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Tonic inhibitory action by nitric oxide on spontaneous mechanical activity in rat proximal colon: involvement of cyclic GMP and apamin-sensitive K^+ channels

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1 The cellular mechanisms by which endogenous nitric oxide (NO) modulates spontaneous motility were investigated in rat isolated proximal colon. The mechanical activity was detected as changes in intraluminal pressure.

2 Apamin (1–100 nM) produced a concentration-dependent increase in the amplitude of the spontaneous pressure waves. The maximal contractile effect was of the same degree as that produced by N_ω-nitro-L-arginine methyl ester (L-NAME) (100 μM) and the joint application of apamin plus L-NAME had no additive effects. Apamin (0.1 μM) reduced the inhibitory effects (i.e. reduction in the amplitude of the pressure waves) induced by sodium nitroprusside (SNP) (1 nM–10 μM) or 8-Br-cyclic GMP (1–100 μM).

3 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) (0.1–5 μM), inhibitor of NO-stimulated guanylate cyclase, produced a concentration-dependent increase of the spontaneous contractions. ODQ (1 μM) in the presence of apamin (0.1 μM) did not produce any further increase in the contraction amplitude, whereas after L-NAME (100 μM) it decreased the spontaneous contractions. ODQ (1 μM) reduced the SNP inhibitory effects.

4 Zaprinast (1–50 μM), inhibitor of cyclic GMP phosphodiesterase, produced a concentration-dependent decrease of the spontaneous contractions. The effects of zaprinast were significantly reduced in the presence of apamin (0.1 μM) or L-NAME (100 μM).

5 These results suggest that small conductance Ca^{2+}-dependent K^+ channels and cyclic GMP are involved in the modulation of the spontaneous contractile activity in rat proximal colon. Cyclic GMP production system and opening of apamin-sensitive K^+ channels appear to work sequentially in transducing an endogenous NO signal.

Keywords: Neural tonic inhibition; nitric oxide; apamin-sensitive K^+ channels; cyclic GMP; ODQ; colon; gastrointestinal motility

Abbreviations: AP, apamin; 8-Br-cyclic GMP, 8-bromo-guanosine 3′,5′-cyclic monophosphate; cyclic GMP, guanosine 3′,5′-cyclic monophosphate; ISO, isoproterenol; L-NAME, N_ω-nitro-L-arginine methyl ester; NO, nitric oxide; ODQ, 1H-[1,2,4]oxadiazolo [4,3-a]quinoxalin-1-one; SNP, sodium nitroprusside; TTX, tetrodotoxin

Introduction

A growing body of evidence suggests a role for endogenous nitric oxide (NO) not only as a non adrenergic, non cholinergic (NANC) inhibitory neurotransmitter released by electrical field stimulation, but also as a tonic neural modulator of the spontaneous motility in a wide range of gastrointestinal tissues (Sanders & Ward, 1992), including rat preparations (Hata *et al*., 1990; Li & Rand 1990; Irie *et al*., 1991; Kanada *et al*., 1992; Suthamnatpong *et al*., 1993a; Postorino *et al*., 1995; Serio *et al*., 1995; Martinez-Cuesta *et al*., 1996; Mulè *et al*., 1998a). In particular, a previous study has indicated that basal neural release of NO exerts a tonic inhibitory influence on circular muscle of rat proximal colon (Mulè *et al*., 1998a).

Different transduction pathways have been proposed for inhibitory actions of NO (see Shuttleworth & Sanders, 1996 for review). One is a pathway involving a guanosine 3′,5′-cyclic monophosphate (cyclic GMP) generating system. According to this hypothesis, production of cyclic GMP, and perhaps phosphorylation of cellular proteins by cyclic GMP-dependent protein kinase, transduces the NO signal and produces relaxation of smooth muscle. Another possible mechanism concerns the enhancement by NO of the open probability of K^+ channels which mediate the hyperpolarization response to inhibitory neurotransmission. In fact, at least a portion of the relaxation induced by NO appears to be due to hyperpolarization of membrane potential or inhibition of electrical activity and subsequent reduction of Ca^{2+} influx through voltage-dependent Ca^{2+} channels. However, the activation of K^+ channels can be due to direct stimulation by NO or mediated indirectly by a cyclic GMP generating system (Bolotina *et al*.,1994; Koh *et al*., 1995). Therefore, biochemical and electrical mechanisms may coexist and together mediate the inhibitory effects of NO.

In particular, we have provided evidence that, in the circular muscle of rat proximal colon, the inhibitory junction potentials (IJPs) are largely dependent on the synthesis of NO and due to the activation of apamin-sensitive Ca^{2+}-dependent K^+ channels (Serio *et al*., 1992, 1995). In contrast, in the same preparation, NO has been reported to mediate NANC relaxation by a mechanism independent of changes in

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membrane potentials (Suthamnatpong *et al*., 1994) and of changes in cyclic GMP content (Takeuchi *et al*., 1996), although the same investigators had previously reported an association between the cyclic GMP level and the NO-induced relaxation (Suthamnatpong *et al*., 1993b; Maehara *et al*.,1994). In any case, the mechanism by which NO produces a tonic inhibition of circular muscle has not been studied in rat colon.

In this report we have investigated the mechanisms involved in the tonic inhibitory action of NO in circular muscle of rat proximal colon. So, our specific objectives were: (1) to verify a possible involvement of the small conductance Ca^{2+}-dependent K^+ channels in the modulation of the spontaneous contractile activity and in the hypermotility induced by N_ω-nitro-L-arginine methyl ester (L-NAME); (2) to determine the role of cyclic GMP in NO tonic inhibition; (3) to analyse the possible interaction among NO basal synthesis, cyclic GMP production system and opening of K^+ channels. Preliminary accounts of part of this work have been given (Mulè *et al*., 1998b).

