OBJECTIVES

In view of the fact exposed in the Introduction we aimed:

1) **To characterize the responses induced by two commonly used NO donors, sodium nitroprusside (SNP) and morpholinosydnonimine hydrochloride (SIN-1), and to compare them with the responses induced by EFS, using the longitudinal smooth muscle of the rat ileum *in vitro*. Additionally, we aimed to test if an excess of NO in physiological conditions can induce changes in the contractile responses to acetylcholine, substance P or KCl.** The experiments undertaken to study these aspects are summarized in chapters 1 and 2.

2) **In view of the lack of information about the changes caused by inflammation on inhibitory responses, we aimed to study the time-course of the changes in the relaxations induced by EFS in inflamed segments of jejunum and non-inflamed worm-free segments of ileum taken from *Trichinella spiralis* infected rats.** The experiments undertaken to study these aspects are summarized in chapter 3.

3) **We also aimed to study the time-course of morphological (inflammation and hypertrophy) and functional changes in responses to electrical field stimulation, acetylcholine, substance P and KCl and its relationship with morphological changes found in *Trichinella spiralis*-infected rats. We also intended to find out if there is a correlation between structural and functional changes and to compare worm-positive inflamed (jejunum) *vs.* worm-free non-inflamed (ileum) areas of the small intestine.** The experiments undertaken to study these aspects are summarized in chapter 4.

The experiments conducted to achieve such objectives were performed in vitro. Full thickness segments were placed in organ bath and motor responses to different kind of stimuli were recorded isometrically. Classical histopathological methods (hematoxilin-eosin staining of formalin fixed paraffin embedded tissue samples) were used to monitor intestinal inflammation. Standard morphometry was used to measure the thickness of

muscle layers. NADPH diaphorase histochemistry was used to measure NOS activity. The amount of NO released by NO donors SNP and SIN-1, was estimated by detection of nitrite levels according to the Griess method.

LACK OF EFFECT OF NITRIC OXIDE ON KCl, ACETYLCHOLINE AND SUBSTANCE P INDUCED CONTRACTIONS IN ILEAL LONGITUDINAL MUSCLE OF THE RAT

Abstract

The aim of this study was to determine whether an excess of nitric oxide (NO) (mimicked by addition of NO donors) might produce by itself changes in the contractile responses to acetylcholine (ACh), substance P (SP) and KCl in the longitudinal muscle of the rat ileum. We also studied the calcium handling properties of this tissue in presence of NO donors. The NO donors assayed sodium nitroprusside (SNP) and 3-morpholinosydnonimine hydrochloride (SIN-1), induced different responses. SNP caused an immediate contraction followed by a sustained relaxation, whereas SIN-1 induced an immediate relaxation followed by a contraction. Even after prolonged incubations (up to 90 min), the NO donors SNP and SIN-1 were unable to modify the ACh- and SP-concentration-response curves, as well as the response to 30mM KCl. The nifedipine-resistant component of the ACh-induced contraction was not modified in presence of SNP. Cyclopiazonic acid (CPA) induced a contraction that was not modified when the tissue was pre-incubated with SNP. Nifedipine caused a sharp relaxation when added during the CPA-induced contraction and, when added previously, it reduced the CPA-induced contractile response. It is concluded that NO excess is not, by itself, responsible for the altered responses to KCl, ACh and SP. The contractility changes observed in the longitudinal muscle of the rat ileum during inflammation could rather be related to the presence of other inflammatory mediators.

Introduction

Nitric oxide has been shown to have a significant role as non-adrenergic non-cholinergic (NANC) transmitter in the gastrointestinal system in several species. Its participation in NANC inhibitory transmission has been documented in many studies concerning different gastrointestinal areas and species, among which the rat colon, the canine stomach, small intestine and colon, the opossum lower esophageal sphincter and the human jejunum (1-3). The so-called constitutive nitric oxide synthase is the enzyme responsible for NO

synthesis in physiological conditions and is found in enteric neurons (4). Electrophysiological studies have shown that NO is released from nerve endings and causes inhibitory junction potentials and relaxation, in some instances together with other inhibitory transmitters such as VIP, PACAP or ATP (1,2,3,5). On the other hand contractile effects of NO have also been reported (6,7,8) and they appear, in some instances, to be due to acetylcholine release (8) or calcium influx through L-type Ca^{2+} channels (9).

