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Role of sarcoplasmic reticulum in the myorelaxant activity of nitric oxide donors in guinea pig gastric fundus

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Abstract

The relaxant effect of two nitric oxide (NO) donors: sodium nitroprusside and 3-morpholino-sydnonimine (SIN-1) on circular smooth muscle strips isolated from guinea pig gastric fundus was studied with the view to elucidating the mechanism, which underlies the NO-induced relaxation of this tissue. Both sodium nitroprusside (10^{-9}–10^{-5} M) and SIN-1 (10^{-9}–10^{-4} M) suppressed the spontaneous fundus tone and hyperpolarized the muscle cells by about 5 mV. They antagonized the acetylcholine (10^{-6} M)-induced tone and exerted their relaxant effects even when Ca^{2+} influx into the cells was triggered through the Na^{+}/Ca^{2+} exchanger. Sodium nitroprusside and SIN-1 antagonized the contraction induced by cyclopiazonic acid (10^{-5} M), a specific inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase. In the presence of high concentrations of sodium nitroprusside or SIN-1, cyclopiazonic acid (10^{-5} M) exerted only a slight if any contractile effect. After the complete relaxation induced by sodium nitroprusside or SIN-1, the K^{+}-channel blockers, tetraethylammonium, apamin and charybdotoxin, as well as the Ca^{2+} ionophore, A 23187, induced high-amplitude contractions, suggesting that the Ca^{2+} sensitivity of the contractile myofilaments was not affected. The results suggest that NO, released from NO donors increases the sarcoplasmic reticulum Ca^{2+} uptake thereby enhancing the vectorial sarcoplasmic reticulum Ca^{2+} release toward the plasmalemma to elicit membrane hyperpolarization and relaxation in guinea pig gastric fundus.

Keywords: SIN-1 (3-morpholino-sydnonimine); Sodium nitroprusside; Nitric oxide (NO); Smooth muscle; Tone; Gastric fundus

1. Introduction

The mechanism by which nitric oxide (NO) relaxes smooth muscle cells has long been a controversial topic (Sanders and Ward, 1992). NO and NO donors, such as sodium nitroprusside and 3-morpholino-syndonimine (SIN-1), are generally thought to cause smooth muscle relaxation by activation of soluble guanylyl cyclase, resulting in an increase in the intracellular content of guanosine 3′:5′-cyclic monophosphate (cyclic GMP), with the subsequent activation of cyclic GMP-dependent protein kinase, leading to relaxation (Sanders and Ward, 1992; Plane et al., 1996). The cyclic GMP-dependent relaxation is associated with a decrease of free Ca^{2+} concentration in the surroundings of the contractile apparatus (Karaki et al., 1988). It is assumed that cyclic GMP, and thus NO, increases the Ca^{2+} uptake into the intracellular Ca^{2+}-stores through activation of sarcoplasmic reticulum Ca^{2+}-ATPase (Raeymaekers et al., 1988; Cornwell et al., 1991; Gibson et al., 1994).

There is evidence that NO is the inhibitory non-adrenergic non-cholinergic neurotransmitter in the gastric fundus of guinea pig (Lefebvre et al., 1992a), pig (Lefebvre et al., 1995) and other animal species. NO, as a highly-permeable molecule, can easily diffuse into the enteric neurons, where it might influence the release of neurotransmitters or its own release intracellularly (Lefebvre et al., 1992b). It has been shown that SIN-1 and other NO donors, when applied at high concentrations, can reduce electrically induced non-adrenergic non-cholinergic relaxation at the prejunctional level in the rat gastric fundus (De Man et al., 1995). However, experiments on pig gastric fundus do not support the assumption that NO can cause prejunctional inhibition of neuronal NO synthase (Lefebvre and Vandekerckhove, 1998).

The gastric fundus smooth muscle develops and maintains a spontaneous tone, which has two components: an L-type Ca^{2+} channel antagonist-sensitive and an L-type

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Ca^{2+} channel antagonist-resistant (Boev et al., 1976; Duridanova et al., 1995). Sodium nitroprusside completely inhibits the spontaneous and acetylcholine-induced tonic and phasic contractions of the stomach (Boev et al., 1976; Petkov et al., 1994). It has been confirmed for the gastric fundus of guinea pig (Jin et al., 1993) and pig (Lefebvre et al., 1995) that the relaxation induced by NO and sodium nitroprusside is mediated via cyclic GMP and cyclic GMP-dependent protein kinase.

