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Mechanism of nitric oxide-induced contraction in the rat isolated small intestine

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1 The contractile response to nitric oxide (NO) in ral ileal myenteric plexus-longitudinal muscle strips was pharmacologically analysed.

2 NO (10^{-7} M) induced only contraction while 10^{-6} M NO induced contraction followed by relaxation. Methylene blue (up to 10^{-4} M) did not affect the NO-induced contractions but significantly reduced the relaxation evoked by 10^{-6} M NO. Administration of 8-bromo-cyclic GMP (10^{-6}–10^{-4} M) only induced relaxation.

3 Sodium nitroprusside (SNP; 10^{-7}–10^{-5} M) induced concentration-dependent contractions *per se*; the contractile response to NO, administered within 10 min after SNP, was concentration-dependently reduced. The guanosine 3′:5′-cyclic monophosphate (cyclic GMP) content of the tissues was not increased during contractions with 10^{-8} M NO and 10^{-6} M SNP; it was increased by a factor of 2 during contraction with 10^{-7} M NO, and by a factor of 12 during relaxation with 3×10^{-6} M NO.

4 The NO-induced contractions were not affected by ryanodine (3×10^{-5} M) but were concentration-dependently reduced by nifedipine (10^{-8}–10^{-7} M) and apamin (3×10^{-9}–3×10^{-8} M).

5 These results suggest that cyclic GMP is not involved in the NO-induced contraction in the rat small intestine. The NO-induced contraction is related to extracellular Ca^{2+} influx through L-type Ca^{2+} channels, that might be activated in response to the closure of Ca^{2+}-dependent K^+ channels.

Keywords: Small intestine; nitric oxide (NO); Ca^{2+} channels; K^+ channels

Introduction

Nitric oxide (NO), derived from L-arginine by NO synthase (NOS), is now generally accepted as a non-adrenergic non-cholinergic (NANC) neurotransmitter (Rand & Li, 1995); in the gastrointestinal tract, it is involved in NANC smooth muscle relaxation, as it can be induced *in vitro* by electrical field stimulation in NANC conditions (Sanders & Ward, 1992; Stark & Szurszewski, 1992). However, in the rat small intestine, electrical field stimulation induced a primary NANC contraction, inhibited by the NOS inhibitor N^G-nitro-L-arginine (Barthó *et al*., 1992) and authentic NO induced contractions mimicked the electrically-induced nitrergic contractions, the optimum concentration for contractions being some 30 times lower than that for relaxation (Barthó & Lefebvre, 1994). A primary contraction to NO was also observed in the opossum oesophageal longitudinal muscle (Saha *et al*., 1993). Furthermore, NOS inhibition was shown to reduce the contraction in response to electrical field stimulation in the rat vas deferens (Vladimirova *et al*., 1994), the 'post-stimulus' or 'rebound' contraction in different tissues such as the opossum oesophagus, canine colon and sheep bladder neck (Thornbury *et al*., 1992; Ward *et al*., 1992; Yamato *et al*., 1992) and the contraction induced by [Met^5]-enkephalin in rat duodenum (Irie *et al*., 1994). These results illustrate that NO can also be involved in the induction of smooth muscle contraction.

The smooth muscle relaxing effects of NO usually involve activation of soluble guanylate cyclase and the production of guanosine 3′:5′-cyclic monophosphate (cyclic GMP) (Schmidt *et al*., 1993). The primary contraction to NO, observed in the opossum oesophageal longitudinal muscle, seems also related to the generation of cyclic GMP as it is inhibited by the guanylate cyclase inhibitor methylene blue and mimicked by the membrane permeable analogue 8-bromo-cyclic GMP (Saha *et al*., 1993). In contrast, the nitrergic rebound contraction in the sheep bladder neck seems not to be related to cyclic GMP but is strongly reduced by ryanodine, an inhibitor of a class of intracellular calcium channels (Thornbury *et al*., 1995).

The aim of the present study in the rat small intestine was (1) to investigate whether cyclic GMP is involved in the NO-induced contraction and (2) to study the influence of substances interfering with the cytosolic calcium concentration. Preliminary accounts of part of this work have been given (Lefebvre *et al*., 1994; Barthó & Lefebvre, 1995).