NO synthesis has been shown to increase in response to a wide variety of inflammatory stimuli. Such stimuli cause the expression of the inducible form of NO synthase (iNOS) (10). This highly effective calcium-independent enzyme appears to be responsible for the NO overproduction found in inflammation. In such situations, marked changes in smooth muscle contractility have been documented, and they occur regardless of the agent used to induce inflammation (10, 11).

NO has some singularities as a transmitter. Among them, the ease to cross the plasma membrane and to trigger a number of effects in the cellular machinery is perhaps the most prominent (1). The fact that in the inflammatory state there are also significant changes in functional parameters such as muscle contractility prompted several groups to hypothesize a causal link between NO overproduction and altered contractility (10, 12-15). The aim of the present study has been to assess to what extent the mere presence of NO may alter the contractile responses of the longitudinal muscle of the rat ileum to different agonists.

Methods

Tissue preparation. We used Sprague-Dawley rats (300-350 g), 8-10 weeks old, kept at a constant temperature (22-23ºC) and with lighting cycle of 12h light/12h dark. The day before the experiments, animals were fasted overnight but allowed *ad libitum* access to water. Rats were killed by decapitation. The abdomen was immediately opened and the ileum was removed and placed in previously aerated (95% O_2 / 5% CO_2) Krebs solution. Whole full thickness segments of ileum were placed in longitudinal direction in a 10ml muscle bath, filled with pre-aerated Krebs solution at 37ºC. This procedure has been approved by the Ethical Committee of the Universitat Autònoma de Barcelona. The upper end of the preparation was tied to an isometric transducer (Harvard UF-1) and preloaded

with 1-1.5g. Tissue was allowed to equilibrate for 1h until a stable baseline was attained. Data corresponding to mechanical activity were displayed and stored in a PC Pentium computer using Datawin 2 software (Panlab-Barcelona), coupled to an ISC-16 A/D card (25 samples/s). Analysis of mechanical activity was performed using this software.

Experimental procedures and data analysis. At the start of each experiment 30mM KCl was added to the bath and the contraction was considered as a reference response (RR). At the end of the experiment, the response to 30mM KCl was measured again. The amplitude of contraction was expressed as a percent of the initial KCl reference response (%RR). When the effects of drugs were to be tested they were added 10 min before agonist addition, unless indicated. The number of repetition (n) stands for the number of experiments performed with tissue samples taken from different animals. All experiments were performed in a paired way. Thus, each preparation served as its own control. To obtain concentration-response curves data were fitted to a Michaelis-Menten equation using a non-linear regression. The equation obtained was used to estimate Emax and pD_2. Statistical analysis was performed using paired Student's t test when two groups were compared, or paired ANOVA test (when more than two groups were matched) followed by Bonferroni post-hoc test. Differences were considered to be significant when $P< 0.05$. All data are expressed as mean ± standard error of the mean (SEM).

Responses to NO donors in the basal state. The response to SNP (10^{-6}-10^{-3}M), and SIN-1 (10^{-5}-10^{-4}M), was obtained under control conditions. To estimate the time-course of the response, challenges with SNP (10^{-4}-10^{-3}M) and SIN-1 (10^{-5}-10^{-4}M) were made for three times at one hour intervals. The release of NO from NO donors was measured as the amount of nitrite (by using the Griess reaction). Briefly, aliquots of incubation buffer-bubbled with carbogen and kept at 37º C and in which the NO donors had been added-were taken at different times and mixed with Griess reagent. The optical density was measured 15 min later. The same procedure was carried out with a series of standard solution of sodium nitrite to calculate the concentration of nitrite in the incubation buffer.

Effects of NO donors on the response to KCl. The response to 30mM KCl was measured in the absence and in the presence of nifedipine 10^{-6}M or TTX 10^{-6}M. The response to KCl was also measured in tissue samples pre-incubated for 10 min with calcium-free Krebs. To

estimate the influence of NO on the 30mM KCl induced contraction, the tissue was incubated for 5 min or 90 min with the NO donors, SNP (10^{-6}-10^{-3}M) and SIN-1 (10^{-5}-10^{-4}M).

