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Antagonism by SR 48692 of mechanical responses to neurotensin in rat intestine

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1 The effects of SR 48692 on neurotensin (NT)-induced mechanical responses were investigated in rat duodenum and proximal colon by use of isometric, isovolumic preparations.

2 SR 48692 inhibited the relaxant responses to NT in duodenal circular and longitudinal muscle. It also antagonized the NT-induced contractile effects in duodenal circular muscle and in proximal colon (both muscular layers).

3 From Schild analysis the pA_2 value for SR 48692 was 8.2 in tissues where NT induced relaxant effects and 7.5 in tissues where NT induced contractile effects and the slope of the regression line was not significantly different from unity, indicating competitive antagonism.

4 SR 48692 did not antagonize the duodenal relaxant effect induced by noradrenaline and the contractile response to carbachol or substance P in duodenum and colon.

5 Our results demonstrate that SR 48692 selectively antagonizes the mechanical actions of NT in rat intestine and confirm the existence of specific NT receptors. Receptors that subserve a relaxant effect seem to be related, but not identical, to those that mediate contractile effects.

Keywords: Neurotensin; neurotensin receptors; SR 48692; non-peptide neurotensin antagonist; neurotensin intestinal effects

Introduction

The gut-brain regulatory peptides have raised considerable interest among researchers over the last decade. Neurotensin (NT), a 13-amino acid neuropeptide, is one of such substances, being widely distributed in the central nervous system and in the digestive tract of mammals (Ferris, 1989). NT, has a broad spectrum of biological activities. In the brain, NT displays neuromodulator actions, in particular on dopaminergic transmission in the nigro-striatal and meso-cortico-limbic system, and it exerts potent hypothermic and analgesic effects when injected in the central nervous system (Bissette *et al.*, 1976; Kasckow & Nemeroff, 1991; Behbehani, 1992; Rostene *et al.*, 1992). In the periphery, when injected intravenously, NT produces several cardiovascular effects, including hypotension and increased vascular permeability, attributable to its ability to stimulate secretion from mast cells (Cochrane, 1990; Prange, 1992). In the gut, NT is localized within specific mucosal endocrine cells (N cells) and nerve fibres in the enteric plexus (Sundler *et al.*, 1977; Schultzberg *et al.*, 1980; Buchan & Barber, 1987) and acts as a modulator of digestive functions (Ferris, 1989). In particular, its potent actions on intestinal smooth muscle activity suggest a possible role for the peptide as a circulating hormone (Al Saffar & Rosell, 1981), neurotransmitter (Goedert *et al.*, 1984; Komori *et al.*, 1986; 1992) or modulator of intestinal motor activity (Mulè *et al.*, 1992; 1995).

The biological effects displayed by NT are mediated by specific membrane receptors. The biochemical and pharmacological properties of these binding sites have been extensively studied in mammalian brain homogenates as well as membrane preparations from neuronal and certain non-neuronal cell lines (Kitabgi *et al.*, 1985). Recently, the characterization of the first nonpeptide NT antagonist, SR 48692, was reported (Gully *et al.*, 1993). The antagonist was shown to inhibit competitively and selectively the interaction of NT with high affinity binding sites in brain membranes while it fails to antagonize NT-induced hypothermia and analgesia in mouse and rat. These observations have raised the hypothesis of the existence of distinct NT receptor subtypes (Dubuc *et al.*, 1994; Labbé-Jullié *et al.*, 1994b). In the periphery, the pharmacological profile of SR 48692 has been investigated only in guinea-pig intestinal tissues and in rat isolated mast-cells (Labbé-Jullié *et al.*, 1994a; Miller *et al.*, 1995).

The present work was designed to investigate the pharmacological properties of SR 48692 in the rat intestine, since duodenum and colon bear functionally distinct NT receptors. In fact, in the duodenum the peptide has been shown to relax the longitudinal smooth muscle and to exert biphasic effects on circular muscle probably acting on two different receptor types (Mulè *et al.*, 1992). In addition, NT contracts the proximal colon (Mulè *et al.*, 1995). Thus, rat intestine offers interesting models for studying and comparing the antagonist effect of SR 48692 on NT receptors.

