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Inhibition of non-adrenergic non-cholinergic relaxations by nitric oxide donors

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Abstract

The effects of pretreatment with the nitric oxide (NO)-releasing substances 3-morpholino-sydnoninime (SIN-1) and nitroglycerin were investigated on relaxations induced by non-adrenergic non-cholinergic (NANC) nerve stimulation, authentic NO and vasoactive intestinal polypeptide (VIP) in the rat gastric fundus. Short periods of electrical stimulation (0.5–16 Hz, 1 ms, pulse trains of 10 s) induced frequency-dependent transient relaxations, previously shown to be mainly mediated by NO. Both SIN-1 (10–100 μM) and nitroglycerin (0.5 mM) pretreatment significantly reduced these electrically induced responses to a similar extent as the inhibitor of the NO biosynthesis L-nitroarginine (30–300 μM). Prolonged periods of electrical stimulation (16 Hz, 1 ms, pulse trains of 180 s) induced a sustained relaxation, previously shown to be mediated by NO and VIP. L-Nitroarginine (30–300 μM) or pretreatment with SIN-1 (100 μM) or nitroglycerin (0.5 mM) did not affect the amplitude of this relaxation but slowed down its onset. Authentic NO (0.01–10 μM) and VIP (0.01–10 nM) induced respectively transient and sustained concentration-dependent relaxations. SIN-1 or nitroglycerin pretreatment had no effect on the concentration-response curves to NO and VIP. These results indicate that prolonged exposure to NO donors inhibits electrically induced nerve-mediated NANC relaxations without affecting the postjunctional response to NO and VIP. As similar results are obtained with NO biosynthesis inhibitors, our results illustrate a prejunctional inhibitory effect of NO on the NANC nerves of the rat gastric fundus and suggest the presence of an autoregulatory mechanism for the nitrergic innervation.

Keywords: Feedback inhibition; Nitrergic nerve; Nitric oxide (NO); NANC (non-adrenergic non-cholinergic); Gastric fundus; (Rat)

1. Introduction

Regulation of neurotransmission involves a variety of communication signals between the presynaptic nerve and the postsynaptic effector cell. In this respect, modulation of the presynaptic nerve terminal is of key importance in the determination of synaptic efficiency. One such modulatory mechanism is feedback inhibition, in which a neurotransmitter depresses its own release by stimulation of specific presynaptic receptors. These so-called autoreceptors have been found on central and peripheral neurons, including α- and β-adrenoceptors in the modulation of adrenergic neurotransmission (for review see Langer, 1981) and muscarinic receptors for modulation of cholinergic neurotransmission (for review see Chesselet, 1984).

We previously proposed nitric oxide (NO) as an inhibitory non-adrenergic non-cholinergic (NANC) neurotransmitter in the gut (Boeckxstaens et al., 1990; Bult et al., 1990a). As NO is a lipophylic molecule which easily diffuses through cell membranes, it might also influence its own release intracellularly in the nerve terminal. Recently, Bult et al. (1990b) demonstrated that chronic treatment of rabbits with the NO donor 3-morpholino-sydnoninime (SIN-1) reduced the release of the endothelium derived relaxing factor from the rabbit thoracic aorta. Also pretreatment of bovine intrapulmonary artery with an NO donor significantly

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inhibited the NO-mediated responses to electrical stimulation (Buga et al., 1993). In addition, the spontaneous decline of NO synthase activity in the murine macrophage cell line J774 was significantly slowed down in a low L-arginine medium (Assreuy et al., 1993) and in the cerebellum, the production of NO was inhibited by addition of authentic NO and by different NO donors (Rogers and Ignarro, 1992; Rengasamy and Johns, 1993; Griscavage et al., 1994). Together, these findings suggest that NO is involved in an autoregulatory mechanism to inhibit its own biosynthesis.