Effects of NO donors on the response to ACh and SP. Cumulative concentration-response curves for ACh (10^{-11}-10^{-4}M) were firstly obtained and used as a control for the remaining experiments. This concentration-response curve was compared to those obtained in the presence of different drugs (TTX 10^{-6}M; nifedipine 10^{-6}M; a combination of nifedipine 10^{-6}M M plus cyclopiazonic acid (CPA, 10^{-5}M)) or to those obtained from tissue samples preincubated for 10 min with calcium-free Krebs. The NO donors, SNP and SIN-1, were used to study the influence of NO on the ACh concentration-response curve. After incubation for 5 or 90 min with SNP (10^{-6}-10^{-3}M) or SIN-1 (10^{-5}-10^{-4}M), the responses to ACh were measured again. The effects of SNP (10^{-5}- 10^{-4} M, 5 min) on the responses to ACh were also measured in the presence of nifedipine 10^{-6}M. The response elicited by 10^{-5}M CPA was measured under control conditions and after 5 min incubation with nifedipine (10^{-6}M) or SNP (10^{-5}-10^{-4}M). We also measured the effect of nifedipine 10^{-6}M on the CPA induced response. Concentration-response curves for SP (10^{-9}- 10^{-5}M) were obtained under control conditions and after 5 or 90 min incubation with either SNP (10^{-6} -10^{-3}M) or SIN-1 (10^{-5}-10^{-4}M). Parallel experiments were carried out to measure the effects of SP in the presence of TTX, nifedipine and atropine. Additional experiments were performed to measure the effects of SNP (10^{-3}M) and SIN-1 (10^{-4}M) on ACh and SP precontracted tissue samples.

Drugs and solutions. The following substances were purchased: acetylcholine (ACh), atropine, substance P (SP), Griess reagent and nifedipine from Sigma (St. Louis, MO); cyclopiazonic acid (CPA), sodium nitroprusside (SNP), 3-morpholinosydnonimine hydrochloride (SIN-1) and tetrodotoxin (TTX) from RBI (Natick, MA). Composition of Krebs solution was (in mM): 115.48 NaCl, 4.61 KCl, 2.5 $CaCl_2$, 1.16 $MgSO_4$, 1.14 NaH_2PO_4, 21.9 $NaHCO_3$, 10.09 glucose, pH 7.4. Composition of calcium-free Krebs was (in mM): 119.23 NaCl, 4.61 KCl, 1.16 $MgSO_4$, 1.14 NaH_2PO_4, 21.9 $NaHCO_3$, 10.09 glucose and β-aminoethylether-N,N'-tetraacetic acid (EGTA) 0.1, in order to chelate residual extracellular calcium. All substances were dissolved in distilled water to make store solutions, except in case of CPA and nifedipine. CPA was dissolved in dimethylsulphoxide (DMSO) as a 10^{-2}M

stock solution. Nifedipine was dissolved in 50% ethanol as a 10^{-2}M stock solution. SIN-1 and SNP were prepared as aqueous solutions immediately before use. The volume that added to the muscle bath never exceeded 5% of its total volume.

Results

Effect of NO donors on the mechanical activity. SNP (10^{-6}-10^{-3}M) caused a biphasic immediate response consisting of a quick concentration-dependent contraction followed by relaxation (Figure 1A and 1B). In contrast, SIN-1 (10^{-5}-10^{-4}M) elicited firstly a concentration-dependent relaxation followed by a contraction (Figure 1C and 1D). Three consecutive challenges with SNP (10^{-4}-10^{-3}M) and SIN-1 (10^{-5}-10^{-4}M), made at intervals of one hour, produced similar responses.

The release of NO from SIN-1 10^{-4}M increased until 1h after addition. In contrast, the release of NO from SNP 10^{-3}M was very quick, and occurred mostly within the first minutes. Figure 2 shows the kinetics of NO release from these compounds.

Effects of NO donors on the contractile responses. KCl 30mM induced a contraction of the longitudinal muscle (2.79±0.31g; n=8). This contraction was not modified in presence of the neural blocker TTX (10^{-6}M; n=6). Incubation of the tissue (5 minutes) with nifedipine abolished the KCl response (0.15±0.11g, $P<0.001$, n=18). In presence of calcium-free Krebs (10 minutes) KCl-induced contraction was strongly inhibited (0.21±0.03g, $P<0.001$, n=12). Incubation of the tissue for 5 or 90 min with SNP (10^{-6}-10^{-3}M, n=6) or SIN-1 (10^{-5}-10^{-4}M, n=6) did not result in measurable changes in the contraction induced by 30mM KCl.