Methods

Male Wistar rats 300–500 g were killed by cervical dislocation. A 2-cm segment of proximal colon was removed from a position just distal to the caecum and placed in Krebs solution. The contents of the excised segment were gently flushed out with Krebs solution. Colonic segments were mounted horizontally in a specially designed organ bath continuously perfused with oxygenated (95% O_2 and 5% CO_2) and heated 37°C Krebs solution.

Recording of mechanical activity

The distal end of each segment was tied around the mouth of a J-tube, which was connected *via* a T catheter to a pressure transducer (Statham Mod. P23XL) and to a syringe for filling the preparation with Krebs solution. The ligated proximal end was secured with a silk thread to an isometric force transducer (Grass FT03). Preparations, distended with 0.5–0.8 ml Krebs solution, were subjected to an initial tension of 1 g and were allowed to equilibrate for at least 30 min. Mechanical activity was detected as changes in intraluminal pressure, which are mainly generated by circular muscle, and recorded on an ink writer polygraph (Grass model 7D). At the beginning of each experiment, the preparation was challenged with KCl (120 mM) until a constant response was achieved. In order to establish NANC conditions, atropine (1 μM) and guanethidine (1 μM) were added to the perfusing Krebs solution at the beginning of each experiment.

Experimental protocols

After the control period, the preparation was incubated with the following drugs: (1) apamin, a selective blocker of small conductance Ca^{2+}- dependent K^+ channels (Blatz & Magleby, 1986); (2) 1H-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), a selective inhibitor of NO-stimulated soluble guanylyl cyclase (Garthwaite *et al*., 1995); (3) zaprinast, a specific inhibitor of cyclic GMP phosphodiesterase (Weishaar *et al*., 1986). These drugs were perfused at least for 30 min, each in consecutively increasing concentrations for evaluating the effects on the spontaneous mechanical activity. Concentration-response curves for sodium nitroprusside (SNP) or isoproterenol were determined in the absence and in the presence of apamin (0.1 μM) or ODQ (1 μM). SNP or isoproterenol were added to the bath for 4 min in concentrations progressively increasing after switching off the perfusion. For the evaluation of the possible mechanism of action involved in the hypermotility induced by blockade of NO synthesis and the possible interactions between different pathways, experiments were performed to test the additive effects between the drugs above the spontaneous contractile activity.

Solutions and drugs

The Krebs solution contained (in mM) NaCl 119, KCl 4.5, $MgSO_4$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.1. The drugs used were: atropine sulphate, guanethidine monosulphate, N_ω-nitro-L-arginine methyl ester (L-NAME), apamin, tetrodotoxin (TTX), isoproterenol hydrochloride, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), sodium nitroprusside (SNP), 8-Br-cyclic GMP, M&B 22948 (zaprinast). All drugs were purchased from SIGMA (St. Louis, MO, U.S.A.). All drugs were dissolved in distilled water, except zaprinast which was dissolved in ethanol and ODQ in dimethyl sulphoxide. Control experiments using solvents alone showed that none had effects on the tissue.

Data analysis and statistical tests

For calculation of spontaneous motility, the mean amplitude of contractile peaks of intraluminal pressure during a 10-min period of time was measured prior to and following drug administration after a new steady state was reached. Results are expressed as the changes in mean amplitude of the phasic contractions and reported as percentages of the values obtained in the control. The inhibitory effects of SNP, isoproterenol, 8-Br-cyclic GMP and zaprinast were expressed as per cent inhibition of contractile activity prior to drug administration, 100% inhibition corresponding to total suppression of spontaneous contractions. In experiments, where the maximal effect of the drug studied could be determined, the concentration (ED_{50}) producing half-max-

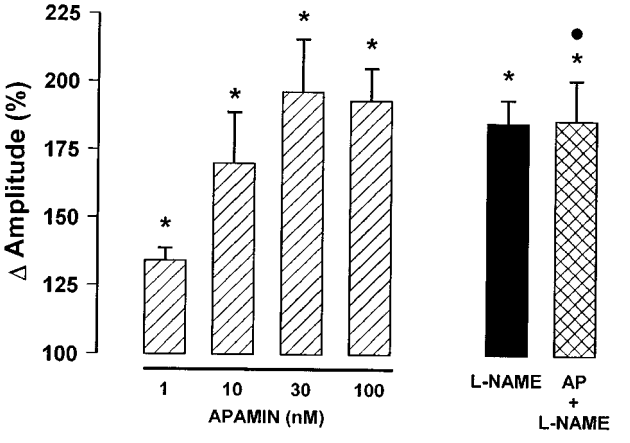

Figure 1 Effects of different concentrations of apamin and of L-NAME (100 μM), alone or in combination with apamin (0.1 μM), on the amplitude of spontaneous mechanical activity, detected as changes in intraluminal pressure, in rat proximal colon. Apamin increased the amplitude of the spontaneous contractions; the maximal effect of apamin was of the same degree of L-NAME (100 μM) and the joint application of apamin (0.1 μM) plus L-NAME (100 μM) had no additive effects. Amplitude of the spontaneous contractions before the addition of apamin ($n=6$) or L-NAME ($n=5$) was taken as 100%. Data are expressed as means ± s.e.mean. *Significantly different from the control. ● Not significantly different from L-NAME.