In the rat gastric fundus, there is evidence that NO and VIP are inhibitory NANC neurotransmitters, with NO mediating the NANC relaxations to low frequency stimulation and both NO and VIP mediating the NANC relaxations to higher frequency stimulation (De Beurme and Lefebvre, 1988; Li and Rand, 1990; Boeckxstaens et al., 1992; D'Amato et al., 1992). The observation that electrically induced NANC relaxations in the rat gastric fundus are reduced by stimulation of prejunctional α_2-adrenoceptors (MacDonald et al., 1990; Lefebvre and Smits, 1992) indicates that the nitrergic NANC innervation in the rat gastric fundus is subject to prejunctional modulation. However, prejunctional inhibition of NANC relaxations by NO has not been demonstrated yet. Therefore, in the present study we investigated the effect of prolonged exposure of the rat gastric fundus to the NO donors SIN-1 and nitroglycerin on relaxations induced by NANC nerve stimulation and on those to authentic NO and VIP.

2. Materials and methods

2.1. Tissue preparation

Male Wistar rats (250–300 g) were fasted for 24 h with free access to water. The animals were anesthetized with an intraperitoneal injection of sodium pentobarbitone (60 mg kg^{-1}) and the stomach was removed via a midline incision. Longitudinal muscle strips of approximately 1.0 cm long and 0.3 cm wide were prepared and mounted in organ baths (25 ml) filled with Krebs-Ringer solution (in mM: NaCl 118.3; KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, CaEDTA 0.026 and glucose 11.1). The solution was maintained at 37°C and aerated with a mixture of 95% O_2 and 5% CO_2.

2.2. Isometric tension recording

One end of the muscle strip was attached to a glass rod and pulled through two platinum ring electrodes. The other end was connected to a strain gauge transducer (Statham UC2) for continuous recording of isometric tension. The strips were brought at their optimal point of length-tension relationship (Boeckxstaens et al., 1991) and then allowed to equilibrate for at least 60 min before experimentation.

2.3. Experimental protocols

All experiments were performed on muscle strips contracted with 0.1 μM 5-hydroxytryptamine (5-HT) and in the presence of 1 μM atropine and 30 μM guanethidine. After each 5-HT-induced contraction, the muscle strips were washed 3 times with an interval of at least 5 min.

In a first series of experiments, the muscle strips were stimulated with short (0.5–16 Hz, 1 ms, pulse trains of 10 s) and prolonged (16 Hz, 1 ms, pulse trains of 180 s) periods of electrical stimulation. The strips were then pretreated for 30 min with SIN-1 (10–100 μM), nitroglycerin (0.5 mM) or saline and after two wash-out periods of 7 min each, the muscle strips were again stimulated electrically. The effect of NO donor pretreatment was compared with the effect of L-nitroarginine (30–300 μM) on the electrically induced NANC relaxations.

In a second series of experiments, the effect of SIN-1 or nitroglycerin pretreatment was investigated on a concentration-response curve to authentic NO (0.01–10 μM) and to VIP (0.01–10 nM).

2.4. Drugs used

The following drugs were used: atropine sulphate, nitroglycerin (Merck, Darmstadt, Germany), guanethidine monosulphate (Ciba Geigy, Switzerland), 5-hydroxytryptamine, L-nitroarginine (Sigma Chemical Co., St. Louis, MO, USA), vasoactive intestinal polypeptide (UCB Bioproducts, Brussels, Belgium). SIN-1 (3-morpholino-sydnoninime) was a gift of Therabel Research (Brussels, Belgium). Solutions of NO were prepared as described by Kelm et al. (1988).

2.5. Presentation of results and statistical analysis

Results are expressed as percentage decrease of the 5-hydroxytryptamine-induced contraction of the rat gastric fundus longitudinal muscle strip.

Values are shown as mean ± S.E.M for the number of rats indicated. For statistical analysis, a Student's *t*-test for paired values was used. *P* values of less than 0.05 were considered to be significant.

