractile transmitter at higher frequencies. The inhibition by L-NNA was stereospecifically prevented by L-arginine and seemed not due to a non-specific effect at the muscular level as the NO- and VIP-induced relaxations were not influenced by L-NNA. This suggests that NO or a related compound is an important component of the inhibitory NANC neurotransmission of the guinea-pig gastric fundus.

The concentration-dependent reduction of the NANC relaxations, induced by short trains (10 s) of stimulation, by L-NNA corresponds to the effect of L-NNA and other NO synthase inhibitors on similar responses in many other

gastrointestinal tissues such as the opossum (Tøttrup *et al.*, 1991) and canine (De Man *et al.*, 1991) lower oesophageal sphincter, the rat gastric fundus (Li & Rand, 1990; Boeckxstaens *et al.*, 1991), the human ileum (Maggi *et al.*, 1991), the canine ileocolonic junction (Boeckxstaens *et al.*, 1990) and the mouse (Gibson *et al.*, 1990) and rat (Gillespie *et al.*, 1989) anococcygeus muscle. It is difficult to correlate the observation that L-NNA completely blocked the NANC relaxations induced by 10 s train stimulation at lower frequencies (0.125–0.5 Hz) with the finding of Grider *et al.* (1985) that these responses are completely blocked in the presence of VIP-antiserum. Our results were similar to those of Li & Rand (1991) who found that NANC relaxations induced by field stimulation at 1 Hz are completely blocked by N^G-nitro-L-arginine methyl ester in the guinea-pig trachea. In sheep cerebral arteries, VIP was proposed as the mediator of neurally mediated vasodilatation, whereby VIP would act via synthesis of NO (Gaw *et al.*, 1991). A similar mechanism in the guinea-pig gastric fundus could explain our findings and those of Grider *et al.* (1985) but this is unlikely

Figure 6 Concentration-response curves to NO (a, b) and vasoactive intestinal polypeptide (VIP, c, d). (a, c) In control tissues, 2 concentration-response curves were constructed without addition of drugs ($\times$, 1st curve; $\circ$, 2nd curve). (b, d) Responses in the absence ($\times$) and in the presence ($\circ$) of 10^{-4} M N^G -nitro-L-arginine (L-NNA). Mean of $n = 6-8$; vertical bars show s.e.mean. * $P < 0.05$: significantly different from the value in the 1st curve (signed-ranks test).

since the VIP-induced relaxations in the guinea-pig gastric fundus are not antagonized by L-NNA. The latter was also observed in the rat gastric fundus (Boeckxstaens *et al.*, 1991) and the opossum lower oesophageal sphincter (Tøttrup *et al.*, 1991). The opposite mechanism, that NO would release VIP, is also unlikely since the VIP-induced relaxations have a different shape from the NANC relaxations induced by train stimulation. In contrast to VIP, the shape and the maximal amplitude of relaxations induced by bolus administration of NO were similar to those induced by the short trains of stimulation. NO has been reported to be more potent in inducing relaxation of vascular than of non-vascular smooth muscle (Buga *et al.*, 1989; Boeckxstaens *et al.*, 1990). Between different non-vascular tissues, the sensitivity can also differ (Buga *et al.*, 1989). In the guinea-pig gastric fundus, the sensitivity to NO was somewhat lower than in the rat gastric fundus (Boeckxstaens *et al.*, 1991) but similar to that in the canine lower oesophageal sphincter (De Man *et al.*, 1991) and ileocolonic junction (Boeckxstaens *et al.*, 1990).

In the rat gastric fundus, the amplitude of NANC relaxations induced by more sustained trains of field stimulation at higher frequencies is not reduced by NO synthase inhibitors (see Li & Rand, 1990, where N^G -monomethyl-L-arginine was tested versus the response induced by stimulation at 5 Hz, and Boeckxstaens, 1991, who tested L-NNA versus the response induced by stimulation at 16 Hz). In the presence of the NO synthase inhibitors, the relaxation developed at a slower rate; VIP antiserum (Li & Rand, 1990) and trypsin (Boeckxstaens, 1991) reduced this type of NANC response, corroborating our suggestion (De Beurme & Lefebvre, 1988) that VIP is involved in this type of NANC relaxation in the rat gastric fundus. Surprisingly, in the guinea-pig gastric fundus, the amplitude of NANC relaxations induced by sustained stimulation was greatly reduced in the presence of L-NNA, even at the higher frequencies of stimulation. The reduction of the responses at higher frequencies was not related to the use of a frequency-response curve as the response to an isolated stimulation at 4 Hz during 3 min was also very clearly antagonized by L-NNA. This result suggests that the role of NO in sustained NANC relaxation is more important in the guinea-pig than in the rat gastric fundus. The observation that infusion of NO is able to induce sustained relaxation corroborates the thesis that NO, when continuously released from nerves to smooth muscle cells, can be responsible for the sustained relaxation observed during continuous field stimulation. Still, our results do not allow us to exclude that NO, released during sustained stimulation, would subsequently release VIP or would be important for the release of VIP as cotransmitter.

