British Journal of Pharmacology (1998) 123, 143–149

Effect of nitroglycerin and long-term electrical stimulation on nitrergic relaxation in the pig gastric fundus

[1]R.A. Lefebvre & K. Vandekerckhove

Heymans Institute of Pharmacology, University of Gent Medical School, De Pintelaan 185, B-9000 Gent, Belgium

1 The effect of incubation with the nitric oxide (NO) donor nitroglycerin and of long-term electrical stimulation on relaxations induced by non-adrenergic, non-cholinergic nerve stimulation, exogenous NO, vasoactive intestinal polypeptide (VIP) and lemakalim was investigated in the pig gastric fundus.

2 In physiological salt solution containing 10^{-6} M atropine and 4×10^{-6} M guanethidine, electrical field stimulation (40 V, 0.1 ms, 0.5–8 Hz) for periods of 10 s at 5 min intervals (train stimulation) and administration of NO (2×10^{-6}–10^{-4} M) at 5 min intervals (NO boli) induced frequency- and concentration-dependent transient relaxations, respectively. Continuous electrical field stimulation with stepwise increase of the frequency (0.5–8 Hz; cumulative stimulation) induced frequency-dependent sustained relaxations. VIP (10^{-7} M), lemakalim (10^{-5} M) and an infusion of NO induced a sustained relaxation.

3 Pretreatment for 30 min with 5×10^{-4} M nitroglycerin reduced the relaxations induced by train and cumulative stimulation, but also the relaxant responses to NO, both when given in boli or as an infusion. The relaxations to VIP and lemakalim were not influenced by pretreatment with nitroglycerin.

4 Long-term electrical stimulation at 4 Hz for 40 min induced a sustained relaxation of the tissues. Administration of 3×10^{-4} M N^G-nitro-L-arginine methyl ester after 10, 20 or 30 min reversed the relaxation to a similar extent (approximately 70%). Previous long-term electrical stimulation at 4 Hz for 30 min did not affect the responses to stimulation, NO and VIP.

5 These results illustrate that nitroglycerin can induce a postjunctional tolerance to nitrergic stimuli in the pig gastric fundus but evidence for a prejunctional inhibition of neuronal NO synthase by NO was not obtained.

Keywords: Gastric fundus; nitrergic neurotransmission; nitric oxide; nitroglycerin; long-term electrical stimulation

Introduction

The concept of the regulation of neurotransmitter release via presynaptic receptors on the axon terminals is well established (Starke, 1981). Neurone terminals may have such receptors for their own neurotransmitter, the so-called presynaptic autoreceptors. Via these receptors, the transmitter can autoregulate its own release. In most cases, feedback inhibition of neurotransmitter release is observed, although also presynaptic facilitation can occur such as via presynaptic β-receptors on peripheral noradrenergic nerve endings (Langer, 1981).

Nitric oxide (NO) is synthesized from L-arginine by a family of iso-enzymes, the NO synthases (NOS). NO is synthesized in endothelial cells by eNOS or NOS3 and is involved in tonic vasodilatation (Moncada *et al*., 1991). In macrophages, iNOS or NOS2 can be induced and the NO released contributes to host defence mechanisms against infectious organisms (Kröncke *et al*., 1995). Both in central and peripheral neurones, NO can be synthesized by nNOS or NOS1 and it is an important peripheral non-adrenergic non-cholinergic (NANC) neurotransmitter (Rand & Li, 1995), especially in the gastrointestinal tract (Brookes, 1993). For the 3 types of NOS, data have been obtained suggesting that NO might be involved in autoregulatory inhibition of its own synthesis. In cultured endothelial cells, NO inhibits eNOS activity (Buga *et al*., 1993) and the NO donor S-nitroso-N-acetylpenicillamine (SNAP) was shown to depress the vasoconstriction by the NOS inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) *in vitro* and the hypertensive effect of L-NAME *in vivo* (Ma *et al*., 1996). Assreuy *et al*. (1993) showed that the spontaneous decline in iNOS activity in the murine macrophage cell line J774 was slowed down when the cells were cultured in a low L-arginine medium and that the NO donor SNAP inhibited iNOS extracted from the cytosol of stimulated J774 cells. As for nNOS, exogenous NO and NO donors inhibit cerebellar NOS activity (Rogers & Ignarro, 1992; Rengasamy & Johns, 1993). In the rat gastric fundus, De Man *et al*. (1995) showed that incubation with the NO donors nitroglycerin (NTG) and 3-morpholino-sydnonimine (SIN-1) reduced transient nitrergic relaxations induced by short periods of electrical stimulation but not those by exogenous NO or vasoactive intestinal peptide (VIP). We have previously shown that in the pig gastric fundus, NO is involved in inhibitory NANC neurotransmission (Lefebvre *et al*., 1995) and the aim of the present study was to investigate whether NO may inhibit NO synthesis in this tissue. To study the effect of exogenous NO, the influence of prolonged incubation with the NO donor NTG on relaxations induced by NANC nerve stimulation, NO and VIP was tested. To study the effect of endogenous NO, the influence of long-term electrical stimulation in NANC conditions on these relaxations was investigated. NO is, indeed, a major contributor to sustained electrically-induced NANC relaxation in the pig gastric fundus (Lefebvre *et al*., 1995); this was confirmed in the present study for stimulation durations up to 40 min.

Methods

Tissue preparation

The stomach was removed from healthy 6 months old male castrated pigs, slaughtered at a local abattoir and transported

[1] Author for correspondence.

to the laboratory in ice-chilled physiological salt solution. The mucosa was removed and strips (15×3 mm) were cut from the fundus in the direction of the circular muscle layer. The tissues were used immediately or stored for a maximum of 24 h in physiological salt solution at 4°C.