3. Results

3.1. Effect of NO donor pretreatment and L-nitroarginine on NANC relaxations to short periods of electrical stimulation

Short periods of electrical stimulation (0.5–16 Hz, 1 ms, pulse trains of 10 s) induced frequency-dependent

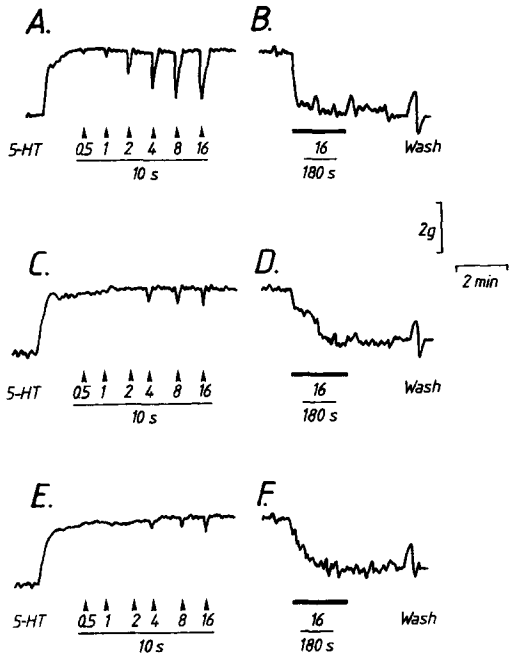

Fig. 1. Typical tracings of the rat gastric fundus showing the effect of electrical stimulation (0.5–16 Hz) with trains of pulses lasting 10 s (A) and 180 s (B) in control conditions and after pretreatment with 100 μM SIN-1 (C) and 0.5 mM nitroglycerin (E). In 4 out of 6 experiments 100 μM SIN-1 (D) and in 4 out of 7 experiments 0.5 mM nitroglycerin (F) inhibited the initial fast response but not the following sustained response induced by 16 Hz electrical stimulation with trains of pulses lasting 180 s.

transient responses which were fast in onset (Fig. 1A) and previously shown to be mediated mainly by NO (Li and Rand, 1990; Boeckxstaens et al., 1991). Pretreatment with SIN-1 (10–100 μM) concentration-dependently inhibited the NANC relaxations to electrical stimulation (Fig. 1C and 2). NANC relaxations to 2 Hz were inhibited from 47 ± 7% to 23 ± 10% and relaxations to 4 Hz from 65 ± 7% to 29 ± 10% by pretreatment with 100 μM SIN-1 (Fig. 2). The same results were obtained after pretreatment with nitroglycerin (0.5 mM) (Fig. 1E and 2): NANC relaxations to 2 Hz were inhibited from 53 ± 6% to 18 ± 8% and those to 4 Hz from 60 ± 5% to 36 ± 6% by pretreatment with 0.5 mM nitroglycerin (Fig. 2). This inhibitory effect of NO donor pretreatment was not reversed after a wash-out period of 60 min. However, after a wash-out period of 90 min during which the strips were washed every 10 min, the inhibition induced by pretreatment with SIN-1 (100 μM) or nitroglycerin (100 μM) was almost completely reversed (results not shown). Incubation of the muscle strips with L-nitroarginine (30–300 μM) significantly inhibited the relaxations to low frequency stimulation with the same potency as pretreatment with NO donors (Fig. 2): relaxations to 2 Hz were inhibited from 55 ± 3% to 17 ± 5% and those to 4 Hz from 62 ± 3% to 28 ± 5% by 30 μM L-nitroarginine. Throughout the incubation period, 100 μM SIN-1 or 0.5 mM nitroglycerin reduced the basal activity of the muscle strips but it reappeared immediately upon wash-out of the NO donors. Pretreatment of the muscle strips with SIN-1 or nitroglycerin or incubation with L-nitroarginine did not affect the contraction to 5-hydroxytryptamine (results not shown).