Methods

General

Adult Wistar rats (300–400 g) were killed by cervical dislocation; the abdomen was opened via an incision in the midline. A duodenal segment just distal to the pylorus and a segment of proximal colon (both about 2 cm in length) were rapidly removed, flushed of luminal contents and placed in 5 ml horizontal organ baths continuously perfused with Krebs solution, gassed (95% O_2 and 5% CO_2) and heated (37°C). The composition of Krebs solution was as follows (mM): NaCl 119, KCl 4.5, $MgSO_4$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11.11. Isometric tension and endoluminal pressure were recorded as previously described (Mulè *et al.*, 1992; 1995). The changes in endoluminal pressure measures mainly circular muscle activity and the modifications of isometric tension are an index of longitudinal muscle responses. Preparations were subjected to an initial tension of 2 g and were filled with Krebs solution until an initial pressure of 10 cmH_2O was obtained.

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Moreover, preparations were allowed to equilibrate for at least 30 min. At the beginning of each experiment, the prepartions were challenged with 1 μM carbachol until a constant response was achieved. Drugs were added to the bath after switching off the perfusion for the testing time. Non-cumulative concentration-response curves for NT were established; thus neurotensin was added to the bath as a single addition at intervals of 30 min. Duodenal segments were incubated with NT for 2 min, but proximal colon for 5 min. The results are expressed as a percentage of the NT maximal response achieved in the absence of antagonist. SR 48692 was incubated at the indicated concentrations for 30 min before the addition of NT or other test drugs (carbachol, substance P and noradrenaline).

Data analysis

Results are expressed as means ± s.e. mean or as mean values with 95% confidence intervals; *n* indicates the number of animals from which intestinal segments were taken. S.e. mean is only presented in the figures if it exceeds the dimension of the symbol. The concentrations of NT eliciting 50% of its own maximum response (EC_{50}) were determined graphically for each curve by linear interpolation. The pA_2 values were determined from a Schild plot with the slope constrained to unity when not significantly differently from unity (Arunlakshana & Schild, 1959). The concentration ratio (CR) (i.e., the ratio of concentrations of neurotensin giving an equal response in the presence and in the absence of the antagonist: always measured as the EC_{50} value) was determined for various concentrations of SR 48692. Statistical differences between two means were determined by Student's *t* test for paired or unpaired observations and one-way analysis of variance (ANOVA) was used when results from duodenum were compared to those from proximal colon. Differences were assumed to be significant when tests gave probability levels $< 5\%$. The least squares method was used for calculating linear regressions. For the Schild plot, differences between the slope and unity were tested with Student's *t* test, under a null hypothesis (slope = 1).

Drugs

The following drugs were used: neurotensin acetate salt (NT), carbamylcholine chloride (carbachol) (CCh), substance P (SP), noradrenaline hydrochloride (NA), all purchased from Sigma. SR 48692 (2[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazol-3-yl)carbonyl asmino]tricyclo (3.3.1.1.^{3,7}) decan-2-carboxylic acid) was generously provided by Dr Danielle Gully, Sanofi Recherche (Montpellier, France). The compound was dissolved in dimethylsulphoxide (DMSO) and successive dilutions were in distilled water. Control tests showed that the solvent had no effect on the preparations. Stock solutions of drugs were stored at −20°C and fresh dilutions with Krebs were made daily.

Results

Duodenum

As described previously (Mulè *et al.*, 1992), NT induced a complex muscular response in rat duodenal segments. In fact NT (1 pM to 100 nM) caused a concentration-dependent relaxation of the circular and longitudinal muscle. This effect was followed by a contractile response, characterized by an increase in tone and amplitude of spontaneous phasic mechanical activity. The contractile effect was also concentration-dependent and was detectable only in endoluminal pressure recordings.

Relaxant effects

No difference was found in the potency or efficacy of NT for inducing a relaxant effect on endoluminal pressure and isometric tension (Table 1) and a maximal response was achieved with 30 nM NT (5 ± 0.3 cmH_2O and 1.9 ± 0.3 g, respectively).

SR 48692 (10–300 nM) had no effect on spontaneous mechanical activity, while it produced a concentration-dependent parallel rightward shift of NT concentration-response curves without altering the peptide maximal response. The EC_{50} values (expressed as pEC_{50}) for NT calculated after different concentrations of antagonist were significantly different (Table 1).