Strips were mounted between 2 platinum plate electrodes under a load of 2 g in 20 ml organ baths, containing physiological salt solution at 37°C and gassed with 95% O_2/5% CO_2. The composition of the physiological salt solution was (mM): Na^+ 137, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, Cl^- 124.1, HCO_3^- 25, $H_2PO_4^-$ 1.2 and glucose 11.5 (Mandrek & Milenov, 1991). To work in NANC conditions, the solution also contained 10^{-6} M atropine and 4×10^{-6} M guanethidine. Changes in length were recorded isotonically via Palmer Bioscience T3 transducers on a Graphtec Linearcorder FWR 3701 or a Graphtec Multicorder MC 6625. Electrical field stimulation was performed by means of a Grass S88 stimulator (40 V, 0.1 ms). The tissues were equilibrated for at least 1 h with replacement of the solution every 15 min.

Protocols

All strips were first contracted with 3×10^{-7} M 5-hydroxytryptamine (5-HT) and subsequently relaxed by 10^{-5} M sodium nitroprusside. After an interval of at least 1 h with regular rinsing, 3×10^{-7} M 5-HT was again administered and when a stable contraction was obtained, electrical field stimulation was performed or a relaxant substance was administered. Frequency-response curves to electrical field stimulation (0.5–8 Hz) were obtained by stimulating the tissues either with 10 s trains at 5 min intervals (train stimulation) or continuously with stepwise increases of the frequency every 3 min (cumulative stimulation). In the first series of experiments, the lowest frequency tested was 0.25 Hz but as this induced very little or no response, this was not maintained. NO was administered in increasing concentrations (2×10^{-6} M – 10^{-4} M) at 5 min intervals (NO boli) or it was continuously administered to the tissue for 10 min by infusing per 10 s the amount yielding 10^{-5} M, when administered in bolus, into the organ bath via a Braun infusion pump (NO infusion). VIP was administered in a concentration of 10^{-7} M. Only one relaxant stimulus (train or cumulative stimulation, NO boli or infusion, or VIP) was studied per tissue and after the relaxant response had been obtained, the tissues were rinsed at 10 min intervals for at least 30 min. The strips were then incubated for 30 min with NTG (5×10^{-4} M) or electrically stimulated at 4 Hz for 30 min (long-term stimulation). In parallel control tissues, the solvent of NTG was administered or there was no stimulation performed. After the tissue had been rinsed 6 times at 3 min intervals, contraction was induced a third time with 3×10^{-7} M 5-HT and the relaxant stimuli were studied again. The effect of NTG on the relaxation induced by 10^{-5} M lemakalim was also studied. As the response to lemakalim clearly declined when studied twice, NTG was incubated for 30 min before the second administration of 5-HT and the response to lemakalim was compared to that in parallel control tissues that received the solvent of NTG.

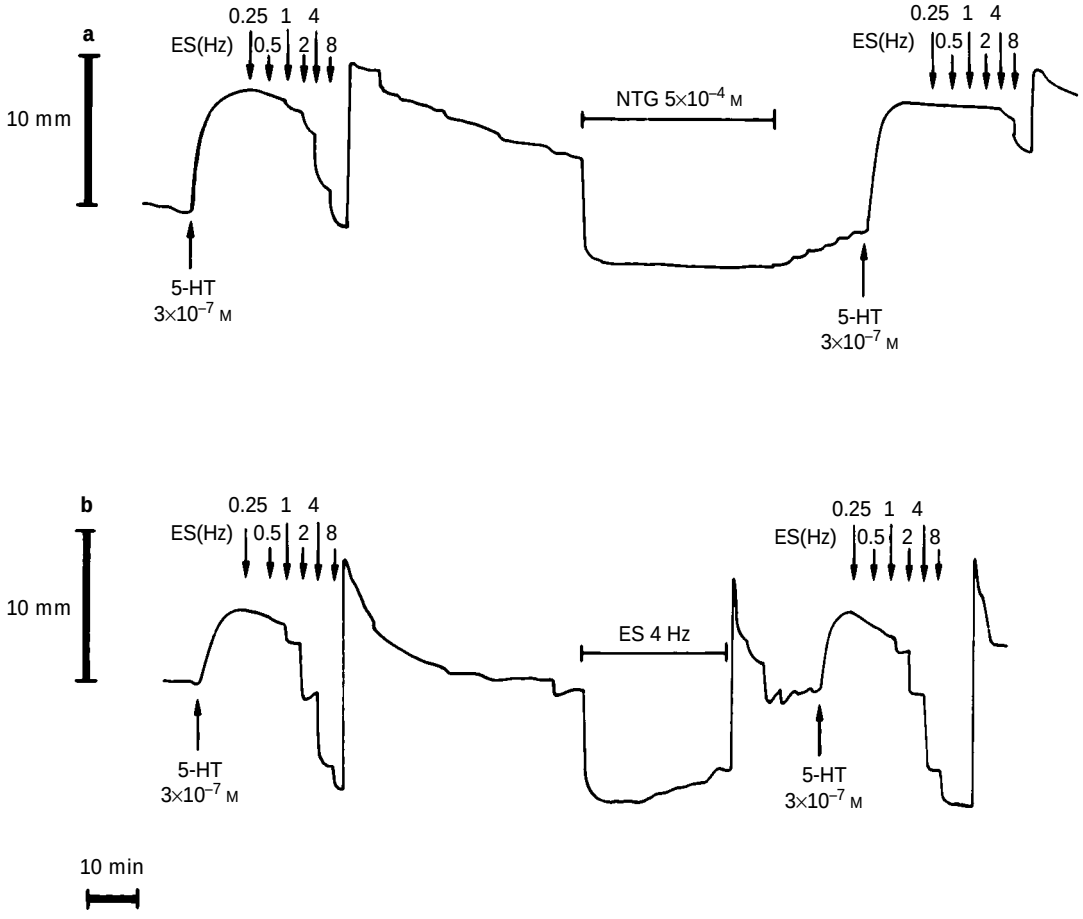

Figure 1 Representative traces showing the relaxation to cumulative electrical stimulation (ES, 40 V, 0.1 ms, 0.25–8 Hz) before and after incubation with 5×10^{-4} M nitroglycerin (NTG; a) and before and after long-term stimulation at 4 Hz (b) for 30 min.