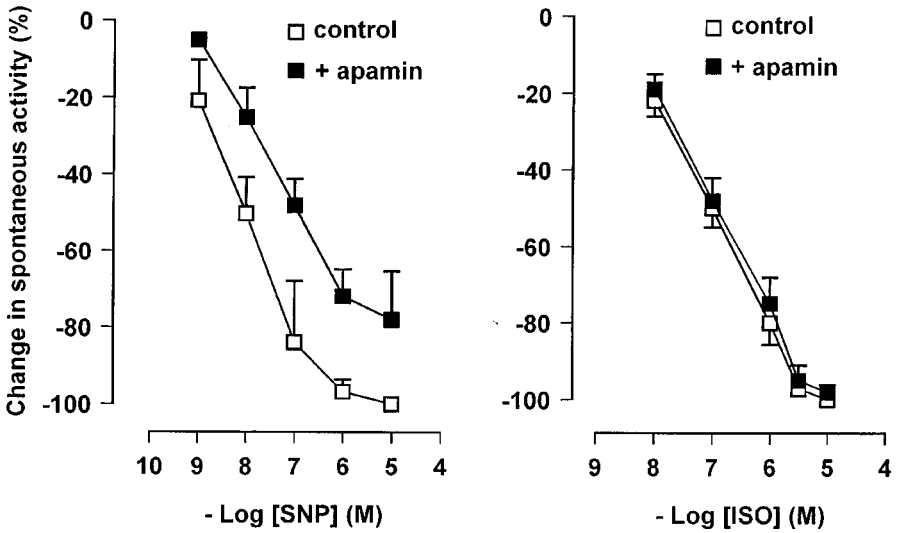

Figure 2 Concentration-response curves for inhibitory effects induced by SNP ($n=5$) or isoproterenol (ISO) ($n=4$) on the spontaneous mechanical activity in the absence or in the presence of apamin (0.1 μM). Apamin reduced the inhibitory effects induced by SNP, without affecting those induced by isoproterenol. Data are expressed as means ± s.e.mean and are reported as a percentage of the total suppression of spontaneous contractions, considered 100%.

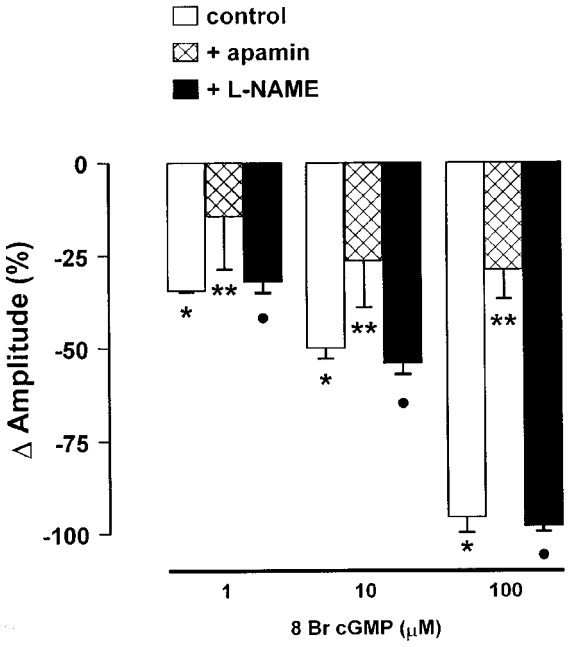

Figure 3 Inhibitory effects induced by different concentrations of 8-Br-cyclic GMP on the amplitude of spontaneous contractions in the control ($n=9$), in the presence of apamin (0.1 μM) ($n=5$) and in the presence of L-NAME (100 μM) ($n=4$). 8-Br-cyclic GMP reduced the amplitude of the spontaneous contractions in a concentration-dependent manner. These effects were significantly reduced in the presence of apamin, but not in the presence of L-NAME. Data are expressed as means ± s.e.mean and are reported as a percentage of the total suppression of spontaneous contractions, considered 100%. *Significantly different from the control. **Significantly different from 8-Br-cyclic GMP. ● Not significantly different from 8-Br-cyclic GMP.

imum response was assessed by linear interpolation on the semilogarithmic concentration-response curve. All data are given as means ± s.e.mean; *n* indicates the number of animals from which the intestinal segments were taken. Statistical analysis was performed by means of Student's *t*-test. A probability value of less than 0.05 was regarded as significant.

Results

Rat proximal colon exibited spontaneous mechanical activity consisting of changes of intraluminal pressure as previously described (Mulè *et al*., 1995; Mulè & Serio, 1997). The rhythm of the spontaneous activity was very regular (1 – 2 c.p.m.) and the amplitude reached about 30% of the contraction evoked by KCl (120 mM). As previously described, L-NAME induced a concentration-dependent increase in the amplitude of the pressure waves and the maximal effect was observed at a concentration of 100 μM (Mulè *et al*., 1998a).

Effects of apamin

Apamin (1 – 100 nM) increased the amplitude of the spontaneous contractions, without affecting the basal tone. The excitatory effect reached a peak within 10 min and then stabilized, more or less maintained throughout the observation period. The enhancement of contractions by apamin was dependent on the concentration reaching a maximum at 30 nM (Figure 1), which was about 65% of the KCl response. The maximal potentiation of spontaneous contractions by apamin was of the same degree as that produced by L-NAME (100 μM) and the joint application of apamin (0.1 μM) plus L-NAME (100 μM) had no additive effect (Figure 1).

The NO donor, SNP (1 nM – 10 μM) produced a concentration-dependent inhibition of the phasic contractile activity of circular muscle (ED_{50} 11 ± 3 nM) and a slight decrease of the resting tone was observed in some preparations at 10 μM. The inhibitory effects induced by SNP (10 μM) were not influenced by TTX (1 μM), indicating that they were not dependent on neural action potentials. Apamin (0.1 μM) reduced the SNP-induced effects, shifting to the right the concentration-response curve (ED_{50} 0.12 ± 0.06 μM), whereas it failed to affect those induced by isoproterenol (ED_{50} 40 ± 6 nM before and ED_{50} 50 ± 2 nM after apamin) (Figure 2).