Methods

General

Male Wistar rats (350–420 g) were killed by a blow on the head and bleeding. After laparotomy, a segment of the distal part of the ileum (the terminal 10 cm was discarded) was removed and 4 longitudinal muscle-myenteric plexus preparations were prepared as described under Paton & Vizi (1969) for the guinea-pig ileum. The strips were mounted under a load of 0.3 g in 20 ml organ baths, containing Krebs solution (composition in mM: NaCl 118.5, KCl 4.8, $CaCl_2$ 1.9, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 10.1), held at 37°C and gassed with 95% O_2/5% CO_2. Changes in length were recorded by means of isotonic transducers (Palmer Bioscience, model T3 or Hugo Sachs B type 368) on Kipp & Zonen recorders (type BD112) or on a Graphtec linearcorder WR 3500. During the equilibration period of 30–80 min, the preparations usually tended to contract in the first 20–30 min and then gradually relaxed to an intermediate tone.

Functional experiments

Responses to 10^{-7} or 10^{-6} M NO were studied in the absence and presence of 3×10^{-5} or 10^{-4} M methylene blue, 10^{-7}, 10^{-6} or 10^{-5} M sodium nitroprusside (SNP), 3×10^{-5} M ryanodine, 10^{-8} or 10^{-7} M nifedipine and 3×10^{-9} or 3×10^{-8} M apamin.

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In parallel control tissues, the responses to NO were repeated without administration of an interfering substance in between. Only one concentration of one interfering substance was studied per tissue, except for nifedipine where the responses to 10^{-7} and 10^{-6} M NO were studied in the presence of 10^{-8} and 10^{-7} M nifedipine. In another set of tissues, the effect of nifedipine was also tested on contractions induced by 2×10^{-2} M KCl or $5\times10^{-7}-1.5\times10^{-6}$ M methacholine. The effect of 8-bromo-cyclic GMP on the tone of the tissues was also investigated, each concentration being tested in different tissues. From any given base line, contractions and relaxations were expressed as percentage of a maximal contraction and relaxation, obtained at the end of the experiment, by administration of 8×10^{-2} M KCl and 10^{-6} M isoprenaline, respectively, except for the experiments with nifedipine (see Results).

Assay of cyclic nucleotides

The strips were mounted in an isotonic setup with 5 ml baths. After an equilibration period of at least 60 min, contractions were induced by the administration of 10^{-8} or 10^{-7} M NO, or 10^{-6} M sodium nitroprusside, relaxations by the administration of 3×10^{-6} M NO. When the maximal contraction or relaxation, respectively, was reached, the tissue was quickly raised out of the organ bath and immediately clamped between 2 liquid nitrogen cooled plates; some tissues were clamped in basal condition (control). The tissue was homogenized, first with a membrane dismembrator (B. Braun Melsungen, 100%) for 45 s, and then with an ultrasonic probe (B. Braun Melsungen) for 4 times 5 s in 6% trichloroacetic acid on ice. The homogenate was centrifuged for 20 min at 2600 *g* and the trichloroacetic acid was extracted 4 times from the supernatant with 5 volumes of water-saturated ether. The samples were then stored at -70°C until assay. The cyclic AMP content was measured with a binding assay based on the method of Tovey *et al.* (1974) and the cyclic GMP content was determined in a radioimmunoassay. The protein content was determined on the pellet by the method of Lowry *et al.* (1951), with bovine serum albumin as standard.

Data analysis

Data are given as mean $\pm$s.e.mean values, *n* referring to tissues obtained from different animals unless otherwise indicated. Comparisons between the responses in the absence and in the presence of inhibitors in the same tissues were done with the Wilcoxon signed-ranks test for paired observations. Responses in parallel tissues (cyclic nucleotide assay) were compared with the Mann-Whitney two-sample rank test for unpaired observations. Differences were considered significant when $P\leqslant0.05$.

Substances used

Apamin (Sigma, St Louis, U.S.A.), 8-bromoguanosine 3′:5′-cyclic monophosphate (8-bromo-cyclic GMP; Sigma), methacholine (O-acetyl-β-methylcholinechloride; Schuchardt, München, Germany), methylene blue (Sigma), nifedipine (Sigma), ryanodine (Alomone Labs, Jerusalem, Israel), sodium nitroprusside (Sigma). Commercially available ampoules of isoprenaline hydrochloride (Sanofi Winthrop, Brussels, Belgium) were used. The cyclic AMP^{3}H-assay system and the cyclic GMP^{125}I-RIA kit were bought from Amersham (Buckinghamshire, U.K.) and DuPont Canada (Ontario, Canada), respectively. Drugs were dissolved in physiological salt solution. A saturated NO solution was prepared as described by Kelm & Schrader (1990), by bubbling argon gas and then NO gas through 3 consecutive in-line connected gas-tight vials, the first 2 containing KOH solutions, the latter deionized water. The concentration of NO in the saturated solution in vial 3 was taken as 2×10^{-3} M. If required, a dilution of this solution was obtained by injecting an aliquot with a Hamilton syringe in a gas-tight vial containing H_2O degassed with argon as described above.