In an additional series, the relaxant response to 10^{-5} M NTG on a contracted tissue was studied before and after incubation with 5×10^{-4} M NTG. To study the involvement of NO in the relaxant response occurring during long-term electrical stimulation, four tissues were stimulated in parallel at 4 Hz for 40 min, either after the tissues had been contracted with 3×10^{-7} M 5-HT or at basal length. L-NAME, 3×10^{-4} M, was administered after 10, 20 or 30 min of stimulation while the fourth control tissue did not receive L-NAME.

Drugs used

Atropine sulphate, guanethidine sulphate, N^G-nitro-L-arginine methyl ester and sodium nitroprusside were obtained from Sigma (St. Louis, U.S.A.), 5-hydroxytryptamine creatinine monosulphate from Janssen Chimica (Geel, Belgium), vasoactive intestinal polypeptide from CRB (Northwich, U.K.), lemakalim from SmithKline Beecham Pharmaceuticals (Betchworth, U.K.) and nitroglycerin from Pohl Boskamp (Hohenlockstadt, Germany). The drugs were dissolved in deionized water, except for nitroglycerin which was a 1 mg ml^{-1} solution in dextrose 5%, PEG 400 0.5% and water, and lemakalim which was dissolved in 70% ethanol. This solvent did not influence tone induced with 5-hydroxytryptamine. A stock solution of VIP (10^{-3} M) was prepared and stored at $-20°C$. All other solutions were prepared on the day of the experiment. A saturated NO solution was prepared as described by Kelm & Schrader (1990), yielding a vial containing NO in a concentration taken to be 2×10^{-3} M.

Data analysis

Relaxations are expressed as percentage of the relaxation induced by 10^{-5} M sodium nitroprusside at the beginning of the experiment. The contraction induced by 3×10^{-7} M 5-hydroxytryptamine after incubation with NTG or after long-term stimulation was expressed as percentage of that induced before, as were changes in tone induced by the procedures. When the effect of L-NAME was studied during long-term stimulation, the relaxant response after 10 min was considered as 100% and the relaxation remaining after 20, 30 or