3.2. Effect of NO donor pretreatment on NANC relaxations to prolonged periods of electrical stimulation

Prolonged periods of electrical stimulation (16 Hz, 1 ms, pulse trains of 180 s) induced a relaxation which was fast in onset and sustained (Fig. 1B) and which was previously shown to be mediated by NO and VIP (Li and Rand, 1990; Boeckxstaens et al., 1992; D'Amato et al., 1992). Pretreatment with SIN-1 (10–100 μM) or with nitroglycerin (0.5 mM) did not significantly inhibit the amplitude of the NANC nerve-induced relaxation (Fig. 2) but in 4 out of 6 experiments SIN-1 (100 μM) and in 4 out of 7 experiments nitroglycerin (0.5 mM)

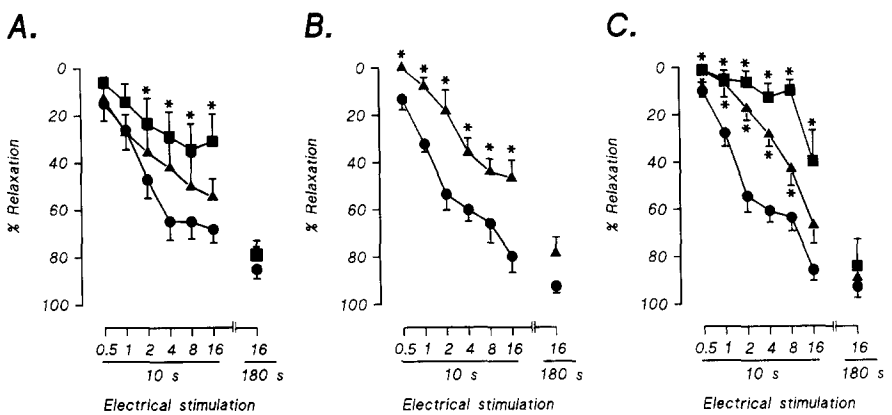

Fig. 2. Frequency-response curves to electrical field stimulation (0.5–16 Hz) with train pulses lasting 10 s and 180 s, in control conditions (●) and after pretreatment of the rat gastric fundus with (A) SIN-1 (▲, 10 μM and ■, 100 μM), (B) nitroglycerin (▲, 0.5 mM) and (C) L-nitroarginine (▲, 30 μM and ■, 300 μM). Results are expressed as percent relaxation of a 0.1 μM 5-hydroxytryptamine-induced contraction and shown as mean ± S.E.M. for $n = 6–7$ experiments. $^*P < 0.05$, significantly different from control.

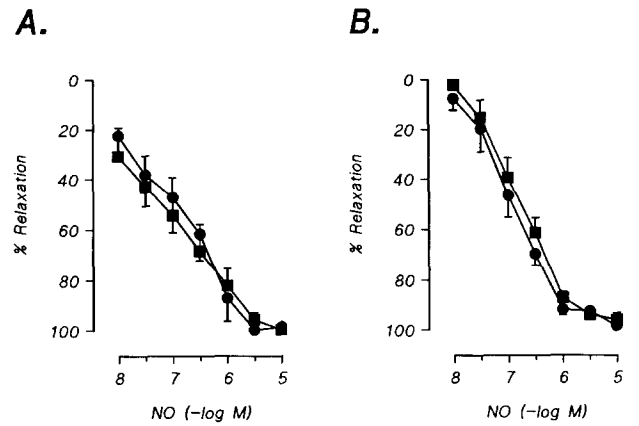

Fig. 3. Concentration-response curves to NO (0.01–10 μM) in control conditions (•) and after pretreatment of the rat gastric fundus with (A) SIN-1 (■, 100 μM) and (B) nitroglycerin (■, 0.5 mM). Results are expressed as percent relaxation of a 0.1 μM 5-hydroxytryptamine-induced contraction and shown as mean ± S.E.M. for $n = 6$ experiments.

pretreatment slowed down the onset of the relaxation (Fig. 1D and F). The effect of SIN-1 and nitroglycerin pretreatment on the electrically induced NANC relaxations was comparable to the effect of L-nitroarginine (30–300 μM) which slowed down the onset of the relaxation induced by long periods of NANC nerve stimulation, without inhibiting the amplitude of the relaxation (Fig. 2).