ACh (10^{-11}-10^{-4}M) elicited a concentration-dependent contraction reaching an Emax of 148±10 %RR, pD_2 6.8±0.5 (n=8). The concentration-response curve was unchanged in presence of the neural blocker TTX 10^{-6}M (n=7). Nifedipine (10^{-6}M) caused a decrease in Emax (155±8 %RR *vs*. treated 81±10 %RR; $P<0.001$) and pD_2 (6.9 ±0.1 *vs*. treated 6.1±0.2; $P<0.007$, n=8). When the tissue was incubated with nifedipine (10^{-6}M) plus CPA (10^{-5}M; n=8), a specific inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, the response to ACh was abolished. After 10 min incubation of the tissue in calcium-free Krebs, a similar effect was observed (n=12). The incubation with the NO donors SNP (10^{-6}-10^{-3}M) or SIN-1

(10^{-5}-10^{-4}M) did not modify the ACh concentration-response curve (n=6). Table I summarizes these results. The nifedipine-resistant component of the ACh concentration-response curve was not affected by SNP (10^{-5}-10^{-4}M).

CPA 10^{-5}M induced a contraction of 103±11 %RR (n=13) (Figure 3). This contraction was not modified when the tissue was preincubated for 5 minutes with SNP (10^{-5}-10^{-4}M; n=6). Nifedipine (10^{-6}M) significantly reduced the CPA-induced contraction (40±5 %RR, P<0.002, n=13) and caused a sharp relaxation when added after CPA. DMSO, which was used as a solvent for CPA, was devoid of measurable mechanical effects.

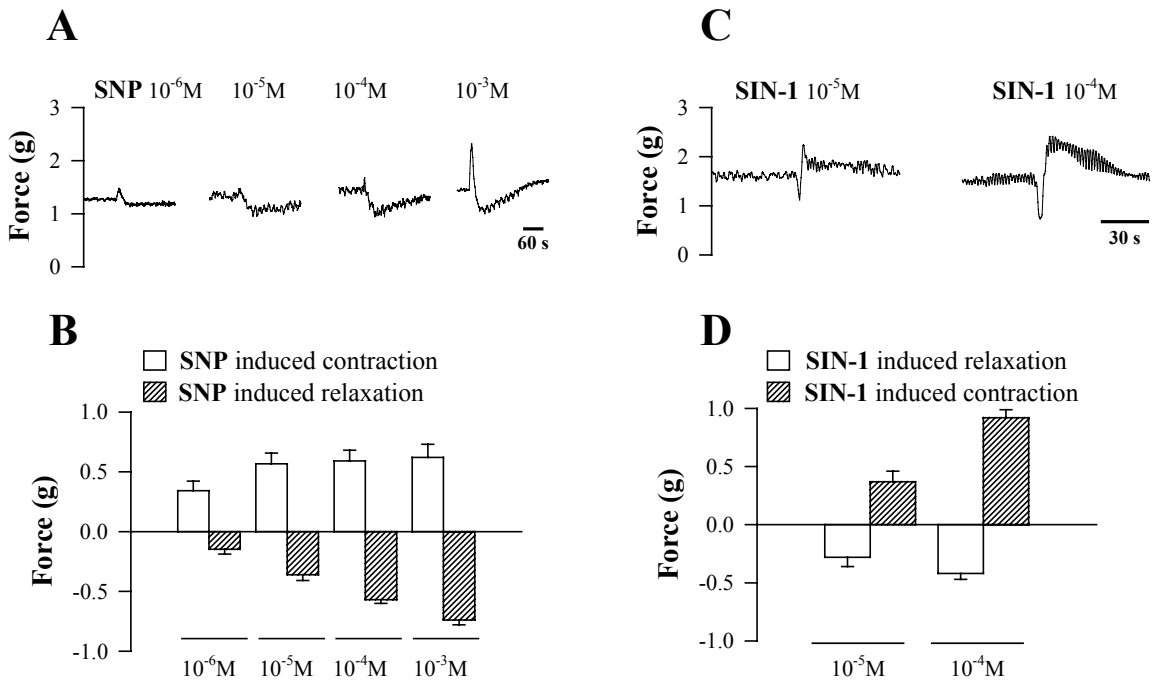

Figure 1. Effect of NO donors on the mechanical activity. Representative recordings of the responses elicited by different concentrations of SNP (A) and the corresponding mean values (B). Representative tracings of the responses elicited by different concentrations of SIN-1 (C) and corresponding mean values (D).