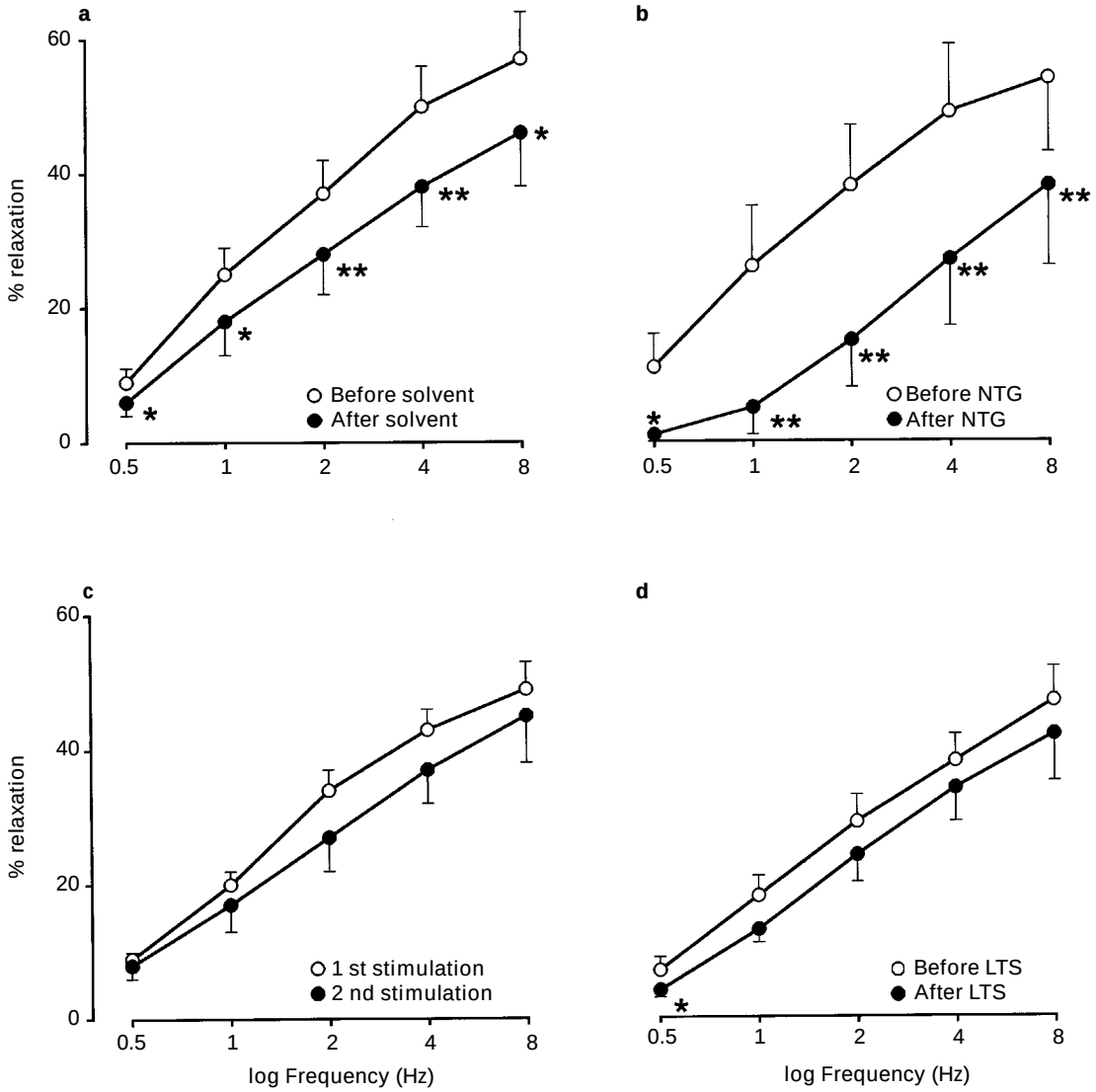

Figure 2 Mean ($n = 7-8$) relaxant responses to train stimulation (ES, 40 V, 0.1 ms, 0.5–8 Hz, 10 s trains) before and after incubation with 5×10^{-4} M nitroglycerin (NTG; b) or its solvent (a) and before and after long-term stimulation (LTS) at 4 Hz (d). In the control tissues for the latter series, the responses were studied twice without LTS in between (c). $*P < 0.05$, $**P < 0.01$: significantly different from the response before. Vertical lines show s.e.mean.

40 min was expressed as percentage of this control value. Results are given as means ± s.e.mean and n refers to strips from different animals. Results within tissues were compared by the paired t test and results between tissues with an unpaired t test. A difference was considered statistically significant at $P<0.05$.

Results

5-HT (3×10^{-7} M) induced stable contractions. This concentration was previously shown to be submaximal, as 10^{-7} M and 10^{-6} M 5-HT induced 59 and 93% of the maximum 5-HT-induced contraction in circular muscle strips of the pig gastric fundus (Lefebvre *et al.*, 1995). Train stimulation (0.5–8 Hz) and NO, administered at 5 min intervals (2×10^{-6} M–10^{-4} M) induced frequency-dependent and concentration-dependent, respectively, short-lasting relaxations. Cumulative stimulation induced frequency-dependent sustained relaxations; when the stimulation was stopped after 3 min at 8 Hz, a quick rebound contraction brought the tone back to its original level (Figure 1). NO infusion (see Methods) and 10^{-7} M VIP induced sustained relaxations. In the control tissues, the relaxant responses to these stimuli were reproducible unless otherwise stated. The relaxant response to 10^{-5} M lemakalim was sustained but was not reproducible.

Effect of NTG on relaxations induced by electrical stimulation, NO, VIP and lemakalim

When NTG, 5×10^{-4} M, was incubated for 30 min in non 5-HT-contracted tissues, it usually induced a relaxation illustrating that these tissues have some intrinsic tone. During the rinsing procedure after the incubation, tone recuperated but often only partly. However, when the tone was still decreased before the next administration of 5-HT, the contractile response to 5-HT at this lower tone level was increased as compared with the response to the previous 5-HT administration, so that the contraction level at which relaxant stimuli were tested after incubation with NTG was similar to that before. When, for example, the effect of incubation with NTG on the responses induced by train stimulation was studied, the administration of NTG induced a relaxation of varying amplitude in the 8 tissues. In 4, the tone fully recovered during the rinsing procedure after the 30 min incubation with NTG and 5-HT induced a similar contraction as before. In the 4 other tissues, the tone was still decreased before the administration of 5-HT again (-17, -167, -120 and -100%) but the response to 5-HT in these tissues was increased to 115, 213, 200 and 167%.

Incubation for 30 min with 5×10^{-4} M NTG abolished the relaxant response to 10^{-5} M NTG (111 ± 9% before and 1 ± 1% after, $n=7$). Incubation with NTG significantly reduced the relaxations induced by train and cumulative stimulation (Figures 1, 2 and 3). In the control tissues of the series where the response to train stimulation was studied, the electrically-induced relaxations significantly declined after incubation with the solvent of NTG (Figure 2a) but the reductions of the responses to train stimulation after incubation with NTG were significantly more pronounced than after its solvent at 0.5, 1, 2 ($P<0.01$) and 4 Hz ($P<0.05$, unpaired t test). Incubation with nitroglycerin also reduced the relaxant responses to NO, both when given at 5 min intervals (Figure 4) or as an infusion (100 ± 8% before and 31 ± 6% after, $n=8$, $P<0.01$). The sustained relaxations to 10^{-7} M VIP (63 ± 18% before and 73 ± 24% after, $n=6$) and 10^{-5} M lemakalim (73 ± 10% after incubation with solvent, $n=11$; 74 ± 7% after incubation with NTG, $n=11$) were not affected by NTG.

Effect of long-term stimulation on relaxations induced by electrical stimulation, NO and VIP

Long-term electrical stimulation at 4 Hz for 40 min induced a sustained relaxation of the tissues, when tissues had been contracted with 5-HT as well as on tissues at basal length. After 10 min of stimulation, the relaxation was 89 ± 5% in contracted tissues ($n=32$) and 33 ± 5% in those at basal length ($n=30$); the relaxations were well maintained for the whole period of stimulation. When administered after 10, 20 or 30 min electrical stimulation in contracted tissues, L-NAME reversed the electrically-induced relaxation by 90 ± 9% ($n=8$), 66 ± 8% ($n=8$) and 66 ± 10% ($n=8$), respectively (Figure 5). When L-NAME was administered during electrical stimulation in tissues at basal length, these values were 66 ± 13% ($n=8$), 74 ± 14% ($n=6$) and 78 ± 20% ($n=8$).