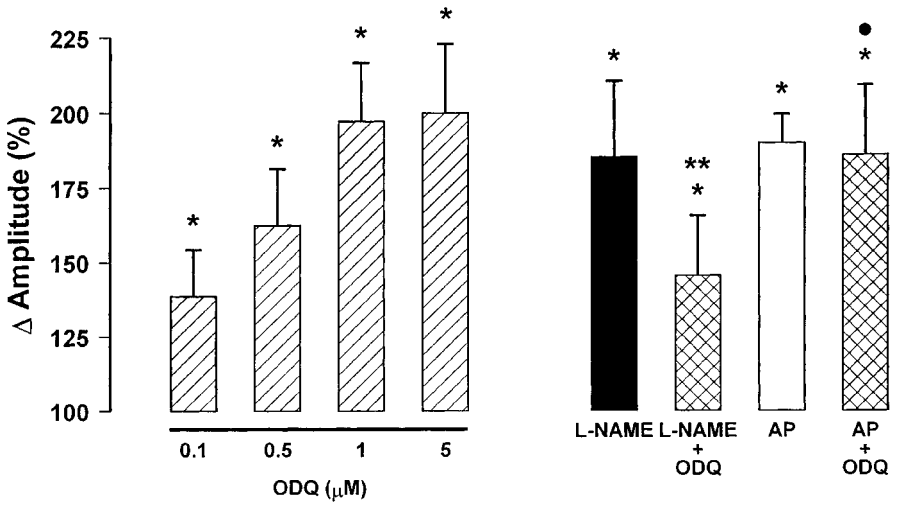

Figure 4 Effects of different concentrations of ODQ on the amplitude of spontaneous mechanical activity in rat proximal colon and effects of ODQ (1 μM) in the presence of L-NAME (100 μM) or apamin (0.1 μM). ODQ increased the amplitude of the spontaneous contractions; the maximal effect of ODQ was of the same degree of L-NAME (100 μM) or apamin (0.1 μM). ODQ (1 μM) in the presence of L-NAME reduced the amplitude of spontaneous contractions, whereas in the presence of apamin did not produce any further modification of spontaneous mechanical activity. Amplitude of the spontaneous contractions before the addition of ODQ ($n=6$), L-NAME ($n=5$) or apamin ($n=5$) was taken as 100%. Data are expressed as means$\pm$s.e.mean. *Significantly different from the control. **Significantly different from L-NAME. ● Not significantly different from apamin.

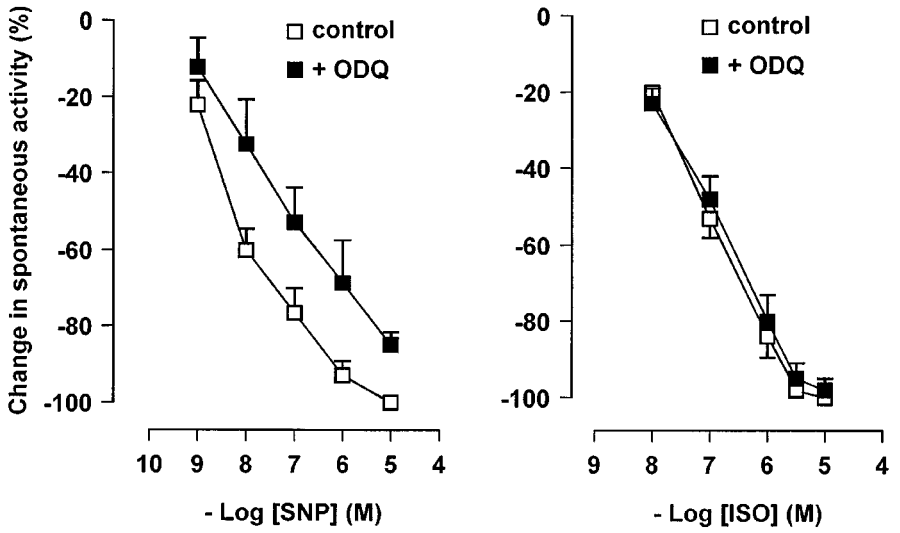

Figure 5 Concentration-response curves for inhibitory effects induced by SNP ($n=6$) or isoproterenol (ISO) ($n=4$) on the spontaneous mechanical activity in the absence or in the presence of ODQ (1 μM). ODQ reduced the inhibitory effects induced by SNP, without affecting those induced by isoproterenol. Data are expressed as means$\pm$s.e.mean and are reported as a percentage of the total suppression of spontaneous contractions, considered 100%.

Effects of 8-Br-cyclic GMP

Proximal colon was exposed to the membrane permeable analogue of cyclic GMP, 8-Br-cyclic GMP, to determine whether an increase of cyclic GMP level would mimic the effects of the NO donor. 8-Br-cyclic GMP (1 – 100 μM) reduced the amplitude of contractions in a concentration-dependent manner and suppressed the phasic waves at 100 μM (Figure 3). Sometimes at the maximal concentration used, a slight reduction in the basal tone was observed. The effects of 8-Br-cyclic GMP were persistent and at least 20 min were required after removal of the drug for full restoration of control activity. 8-Br-cyclic GMP inhibitory effect was largely and significantly reduced in the presence of apamin (0.1 μM), but not in the presence of L-NAME (100 μM) (Figure 3).

