Effect of neuropeptide Y on adrenergic and non-adrenergic, non-cholinergic responses in the rat anococcygeus muscle

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1 The effects of neuropeptide Y (NPY) were examined on adrenergic and non-adrenergic, non-cholinergic (NANC) neurotransmission in the rat anococcygeus muscle.

2 NPY (0.1–0.3 μ M) greatly potentiated the contractile responses induced by field stimulation. Prazosin (0.1 μ M) completely abolished the stimulation-induced responses either in the absence or presence of NPY.

3 NPY (0.1–0.3 μ M) enhanced only the contractile responses to low doses of noradrenaline (NA, 0.003–0.01 μ M). Responses to tyramine were unaffected by the same concentrations of NPY.

4 In superfused anococcygeus, previously loaded with [³H]-NA, NPY (0.1–0.3 μ M) failed to modify the basal, as well as the stimulation-evoked, release of tritium at 2 and 4 Hz.

5 NANC relaxations induced by electrical stimulation were significantly reduced, in a concentration-related manner, by 0.1–0.3 μ M NPY.

6 L-N^G-nitro-arginine (L-NOARG, 30 μ M) enhanced the stimulation (0.25–1 Hz)-induced motor responses. In the presence of L-NOARG (30 μ M), NPY (0.1 μ M) did not modify the motor responses induced by field stimulation (0.25–0.5 Hz). L-Arginine did not reverse the NPY-induced potentiation of stimulation-induced motor responses.

7 The relaxations of anococcygeus muscle induced by sodium nitroprusside (SNP, 0.01–0.3 μ M) were diminished by NPY (0.1–0.3 μ M).

8 Our study suggests that NPY, at concentrations devoid of contractile effect, potentiates the motor responses of rat anococcygeus muscle as a consequence, at least in part, of the inhibition of NANC relaxing responses by a different mechanism from L-NOARG.

Keywords: NPY, Neuropeptide Y, NANC transmission; rat anococcygeus muscle; L-N^G-nitroarginine

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide originally isolated from porcine brain (Tatemoto, 1982). The coexistence and corelease of NPY with noradrenaline (NA) in adrenergic nerve terminals in the sympathetic nervous system has been demonstrated in several tissues (for review see: Lundberg *et al.*, 1990). In peripheral smooth muscle tissues, the effects of NPY that seem to vary with species and tissues include: (i) direct smooth muscle contraction at relatively high concentrations of NPY, in some but not all tissues; (ii) potentiation of contractile responses, at subthreshold concentrations of NPY, induced by other agents including NA and (iii) modulation of its own release as well as the release of NA (Potter, 1988; Lundberg *et al.*, 1990).

The anococcygeus muscle has a motor noradrenergic and an inhibitory non-adrenergic, non-cholinergic (NANC) innervation (Gillespie, 1972). The neuronal release of NA produces contraction mainly via α_1 -adrenoceptors (Docherty & Starke, 1981), demonstrated by the fact that motor responses were blocked by prazosin, a selective α_1 -adrenoceptor antagonist, but not by rauwolscine, a selective α_2 -adrenoceptor antagonist. Although the identity of the NANC neurotransmitter has not yet been clearly elucidated, the results obtained by Gillespie & Sheng (1990) suggest that the NANC neurotransmitter could be nitric oxide (NO) or a substance that can liberate NO.

The aim of this study was to characterize the effects of NPY on both noradrenergic and NANC transmission in the rat anococcygeus muscle.

Methods

Male Sprague-Dawley rats (300–500 g) were stunned and exsanguinated. The paired anococcygeus muscles were dissected as described by Gillespie (1972) and set up in isolated organ baths containing 20 ml of Krebs bicarbonate solution of the following composition (in mM): NaCl 112.0, KCl 4.7, CaCl₂ 2.5, KH₂PO₄ 1.1, MgSO₄ 1.2, NaHCO₃ 25.0 and glucose 11.1. The solution was maintained at 37 $\pm$ 0.5°C and continuously gassed with 95% O₂, 5% CO₂. The organ-responses against 0.5 g tension were recorded by means of an isometric transducer (UF-1) on an Omniscribe pen-recorder. The preparations were allowed to equilibrate for at least 30 min during which time the Krebs solution was replaced repeatedly. Following equilibration, the preparation was primed with 30 μ M NA.