When the effect of long-term stimulation for 30 min versus the relaxant stimuli was investigated, there was no decrease in

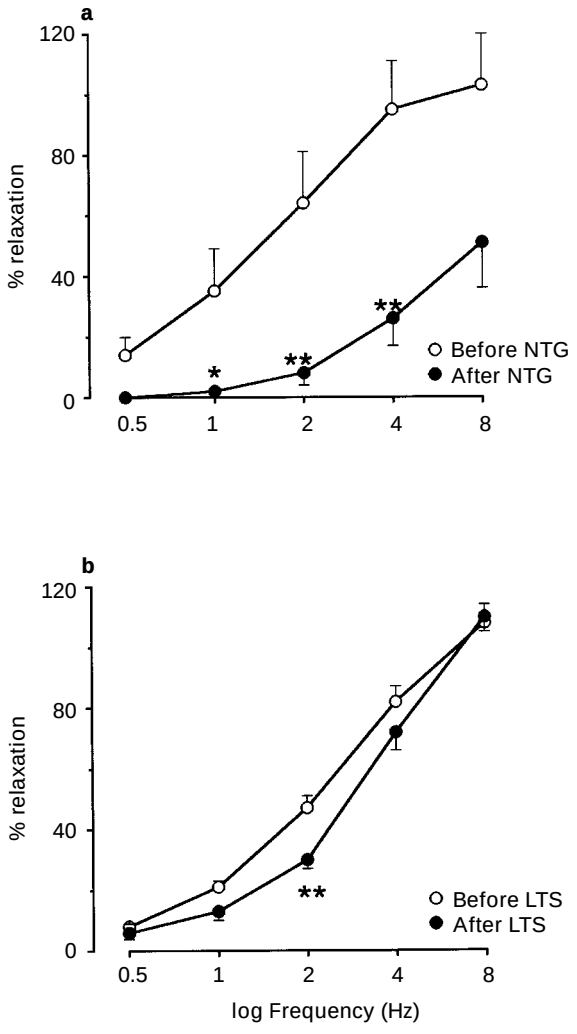

Figure 3 Mean ($n=5$–8) relaxant responses to cumulative electrical stimulation (ES, 40 V, 0.1 ms, 0.5–8 Hz, 3 min at each frequency) before and after incubation with 5×10^{-4} M nitroglycerin (NTG; a) and before and after long-term stimulation (LTS) at 4 Hz (b). *$P<0.05$, **$P<0.01$: significantly different from the response before. Vertical lines show s.e.mean.

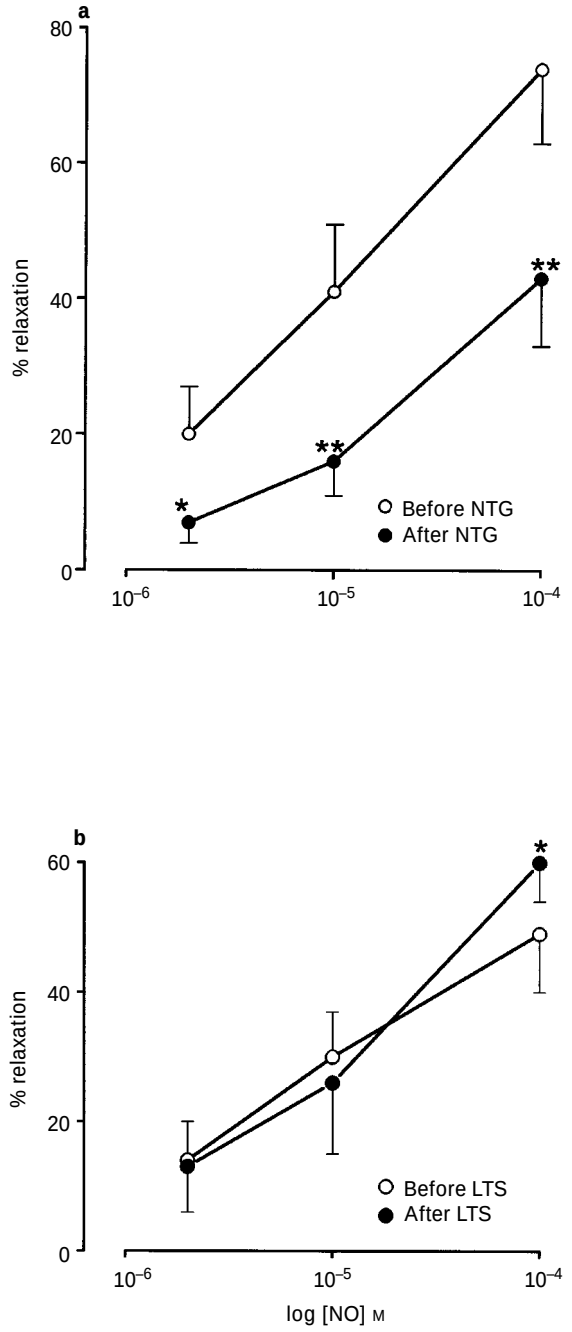

Figure 4 Mean ($n=8$) relaxant responses to NO (2×10^{-6}–10^{-4} M, administered at 5 min intervals) before and after incubation with 5×10^{-4} M nitroglycerin (NTG; a) and before and after long-term stimulation (LTS) at 4 Hz (b). *$P<0.05$, **$P<0.01$: significantly different from the response before. Vertical lines show s.e.mean.