Table 1 Effects of SR 48692 on the relaxant and contractile responses induced by NT in rat duodenum and colon

	Endoluminal pressure	*Isometric tension*
Duodenal relaxant effect	pEC_{50}	pEC_{50}
NT	9.5 ± 0.05	9.5 ± 0.07
NT after SR 10 nM	9.0 ± 0.07	9.0 ± 0.07
NT after SR 30 nM	8.0 ± 0.08*	8.4 ± 0.06*
NT after SR 100 nM	7.3 ± 0.08*	7.7 ± 0.05*
NT after SR 300 nM	6.6 ± 0.07*	7.0 ± 0.05*
Duodenal contractile effect		
NT	8 ± 0.04	
NT after SR 10 nM	8 ± 0.05	
NT after SR 30 nM	7.7 ± 0.06*	
NT after SR 100 nM	7.3 ± 0.08*	
NT after SR 300 nM	6.9 ± 0.05*	
Colon contractile effect		
NT	9.1 ± 0.04	9.3 ± 0.07
NT after SR 10 nM	9 ± 0.05	9.1 ± 0.05
NT after SR 30 nM	8.6 ± 0.07*	8.8 ± 0.06*
NT after SR 100 nM	8.0 ± 0.05*	8.3 ± 0.07*
NT after SR 300 nM	6.6 ± 0.07*	7.3 ± 0.05*

The values are given as means ± s.e.mean from 4 animal preparations. *$P < 0.05$ as compared to the control value. pEC_{50} = −log concentration of neurotensin producing half maximum effect.

The Schild analysis yielded a straight line with a slope not significantly different from unity and an intercept with the abscissa scale (pA_2) of 8.2 (CL 7.9–8.6) equivalent to a K_B of 6 (CL 1.2–25) nM. No difference was found when data from circular or longitudinal muscle were submitted to Schild analysis and therefore only data relating to endoluminal pressure are presented (Figure 1).

Contractile effects

The contractile effect on circular muscle induced by NT appeared and reached a maximum at concentrations higher than those required for inducing relaxant effects. pEC_{50} value calculated was 8 ± 0.04 nM and maximal response (10.3 ± 1.2 cmH_2O) was achieved with 300 nM NT (Figure 2). SR 48692 (10 to 300 nM) produced parallel shifts to the right in the peptide concentration-response curve (Figure 2). When the data were submitted to Schild analysis a straight line was obtained with a slope not significantly different from unity and an intercept with the abscissa scale (pA_2) of 7.5 (CL 7.1–8) and K_B 30 (CL 10–80) nM (Figure 2).

Proximal colon

As previously described (Mulè *et al.*, 1995), NT (1 pM to 0.1 μM) induced a concentration-dependent contractile effect on both muscular layers. No difference was found in the potency of NT for inducing the excitatory effects on endoluminal pressure or isometric tension (Table 1). Maximal contractile effects of 14 ± 0.4 cmH_2O and 2.5 ± 0.3 g, were obtained at a concentration of 100 nM. Figure 3 illustrates the concentration-response curves for the contraction induced by NT only on circular muscle in the control and after various SR 48692 concentrations. A gradual and parallel shift to the right of the NT concentration-response curve was observed after the antagonist. The Schild plot appeared to be well-fitted by a