Results

Functional data

NO induced contraction at 10^{-7} M and a biphasic response, contraction followed by relaxation, at 10^{-6} M (Figure 1). These responses were reproducible although the contractile responses tended to decrease slightly. The contractile responses upon a first and second administration were $18.5\pm1.6\%$ and $17.3\pm1.3\%$ ($n=12$ from 7 animals) for 10^{-7} M NO, and $22.9\pm1.5\%$ and $20.7\pm1.2\%$ ($n=18$, $P<0.05$) for 10^{-6} M NO. The relaxant response to 10^{-6} M NO was well maintained ($41.6\pm2.7\%$ and $41.9\pm2.6\%$; $n=17$).

Methylene blue (contact time 10–15 min) induced a slow and moderate contraction, that faded away within 10 min ($11.0\pm2.2\%$ for 3×10^{-5} M; $n=8$ and $9.4\pm2.0\%$ for 10^{-4} M; $n=8$ from 5 animals). Methylene blue (3×10^{-5} M) was ineffective on either the contractile (Table 1) or relaxant action of NO. Methylene blue (10^{-4} M) significantly reduced the relaxant response to 10^{-6} M NO from $46.0\pm7.2\%$ to $26.3\pm6.9\%$ ($n=8$ from 5 animals, $P<0.01$), but it hardly influenced the contractile responses to NO (Figure 1, Table 1). The decrease of the NO (10^{-6} M)-induced contraction by 10^{-4} M methylene blue (from 24.3 ± 2.5 to $20.0\pm1.9\%$) was significant but was comparable to the spontaneous decline of the contraction by 10^{-6} M NO. SNP induced a short-lasting contraction *per se* ($8.6\pm1.8\%$ during 16 ± 6 s for 10^{-7} M, $16.6\pm4.0\%$ during

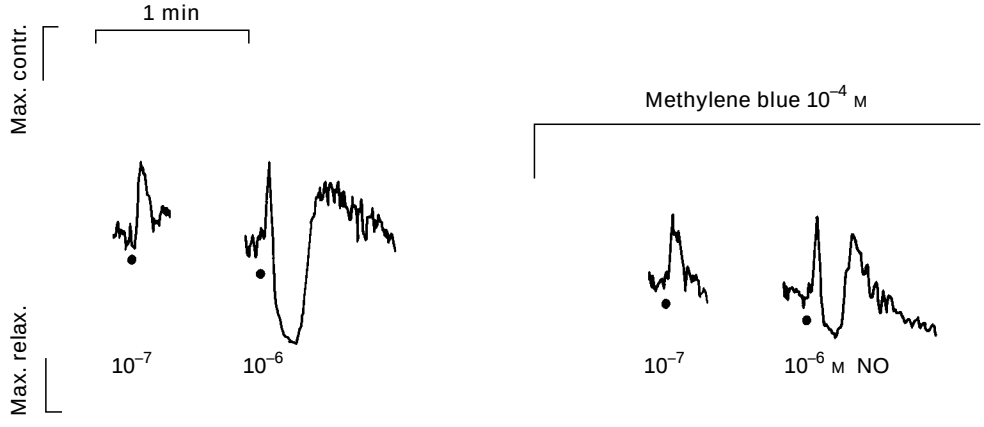

Figure 1 Responses to 10^{-7} and 10^{-6} M NO before and in the presence of 10^{-4} M methylene blue. Maximal contraction and relaxation were determined by administration of 8×10^{-2} M KCl and 10^{-6} M isoprenaline at the end of the experiment.