Our earlier data, in agreement with the 'superficial buffer barrier' hypothesis (for review, see Van Breemen et al., 1995), suggest an important role of sarcoplasmic reticulum and sarcoplasmic reticulum Ca^{2+}-ATPase in the control of gastric fundus tone (Petkov and Boev, 1996a,b). A preliminary study (Duridanova et al., 1995) has also shown that sodium nitroprusside and cyclic GMP analogues increase the Ca^{2+}-activated K^+ currents in single smooth muscle cells of guinea-pig gastric fundus and that this effect is abolished in the presence of a specific inhibitor of the sarcoplasmic reticulum Ca^{2+}-ATPase—thapsigargin.

The purpose of the present study was to clarify the mechanism by which NO, released from NO donors, could cause relaxation of the guinea pig gastric fundus. The effects of two NO donors: the organic SIN-1 and the inorganic sodium nitroprusside, on the spontaneous and the induced tone were studied. The specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase cyclopiazonic acid (Seidler et al., 1989), was used to evaluate the role of the intracellular Ca^{2+} stores in the relaxant effects of the NO donors. In this study we preferred to use cyclopiazonic acid instead of thapsigargin, because, unlike cyclopiazonic acid, thapsigargin is an inreversible inhibitor (Inesi and Sagara, 1994). Moreover, thapsigargin is light sensitive, sensitive to oxidation, adheres to the walls of the bath, and directly blocks the L-type Ca^{2+} channels (Buryi et al., 1995), while cyclopiazonic acid does not (Nelson et al., 1994).

Recent studies have shown that NO and NO donors can also cause membrane hyperpolarization in smooth muscle cells (Cayabyab and Daniel, 1995, 1996), including those of the gastric fundus (Kitamura et al., 1993; Ohno et al., 1996). This was the approach to investigate the effects of SIN-1 and sodium nitroprusside on membrane potential.

2. Materials and methods

2.1. Tissue preparation

Male adult guinea pigs weighing 250–350 g were stunned and exsanguinated. Through a midline incision in the abdomen, the entire stomach was removed and immediately placed in a modified Ca^{2+}-containing physiological Krebs solution (composition in mM: 137.5 Na^+, 5.9 K^+, 2.5 Ca^{2+}, 1.2 Mg^{2+}, 134.2 Cl^-, 15.5 HCO_3^-, 1.2 $H_2PO_4^-$, 11.5 glucose) at room temperature (21–23°C). After being opened along the longitudinal axis of the greater curvature, the stomach was pinned flat in a Petri dish with the muscle side up and stretched to its in vivo length. It was carefully scraped free of fat and connective tissue.

2.2. Organ bath experiments

Circular smooth muscle strips (2-mm wide and 10-mm long) were cut out from the fundus region of the stomach, and the mucosal layer was removed. The strips were then suspended vertically in 10-ml organ baths (two strips per bath). One end of each strip was anchored to the bottom of the bath and the other was connected to a force-displacement transducer (FT03, Grass) coupled to a pen recorder for isometric tension recording. The strips were suspended under 10-mN tension. These procedures were carried out in a Ca^{2+}-free Krebs solution, which was prepared like the Ca^{2+}-containing solution (see above) but by substituting Na^+ for Ca^{2+}. Ten minutes later the bath solution was replaced by a Ca^{2+}-containing physiological Krebs solution to initiate contractions. The temperature of the bath solutions was thermostatically controlled (37°C) and the solutions were continuously bubbled with a mixture of 95% O_2 and 5% CO_2 to achieve a pH of 7.4. There was a 90- to 120-min equilibration period. During this period the bath solution was changed every 15 min.

Concentration-response curves for the effects of nifedipine, sodium nitroprusside, SIN-1 and 8-bromo cyclyc GMP were obtained by cumulative application of the drugs.