In the mouse anococcygeus muscle, L-arginine increased the electrically induced NANC relaxations (Gibson *et al.*, 1990), suggesting that the amount of L-arginine available for NO synthesis during nerve stimulation is limited. No such effect of L-arginine was observed in the guinea-pig gastric fundus. L-NNA did not influence the basal tone of the tissue. The influence of NO synthesis inhibitors on the basal tone of non-vascular smooth muscle preparations differs between tissues, e.g. in the rat anococcygeus muscle (Gillespie *et al.*, 1989), the canine ileocolonic junction (Boeckxstaens *et al.*, 1990) and the rat gastric fundus (Li & Rand, 1990; Boeckxstaens *et al.*, 1991), the NO-synthesis inhibitors induce contraction; inhibition of a tonic production of NO is a possible mechanism. In contrast, the NO synthesis inhibitors do not influence basal tone in the guinea-pig trachea (Li & Rand, 1991), canine lower oesophageal sphincter (De Man *et al.*, 1991) and the rat duodenum (Irie *et al.*, 1991).

In conclusion, our results suggest that NO or a related compound is involved in NANC inhibitory neurotransmission in the guinea-pig gastric fundus, both when elicited by brief periods of stimulation and when stimulation was sustained. These results corroborate the finding that NO is important for the adaptive relaxation of the guinea-pig stomach (Desai *et al.*, 1991).