Substance P (10^{-9}-10^{-5}M) elicited concentration-dependent contractions reaching an Emax of 146±8 %RR and pD_2 6.7±0.1 (n=8). TTX 10^{-6}M did not cause any significant change in Emax, but the pD_2 was decreased (6.92±0.12 control *vs*. 6.11±0.2 treated, n=7, P<0.003).

Atropine caused similar effects (pD_2 values: 6.69±0.2 control *vs*. 6.33±0.2 treated, n=6, P< 0.04). Nifedipine (10^{-6}M) caused a decrease in Emax (127.9±7.8 %RR in control *vs.* 36.12± 9.4 in treated, n=6, P<0.0001) and pD_2 (6.88 ± 0.2 in control *vs*. 5.7±0.12 in treated, n=6; P<0.003). Incubation for 5 min or 90 min with SNP (10^{-6}-10^{-3}M; n=6) and SIN-1 (10^{-5}-10^{-4}M; n=6) did not modify the SP concentration-response curve (Table I).

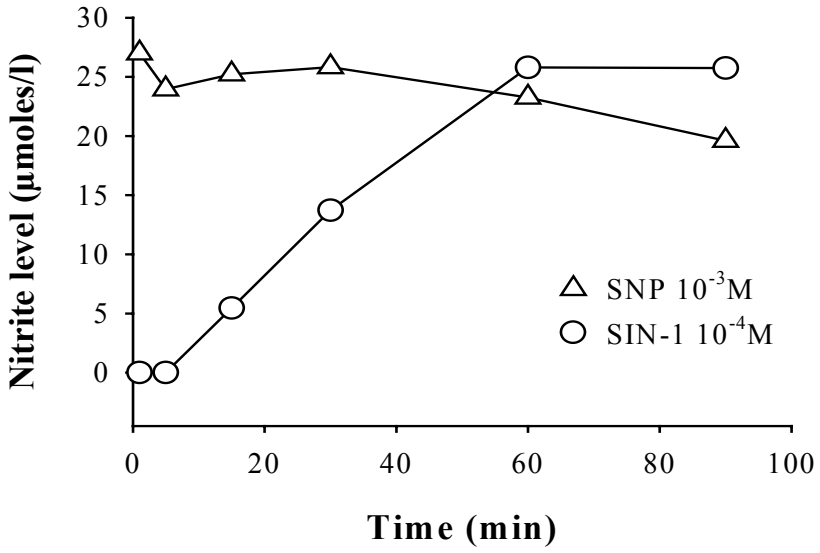

Figure 2. Time-course of NO release from the NO donors SNP and SIN-1. NO released was estimated as the nitrite produced when 10^{-3}M SNP or 10^{-4}M SIN-1 were added to Krebs solution kept at 37ºC and continuously bubbled with CO_2 5% / O_2 95%.

Effects of SNP 10^{-3}M and SIN-1 10^{-4}M on tissue samples precontracted with ACh or SP. The responses to SIN-1 and SNP in tissue precontracted with ACh or SP showed similar profiles than those obtained in control conditions, though the maximal relaxation attained was increased (SNP: P<0.03 for ACh-precontracted, P<0.001 for SP-precontracted; SIN-1: P<0.033 for ACh-precontracted and P<0.001 for SP-precontracted). Figure 4 shows these results.