Ca^{2+} entry into the smooth muscle cells through the Na^+/Ca^{2+} exchanger was potentiated as described elsewhere (Petkov and Boev, 1996b). In the present experiments, the Ca^{2+} influx was activated by replacing the standard 2.5 mM Ca^{2+}-containing solution by a solution in which Na^+ was replaced by Li^+ and 10 mM Ca^{2+} was added. Ouabain (10^{-5} M) was added to the solution throughout the experiments in order to inhibit the Na^+/K^+ pump thereby intensifying the activity of the Na^+/Ca^{2+} exchanger by loading the cell with Na^+. Nifedipine (3×10^{-6} M) and atropine (10^{-6} M) were also included in the solution in order to block the other pathways of Ca^{2+} entry into the cells, such as the L-type Ca^{2+} channels and the acetylcholine-induced pathway. Pre-incubation of the strips in this solution resulted in powerful tonic contraction, suggesting an extensive Ca^{2+} penetration via the Na^+/Ca^{2+} exchanger and possibly also activation of a non-selective cation channel (Petkov et al., 1996).

To affect the buffering capacity of intracellular Ca^{2+} stores, either of the selective sarcoplasmic reticulum Ca^{2+}-ATPase inhibitor, cyclopiazonic acid (10^{-5} M) and/or the sarcoplasmic reticulum Ca^{2+} release channel activator ryanodine (3×10^{-5} M), was added to the bath solution before exposure to NO donors. The individual concentrations of cyclopiazonic acid and ryanodine used in the study were the 'standard' maximal concentrations of

these drugs used for functional studies (Lou et al., 1993; Cayabyab and Daniel, 1996). The concentration-response effects of cyclopiazonic acid and ryanodine in gastric fundus smooth muscle have been also investigated (Petkov and Boev, 1996a, 1998).

2.3. Electrical recordings

The effects of SIN-1 and sodium nitroprusside on the electrical activity of the fundus preparations was studied by the method of Woodbury and Brady (1956). The membrane potential was recorded by floating glass intracellular microelectrodes with a diameter < 1 μm at the tip, filled with 2.5 M KCl solution. An amplifier B-1623 (MIKI, Hungary) with high input impedance (10^{12} Ω) was used. The electrical responses were monitored on a storage oscilloscope (Tektronix 5111; USA).

2.4. Drugs

The drugs used were: acetylcholine, apamin, charybdotoxin, cyclopiazonic acid, 8-bromo cyclyc GMP, N^{ω}-nitro-L-arginine (L-NNA), nifedipine, sodium nitroprusside, tetraethylammonium, tetrodotoxin (Sigma); A 23187, ryanodine (Calbiochem); ouabain (Merck); SIN-1 was a generous gift from Dr. Schönafinger (Cassella). All other compounds were of analytical grade. SIN-1 and sodium nitroprusside were prepared as aqueous solutions immediately before use. Cyclopiazonic acid was dissolved in dimethylsulfoxide in a concentration of 10 mM as stock solutions. Ryanodine and nifedipine were dissolved in ethanol. L-NNA was dissolved in 65 mM HCl. Dimethylsulfoxide, HCl and ethanol in the concentrations used had no permanent effect on the contractility of the guinea pig gastric fundus smooth muscles.

2.5. Statistics

All the responses are expressed as means ± S.E.M. for *n*, the number of preparations. The data were assessed for statistical significance using Student's *t*-test at $P < 0.05$.