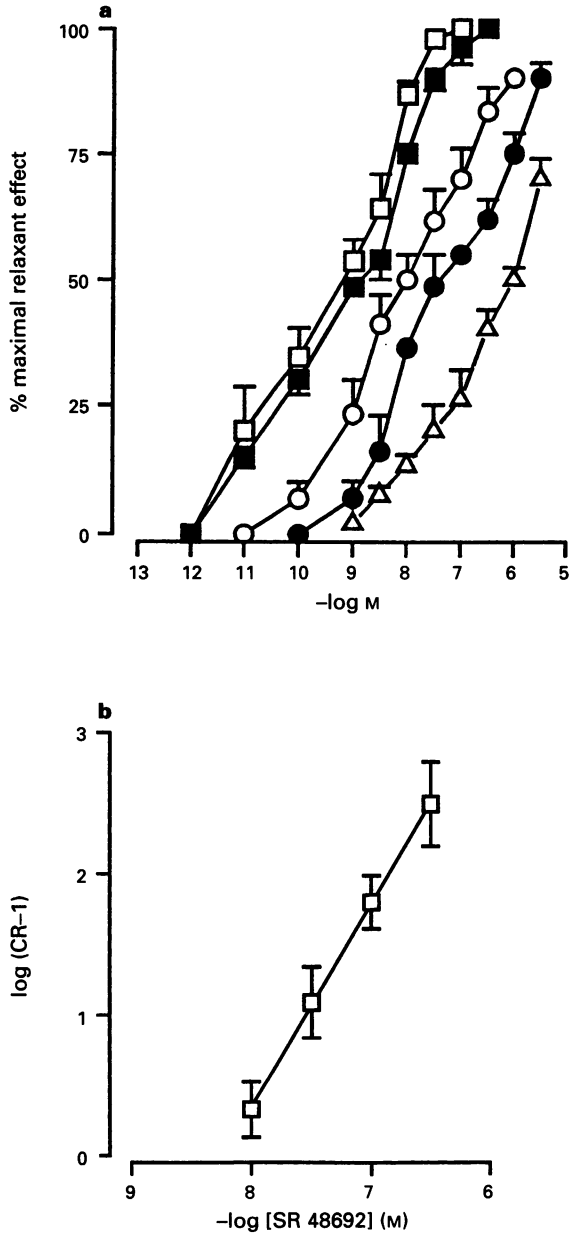

Figure 1 (a) Concentration-response curves for the relaxant effect induced by NT on rat duodenal endoluminal pressure in the absence (□) and in the presence of various concentrations of SR48692 (■, 10 nM; ○, 30 nM; ●, 100 nM; △, 300 nM). Each point represents the mean value with s.e.mean ($n=4$). (b) Schild plot for the antagonist effects of SR48692. The intercept on the abscissa scale gives the pA_2 value. $y = 1.44x + 11.89$; $r = 0.99$; $pA_2 = 8.2$.

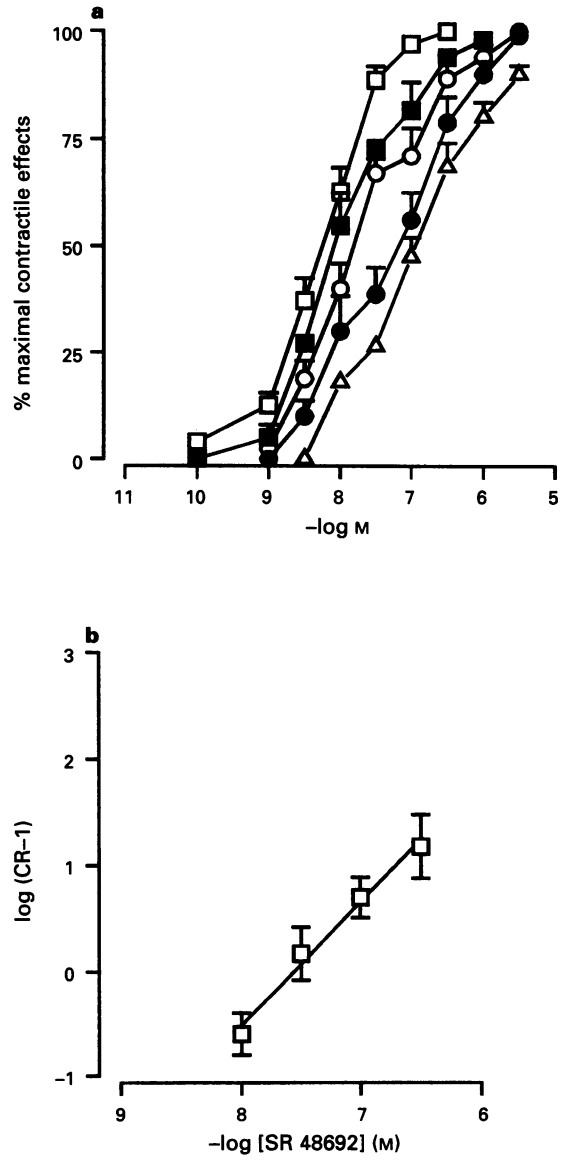

Figure 2 (a) Concentration-response curves for the contractile effect induced by NT on rat duodenal endoluminal pressure in the absence (□) and in the presence of various concentrations of SR48692 (■, 10 nM; ○, 30 nM; ●, 100 nM; △, 300 nM). Each point represents the mean value with s.e.mean ($n=4$). (b) Schild plot for the antagonist effects of SR48692. The intercept on the abscissa scale gives the pA_2 value. $y = 1.18x + 8.95$; $r = 0.99$; $pA_2 = 7.5$.