26 ± 9 s for 10^{-6} M and $19.3 \pm 3.0\%$ during 16 ± 6 s for 10^{-5} M SNP; $n = 7$ from 5 animals). When tone had returned to base line, no relaxant effect of SNP was observed but when NO was administered within 10 min after SNP, the contractile responses to NO were concentration-dependently reduced, being abolished after 10^{-5} M SNP (Figure 2, Table 1). In contrast, the relaxant response to 10^{-6} M NO was not affected by prior administration of SNP and in a few tissues, a relaxant response (varying from 8 to 44%) to 10^{-7} M NO occurred (in 1 out of 6 after 10^{-7} M, in 2 out of 6 after 10^{-6} M and in 2 out of 5 after 10^{-5} M SNP). Even when tone was increased to 50–75% of the KCl-induced reference contraction by use of methacholine, a small relaxant effect was observed in only 2 out of 5 preparations with 10^{-5} M SNP. 8-Bromo-cyclic GMP had no effect on the tone of tissues at 10^{-6} M ($n = 3$), while it induced slowly developing concentration-dependent relaxations at higher concentrations (10^{-5} M: $37.8 \pm 7.9\%$; 10^{-4} M: $73.8 \pm 7.1\%$; $n = 6$ from 4 animals).

Ryanodine (3×10^{-5} M) induced a pronounced contraction ($45.4 \pm 3.1\%$; $n = 7$), that developed slowly and disappeared at a still slower rate. When tone had returned to the original level (taking from 20 to 50 min), another 10 min was allowed before NO was readministered. The NO-induced responses were not affected by the prior administration of ryanodine (Table 1). Nifedipine (10^{-8} and 10^{-7} M) was added cumulatively within the same tissues and the contact time was at least 10 min for

Table 1 Effect of methylene blue, SNP, ryanodine, nifedipine and apamin on the contractions to NO

	NO 10^{-7} M		*NO* 10^{-6} M	
	n	*Contraction* (%)	n	*Contraction* (%)
Predrug	5	15.9 ± 1.1	8	20.8 ± 1.4
Methylene blue (3×10^{-5} M)	5	17.0 ± 1.8	8	17.8 ± 1.4
Predrug	6 (4)	20.9 ± 2.8	8 (5)	24.3 ± 2.5
Methylene blue (10^{-4} M)	6 (4)	16.0 ± 3.2	8 (5)	20.0 ± 1.9*
Predrug	6 (4)	18.0 ± 2.1	7 (5)	16.4 ± 1.8
SNP (10^{-7} M)	6 (4)	8.8 ± 2.5*	7 (5)	6.2 ± 1.5*
Predrug	6 (4)	18.3 ± 3.0	7 (5)	21.1 ± 2.4
SNP (10^{-6} M)	6 (4)	2.3 ± 1.2*	7 (5)	1.5 ± 1.0*
Predrug	5 (4)	18.2 ± 2.8	7 (5)	19.4 ± 2.0
SNP (10^{-5} M)	5 (4)	0*	7 (5)	1.0 ± 1.0*
Predrug	6	23.6 ± 2.9	6	24.9 ± 2.9
Ryanodine (3×10^{-5} M)	6	23.8 ± 4.9	6	20.4 ± 3.5
Predrug	7	21.4 ± 2.6	7	18.3 ± 1.7
Nifedipine (10^{-8} M)	6	6.3 ± 0.9*	6	5.9 ± 1.1*
Nifedipine (10^{-7} M)	6	0*	6	0*
Predrug	6 (5)	17.2 ± 1.8	7 (5)	22.2 ± 2.3
Apamin (3×10^{-9} M)	6 (5)	1.9 ± 1.9*	7 (5)	3.2 ± 1.4*
Predrug	7 (5)	23.6 ± 1.8	8 (6)	26.3 ± 3.3
Apamin (3×10^{-8} M)	7 (5)	0*	8 (6)	0**

Values are means $\pm$ s.e.mean. $*P < 0.05$, $**P < 0.01$: significantly different from the predrug value (Wilcoxon signed-ranks test). The figures in parentheses after the n values give the number of animals if smaller than the number of tissues.

Table 2 Cyclic nucleotide content (pmol mg^{-1} protein) in control conditions and after administration of NO or SNP

Stimulus	n	*Cyclic GMP*	n	*Cyclic AMP*
Control	12 (9)	0.295 ± 0.020	11 (9)	6.09 ± 0.74
NO (10^{-8} M, contraction)	6 (5)	0.409 ± 0.062	4 (4)	4.57 ± 1.25
NO (10^{-7} M, contraction)	6 (5)	0.577 ± 0.137*	5 (4)	4.98 ± 0.97
NO (3×10^{-6} M, relaxation)	8 (5)	3.570 ± 0.400**	7 (5)	4.47 ± 0.39
SNP (10^{-6} M, contraction)	6 (5)	0.374 ± 0.036	6 (5)	5.70 ± 0.89

Values are means $\pm$ s.e.mean. $*P < 0.05$, $**P < 0.01$: significantly different from the control value (Mann-Whitney two-sample rank test). The figures in parentheses after the n values give the number of animals.