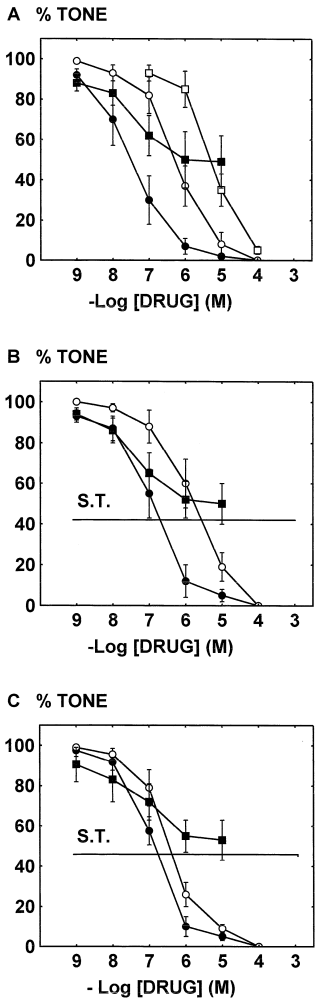

Fig. 1. Cumulative concentration-response curves for the relaxant effect of sodium nitroprusside (closed circles), SIN-1 (open circles), nifedipine (closed squares), 8-bromo cyclic GMP (open squares) on the spontaneous (A), acetylcholine (10^{-6} M)-induced (B) and cyclopiazonic acid (10^{-5} M)-induced (C) tone of guinea pig gastric fundus strips. In panel (A), the spontaneous tone was taken to be 100%. In panel (B), acetylcholine (10^{-6} M)-induced tone together with spontaneous tone was taken to be 100%. In panel (C), cyclopiazonic acid (10^{-5} M)-induced tone together with the spontaneous tone was taken to be 100%. The line indicates the level of the spontaneous tone (S.T.) in panels B and C. Values are means ± S.E.M. for $n = 6$–10.

3. Results

3.1. Effect of SIN-1 on the spontaneous tone of guinea pig gastric fundus

SIN-1 concentration dependently (10^{-9}–10^{-4} M) decreased the spontaneous tone and even completely suppressed it (Fig. 1A). SIN-1 at low concentrations (10^{-9}–10^{-7} M) caused a transient relaxation of the fundus. In the presence of higher concentrations of SIN-1 (10^{-6}–10^{-4} M), the relaxation was sustained, persisting for hours, i.e., until SIN-1 was washed out. Sodium nitroprusside proved to be a more potent myorelaxant than SIN-1, while the membrane permeable analogue of cyclic GMP, 8-bromo cyclyc GMP was less potent (Fig. 1A). Nifedipine, a typical L-type Ca^{2+} channel antagonist, applied cumulatively (10^{-9}–10^{-5} M) only partly suppressed the spontaneous tone of the fundus strips (Fig. 1A). In the presence of a supramaximal concentration of nifedipine (10^{-5} M), sodium nitroprusside (10^{-6} M) as well as SIN-1 (10^{-5} M), inhibited the rest of the tone to the baseline zero level.

We used tetrodotoxin as well as the NO synthase inhibitor—L-NNA—to study the possible involvement of nervous structures in the relaxant effect of SIN-1. The concentration-response curve for the effect of SIN-1 in the presence of 10^{-6} M tetrodotoxin did not differ signifi-

cantly from the concentration-response curve obtained under control conditions. In the presence of 10^{-4} M L-NNA, the response to SIN-1 was not significantly changed either (results not shown). All this suggests that the effect of SIN-1 is not mediated via the release of a neurotransmitter and that the effect should be considered a myogenic one.

3.2. Effect of SIN-1 and sodium nitroprusside on the membrane potential

The resting membrane potential of the smooth muscle cells from the circular layer of the guinea pig gastric fundus was about −55 mV and most of the cells were electrically quiescent. SIN-1 and sodium nitroprusside, applied at the concentration in which they completely suppressed the spontaneous tone, i.e., 10^{-4} M SIN-1 and 10^{-5} M sodium nitroprusside, hyperpolarized the membrane by about 5 mV (Table 1). The membrane potential rapidly returned to its initial levels after washout of the drugs.

3.3. Effect of SIN-1 and sodium nitroprusside on acetylcholine-induced tone

To establish whether SIN-1 and sodium nitroprusside could inhibit the contractions in response to acetylcholine which activates the Ca^{2+} influx from the extracellular space and releases Ca^{2+} from the intracellular stores in the guinea pig gastric fundus, we evaluated the effect of SIN-1 and sodium nitroprusside on acetylcholine-induced tone.

As illustrated in Fig. 1B, the acetylcholine(10^{-6} M)-induced tone was only partly reduced by nifedipine. Thus, acetylcholine-induced tonic contraction, like the spontaneous tone has two components, a nifedipine-sensitive and a nifedipine-resistant one. Unlike nifedipine, SIN-1 and sodium nitroprusside completely suppressed the contraction induced by 10^{-6} M acetylcholine and the spontaneous tone. Here again sodium nitroprusside was a more potent myorelaxant than SIN-1. (Fig. 1B). 8-bromo cyclic GMP (10^{-6}–10^{-3} M) also completely suppressed the contraction induced by 10^{-6} M acetylcholine and the spontaneous tone (not illustrated).