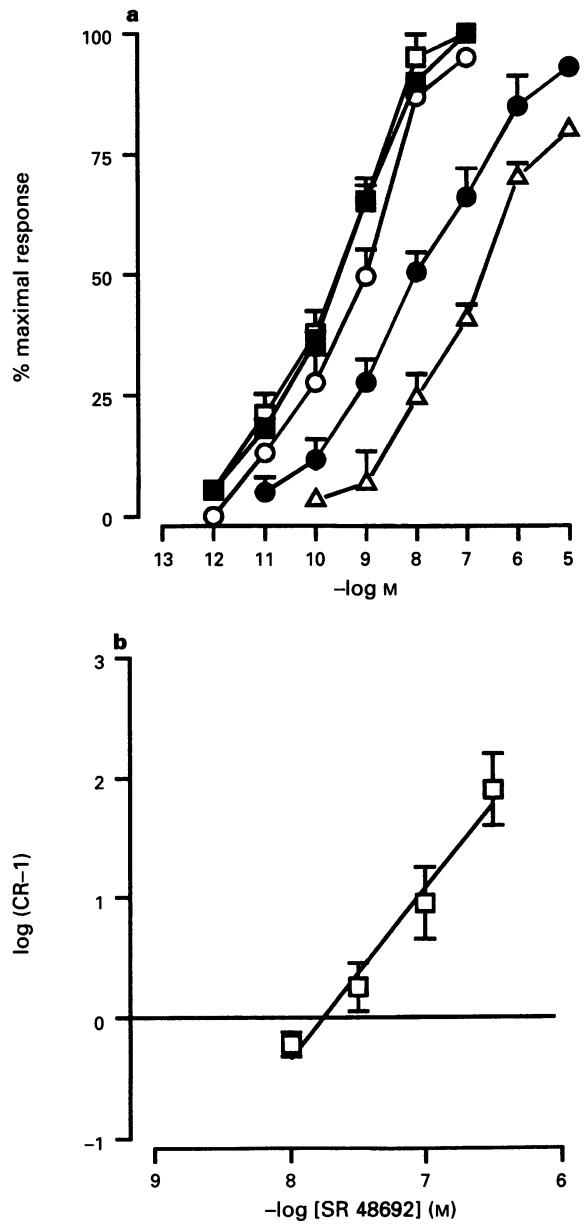

Figure 3 (a) Concentration-response curves for the contractile effect induced by NT in rat colon endoluminal pressure in the absence (□) and in presence of various concentrations of SR48692 (■, 10 nM; ○, 30 nM; ●, 100 nM; △, 300 nM). Each point represents the mean with s.e.mean ($n=4$). (b) Schild plot for the antagonist effects of SR48692. The intercept on the abscissa scale gives the pA_2 value. $y=1.4x+10.95$; $r=0.99$; $pA_2=7.7$.

straight regression line not significantly different in slope from unity and gave a pA_2 value of 7.7 (CL 7.1–8.1) equivalent to a K_B of 20 (CL 8–80) nM (Figure 3). Such a value of K_B was similar to that calculated for the contractile effect of NT in the duodenum, but it was significantly different from that obtained for the duodenal relaxant effect.

Specificity of the SR 48692 effects

To test the selectivity of the antagonism we examined the effect of SR 48692 on the responses to some neurochemicals active on rat duodenum and colon. Pretreatment for 30 min of the preparations with SR 48692 (300 nM) did not affect the relaxant effect of NA or contractile response elicited by CCh in the duodenum as well as the contraction to CCh and SP in the colon, whereas it markedly reduced the NT response (Figure 4). In addition, the antagonistic effects of SR 48692 on the

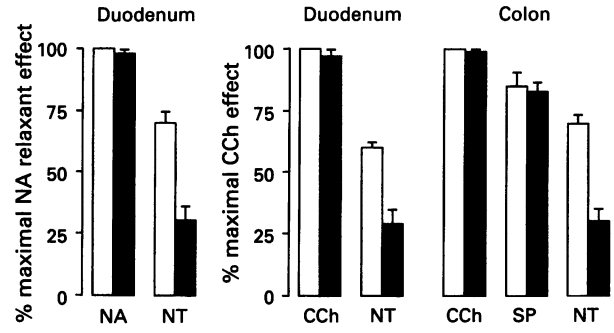

Figure 4 Responses elicited by noradrenaline (NA) (10 μM), neurotensin (NT) (100 nM), carbachol (CCh) (1 μM), substance P (SP) (100 nM) in rat duodenum and colon preparations that were not exposed (open columns) or exposed for 30 min (solid columns) to 300 nM SR48692. For each agonist the tested concentration was near maximally effective. The results are expressed as the percentage of the response elicited by 10 μM NA or 1 μM CCh in the absence of SR48692. The values are means ± s.e.mean from 4 experimental preparations.