3.3. Effect of NO donor pretreatment on the relaxations to NO and to VIP

Authentic NO (0.01–10 μM) and VIP (0.01–10 nM) induced respectively fast and slow but sustained concentration-dependent relaxations (Figs. 3 and 4). SIN-1 (100 μM) or nitroglycerin (0.5 mM) did not affect the concentration-response curve to NO (Fig. 3) or to VIP (Fig. 4). As previously shown (Boeckxstaens et al., 1991), L-nitroargine had no effect on the relaxations to NO or VIP. Similar to L-nitroarginine, SIN-1 or nitroglycerin pretreatment did not affect the rate of development of the relaxations to NO or to VIP.

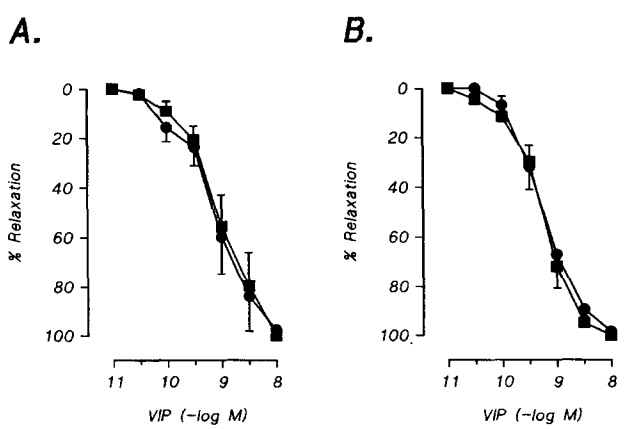

Fig. 4. Concentration-response curves to VIP (0.01–10 nM) in control conditions (•) and after pretreatment of the rat gastric fundus with (A) SIN-1 (■, 100 μM) and (B) nitroglycerin (■, 0.5 mM). Results are expressed as percent relaxation of a 0.1 μM 5-hydroxytryptamine-induced contraction and shown as mean ± S.E.M. for $n = 4–5$ experiments.

4. Discussion

NO fulfills a wide range of biological functions, including neurotransmission. NO not only functions as a messenger between neurons and neurons and their effector cells, but it also modulates the release of other neurotransmitters, such as acetylcholine and noradrenaline (Lefebvre et al., 1992; Rattan and Thatikunta, 1993; Baccari et al., 1994; Hryhorenko et al., 1994). In the present study, we provided evidence illustrating a prejunctional inhibitory effect of NO on the NANC innervation of the rat gastric fundus, suggesting the presence of an autoregulatory mechanism for the nitrergic innervation.

In the rat gastric fundus, both NO and VIP are released by inhibitory NANC nerves (De Beurme and Lefebvre, 1988; Li and Rand, 1990; Boeckxstaens et al., 1992; D'Amato et al., 1992). NO has been shown to mediate the NANC relaxations to low frequency stimulation, whereas both NO and VIP are released by prolonged stimulation at higher frequencies. It was demonstrated that NO mediates the initial fast component of the NANC response to prolonged high frequency stimulation whereas the sustained response mainly results from the release of VIP (Li and Rand, 1990; Boeckxstaens et al., 1992). To investigate the effect of NO pretreatment on the nitrergic component, we studied the effect of the NO-releasing compounds SIN-1 and nitroglycerin on the transient, NO-mediated relaxations to short trains of electrical stimulation. The effect on the peptidergic component of the NANC response was evaluated by studying the NANC responses induced by prolonged stimulation at high frequency. We demonstrated that pretreatment with SIN-1 and nitroglycerin significantly inhibited the transient relaxations to short periods of electrical stimulation. Since the degree of inhibition was comparable to that obtained after blockade of the NO biosynthesis with L-nitroarginine and since the concentration-response curves to NO or VIP were not affected, we conclude that this inhibition results from a prejunctional action of NO on the nitrergic component. This was further illustrated by the finding that SIN-1 and nitroglycerin pretreatment had a similar effect on the NANC response to prolonged stimulation at high frequency as previously shown for L-nitroarginine: the initial fast,