Table 1
Changes in the membrane potential of guinea pig gastric fundus smooth muscle induced by sodium nitroprusside and SIN-1

Control	55.8 ± 2.3 mV
Sodium nitroprusside (10^{-5} M)	61.0 ± 1.8 mV[a]
After washout	56.1 ± 2.4 mV
Control	56.1 ± 2.0 mV
SIN-1 (10^{-4} M)	61.2 ± 2.5 mV[a]
After washout	57.0 ± 2.4 mV

Values are means ± S.E.M., for $n = 10$–12.
[a] $P < 0.05$.

3.4. Effect of SIN-1 and sodium nitroprusside on the guinea pig gastric fundus contracted by the Ca^{2+} influx via the Na^{+}/Ca^{2+} exchanger

Experiments were performed to find whether SIN-1 and sodium nitroprusside can cause relaxation of fundus strips contracted by activation of a non-specific Ca^{2+} entry into the muscle cells, via a reversed mode of functioning of the Na^{+}/Ca^{2+} exchanger. This was achieved by preincubation of the muscle in a solution (described in Section 2), which resulted in the appearance of a powerful tonic contraction. Addition of SIN-1 (10^{-7}–10^{-4} M), sodium nitroprusside (10^{-8}–10^{-5} M) or 8-bromo cyclic GMP (10^{-6}–10^{-3} M), to the bath solution caused concentration-dependent relaxation of the contracted fundus strips and led to complete suppression of the induced contraction and spontaneous tone ($n = 4$–6). Obviously, SIN-1, sodium nitroprusside and 8-bromo cyclic GMP can exert their relaxant effects irrespective of the pathways of Ca^{2+} entry into the fundus smooth muscle cells.

3.5. The role of sarcoplasmic reticulum Ca^{2+} stores in the relaxant effects of SIN-1 and sodium nitroprusside

At high concentrations, SIN-1 (3×10^{-4} M; $n = 12$), sodium nitroprusside (10^{-4} M; $n = 10$) and 8-bromo cyclyc GMP (10^{-3} M; $n = 3$) completely suppressed the contraction induced by 10^{-5} M cyclopiazonic acid and the spontaneous tone. The inhibitory effects of sodium nitroprusside and SIN-1 on the cyclopiazonic acid (10^{-5} M)-induced tonic contraction were concentration-dependent (Fig. 1C). Unlike SIN-1 and sodium nitroprusside, nifedipine (10^{-9}–10^{-5} M) failed to suppress the cyclopiazonic acid-induced contraction (Fig. 1C). Thus, cyclopiazonic acid-induced tone, like the spontaneous tone, has two components: nifedipine-sensitive and nifedipine-resistant.

In another series of experiments, the fundus strips were pre-incubated with sodium nitroprusside, SIN-1 or 8-bromo cyclic GMP and then 10^{-5} M cyclopiazonic acid was added to see whether it would elicit contraction in the presence of these agents. When the drugs were applied at concentrations at which they suppressed the spontaneous tone, i.e., 10^{-4} M SIN-1 ($n = 8$), 10^{-5} M sodium nitroprusside ($n = 6$), or 10^{-4} M 8-bromo cyclyc GMP ($n = 4$), the subsequent addition of 10^{-5} M cyclopiazonic acid induced only a slight tonic contraction, but in no case did muscle tension recover to level of the spontaneous tone. In the presence of 3×10^{-4} M SIN-1 ($n = 6$) or 10^{-4} M sodium nitroprusside ($n = 6$), cyclopiazonic acid (10^{-5} M) failed to contract the muscle strips.

The combination of cyclopiazonic acid and ryanodine, an activator of sarcoplasmic reticulum Ca^{2+}-induced Ca^{2+} release, was also used to fully destroy the intracellular Ca^{2+} stores. In the presence of both cyclopiazonic acid (10^{-5} M) and ryanodine (3×10^{-5} M), SIN-1 and sodium