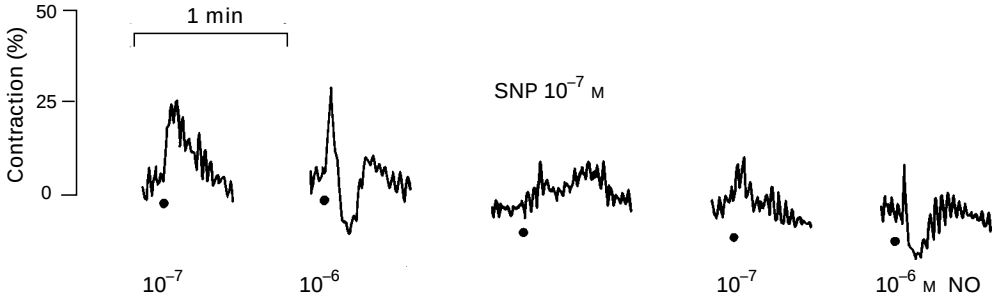

Figure 2 Responses to 10^{-7} and 10^{-6} M NO before and after administration of 10^{-7} M sodium nitroprusside (SNP). Contraction as % of the KCl (8×10^{-2} M)-induced contraction at the end of the experiment.

each concentration. Nifedipine concentration-dependently reduced the basal tone of the tissues (Figure 3) and in the presence of 10^{-7} M nifedipine, the preparations were as relaxed as with 10^{-6} M isoprenaline (as tested in preliminary experiments). Even after nifedipine had been rinsed out and 30–40 min had elapsed, no full contractile response to KCl could be obtained. In the experiments with nifedipine, responses were therefore expressed as percentage of a maximal contraction obtained with 5×10^{-5} M methacholine at the end of the experiment, 30–40 min after nifedipine had been removed from the organ bath by rinsing (in preliminary tests, this concentration of methacholine induced contractions comparable to those evoked with 8×10^{-2} M KCl). Nifedipine concentration-dependently reduced the contractions induced by 10^{-7} and 10^{-6} M NO (Figure 3, Table 1). Relaxations to 10^{-6} M NO were only occasionally observed in the presence of 10^{-8} M nifedipine and were never seen in the presence of 10^{-7} M nifedipine, which is most probably due to the relaxed state of the tissues. The contractions induced by 2×10^{-2} M KCl were affected by nifedipine in a similar way as those induced by NO ($43.0\pm7.5\%$, $12.5\pm3.7\%$ and 0% before and in the presence of 10^{-8} and 10^{-7} M nifedipine, respectively; $n=5$, $P<0.05$), while the contractions induced by methacholine were less sensitive to nifedipine, although still being significantly reduced (5×10^{-7} M methacholine: from $64.5\pm8.9\%$ to $48.2\pm8.2\%$ and $14.4\pm3.9\%$; 1.5×10^{-6} M methacholine: from $88.3\pm2.1\%$ to $70.4\pm10.2\%$ and $52.8\pm10.9\%$; $n=5$, $P<0.05$). Especially in the presence of 10^{-7} M nifedipine, the contractions to methacholine (normally reaching maximum within 30 s) developed at a slower speed (maximum reached after more than 1 min). After nifedipine had been rinsed out and 30–40 min had elapsed, responses to KCl were still suppressed while responses to methacholine were fully restored.

Apamin (contact time 10–15 min) induced a slow contraction, reaching maximum within 2–4 min and fading away within the contact time ($5.7\pm0.8\%$ contraction for 3×10^{-9} M; $n=7$ and $21.0\pm3.2\%$ for 3×10^{-8} M; $n=8$). Apamin strongly reduced the primary contractions to NO at a concentration of