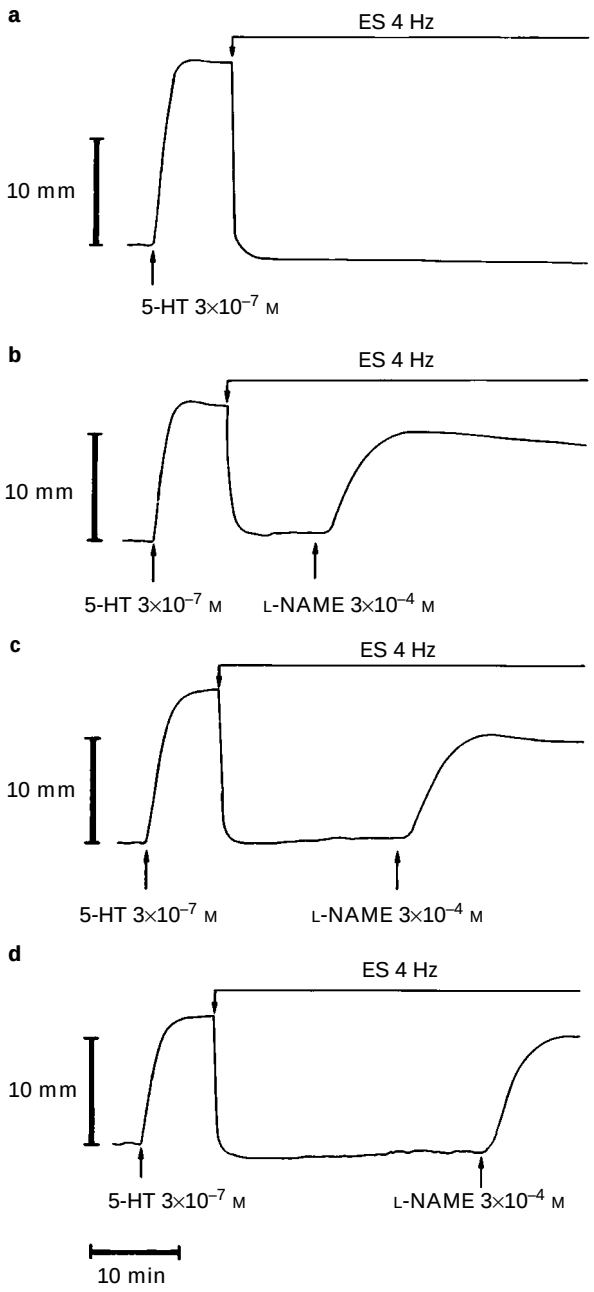

Figure 5 Representative traces showing the effect of 3×10^{-4} M L-NAME administered after 10 (b), 20 (c) or 30 min (d) of long-term stimulation at 4 Hz (ES) in contracted tissues. The control tissue (a) was stimulated for 40 min in the absence of L-NAME.

tone when 5-HT was again administered, as the rebound contraction occurring after stimulation had ceased brought tone back to its original level. The response to 5-HT was maintained so that the relaxant responses to NO, VIP and train and cumulative stimulation were studied at the same level of contraction as before long-term stimulation. The electrically-induced relaxant responses were not systematically depressed by long-term stimulation (Figures 1, 2 and 3). The significant reduction of the response to a train of stimulation at 0.5 Hz was minimal, and the significant reduction of the response to cumulative stimulation at 2 Hz after long-term stimulation was also observed in a parallel control series (decrease from $39\pm6\%$ to $29\pm4\%$, $n=8$, $P<0.05$). Long-term stimulation also did not affect the responses to NO boli (Figure 4) and to NO infusion ($84\pm6\%$ before and $108\pm12\%$ after, $n=8$). In addition, the relaxation to VIP was not changed by long-term stimulation ($34\pm8\%$ before and $40\pm11\%$ after, $n=8$).

Discussion

Data in the literature suggest that NO might be involved in autoregulatory inhibition of its own synthesis. In the rat gastric fundus, De Man *et al.* (1995) showed that pretreatment with NTG and SIN-1 reduced the NANC relaxations induced by short periods of electrical stimulation, without affecting the responses to NO and VIP. They concluded that this inhibition results from a prejunctional action of NO on the nitrergic

component of the NANC response, most likely due to downregulation of the neuronal NO synthase, and suggested the presence of an autoregulatory mechanism for the nitrergic innervation. In the pig gastric fundus, NO is involved in both short-lasting and sustained NANC relaxation (Lefebvre *et al*., 1995) and we, therefore, studied the effect of NTG on NANC relaxations in the pig gastric fundus. As an autoregulatory mechanism should be functional during endogenous NO release, we also studied the effect of long-term stimulation. Although the tissues have some intrinsic tone, we have previously shown that tone has to be raised to obtain reproducible responses to the relaxant stimuli (Lefebvre *et al*., 1995). 5-Hydroxytryptamine induces stable plateau contractions that are due to direct smooth muscle activation as the responses are not influenced by tetrodotoxin (unpublished results). No evidence for a prejunctional inhibition of NOS was obtained.

Incubation with NTG inhibited the nitrergic relaxations induced by train and cumulative stimulation, but also the relaxations to exogenous NO, pointing to a postjunctional site of interaction. Tolerance to the relaxant effect of organic nitrates after prolonged exposure to NTG is well known in vascular smooth muscle. A diminished metabolism of the organic nitrates at the level of the smooth muscle cells by depletion of the intracellular thiol pool has been proposed as the underlying mechanism (Needleman *et al*., 1973; Axelsson & Ahlner, 1987). In many studies, the response to agents that generate NO in a non-enzymatic way such as SIN-1 and sodium nitroprusside is, indeed, not reduced in vessels tolerant to NTG (Berkenboom *et al*., 1988; Kühn & Förstermann, 1989; Kowaluk & Fung, 1990). Still, tolerance to this type of NO donor or NO itself in vascular preparations has been described (Kukovetz & Holzmann, 1989; Zhang *et al*., 1993) and decreased soluble guanylate cyclase activity or increased hydrolysis of cyclic GMP by phosphodiesterases might explain these observations (Ahlner *et al*., 1986; Romanin & Kukovetz, 1989; Papapetropoulos *et al*., 1996). As incubation with NTG reduced the responses to exogenous NO and to the endogenous nitrergic neurotransmitter, a similar mechanism might prevail in the pig gastric fundus. The reduced effectiveness is limited to procedures inducing relaxation via the guanylate cyclase-cyclic GMP pathway, as the responses to VIP, that activates adenylate cyclase in smooth muscle cells (Bitar & Makhlouf, 1982) and lemakalim, a K^+ channel opener (Quast & Cook, 1989), were not affected by incubation with NTG, excluding a non-selective interaction with the relaxant capacity of the smooth muscle cells. It has been suggested that the relaxation of gastric smooth muscle cells by VIP is partly due to generation of NO in the smooth muscle (Jin *et al*., 1993; Murthy *et al*., 1993). In the pig gastric fundus, a reduced effect of VIP should then be expected after incubation with NTG, which was not the case.