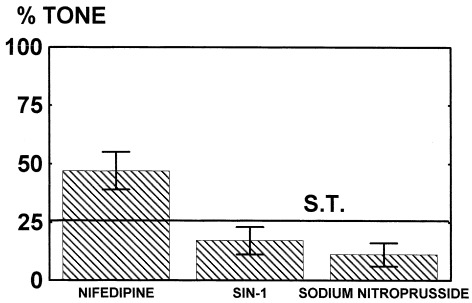

Fig. 2. The relaxant effect of nifedipine (10^{-5} M), SIN-1 (10^{-3} M) and sodium nitroprusside (10^{-4} M) on fundus strips contracted by the combination of cyclopiazonic acid (10^{-5} M) and ryanodine (3×10^{-5} M). The tone induced by the combination of cyclopiazonic acid and ryanodine together with the spontaneous tone was taken to be 100%. The line indicates the level of the spontaneous tone (S.T.). Values are means ± S.E.M. for $n = 4–6$.

nitroprusside, even at high concentrations, did not cause complete relaxation of the fundus strips ($n = 4–6$). Fig. 2 shows a comparison of the inhibitory effect of nifedipine (10^{-5} M) on the tone induced by both ryanodine (3×10^{-5} M) and cyclopiazonic acid (10^{-5} M) and the inhibitory effect of SIN-1 (10^{-3} M) and sodium nitroprusside (10^{-4} M).

3.6. Effects of the K^+ channel blockers and the calcium ionophore A 23187

In order to find whether SIN-1 and sodium nitroprusside could affect the Ca^{2+} sensitivity of the contractile myofilaments, we performed experiments with blockers of the K^+ channels, which cause membrane depolarization and indirectly activate the L-type Ca^{2+} channels thus increasing Ca^{2+} influx into the cells. To exclude any possible release of neurotransmitters, all experiments with K^+ channel blockers were performed in the presence of 10^{-6} M tetrodotoxin. We also used the Ca^{2+} ionophore, A 23187, which transferred Ca^{2+} from the extracellular space through the membrane and destroyed the intracellular Ca^{2+} stores.

After complete inhibition of the spontaneous tone by 10^{-4} M SIN-1 or 10^{-5} M sodium nitroprusside, tetraethylammonium (10^{-2} M), a typical blocker of K^+ channels, induced tonic and powerful phasic contractions with an amplitude greater than that of the spontaneous tone ($n = 8–10$; Fig. 3A). In the presence of 10^{-4} M SIN-1 or 10^{-5} M sodium nitroprusside, the selective blocker of large conductance Ca^{2+}-activated K^+ channels, charybdotoxin (10^{-6} M), slightly increased the tone and caused the appearance of phasic contractions ($n = 6$; Fig. 3B), The selective blocker of Ca^{2+}-activated K^+ channels of small conductance, apamin (10^{-6} M), led to the appearance of tonic and high-amplitude phasic contractions ($n = 4–6$). Moreover, the effects of charybdotoxin and apamin on the contractions were additive (Fig. 3B).