NO-mediated component was inhibited whereas the amplitude of the sustained peptidergic component was unaffected or slightly reduced (Boeckxstaens et al., 1992). Inhibition of VIP release should also be considered as possible underlying mechanism but the effect of exogenous NO on the sustained component was comparable to that of L-nitroarginine. In addition, NO was suggested to enhance rather than inhibit the release of VIP in gastric muscle strips (Grider et al., 1992). Although the actual release of NO or VIP was not measured, we conclude that NO exerts a prejunctional inhibitory effect on the nitrergic but not the peptidergic component of the NANC innervation in the rat gastric fundus.

Recently, a similar inhibitory effect of nitroglycerin pretreatment was reported in the rat gastric fundus (Barbier and Lefebvre, 1994). The authors suggested that depletion of endogenous thiols by nitroglycerin might account for this observation. However, SIN-1, which releases NO spontaneously without consumption of thiols, had a similar inhibitory effect on the NANC responses making it less likely that depletion of thiols or tolerance to nitroglycerin explains the inhibitory effect of NO donors. Although the relaxations to authentic NO and to VIP were not altered by pretreatment with NO donors, excluding a postjunctional toxic effect, a neurotoxic effect which results in a persistent reduction of NO release cannot be excluded. However, as the inhibitory effect of the NO donors was reversed after a wash-out period of 90 min, we favour the hypothesis that the prolonged inhibition of the NANC responses results from a persistent effect of NO on NO synthase. NO was previously shown to downregulate the activity of NO synthase, the enzyme which catalyzes NO from L-arginine (Rogers and Ignarro, 1992; Assreuy et al., 1993; Buga et al., 1993; Rengasamy and Johns, 1993; Griscavage et al., 1994). In addition, NO is thought to bind with high affinity to the heme group of NO synthase, thereby forming a stable complex with a low dissociation constant (Tsai, 1994). This might explain the persistent effect of the NO donors, also observed in other studies (Rengasamy and Johns, 1993; Bult et al., 1995). Compared to the studies in which enzyme activity was measured, we had to use higher concentrations of NO donors to inhibit NANC relaxations. However, the measurement of enzyme activity is more sensitive compared to the measurement of a mechanical response in our study, requiring more exogenous NO to reveal an effect on the NANC responses. As such, lower concentrations of NO donors will be more effective on isolated vesicles than on muscle strips. As also evidence is growing suggesting feedback inhibition of endogenous NO synthesis in vivo (Petros, 1994; Bult et al., 1995), we conclude that the prejunctional inhibition of NANC relaxations by exposure to NO donors most likely results from feedback inhibition of NO synthase. Physiologically, this feedback inhibition of NO release might function as a brake on the NO release from the nerve terminal, allowing fine regulation of neurotransmission and preventing excessive release of toxic quantities of NO. As such, treatment of patients with nitroderivates, for example in cardiovascular disease, or enhanced synthesis of large amounts of NO, e.g. in inflammation, might interfere with normal neurotransmission, possibly explaining to some extent the motility disturbances observed under these conditions.

In summary, we demonstrated that pretreatment with the NO donors SIN-1 or nitroglycerin significantly inhibited the NANC nerve-mediated relaxations in the rat gastric fundus, without altering the postjunctional response to NO or VIP. This inhibitory effect was comparable to that of inhibition of the NO biosynthesis with L-nitroarginine. These results suggest that exogenous NO has a prejunctional inhibitory effect on the nitrergic component of the NANC response, most likely resulting from a down-regulation of the neuronal NO synthase.

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