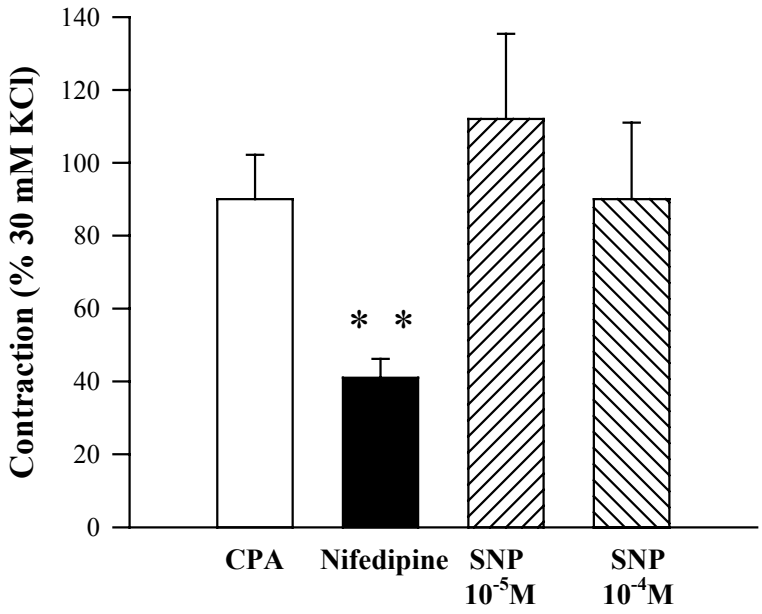

Figure 3. Mean values obtained when CPA 10^{-5}M was added in control conditions (open bar), in presence of nifedipine 10^{-6}M (solid bar) or SNP at the indicated concentration (hatched bars).

Table 1

Emax and pD_2 values corresponding to the agonists ACh and SP under control conditions and in the presence of the NO donors SNP and SIN-1 (10^{-5}-10^{-4}M). In each case the incubation time is stated. Figures represent mean ± S.E.M.

ACh		Emax (%RR)	pD_2	**SP**		Emax (%RR)	pD_2
CONTROL	0	148±10.0	6.80±0.5	CONTROL	0	130±10	6.70±0.2
+ SNP 5 min	10^{-4}M	149±10.2	6.70±0.32	+ SNP 5 min	10^{-4}M	130±7.7	6.53±0.16
	10^{-5}M	156±12.4	7.20±0.37		10^{-5}M	121±12	6.6±0.11
+ SNP 90 min	10^{-4}M	151±4.2	7.06±0.15	+ SNP 90 min	10^{-4}M	120±14	6.7±0.2
	10^{-5}M	147±14.0	7.23±0.4		10^{-5}M	140±10	6.7±0.18
+ SIN1 5 min	10^{-4}M	142±8.2	7.17±0.2	+ SIN1 5 min	10^{-4}M	118±10	6.44±0.2
	10^{-5}M	146±12.0	7.24±0.2		10^{-5}M	121±7	6.40±0.1
+ SIN1 90 min	10^{-4}M	145±7.8	7.22±0.22	+ SIN1 90 min	10^{-4}M	132±6.7	6.87±0.1
	10^{-5}M	148±3.7	7.21±0.25		10^{-5}M	137±5.1	6.91±0.1

Discussion

The primary aim of this work has been to determine whether the presence of high concentrations of NO, provided by incubation with NO donors, is able to modify the contractile responses of longitudinal ileal muscle to substances acting essentially at the muscular level. NO is usually regarded as an inhibitory neurotransmitter having a chief role in the relaxation of the circular muscle of the intestine in response to electrical field stimulation of enteric nerves. A transient relaxation is also observed when NO donors are applied to the longitudinal muscle of the rat ileum, at least when the tissue is in a precontracted state (16). However, in our study the NO donors SNP and SIN-1 caused biphasic responses. In other tissues, contractile responses to NO donors have also been reported (6, 17). Such contractile responses have been related to an effect of NO on basal ACh release from nerve endings (8, 18) or to calcium influx through L-type Ca^{2+} channels (9). The fact that the effects of NO donors are commonly tested on precontracted preparations appears as one of the causes of such differences for it has been reported that NO donors and other inhibitory mediators induce opposite effects on quiescent or precontracted tissue (7). Other groups have reported different actions of different NO donors, which have been attributed to the generation of NO derivatives such as superoxide ions or peroxynitrites (19, 20). In the present study, the sequence of events in the response to SNP and SIN-1 is similar for precontracted and control tissue.