Effects of ODQ

The effects of ODQ were tested in order to verify whether a tonic biosynthesis of cyclic GMP triggered by NO would be involved in the modulation of the spontaneous mechanical activity. ODQ (0.1 – 5 μM) produced a concentration-dependent increase of the spontaneous contraction amplitude which

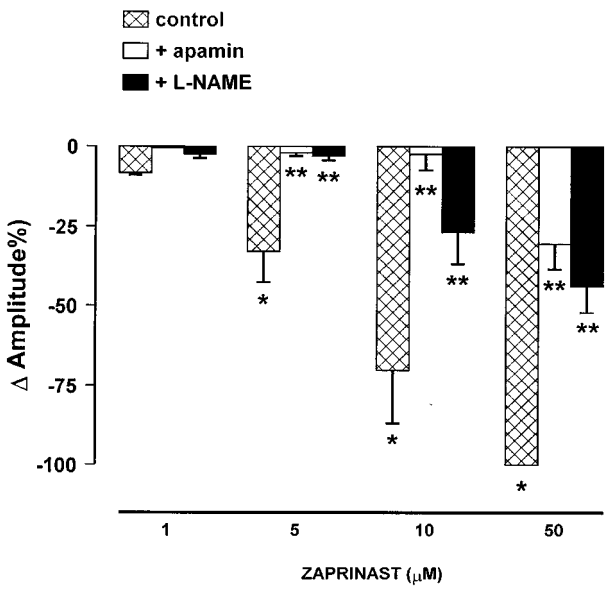

Figure 6 Effects of different concentrations of zaprinast on the amplitude of spontaneous mechanical activity in the control ($n=8$), in the presence of apamin (0.1 μM) ($n=4$) and in the presence of L-NAME (100 μM) ($n=4$). Zaprinast reduced the amplitude of the spontaneous contractions in a concentration-dependent manner. These effects were significantly reduced in the presence of apamin or in the presence of L-NAME. Data are expressed as means $\pm$s.e.mean and are reported as a percentage of the total suppression of spontaneous contractions, considered 100%. *Significantly different from the control. ** Significantly different from zaprinast.

reached a peak within 10 min and was then maintained. Maximal effect was produced by 1 μM ODQ and this was about 60% of the KCl response (Figure 4). When the experiments were performed in tissues pretreated with L-NAME (100 μM), ODQ (1 μM) did not induce enhancement of the contraction amplitude but produced a significant decrease in the amplitude of spontaneous contractions (Figure 4). The administration of ODQ after apamin (0.1 μM) treatment did not produce any further increase in the contraction amplitude (Figure 4). In addition, ODQ (1 μM) reduced the inhibitory effect produced by SNP, shifting to the right the concentration-response curve (ED_{50} 17.8$\pm$5 nM before and 0.12$\pm$0.05 μM after ODQ), whereas it did not influence the isoproterenol-induced inhibitory effects (ED_{50} 43$\pm$7 nM before and ED_{50} 48$\pm$ 2 nM after ODQ) (Figure 5).

Effects of zaprinast

Zaprinast was tested to verify whether increasing the endogenous level of cyclic GMP would reproduce the effects of the NO donor or 8-Br-cyclic GMP.

Zaprinast (1–50 μM) produced a decrease in the spontaneous contraction amplitude. The effect occurred after 10–15 min and persisted more or less maintained when the drug was present but it was reversible upon washout. The reduction induced by zaprinast was related to the concentration and at 50 μM it essentially abolished all mechanical activity (Figure 6). In the presence of apamin (0.1 μM) or L-NAME (100 μM) zaprinast caused much less reduction in contractile amplitude than in untreated preparations (Figure 6), whereas isoproterenol was still able to induce the same inhibitory effects (ED_{50} 40$\pm$6 nM before and ED_{50} 50$\pm$2 nM after apamin; ED_{50} 42$\pm$6 nM before and ED_{50} 45$\pm$5 nM after L-NAME).

Discussion

The results of this study provide support for the hypothesis that, in rat proximal colon, cyclic GMP production system and opening of small conductance Ca^{2+}-dependent K^+ channels are involved sequentially in transducing the endogenous NO signal responsible for tonic inhibition.

It has previously been shown that NO is tonically released to maintain a certain degree of suppression of colonic circular muscle activity (Ward *et al*., 1992; Middleton *et al*.,1993; Keef *et al*., 1997; Mulè *et al*., 1998a). Since the cellular mechanism by which NO tonic suppression occurs is not known, the aim of the present study was to verify whether activation of apamin-sensitive K^+ channels is involved in the NO tonic inhibitory influence and whether the effect is dependent on a cyclic GMP generating system.

Although apamin-sensitive K^+ channels are not typically involved in NO-dependent hyperpolarizations (e.g., Keef *et al*., 1993), apamin has been shown to inhibit nitrergic nerve-induced IJPs as well as relaxation in different preparations (Cristink *et al*., 1991; Osthaus & Galligan, 1992; Kitamura *et al*., 1993) including rat duodenum (Martins *et al*., 1995) and proximal colon (Serio *et al*., 1992, 1995). In our study the contractile effects of apamin, *per se*, suggest that apamin-sensitive K^+ channels are involved in modulation of spontaneous activity of the circular smooth muscle in the rat proximal colon. This seems to be a peculiarity of rat intestine. In fact also in rat small intestine and caecum, these channels modulate the spontaneous mechanical activity (Mulè *et al*., 1992; Serio *et al*., 1996; Lefebvre & Barthò, 1997), even if observations showing that apamin causes or potentiates contractions in smooth muscles under physiological conditions are very rare (Maas & Den Hertog 1979; Suzuki *et al*., 1993). The observation that apamin was able to reduce SNP-induced inhibitory effects, without affecting those induced by isoproterenol, is consistent with the idea that NO is able to activate apamin-sensitive K^+ channels in our preparation. Moreover, the increase in the contraction amplitude induced by apamin was not additive to that induced by L-NAME. This could suggest that the two drugs induce hypermotility through a common pathway and, therefore, it seems likely that the activation of apamin-sensitive K^+ channels is dependent on NO production. One might object that the lack of additive effects between L-NAME and apamin could be due to the reaching of a maximal contractile state. However, this hypothesis can be ruled out, since KCl test indicated that the preparation was able to produce contractions greater in amplitude than those induced by L-NAME and apamin.