Long-term electrical stimulation on tissues contracted with 5-HT induced a sustained relaxation that was largely nitrergic in nature, as even after 30 min the relaxation was reversed for approximately 70% by the NOS inhibitor L-NAME. This illustrates that in the pig gastric fundus, NO is also largely involved in the sustained NANC relaxation induced by electrical stimulation. This contrasts with data in the rat and ferret stomach, where NO seems to be mainly important for initiating NANC relaxations (Li and Rand, 1990; Boeckxstaens *et al*., 1992; Grundy *et al*., 1993) but is similar to the findings in the guinea-pig and cat stomach (Lefebvre *et al*., 1992; Barbier & Lefebvre, 1993). In the rat anococcygeus muscle, it was shown that NO release can be maintained for 2 h of electrical stimulation (Kasakov *et al*., 1995). After having checked that the smaller relaxation induced by long-term stimulation in tissues at basal length was largely nitrergic, we investigated whether this procedure influenced the NANC responses to electrical field stimulation. The electrically-induced NANC relaxations were not affected nor were the relaxations induced by NO and VIP. This suggests, firstly, that the NO released during long-term stimulation does not suppress neuronal NO synthase activity at the prejunctional level. Secondly, it means that the postjunctional changes induced in the pig gastric smooth muscle cells by NO, administered via NTG, are not mimicked by endogenous NO. It should be borne in mind that a peptide such as VIP may be released, as well as NO, during long-term stimulation (Lefebvre *et al*., 1995). In how far an increase in cyclic AMP might interfere with the development of tolerance to nitrergic relaxations is not known, but cyclic AMP-cyclic GMP interactions at the level of the phosphodiesterases have been demonstrated (Jang *et al*., 1993; Eckly & Lugnier, 1994; Wright *et al*., 1994).

In conclusion, incubation of circular muscle strips of the pig gastric fundus with NTG significantly reduced the relaxant responses to electrical stimulation of the NANC nerves but also those to exogenous NO. These relaxations were not affected by long-term electrical stimulation. The results illustrate that nitroglycerin can induce a postjunctional tolerance to nitrergic stimuli but do not provide evidence for the prejunctional inhibition of neuronal NOS by NO.

The authors thank Mr Valère Geers for technical assistance. The study was financially supported by grant No. 3G0031.96 from the Fund for Scientific Research Flanders and grant 011A1696 from the Special Investigation Fund of the Gent University.

References

AHLNER, J., ANDERSSON, R.G.G., AXELSSON, K.L., DAHLSTRÖM, U. & RYDELL, E.L. (1986). Development of tolerance to glyceryl trinitrate in an isolated human peripheral vein and its relation to cyclic GMP metabolism. *Acta Pharmacol. Toxicol*., **59,** 123–128.

ASSREUY, J., CUNHA, F.Q., LIEW, F.Y. & MONCADA, S. (1993). Feedback inhibition of nitric oxide synthase activity by nitric oxide. *Br. J. Pharmacol*., **108,** 833–837.

AXELSSON, K.L. & AHLNER, J. (1987). Nitrate tolerance from a biochemical point of view. *Drugs*, **33** (Suppl. 4), 63–68.

BARBIER, A.J. & LEFEBVRE, R.A. (1993). Involvement of the L-arginine:nitric oxide pathway in nonadrenergic noncholinergic relaxation of the cat gastric fundus. *J. Pharmacol. Exp. Ther*., **266,** 172–178.

BERKENBOOM, G., FONTAINE, J. & DEGRE, S. (1988). Persistence of the response to SIN-1 on isolated coronary arteries rendered tolerant to nitroglycerin in vitro or in vivo. *J. Cardiovasc. Pharmacol*., **12,** 345–349.

BITAR, K.N. & MAKHLOUF, G.M. (1982). Relaxation of isolated gastric muscle cells by vasoactive intestinal peptide. *Science*, **216,** 531–533.

BOECKXSTAENS, G.E., PELCKMANS, P.A., DE MAN, J.G., BULT, H., HERMAN, A.G. & VAN MAERCKE, Y.M. (1992). Evidence for a differential release of nitric oxide and vasoactive intestinal polypeptide by nonadrenergic noncholinergic nerves in the rat gastric fundus. *Arch. Int. Pharmacodyn*., **318,** 107–115.

BROOKES, S.J.H. (1993). Neuronal nitric oxide in the gut. *J. Gastroenterol. Hepatol.*, **8,** 590–603.

BUGA, G.M., GRISCAVAGE, J.M., ROGERS, N.E. & IGNARRO, L.J. (1993). Negative feedback regulation of endothelial cell function by nitric oxide. *Circ. Res.*, **73,** 808–812.

DE MAN, J.G., BOECKXSTAENS, G.E., DE WINTER, B.Y., MOREELS, T.G., HERMAN, A.G. & PELCKMANS, P.A. (1995). Inhibition of non-adrenergic non-cholinergic relaxations by nitric oxide donors. *Eur. J. Pharmacol.*, **285,** 269–274.

ECKLY, A.E. & LUGNIER, C. (1994). Role of phosphodiesterases III and IV in the modulation of vascular cyclic AMP content by the NO/cyclic GMP pathway. *Br. J. Pharmacol.*, **113,** 445–450.

GRUNDY, D., GHARIB-NASERI, M.K. & HUTSON, D. (1993). Role of nitric oxide and vasoactive intestinal polypeptide in vagally mediated relaxation of the gastric corpus in the anaesthetized ferret. *J. Autonom. Nerv. Syst.*, **43,** 241–246.

JANG, E.K., DAVIDSON, M.M.L., CRANKSHAW, D. & HASLAM, R.J. (1993). Synergistic inhibitory effects of atriopeptin II and isoproterenol on contraction of rat aortic smooth muscle: roles of cGMP and cAMP. *Eur. J. Pharmacol.*, **250,** 477–481.