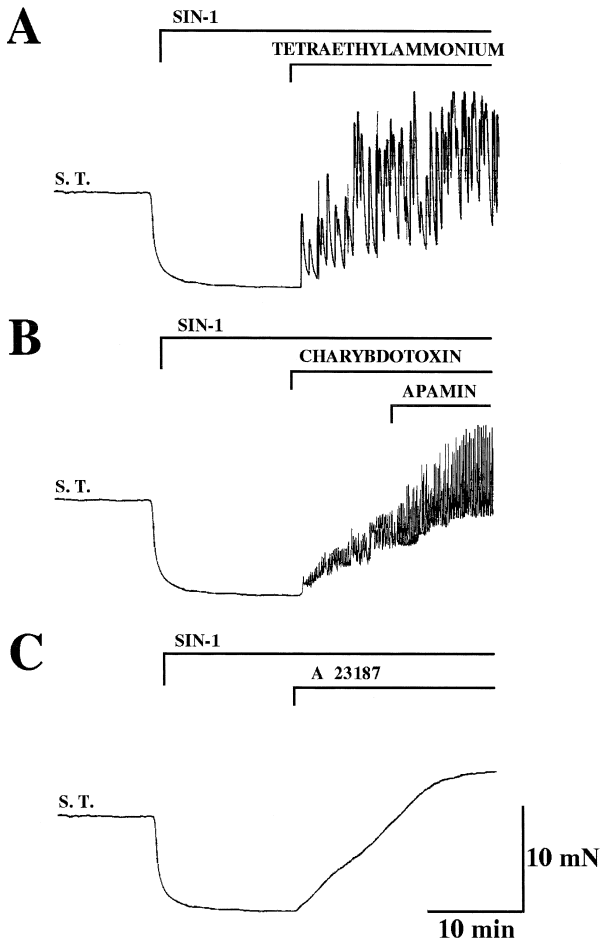

Fig. 3. Recordings of changes in spontaneous tone (S.T.) of guinea pig gastric fundus smooth muscle strips, induced by 10^{-4} M SIN-1. (A) Tetraethylammonium (10^{-2} M) caused the appearance of tonic and high-amplitude phasic contractions in the presence of SIN-1; (B) Charybdotoxin (10^{-6} M) and apamin (10^{-6} M) also caused the appearance of tonic and phasic contractions in the presence of SIN-1; (C) The calcium ionophore, A 23187 (10^{-5} M), restored the tone after SIN-1. The lines indicate the presence of the drugs throughout the experiment.

After complete inhibition of the spontaneous tone by 10^{-4} M SIN-1 ($n = 5$) or 10^{-5} M sodium nitroprusside ($n = 6$), the Ca^{2+} ionophore A 23187 (10^{-5} M) induced tonic contractions with an amplitude greater than that of the spontaneous tone (Fig. 3C).

4. Discussion

The present study showed that the NO donors SIN-1 and sodium nitroprusside as well as 8-bromo cyclic GMP completely suppressed both components of the spontaneous tone of the guinea pig gastric fundus, i.e., the L-type Ca^{2+} channel antagonist-sensitive one, which is potential-dependent and the L-type Ca^{2+} channel antagonist-resistant one, which is potential-independent (Boev et al.,

1976). The experiments also showed that SIN-1 and sodium nitroprusside, applied in the concentrations at which they completely suppressed the spontaneous tone, hyperpolarized the membrane by about only 5 mV. This is consistent with the results of Ohno et al. (1996), who found that sodium nitroprusside exerts a similar hyperpolarizing effect on the guinea pig gastric fundus. A hyperpolarizing action of 8-bromo cyclic GMP on gastric fundus smooth muscle has also been reported (Kitamura et al., 1993).

Preliminary studies (Boev, unpublished) have shown that direct electrical stimulation even by supramaximal hyperpolarizing currents, partly reduces the spontaneous tone of the gastric fundus muscle, approximately to the level reached with L-type Ca^{2+} channel antagonists. In contrast, the NO donors completely abolished the muscle tone, i.e., both the potential-dependent and the potential-independent components. Although membrane hyperpolarization is involved in the effects of NO and NO donors, which act either directly on the membrane ion channels (Bolotina et al., 1994) or indirectly on soluble guanylyl cyclase and cyclic GMP-dependent kinase (Jin et al., 1993; Murray et al., 1995; Cayabyab and Daniel, 1995; Lefebvre et al., 1995; Plane et al., 1996), it could not be the only reason for their inhibitory effects on the spontaneous tone.

Sodium nitroprusside does not affect Ca^{2+} sensitivity of the contractile myofilaments in the cat stomach (Petkov et al., 1994), while in some other smooth muscles the relaxation induced by high concentrations of sodium nitroprusside is suggested to involve a direct inhibitory effect on the contractile proteins (Karaki et al., 1988; Gibson et al., 1994). It seems unlikely that SIN-1 and sodium nitroprusside decrease the Ca^{2+} sensitivity of the contractile apparatus in the guinea pig fundus, since after SIN-1 or sodium nitroprusside, the K^+ channel blockers and the Ca^{2+} ionophore, A 23187, restored the contractions to an amplitude higher than that of the spontaneous tone.

The results obtained in this study could be interpreted in terms of the 'superficial buffer barrier' hypothesis (for review, see Van Breemen et al., 1995). According to the hypothesis, part of the Ca^{2+} entering the smooth muscle cell through the plasma membrane is actively taken up by the sarcoplasmic reticulum Ca^{2+}-ATPase into Ca^{2+} stores before it can reach the contractile proteins and is released preferentially towards the plasmalemma. This flux of Ca^{2+} from the sarcoplasmic reticulum to the plasma membrane has been termed 'vectorial Ca^{2+} release'. The latter provides a negative feedback regulation of plasma membrane excitability through activation of Ca^{2+}-sensitive K^+ channels, which leads to membrane hyperpolarization.