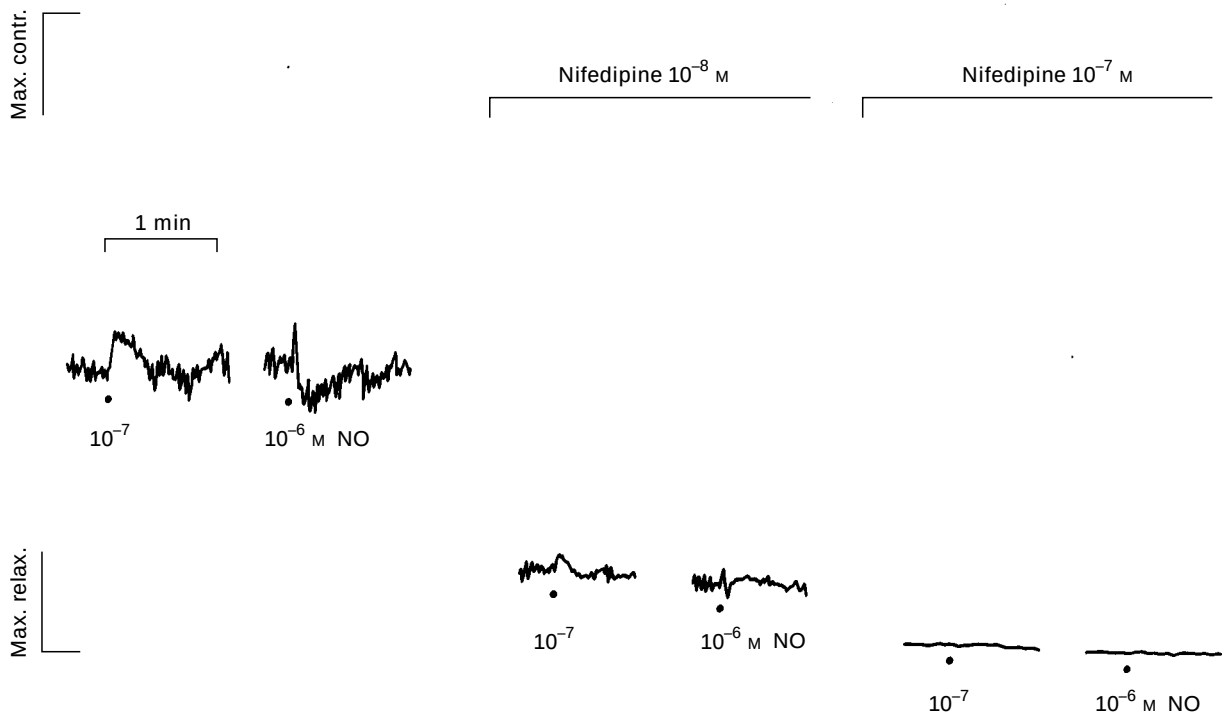

Figure 3 Responses to 10^{-7} and 10^{-6} M NO before and in the presence of 10^{-8} M and 10^{-7} M nifedipine. Maximal contraction and relaxation were determined by administration of 5×10^{-5} M methacholine and 10^{-6} M isoprenaline at the end of the experiment.

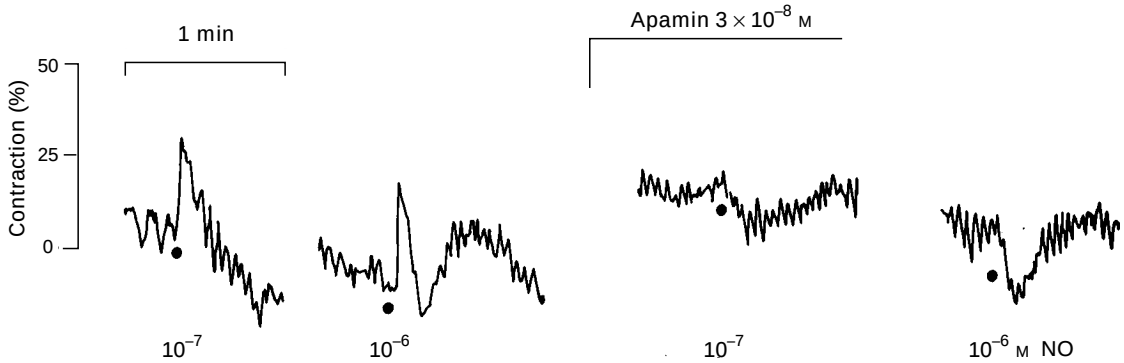

Figure 4 Responses to 10^{-7} and 10^{-6} M NO before and in the presence of 3×10^{-8} M apamin. Contraction as % of the KCl (8×10^{-2} M)-induced contraction at the end of the experiment.

3×10^{-9} M and abolished them at a concentration of 3×10^{-8} M (Figure 4, Table 1). In 1 tissue out of 7, a relaxation (12.5%) to 10^{-7} M NO occurred in the presence of 3×10^{-8} M apamin. The latter concentration significantly reduced the relaxant response in the 6 tissues out of 8, that showed a relaxation to 10^{-6} M NO (from $42.2 \pm 8.4\%$ to $31.2 \pm 6.7\%$; $P < 0.05$); 2 tissues did not relax in response to 10^{-6} M NO before the administration of 3×10^{-8} M apamin, but did so in its presence (15 and 25%).