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References

- ABRAHAMSSON, H. (1986). Non-adrenergic non-cholinergic nervous control of gastrointestinal motility patterns. *Arch. Int. Pharmacodyn. Ther.*, **280**, Suppl., 50–61.
- BOECKXSTAENS, G.E. (1991). In vitro study on the nature of the inhibitory non-adrenergic non-cholinergic neurotransmitter in the gut. *Ph. D. Thesis, University of Antwerp, Belgium*.
- BOECKXSTAENS, G.E., PELCKMANS, P.A., BOGERS, J.J., BULT, H., DE MAN, J.G., OOSTERBOSCH, L., HERMAN, A.J. & VAN MAERCKE, Y.M. (1991). Release of nitric oxide upon stimulation of nonadrenergic noncholinergic nerves in the rat gastric fundus. *J. Pharmacol. Exp. Ther.*, **256**, 441–447.
- BOECKXSTAENS, G.E., PELCKMANS, P.A., BULT, H., DE MAN, J.G., HERMAN, A.G. & VAN MAERCKE, Y.M. (1990). Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction. *Eur. J. Pharmacol.*, **190**, 239–246.
- BREDT, D.S., HWANG, P.M. & SNYDER, S.H. (1990). Localization of nitric oxide synthase indicating a neural role for nitric oxide. *Nature*, **347**, 768–770.
- BUGA, G.M., GOLD, M.E., WOOD, K.S., CHAUDHURI, G. & IGNARRO, L.J. (1989). Endothelium-derived nitric oxide relaxes nonvascular smooth muscle. *Eur. J. Pharmacol.*, **161**, 61–72.
- BULT, H., BOECKXSTAENS, G.E., PELCKMANS, P.A., JORDAENS, F.H., VAN MAERCKE, Y.M. & HERMAN, A.G. (1990). Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature*, **345**, 346–347.
- DE BEURME, F.A. & LEFEBVRE, R.A. (1987). Influence of α -chymotrypsin and trypsin on the non-adrenergic non-cholinergic relaxation in the rat gastric fundus. *Br. J. Pharmacol.*, **91**, 171–177.
- DE BEURME, F.A. & LEFEBVRE, R.A. (1988). Vasoactive intestinal polypeptide as possible mediator of relaxation in the rat gastric fundus. *J. Pharm. Pharmacol.*, **40**, 711–715.
- DE MAN, J.G., PELCKMANS, P.A., BOECKXSTAENS, G.E., BULT, H., OOSTERBOSCH, L., HERMAN, A.G. & VAN MAERCKE, Y.M. (1991). The role of nitric oxide in inhibitory non-adrenergic non-cholinergic neurotransmission in the canine lower oesophageal sphincter. *Br. J. Pharmacol.*, **103**, 1092–1096.
- DESAI, K.M., SESSA, W.C. & VANE, J.R. (1991). Involvement of nitric oxide in the reflex relaxation of the stomach to accommodate food or fluid. *Nature*, **351**, 477–479.
- GAW, A.J., ABERDEEN, J., HUMPHREY, P.P.A., WADSWORTH, R.M. & BURNSTOCK, G. (1991). Relaxation of sheep cerebral arteries by vasoactive intestinal polypeptide and neurogenic stimulation: inhibition by L-N^G-monomethyl arginine in endothelium-denuded vessels. *Br. J. Pharmacol.*, **102**, 567–572.
- GIBSON, A., MIRZAZADEH, S., HOBBS, A.J. & MOORE, P.K. (1990). L-N^G-monomethyl arginine and L-N^G-nitro arginine inhibit non-adrenergic non-cholinergic relaxation of the mouse anococcygeus muscle. *Br. J. Pharmacol.*, **99**, 602–606.
- GILLESPIE, J.S., LIU, X. & MARTIN, W. (1989). The effects of L-arginine and N^G-monomethyl L-arginine on the response of the rat anococcygeus muscle to NANC nerve stimulation. *Br. J. Pharmacol.*, **98**, 1080–1082.
- GRIDER, J.R. (1990). Identification of neurotransmitters by selective protection of postjunctional receptors. *Am. J. Physiol.*, **258**, G103–G106.
- GRIDER, J.R., CABLE, M.B., SAID, S.I. & MAKHLOUF, G.M. (1985). Vasoactive intestinal peptide as a neural mediator of gastric relaxation. *Am. J. Physiol.*, **248**, G73–G78.
- GRIDER, J.R. & MAKHLOUF, G.M. (1987). Prejunctional inhibition of vasoactive intestinal peptide release. *Am. J. Physiol.*, **253**, G7–G12.
- IRIE, K., MURAKI, T., FURUKAWA, K. & NOMOTO, T. (1991). L-N^G-nitro-arginine inhibits nicotine-induced relaxation of isolated rat duodenum. *Eur. J. Pharmacol.*, **202**, 285–288.
- ISHII, K.B., CHANG, B., KERWIN, J.F., JR, HUANG, Z.-J. & MURAD, F. (1990). N^G-nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. *Eur. J. Pharmacol.*, **176**, 219–223.
- KELM, M. & SCHRADER, J. (1990). Control of coronary vascular tone by nitric oxide. *Circ. Res.*, **66**, 1561–1575.
- LEFEBVRE, R.A., BAERT, E. & BARBIER, A.J. (1991). Influence of N^G-nitro-L-arginine on non-adrenergic non-cholinergic relaxation in the guinea-pig gastric fundus, Abstract book, *Second Int. Meeting on Biology of Nitric Oxide*, London, 30.9–2.10, p. 149.
- LI, C.G. & RAND, M.J. (1990). Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. *Eur. J. Pharmacol.*, **191**, 303–309.
- LI, C.G. & RAND, M.J. (1991). Evidence that part of the NANC relaxant response of guinea-pig trachea to electrical field stimulation is mediated by nitric oxide. *Br. J. Pharmacol.*, **102**, 91–94.
- MAGGI, C.A., BARBANTI, G., TURINI, D. & GIULIANI, S. (1991). Effect of N^G-monomethyl L-arginine (L-NMMA) and N^G-nitro L-arginine (L-NOARG) on non-adrenergic non-cholinergic relaxation in the circular muscle of the human ileum. *Br. J. Pharmacol.*, **103**, 1970–1972.
- McKIRDY, H.C. (1988). Novel autonomic neurotransmitters and upper gastrointestinal function. *Pharmacol. Ther.*, **38**, 429–452.
- MONCADA, S., PALMER, R.M.J. & HIGGS, E.A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43**, 109–142.
- MOORE, P.K., ALSWAYEH, O.A., CHONG, N.W.S., EVANS, R.A. & GIBSON, A. (1990). L-N^G-nitro arginine (L-NOARG), a novel, L-arginine reversible inhibitor of endothelium-dependent vasodilatation in vitro. *Br. J. Pharmacol.*, **99**, 408–412.
- MÜLSCH, A. & BUSSE, R. (1990). N^G-nitro-L-arginine (N^G-[imino(nitroamino)methyl]-l-ornithine) impairs endothelium-dependent dilations by inhibiting cytosolic nitric oxide synthesis from L-arginine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **341**, 143–147.
- SAHYOUN, H.A., COSTALL, B. & NAYLOR, R.J. (1982). On the ability of domperidone to selectively inhibit catecholamine-induced relaxation of circular smooth muscle of guinea-pig stomach. *J. Pharm. Pharmacol.*, **34**, 27–33.
- TAYLOR, G.S. & BYWATER, R.A.R. (1988). Novel autonomic neurotransmitters and intestinal function. *Pharmacol. Ther.*, **40**, 401–438.
- TØTTRUP, A., SVANE, D. & FORMAN, A. (1991). Nitric oxide mediating NANC inhibition in opossum lower esophageal sphincter. *Am. J. Physiol.*, **260**, G385–G389.
- TUCKER, J.F., BRAVE, S.R., CHARALAMBOUS, L., HOBBS, A.J. & GIBSON, A. (1990). L-N^G-nitro arginine inhibits non-adrenergic, non-cholinergic relaxations of guinea-pig isolated tracheal smooth muscle. *Br. J. Pharmacol.*, **100**, 663–664.

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