different response of duodenum and proximal colon were removed by washing out the preparations. However, the recovery of the response to NT took progressively longer as the concentration of SR 48692 was increased.

Discussion

The results obtained in the present investigation show that SR 48692, a non-peptide antagonist of NT-receptors (Gully *et al.*, 1993) inhibits the actions of NT in rat intestine and confirm our previous suggestion that NT acts by a specific receptor-dependent mechanism.

In the preparations examined (duodenal and colonic segments) NT concentration-response curves obtained in the presence of increasing concentrations of SR 48692 indicate that the antagonist did not modify the efficacy of the agonist and yielded a slope of the Schild plot not different from unity showing that the antagonism is competitive and, therefore, the obtained pA_2 values can be taken as the $-\log K_B$ values (Arunlakshana & Schild, 1959). This agrees with the pharmacological profile reported in the guinea-pig intestine, where SR 48692 was able to antagonize competitively neurogenic contractions in the ileum and myogenic relaxations in the colon (Labbè-Jullié *et al.*, 1994a). The observation that SR 48692 at all concentrations tested failed to induce any response by itself or to modify the spontaneous mechanical activity in the preparations examined suggests that the compound is devoid of any intrinsic agonist activity and excludes the possibility of a tonic endogenous release of NT. Moreover, SR 48692 appears to be selective for NT receptors since it did not antagonize the contractile response to CCh and SP, and the relaxant effect of NA in the preparations used.

K_B values derived in circular and longitudinal muscles of duodenum and colon (both muscular layers) were in the low-medium nanomolar range in good agreement with K_I values reported for the binding of SR 48692 in rat brain (Gully *et al.*, 1993), but they were higher than in guinea-pig intestine (Labbè-Jullié *et al.*, 1994a). Therefore, SR 48692 shows some degree of species specifity, as is often observed for nonpeptide antagonists of other neuropeptide receptors (Hall *et al.*, 1993).

SR 48692 has been shown to inhibit the interaction of NT with high affinity binding sites in brain membrane and prevented a variety of NT-induced celluar responses (Gully *et al.*, 1993; Miller *et al.*, 1995), whereas it fails to antagonize NT-induced hypothermia and analgesia in rat (Labbé-Jullié *et al.*, 1994b; Dubuc *et al.*, 1994). Thus, SR 48692 could be used to reveal putative NT receptor subtypes. In view of this, we tested the compound on the different mechanical responses to NT in

various parts of rat intestine. Our previous investigation on dual motor effects of NT led us to propose that two different types of receptor are present in rat duodenum, since NT showed different potency for inducing relaxant or contractile effects and differences in sensitivity to desensitization and in transduction mechanisms of NT receptors were found (Mulè *et al.*, 1992). SR 48692 antagonized both relaxant and contractile effects in rat intestine, but with a difference in the apparent affinity. K_B values calculated for the putative receptors mediating relaxation in longitudinal and circular muscle of rat duodenum are significantly lower than values obtained for contractile effects. The observed difference in pA_2 values for SR 48692 suggests that two receptor sites with different affinity for SR 48692 are present in rat intestine: the higher affinity receptor sites are involved in the relaxant effect and the lower affinity receptor sites participate in the contractile response. Thus, this finding could be further evidence, even if not definitive, for the presence of pharmacologically distinct receptors for NT in rat intestine. On the other hand, K_B values calculated for the putative receptors mediating contraction in circular muscle of rat duodenum and colon (both muscular layers), were almost identical and, thus, it is possible to argue that the NT receptors present in these intestinal segments and responsible for the contractile response are the same.

In conclusion, SR 48692 selectively and competitevely antagonizes both the relaxant and the contractile actions of NT in rat isolated intestine and can be regarded as a novel tool with which to explore the physiological and pathological roles of NT in gastrointestinal motility.

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