Cyclic nucleotide responses

The cyclic GMP content of the tissues in control conditions was 0.295 ± 0.020 pmol mg^{-1} protein (Table 2). After the tissues were clamped when the contraction by 10^{-8} M NO or 10^{-6} M SNP reached its maximum, the cyclic GMP content was not significantly changed. The contraction by 10^{-7} M NO was accompanied by a significant (about two fold) increase of the cyclic GMP content; the cyclic GMP content during relaxation with 3×10^{-6} M NO was increased by a factor of 12. The cyclic AMP content was not affected by any of the stimuli.

Discussion

The aim of this study was to investigate the mechanism of the NO-induced primary contraction in the rat small intestine. Our data do not provide evidence for an involvement of cyclic GMP in the NO-induced contraction, in contrast to what has been found for the opossum oesophageal longitudinal muscle (Saha *et al*., 1993), for the following reasons: (1) The membrane permeable analogue of cyclic GMP, 8-bromo-cyclic GMP, only induced relaxation, in concentrations that are also required to obtain relaxation in other gastrointestinal preparations such as the opossum internal anal sphincter (Moummi & Rattan, 1988) and the rat gastric fundus (Smits & Lefebvre, 1995). (2) Methylene blue did not affect NO-induced contractions but significantly reduced the NO-induced relaxation, corresponding to its inhibitory effect on relaxation induced by NO and NO-donors in vascular smooth muscle preparations (Gruetter *et al*., 1981; Martin *et al*., 1985); this effect is ascribed to inhibition of soluble guanylate cyclase (Ignarro *et al*., 1986). The concentration of methylene blue required to reduce the NO-induced relaxation in the rat small intestine was higher than in vascular preparations, where 10^{-5} M has a pronounced effect (Gruetter *et al*., 1981; Martin *et al*., 1985). A similar moderate inhibitory effect of methylene blue on nitrergic relaxations has been observed in other gastrointestinal preparations (Moummi & Rattan, 1988; Rattan & Moummi, 1989; Huizinga *et al*., 1992; Lefebvre *et al*., 1995). The contractile effect of methylene blue might be related to the inhibition of basically active guanylate cyclase and the reduction of the basal level of cyclic GMP, as observed in the rabbit aorta (Martin *et al*., 1985). (3) There was no significant increase in cyclic GMP content upon contraction of the tissues with 10^{-8} M NO and 10^{-6} M SNP, while a pronounced increase was observed upon relaxation with 3×10^{-6} M NO, the optimal concentration for relaxation (Barthó & Lefebvre, 1994). The moderate though significant increase in cyclic GMP, observed with 10^{-7} M NO, might be related to a threshold stimulation of guanylate cyclase as 10^{-7} M is the threshold concentration to induce relaxation in rat small intestine, where tone is increased (Barthó & Lefebvre, 1994). Although it has been proposed that nitrergic relaxations are cyclic GMP-independent in the rat duodenum and proximal colon (Martins *et al*., 1995; Takeuchi *et al*., 1996), our results corroborate that the NO-induced relaxation in the rat small intestine is related to the activation of soluble guanylate cyclase and an increased level of cyclic GMP (Kanada *et al*., 1991; 1993).