We have previously shown that the incubation of rat colonic circular muscle with SNP results in a hyperpolarization and a cessation of the spontaneous cyclic mechanical activity of the tissue (2). Both effects appear immediately upon addition of the NO donor and last for as long time as the NO donor is kept in contact with the tissue, even when its concentration is relatively small (10^{-5}M SNP). In view of this result, we intended to study whether the presence of NO in the medium could modify the contractile properties or the responses to different agonists. For this purpose we incubated ileal muscle with two NO donors which differ in the NO release kinetics. Thus, SNP provides a high concentration of NO in a short time, whereas SIN-1 is able to release NO gradually. Thus, the use of SNP allowed us to study the influence of exposure to a high concentration of NO for a short time on the contractile responses of the tissue after 5 min or after a longer period (90 min). The use of SIN-1 allowed us to study the influence of a sustained release of NO on muscle

contractility. We did not find any substantial change in the response to KCl, ACh, and SP, in any of the experiments performed. These results are in agreement with other works where NO did not modify the response to ACh in rabbit stomach (18) or canine ileum (21), though in these studies high concentrations of NO donors were not used.

Several studies have been addressed to check if an excess of NO might modify other properties of the gastrointestinal tract such as vascular and mucosal function integrity, which could, in turn, modify the motor responses. Those results showed that high concentrations of NO do not modify the mucosal barrier and vascular integrity (22). In contrast, other groups have reported remarkable changes in contractile properties of inflamed tissues. Thus, in several model of colitis, KCl, SP and carbachol-induced contractions are markedly reduced (10, 11). In some models, such as TNB-induced colitis, high NO production has been considered as a cause of the observed reduction in KCl-induced contraction (10). Despite the fact that NO is considered one of the more prominent NANC inhibitory transmitters in the gastrointestinal tract, the effect of this easily diffusing molecule in the long run have not been clearly elucidated. During inflammation, the similarities in the time course of NO overproduction and of the functional changes in contractility were suggestive of a causal link between both events (12-15), supporting the idea that NO overproduction is one of the major events leading to functional changes. The results obtained in this study do not provide support to this view. In some of the studies in which NO overproduction is related to inflammation, homogenates of whole thickness pieces of intestinal tissue have been used (10). In such conditions, it is hazardous to assume that NO overproduction is specifically affecting the muscle and should be dependent on iNOS activity. The availability of NOS inhibitors with an increasing selectivity for iNOS will probably help ascertain this point. Our results suggest that NO does not modify motor responses, at least in the absence of an underlying inflammatory background. Such view gets further support from other works, which have shown that in the rat ileum incubation for 90 min with interleukin-1-beta inhibits ACh-induced contractions regardless of the rate of NO synthesis (23).

The amount of NO actually released by SNP and SIN-1 in the conditions under which the functional studies are performed, have been estimated by detection of nitrite levels according to the Griess method. When authentic NO is introduced in an aqueous medium

in the presence of oxygen, it is converted almost exclusively in nitrite (24). The estimated half-life of NO in such aqueous solutions averages a few seconds (25). Thus, the measure of nitrite levels may be considered a good estimate of the time course of NO release from NO donors. The levels of NO provided by incubation with the NO donors used here are about 30 μM. Such levels are attained within a very short time (in the case of SNP) or in about 1h (in the case of SIN-1). Thus, as neither of them is able to affect measurably the contractile properties of the longitudinal muscle, one should conclude that the presence of increased NO levels is not the chief causal factor to explain altered muscle function in inflammatory states. This result was actually unexpected for NO has been shown to affect cellular mechanisms potentially important for muscle contraction, such as membrane potential, guanylate cyclase activity and potassium channel function (1, 26). In fact, as stated above, we have found that the rat colonic circular muscle responds to SNP with a sustained inhibition of spontaneous cyclic contractions, even at lower concentration (10^{-5}M) (2).