To test the involvement of cyclic GMP in NO-dependent tonic inhibition, we used ODQ, which has been identified as a potent and selective inhibitor of NO-stimulated soluble guanylate cyclase (Garthwaite *et al*., 1995), rather than methylene blue and LY 83583, which are reported to cause non-specific effects (Sanders *et al*., 1989; Barbier & Lefebvre, 1992; Liu *et al*., 1994). ODQ has been reported to block actions of SNP and partially or totally antagonize NO-dependent responses to nerve stimulation in mouse caecum (Young *et al*., 1996) or canine proximal colon (Franck *et al*., 1997). In our preparation, the ability of SNP to inhibit spontaneous contractions was affected by ODQ, which failed to modify the relaxing effect of isoproterenol, acting *via* stimulation of adenylate cyclase. This observation indicates that the effect of exogenous NO on circular muscle of rat proximal colon can be mediated by a mechanism dependent on cyclic GMP. ODQ was effective at concentrations similar to those which abolish NO-stimulated cyclic GMP formation in a

variety of other tissues (Garthwaite *et al*., 1995; Cellek *et al*., 1996). The hypothesis that cyclic GMP is a second messenger of NO action in rat proximal colon is strengthened by the following: (i) cyclic GMP (delivered as its membrane permeable analogue) mimicked the mechanical effects of the NO donor, SNP; (ii) both SNP- and 8-Br-cyclic GMP-induced effects were affected by the same agent, apamin. The possibility that the reduction of the SNP or 8-Br-cyclic GMP effects observed in the presence of apamin occurs as a consequence of the increased mechanical activity can be ruled out since SNP or 8-Br-cyclic GMP effects were unaffected when the phasic contractions were otherwise increased (i.e. by TTX or by L-NAME). So, our results corroborate the suggestion that NO action is related to the activation of soluble guanylate cyclase, although in rat duodenum and proximal colon nitrergic relaxation has been proposed to be cyclic GMP-independent (Martins *et al*.,1995; Takeuchi *et al*.,1996). Furthermore, our findings that ODQ raised the contraction amplitude suggest that basal production of cyclic GMP maintains a suppression of the contractility. A contractile effect by ODQ due to an increase of release of acetylcholine, as reported in guinea-pig ileum (Hebeiss & Kilbinger, 1998) can be excluded in our experiments because they were performed in the presence of atropine. Moreover, the excitatory action of ODQ was no more detectable after inhibition of NOS by L-NAME indicating that, in our preparation, cyclic GMP production is presumably due to continuous synthesis of NO. Actually, in the presence of L-NAME, an inhibitory effect of ODQ on contractile activity was unmasked. We have no data to explain the mechanism of the inhibitory action, but a decrease in the amplitude of spontaneous contractions by ODQ in the presence of L-NAME was observed also by Franck *et al*. (1997) in canine proximal colon.

Several studies on NANC inhibitory innervation have employed zaprinast, an inhibitor of the cyclic GMP specific phosphodiesterase isoenzyme (phosphodiesterase V), to potentiate the relaxant response to nitrergic nerve stimulation, since it increases the availability of cyclic GMP (Barbier & Lefebvre, 1995; Williams & Parsons, 1995). In our preparation, zaprinast decreased in a concentration-dependent manner the spontaneous contractions, confirming our hypothesis that cyclic GMP levels are inversely related to the contraction amplitude in rat proximal colon. In agreement with the observations by Ward *et al*. (1992), we found that zaprinast effects, but not isoproterenol, were significantly reduced in the presence of L-NAME, suggesting that they depend on NO synthesis.

Lastly, our results allow us to hypothesize an interaction between cyclic GMP production system and opening of K^+ channels. In fact, apamin was able to reduce the inhibitory responses of the preparation to 8-Br-cyclic GMP or to endogenous increase of cyclic GMP obtained with zaprinast, without affecting those to isoproterenol. Furthermore, the lack of any additive effects between apamin and ODQ strengthens the hypothesis that the opening of the small conductance Ca^{2+}-dependent K^+ channels is due to a cyclic GMP-dependent mechanism.

Taken together, our results suggest that tonic inhibitory action of NO on spontaneous activity in circular muscle of rat proximal colon is mediated by apamin-sensitive K^+ channels through an increase in the level of cyclic GMP. Studies in other preparations provided evidence for the hypothesis that NO activates Ca^{2+}- dependent K^+ current and that guanyl cyclase and protein kinase G are involved in the signal transduction pathway (e.g. Koh *et al*., 1995; Murray *et al*., 1995).

In conclusion, small conductance Ca^{2+}-dependent K^+ channels and cyclic GMP are involved in the modulation of the spontaneous contractile activity of circular muscle in rat proximal colon. The opening of apamin-sensitive K^+ channels and guanylate cyclase stimulation are dependent mechanisms, which appear to work in series in order to mediate the tonic inhibitory action of NO.

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References

BARBIER, A.J. & LEFEBVRE, R.A. (1992). Effect of Ly83583 on relaxation induced by non-adrenergic non-cholinergic nerve stimulation and exogenous nitric oxide in the rat gastric fundus. *Eur. J. Pharmacol.,* **21,** 331 – 334.

BARBIER, A.J. & LEFEBVRE, R.A. (1995). Relaxant influence of phosphodiesterase inhibitors in the cat gastric fundus. *Eur. J. Pharmacol.,* **276,** 41 – 47.

BLATZ, A.L. & MAGLEBY, K.L. (1986). Single apamin-blocked Ca^{++}-activated K^+ channels of small conductance in cultured rat skeletal muscle. *Nature,* **323,** 718 – 720.

BOLOTINA, V.M., NAJIBI, S., PALACINO, J.J., PAGANO, P.J. & COHEN, R.A. (1994). Nitric oxide directly activates calcium-depentent potassium channels in vascular smooth muscle. *Nature,* **368,** 850 – 853.

CELLEK, S., KASALOV, L. & MONCADA, S. (1996). Inhibition of nitrergic relaxations by a selective inhibitor of the soluble guanylate cyclase. *Br. J. Pharmacol.,* **118,** 137 – 140.