In the sheep urinary bladder neck, the NO-mediated rebound contraction was almost abolished by ryanodine and only weakly reduced by nifedipine (Thornbury *et al*., 1995), but exactly the opposite was found for the primary contraction in the rat small intestine (present study) suggesting that the NO-mediated contraction is related to Ca^{2+} influx through L-type Ca^{2+} channels. In concentrations above 10^{-5} M, ryanodine is an inhibitor of the ryanodine receptor, one of the 2 classes of intracellular Ca^{2+} release channels (Ehrlich *et al*., 1994) and the gene for the third subtype of ryanodine receptor is also widely expressed in the gastrointestinal tract (Sorrentino & Volpe, 1993). However, the ineffectiveness of ryanodine at reducing the NO-induced contraction in the rat small intestine illustrates that it is not due to Ca^{2+} release from the ryanodine-sensitive intracellular Ca^{2+} store. In contrast to the sheep urinary bladder neck, where ryanodine had little effect on base line tension (Thornbury *et al*., 1995), it induced a slow contraction in the rat small intestine. This type of contraction has also been observed with ryanodine in other tissues (Hillyard & Procita, 1958; Cortijo *et al*., 1994); in the guinea-pig trachea, it was suggested that this contraction is due to ryanodine-induced Ca^{2+} release from the intracellular store (Cortijo *et al*., 1994). The L-channel-selective dihydropyridine calcium entry blocker nifedipine (Spedding & Paoletti, 1992) concentration-dependently reduced the basal tone of the rat small intestine, illustrating active Ca^{2+} channels in resting conditions. As has been shown before in other gastrointestinal smooth muscle preparations (Maggi *et al*., 1985; Fontaine & Lebrun, 1988), the contractile response to KCl was more sensitive to nifedipine than the contraction induced by the cholinomimetic drug methacholine. This illustrates that receptor-operated Ca^{2+} channels are less sensitive to the dihydropyridines than voltage-operated Ca^{2+} channels, as also observed in vascular smooth muscle preparations (Kondo *et al*., 1980; Godfraind, 1983). The NO-induced contractions were as sensitive to nifedipine as the KCl-induced ones, corresponding with the involvement of voltage-operated Ca^{2+} channels.

Surprisingly, the contractile effect of NO was also blocked by apamin, a blocker of small conductance Ca^{2+}-activated K^+ channels (Kolb, 1990). Indeed, in some gastrointestinal preparations, apamin-sensitive K^+ channels have been suggested to be involved in the relaxant effect of NO and also in this study, the relaxant effect of NO was reduced by apamin. The electrophysiological correlate of NO-induced relaxation is hyperpolarization, corresponding to the inhibitory junction potential observed upon electrical field stimulation of the inhibitory NANC neurones. In the opossum oesophagus, the canine pyloric sphincter and the rat proximal colon, apamin reduces the electrically-induced inhibitory junction potential and the hyperpolarization by NO or NO-donors (Bayguinov & Sanders, 1993; Cayabyab & Daniel, 1995; Serio *et al*., 1995). The proposed mechanism is that NO induces an increase in cyclic GMP, which leads to activation of apamin-sensitive K^+ channels; the efflux of K^+ leads to hyperpolarization and closure of voltage-dependent Ca^{2+} channels. However, NO is also able to open K^+ channels by a direct mechanism in vascular smooth muscle (Bolotina *et al*., 1994). Our data with apamin and nifedipine in the rat small intestine suggest that NO, in lower concentrations than required to induce relaxation, might evoke closing of apamin-sensitive K^+ channels by a mechanism not dependent on cyclic GMP, leading to depolarization and activation of voltage-dependent Ca^{2+} channels. Depolarization due to a decreased K^+ conductance has been observed with acetylcholine in smooth muscle cells of the toad and guinea-pig stomach, although no inward Ca^{2+} current was observed (Sims *et al*., 1985; Lammel *et al*., 1991). The contractile effect of apamin *per se* also suggests that closure of basically active K^+ channels can lead to contraction. Apamin-induced depolarization of the membrane potential, spike discharge and contraction have all been observed in other gastrointestinal tissues (Hills *et al*., 1983; Lim & Muir, 1986; Kamata *et al*., 1988; He & Goyal, 1993; Maggi & Giuliani, 1993). As the contraction by apamin faded away, the tissue seems to adapt to the permanent blockade of the apamin-sensitive K^+ channels. With regard to the opposing effects of

low (excitatory) and higher (inhibitory) concentrations of NO in the rat small intestine, similar observations have been made with the NO-donor 3-morpholino-sydnonimine (SIN-1) as to its effects on cardiac Ca^{2+} current (Méry *et al*., 1993) and reactive oxygen production by isolated polymorphonuclear leukocytes (Pieper *et al*., 1994).

The contractile response to NO was mimicked by SNP, as described previously (Barthó *et al*., 1992). Although the contractile response to NO was reproducible within 10 min, it was concentration-dependently reduced by prior administration of SNP and relaxation was promoted. It seems that the presence of low, sub-relaxant amounts of NO somehow desensitizes the cellular components involved in the contractile effect, but not those mediating relaxation. This could also be responsible for the quick fading of the contractile action of SNP, although this drug, unlike the administration of the NO-saturated solution, probably yields more constant NO concentrations in the organ bath.

In conclusion, NO-induced contractions in the rat small intestine are related to extracellular Ca^{2+} influx through L-type Ca^{2+} channels; apamin-sensitive K^+ channels are also involved.

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