JIN, J.-G., MURTHY, K.S., GRIDER, J.R. & MAKHLOUF, G.M. (1993). Activation of distinct cAMP- and cGMP-dependent pathways by relaxant agents in isolated gastric muscle cells. *Am. J. Physiol.*, **264,** G470–G477.

KASAKOV, L., CELLEK, S. & MONCADA, S. (1995). Characterization of nitrergic neurotransmission during short- and long-term electrical stimulation of the rabbit anococcygeus muscle. *Br. J. Pharmacol.*, **115,** 1149–1154.

KELM, M. & SCHRADER, J. (1990). Control of coronary vascular tone by nitric oxide. *Circ. Res.*, **66,** 1561–1575.

KOWALUK, E.A. & FUNG, H.-L. (1990). Dissociation of nitrovasodilator-induced relaxation from cyclic GMP levels during in vitro nitrate tolerance. *Eur. J. Pharmacol.*, **176,** 91–95.

KRÖNCKE, K.-D., FEHSEL, K. & KOLB-BACHOFEN, V. (1995). Inducible nitric oxide synthase and its product nitric oxide, a small molecule with complex biological activities. *Biol. Chem. Hoppe-Seyler*, **376,** 327–343.

KUHN, M. & FÖRSTERMANN, U. (1989). Endothelium-dependent vasodilatation in human epicardial coronary arteries: effect of prolonged exposure to glyceryl trinitrate or SIN-1. *J. Cardiovasc. Pharmacol.*, **14** (Suppl. 11), S47–S54.

KUKOVETZ, W.R. & HOLZMANN, S. (1989). Tolerance and cross-tolerance between SIN-1 and nitric oxide in bovine coronary arteries. *J. Cardiovasc. Pharmacol.*, **14** (Suppl. 11), S40–S46.

LANGER, S.Z. (1981). Presynaptic regulation of the release of catecholamines. *Pharmacol. Rev.*, **32,** 337–362.

LEFEBVRE, R.A., BAERT, E. & BARBIER, A.J. (1992). Influence of N^G-nitro-L-arginine on non-adrenergic non-cholinergic relaxation in the guinea-pig gastric fundus. *Br. J. Pharmacol.*, **106,** 173–179.

LEFEBVRE, R.A., SMITS, G.J.M. & TIMMERMANS, J.-P. (1995). Study of NO and VIP as non-adrenergic non-cholinergic neurotransmitters in the pig gastric fundus. *Br. J. Pharmacol.*, **116,** 2017–2026.

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.

MA, X.-L., LOPEZ, B.L., CHRISTOPHER, T.A., BIRENBAUM, D.S. & VINTEN-JOHANSEN, J. (1996). Exogenous NO inhibits basal NO release from vascular endothelium in vitro and in vivo. *Am. J. Physiol.*, **271,** H2045–H2051.

MANDREK, K. & MILENOV, K. (1991). Responses of porcine gastric and duodenal smooth muscle to VIP. *J. Autonom. Pharmacol.*, **11,** 353–364.

MONCADA, S., PALMER, R.M.J. & HIGGS, A. (1991). Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **43,** 109–142.

MURTHY, K.S., ZHANG, K.-M., JIN, J.-G., GRIDER, J.R. & MAKHLOUF, G.M. (1993). VIP-mediated G protein-coupled Ca^{2+} influx activates a constitutive NOS in dispersed gastric muscle cells. *Am. J. Physiol.*, **265,** G660–G671.

NEEDLEMAN, P., JAKSCHIK, B. & JOHNSON, E.M., Jr, (1973). Sulfhydryl requirement for relaxation of vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, **187,** 324–331.

PAPAPETROPOULOS, A., GO, C.Y., MURAD, F. & CATRAVAS, J.D. (1996). Mechanisms of tolerance to sodium nitroprusside in rat cultured aortic smooth muscle cells. *Br. J. Pharmacol.*, **117,** 147–155.

QUAST, U. & COOK, N.S. (1989). Moving together: K^+ channel openers and ATP-sensitive K^+ channels. *Trends Pharmacol. Sci.*, **10,** 431–435.

RAND, M.J. & LI, C.G. (1995). Nitric oxide as a neurotransmitter in peripheral nerves: Nature of transmitter and mechanism of transmission. *Ann. Rev. Physiol.*, **57,** 659–682.

RENGASAMY, A. & JOHNS, R.A. (1993). Regulation of nitric oxide synthase by nitric oxide. *Mol. Pharmacol.*, **44,** 124–128.

ROGERS, N.E. & IGNARRO, L.J. (1992). Constitutive nitric oxide synthase from cerebellum is reversibly inhibited by nitric oxide formed from L-arginine. *Biochem. Biophys. Res. Commun.*, **189,** 242–249.

ROMANIN, C. & KUKOVETZ, W.R. (1989). Tolerance to nitroglycerin is caused by reduced guanylate cyclase activation. *J. Mol. Cell. Cardiol.*, **21,** 41–48.

STARKE, K. (1981). Presynaptic receptors. *Ann. Rev. Pharmacol. Toxicol.*, **21,** 7–30.

WRIGHT, I.K., AMIRCHETTY-RAO, S. & KENDALL, D.A. (1994). Potentiation by forskolin of both SNP- and ANP-stimulated cyclic GMP accumulation in porcine isolated palmar lateral vein. *Br. J. Pharmacol.*, **112,** 1146–1150.

ZHANG, L.M., CASTRESANA, M.R., STEFANSSON, S. & NEWMANN, W.H. (1993). Tolerance to sodium nitroprusside. Studies in cultured porcine vascular smooth muscle cells. *Anesthesiology*, **79,** 1094–1103.

(Received June 2, 1997
Revised August 4, 1997
Accepted October 1, 1997)