CHRISTINCK, F., JURY, J., CAYABYAB, F. & DANIEL, E.E. (1991). Nitric oxide may be the final mediator of nonadrenergic, noncholinergic inhibitory junction potentials in the gut. *Can. J. Physiol. Pharmacol.,* **69,** 1448 – 1453.

FRANCK, H., SWEENEY, K.M., SANDERS, K.M. & SHUTTLEWORTH, C.W.R. (1997). Effects of a novel guanylate cyclase inhibitor on nitric oxide-dependent inhibitory neurotransmission in canine proximal colon. *Br. J. Pharmacol.,* **122,** 1223 – 1229.

GARTHWAITE, J., SOUTHAM, E., BOULTON, C.L., NIELSEN, E.B., SCHMIDT, K. & MAYER, B. (1995). Potent and selective inhibition of nitric oxide-sensitive guanylyl cyclase by 1-H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one. *Mol. Pharmacol.,* **48,** 184 – 188.

HATA, F., ISHII, T., KANADA, A., YAMANO, N., KATAOKA, T., TACHEUCHI, T. & YAGASAKI, O. (1990). Essential role of nitric oxide in descending inhibition in the rat proximal colon. *Biochem. Biophys. Res. Commun.,* **172,** 1400 – 1406.

HEBEISS, K. & KILBINGER, H. (1998). Nitric oxide-sensitive guanylyl cyclase inhibits acetylcholine release and excitatory motor transmission in the guinea-pig ileum. *Neuroscience,* **82,** 623 – 629.

IRIE, K., MURAKI, T., FURUKAWA, K. & NOMOTO, T. (1991). L-Nitro-arginine inhibits nicotine-induced relaxation of isolated rat duodenum. *Eur. J. Pharmacol.,* **202,** 285 – 288.

KANADA, A., HATA, F., SUTHAMNANTPONG, N., MAEHARA, T., ISHII, T., TAKEUCHI, T. & YAGASAKI, O. (1992). Key roles of nitric oxide and cyclic GMP in nonadrenergic and noncholinergic inhibition in rat ileum. *Eur. J. Pharmacol.,* **216,** 287 – 292.

KEEF, K.D., DU, C., WARD, S.M., McGREGOR, B. & SANDERS, K.M. (1993). Enteric inhibitory neural regulation of human colonic circular muscle: role of nitric oxide. *Gastroenterology,* **105,** 1009 – 1016.

KEEF, K.D., MURRAY, D.C., SANDERS, K.M. & SMITH, T.K. (1997). Basal release of nitric oxide induces an oscillatory motor pattern in canine colon. *J. Physiol.,* **499,** 773 – 786.

KITAMURA, K., LIAN, Q., CARL, A. & KURIYAMA, H. (1993). S-nitrosocysteine, but not sodium nitroprusside, produces apamin-sensitive hyperpolarization in rat gastric fundus. *Br. J. Pharmacol.,* **109,** 415–423.

KOH, S.D., CAMPBELL, J.D., CARL, A., SANDERS, K.M. (1995). Nitric oxide activates multiple channels in canine colonic smooth muscle. *J. Physiol.,* **489,** 735–743.

LEFEBVRE, R.A. & BARTHO', L. (1997). Mechanism of nitric oxide-induced contraction in rat isolated small intestine. *Br. J. Pharmacol.,* **120,** 975–981.

LI, C.G. & RAND, M.J. (1990). Nitric oxide and vasoactive intestinal polypeptide mediate non-adrenergic, non-cholinergic inhibitory transmission to smooth muscle of the rat gastric fundus. *Eur. J. Pharmacol.,* **191,** 303–309.

LIU, L.W.C., THUNEBERG, L. & HUIZINGA, J.D. (1994). Selective lesioning of interstitial cells of Cajal by methylene blue and light leads to loss of slow waves. *Am. J. Physiol.,* **266,** G485–G496.

MAAS, A.J.J. & DEN HERTOG, A. (1979). The effect of apamin on the smooth muscle cells of the guinea-pig taenia coli. *Eur. J. Pharmacol.,* **58,** 151–156.

MAEHARA, T., FUJITA, A., SUTHAMNATPONG, N., TAKEUCHI, T. & HATA, F. (1994). Differences in relaxant effects of cyclic GMP on skinned muscle preparations from the proximal and distal colon of rats. *Eur. J. Pharmacol.,* **261,** 163–170.

MARTINEZ-CUESTA, M.A., ESPLUGUES, J.V. & WHITTLE, B.J.R. (1996). Modulation by nitric oxide of spontaneous motility of rat isolated duodenum: role of tachykinins. *Br. J. Pharmacol.,* **118,** 1335–1340.

MARTINS, S.L.R., DE OLIVEIRA, R.B. & BALLEJO, G. (1995). Rat duodenum nitrergic-induced relaxations are cGMP-independent and apamin-sensitive. *Eur. J. Pharmacol.,* **284,** 265–270.

MIDDLETON, S.J., CUTHBERT, A.W., SHORTHOUSE, M. & HUNTER, J.O. (1993). Nitric oxide affects mammalian distal colonic smooth muscle by tonic neural inhibition. *Br. J. Pharmacol.,* **108,** 974–979.

MULÈ, F., D'ANGELO, S., AMATO, A., CONTINO, I. & SERIO, R. (1998a). Modulation by nitric oxide of spontaneous mechanical activity in rat proximal colon. *J. Auton. Pharmacol.,* **18,** 1–6.

MULÈ, F., GERACI, A., SERIO, R. & POSTORINO, A. (1992). On the peptidergic hypothesis for non-adrenergic non-cholinergic innervation in the rat duodenum. *J. Auton. Pharmacol.,* **12,** 81–88.