In earlier experiments with the patch–clamp method, we found that sodium nitroprusside and cyclic GMP analogues increase membrane K^+ currents in single smooth muscle cells of the guinea pig gastric fundus (Duridanova et al., 1995). This effect depends on the Ca^{2+} buffering capacity of the pipette solutions and could be effectively antagonized by thapsigargin, which causes irreversible inhibition of the sarcoplasmic reticulum Ca^{2+}-ATPase (Inesi and Sagara, 1994). Other authors have also demonstrated that NO donors activate Ca^{2+}-activated K^+ channels (Cayabyab and Daniel, 1995; Murray et al., 1995; Jury et al., 1996; Plane et al., 1996). This effect is prevented by inhibition of cyclic GMP-dependent protein kinase (Murray et al., 1995), activated by cyclic GMP analogues (Duridanova et al., 1995) and decreased by sarcoplasmic reticulum Ca^{2+}-ATPase inhibitors (Jury et al., 1996). These data support the assumption that the membrane hyperpolarization induced by NO and NO donors requires functional sarcoplasmic reticulum Ca^{2+}-ATPase and involves cyclic GMP-dependent protein kinase.

It is suggested that NO and NO donors exert their relaxant effects on smooth muscles by altering the intracellular Ca^{2+} homeostasis (Gibson et al., 1994; Petkov et al., 1994; Duridanova et al., 1995; Murray et al., 1995; Wayman et al., 1996). We observed that, in the guinea pig gastric fundus, sodium nitroprusside and SIN-1 caused relaxation irrespective of the pathway by which the intracellular Ca^{2+} concentration was increased. Unlike nifedipine, SIN-1 and sodium nitroprusside completely suppressed the acetylcholine-induced tone.

The present study also showed that the relaxation induced by NO donors depended upon the status of internal Ca^{2+} stores. When both sarcoplasmic reticulum Ca^{2+} uptake and Ca^{2+} release were blocked by cyclopiazonic acid and ryanodine, SIN-1 and sodium nitroprusside even at high concentrations, failed to cause complete relaxation. Sodium nitroprusside and SIN-1 antagonized the contractile effect of the inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase—cyclopiazonic acid but there was a shift of the concentration-response curves to the right. In the presence of high concentrations of sodium nitroprusside, SIN-1 or 8-bromo cyclic GMP, cyclopiazonic acid failed to contract the fundus smooth muscle. Other authors presented evidence that, in the rabbit aorta, blockade of Ca^{2+}-ATPase by cyclopiazonic acid inhibited the cyclic GMP-dependent relaxation induced by NO-releasing nitroglycerine (Lou et al., 1993). The question arises as to whether NO released from NO donors could overcome the action of cyclopiazonic acid and allow the Ca^{2+} stores to refill.

One of the substrates of the cyclic GMP-dependent protein kinase is phospholamban, a small regulatory protein associated with sarcoplasmic reticulum Ca^{2+}-ATPase. This protein has been identified in sarcoplasmic reticulum membranes from gastric muscles (Raeymaekers and Jones, 1986) and its phosphorylation has been found to stimulate the sarcoplasmic reticulum Ca^{2+}-ATPase activity in smooth muscle (Raeymaekers et al., 1988; Cornwell et al., 1991). Phosphorylation of phospholamban could underlie the ability of the NO donors to stimulate Ca^{2+} uptake into sarcoplasmic reticulum (Cornwell et al., 1991; Gibson et al., 1994; Wayman et al., 1996). However, whether or not the NO donors are able to overcome the action of cyclopiazonic acid remains to be clarified.

In conclusion, these results support the hypothesis that, in guinea pig gastric fundus smooth muscle, the relaxation induced by NO is due to an increase of Ca^{2+} accumulation in the sarcoplasmic reticulum, which in turn leads to activation of the vectorial Ca^{2+} release from the sarcoplasmic reticulum toward the plasmalemma. Thus, decreased intracellular free calcium concentration is coupled with elevation of subplasmalemmal calcium, which in turn causes cell membrane hyperpolarization. Acting together both processes may provide for the sustained relaxation induced by NO donors in the guinea pig gastric fundus.

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