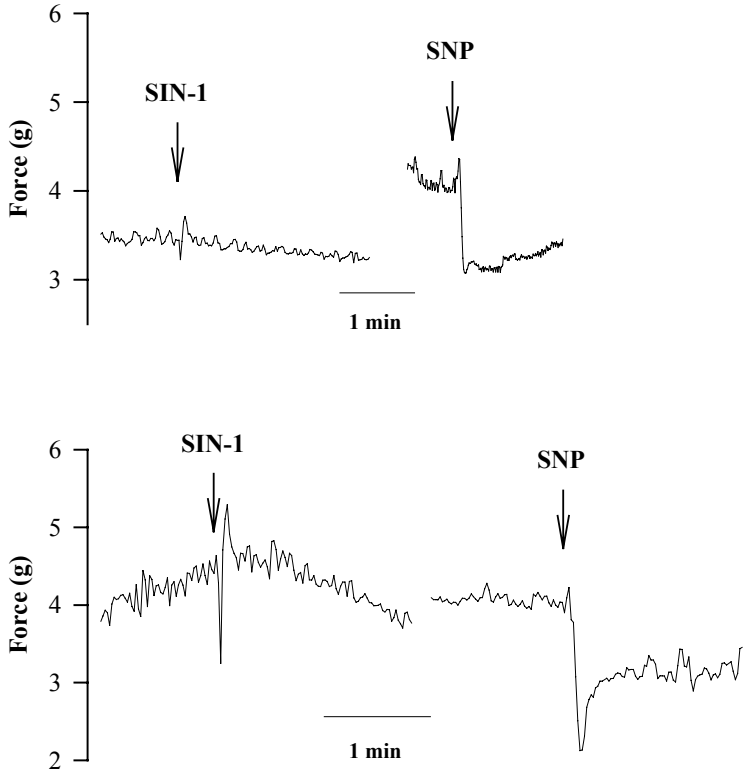

Figure 4. Response of precontracted ileal muscle to NO donors. Precontraction was achieved with ACh (top) and SP (bottom). Left figures show the effects of 10^{-4}M SIN-1, right panels show the effects of 10^{-3}M SNP addition.

It is accepted that the rise in intracellular calcium required for the contraction in response to muscarinic receptor activation is provided both by calcium influx through L-type Ca^{2+} channels and by calcium release from intracellular calcium stores (27). We have intended to estimate to what extent this contractile response is dependent on each source of calcium using nifedipine as an extracellular calcium entry blocker and CPA as blocker of the Ca^{2+}-ATPase in sarcoplasmic reticulum. In our study, the presence of nifedipine resulted in a 50% reduction of the maximal contraction in response to ACh. After incubation with CPA plus nifedipine, ACh-induced contraction was completely abolished, suggesting that intracellular calcium stores were depleted. These results provide an estimate of the relative contribution of both sources of calcium. In the present study we have no evidence that NO might modify calcium handling. This is in agreement with other studies showing that the induction of iNOS by bacterial lipopolysaccharide is unable to modify IP_3-related calcium release in ACh-induced contraction (21).

Prolonged exposure to CPA would be expected to reduce intracellular calcium stores by inhibiting calcium pumping into the sarcoplasmic reticulum. It would result in a small and sustained increase in cytosolic calcium levels, which could, in turn, elicit a sustained contraction. However, in our experiments CPA induced a strong, immediate and sustained contraction. When the tissue was preincubated with nifedipine, a slow long lasting contraction was recorded, but the immediate response did not occur and the maximal level of contraction reached was lower. This suggests that depletion of calcium stores activates L-type Ca^{2+} channels. When nifedipine was added to the bath at the top of CPA-induced contraction, a sharp relaxation immediately followed. Similar effects have been reported in the canine ileum (28) and in the cat gastric fundus (29). In the canine ileum, CPA induced spiking activity at the top of slow waves without a significant change in the membrane potential. Spikes were prevented by incubation with nifedipine (28). In the cat gastric fundus, nifedipine or calcium-free Krebs decreased CPA induced contraction (29, 30). Nifedipine also caused a sharp relaxation during the sustained contraction induced by CPA or thapsigargin. According to the so-called *capacitive calcium entry* (29, 30), depletion of calcium stores may provide a signal for activation of calcium influx through the plasma membrane. However, in rat ileal smooth muscle, emptying of intracellular calcium stores may activate calcium influx not associated to voltage-dependent calcium channels

(31). In mouse anococcygeous muscle, CPA-induced contraction may be mediated by a transient calcium-dependent chloride current, as a consequence of calcium release from intracellular stores. The chloride current depolarizes the cells, which leads to the opening of voltage-operated L-type calcium channels (32).

In conclusion, our results suggest that incubation of healthy intestinal muscle with high concentrations of NO does not reproduce the changes in contractility found in inflamed tissues. Accordingly, in the basal state, exogenously added NO does not modify the pathways involved in KCl-, ACh- and SP-induced contractions, including calcium release from intracellular stores and influx of extracellular calcium through L-type Ca^{2+} channels. Thus, NO overproduction alone does not seem to be the chief cause of altered muscle function during inflammation.

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