MULÈ, F. & SERIO, R. (1997). Inhibition of mechanical activity by neurotensin in rat proximal colon: involvement of nitric oxide. *Am. J. Physiol.,* **273,** G491–G497.

MULÈ, F., SERIO, R. & CONTINO, I. (1998b). Mechanisms involved in the modulation of colonic motility by nitric oxide. *Neurogastroenterol. Mot.,* **10,** 88.

MULÈ, F., SERIO, R. & POSTORINO, A. (1995). Motility pattern of isolated rat proximal colon and excitatory action of neurotensin. *Br. J. Pharmacol.,* **275,** 131–137.

MURRAY, J.A., SHIBATA, E.F., BURESH, T.L., PICKEN, H., O'MEARA, B.W. & CONKLIN, J.L. (1995). Nitric oxide modulates a calcium-activated potassium current in muscle cells from opossum esophagus. *Am. J. Physiol.,* **269,** G606–G612.

OSTHAUS, L.E. & GALLIGAN, J.J. (1992). Antagonists of nitric oxide synthesis inhibit nerve-mediated relaxations of longitudinal muscle in guinea pig ileum. *J. Pharmacol. Exp. Ther.,* **260,** 140–145.

POSTORINO, A., SERIO, R. & MULÈ, F. (1995). Nitric oxide is involved in non adrenergic, non cholinergic inhibitory neurotransmission in rat duodenum. *J. Auton. Pharmacol.,* **15,** 65–71.

SANDERS, K.M., BURKE, E.P. & STEVEN, R.J. (1989). Effects of methylene blue on rhythmic activity and membrane potential in the canine proximal colon. *Am. J. Physiol.,* **256,** G779–G784.

SANDERS, K.M. & WARD, S.M. (1992). Nitric oxide as a mediator of nonadrenergic noncholinergic neurotransmission. *Am. J. Physiol.,* **262,** G379–G392.

SERIO, R., MULÈ, F. & POSTORINO, A. (1992). Non-adrenergic non-cholinergic inhibitory responses to nerve stimulation in rat colonic circular muscle. *Exp. Physiol.,* **77,** 119–127.

SERIO, R., MULÈ, F. & POSTORINO, A. (1995). Nonadrenergic, noncholinergic inhibitory junction potentials in rat proximal colon: role of nitric oxide. *Can. J. Physiol. Pharmacol.,* **73,** 79–84.

SERIO, R.,MULÈ, F., POSTORINO, A., VETRI, T. & BONVISSUTO, F. (1996). Apamin-sensitive and -insensitive components of inhibitory junction potentials in rat caecum: role of nitric oxide. *J. Auton. Pharmacol.,* **16,** 183–189.

SHUTTLEWORTH, C.W.R. & SANDERS, K.M. (1996). Involvement of nitric oxide in neuromuscular transmission in canine proximal colon. *Proc. Soc. Exp. Biol. Med.,* **21,** 16–23.

SUTHAMNATPONG, N., HATA, F., KANADA, A., TAKEUCHI, T. & YAGASAKI, O. (1993a). Mediators of nonadrenergic, noncholinergic inhibition in the proximal, middle and distal regions of rat colon. *Br. J. Pharmacol.,* **108,** 348–355.

SUTHAMNATPONG, N., HOSOKAWA, M., TAKEUCHI, T., HATA, F. & TAKEWAKI, T. (1994). Nitric oxide-mediated inhibitory response of rat proximal colon: independence from changes in membrane potential. *Br. J. Pharmacol.,* **112,** 676–682.

SUTHAMNATPONG, N., MAEHARA, T., KANADA, A., TAKEUCHI, T. & HATA, F. (1993b). Dissociation of cGMP level from relaxation of the distal, but not the proximal colon of rats. *Jpn. J. Pharmacol.,* **62,** 387–393.

SUZUKI, K., ITO, K.M., MINAYOSHI, Y., SUZUKI, H., ASANO, M. & ITO, K. (1993). Modification by charybdotoxin and apamin of spontaneous electrical and mechanical activity of the circular smooth muscle of guinea-pig stomach. *Br. J. Pharmacol.,* **109,** 661–666.

TAKEUCHI, T., KISHI, M., ISHII, T., NISHIO, H. & HATA, F. (1996). Nitric oxide-mediated relaxation without concomitant changes in cyclic GMP content of rat proximal colon. *Br. J. Pharmacol.,* **117,** 1204–1208.

WARD, S.M., DALZIEL, H.H., BRADLEY, M.E., BUXTON, I.L.O., KEEF, K., WESTFALL, D.P. & SANDERS, K.M. (1992). Involvement of cyclic GMP in non-adrenergic, non-cholinergic inhibitory neurotransmission in dog proximal colon. *Br. J. Pharmacol.,* **107,** 1075–1082.

WEISHAAR, R.E., BURROWS, S.D., KOBYLARZ, D.C., QUADE, M.M. & EVANS, D.B. (1986). Multiple molecular forms of cyclic nucleotide phosphodioesterase in cardiac and smooth muscle and in platelets. Isolation, characterization, and effects of various reference phosphodiesterase inhibitors and cardiotonic agents. *Biochem. Pharmacol.,* **35,** 787–800.

WILLIAMS, S.J. & PARSONS, M.E. (1995). Nitric oxide, an enteric nonadrenergic-noncholinergic relaxant transmitter: evidence using phosphodiesterase V and nitric oxide synthase inhibition. *Br. J. Pharmacol.,* **116,** 1789–1796.

YOUNG, H.M., CIAMPOLI, D., JOHNSON, P.J. & STEBBING, M.J. (1996). Inhibitory transmission to the longitudinal muscle of the mouse caecum is mediated largely by nitric oxide acting via soluble guanylyl cyclase. *J. Auton. Nerv. Syst.,* **61,** 103–108.

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