Effect of neuropeptide Y on stimulation-induced contractile responses

Electrical field stimulation was carried out via platinum ring electrodes, between which the muscle was freely suspended. Square wave pulses of 0.1 ms duration and supramaximal voltage at 0.25 to 5 Hz were applied for 10 s every 3 min with a Harvard stimulator. Frequency-response curves were generated and the tissues washed for 15 min. NPY (0.1–0.3 μ M) or bovine serum albumin (BSA, 0.1%) was then added and after 6 min the frequency-response curve was repeated in the same muscle. Responses were calculated as percentage of the control response elicited by the highest frequency of stimulation used (5 Hz). Only one concentration of NPY was applied per muscle. In some experiments desmethylinipramine (DMI, 0.1 μ M) was present in the Krebs solution.

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Effect of neuropeptide Y on noradrenaline and tyramine contractile responses

Thirty min after the contraction with 30 μM NA, concentration-response curves to NA were constructed in a cumulative manner. After a washout period of 30 min, NPY (0.01–0.3 μM) or BSA (0.1%) was applied 6 min before the cumulative addition of NA was repeated. Each tissue was exposed to only one concentration of NPY. In preliminary experiments we found that, after a second concentration-response curve to NA, responses were always stabilized. Therefore, since the maximum contraction with NA in the presence of the vehicle or NPY was always higher than the maximum contraction obtained in the first curve, the results for NA were calculated as a percentage of the maximum response obtained in the second curve in the presence or absence of NPY. DMI (0.1 μM), normetanephrine (1 μM), propranolol (1 μM) and Na_2EDTA (23 μM) were present in the Krebs solution throughout the NA experiments to block neuronal and extraneuronal uptake, β -adrenoceptors, and to prevent oxidative degradation of NA respectively. When the effect of NPY on tyramine responses was studied, only one concentration-response curve per muscle in the absence or presence of NPY was recorded. The responses were expressed as a percentage of the maximum response to NA (30 μM) applied after 30 min of tyramine washout.

Effect of neuropeptide Y on NANC relaxations

To record NANC relaxations, tone was raised by addition of guanethidine (30 μM). When tone had reached a plateau a frequency-response, or sodium nitroprusside (SNP, 0.01–10 μM) concentration-response, curve was constructed. After a washout of 15 min, NPY (0.1–0.3 μM) or vehicle (BSA 0.1%) was applied during 6 min and a second frequency or SNP response curve was repeated. Field stimulation was applied at supramaximal voltage, 1 ms duration at 0.25 to 5 Hz applied for 10 s every 3 min. SNP (0.01–10 μM) was cumulatively added to the bath. Responses were calculated as the percentage reduction in the degree of tone existing at the moment of nerve stimulation or before applying SNP.

Effect of L-N^G-nitroarginine (L-NOARG) on stimulation-induced contractile responses in presence or absence of NPY

Frequency-response curves were generated and the tissues were washed for 15 min. L-NOARG (30 μM) or L-NOARG (30 μM) plus NPY (0.1 μM) was then added and after 18 min (L-NOARG) and 6 min (NPY) the frequency-response curve was repeated in the same muscle. Responses were calculated as a percentage of the response elicited by the highest frequency of stimulation used (5 Hz).

Comparison of L-N^G-nitroarginine and neuropeptide effects on noradrenergic and NANC responses

Contraction and relaxation to electrical stimulation (0.5 and 2.5 Hz) were studied in preparations from the same anococcygeus muscle. In one of them tone was raised as previously described. After responses to electrical stimulation were stabilized, NPY (0.1–0.3 μM , 6 min) or L-NOARG (3–10 μM , 18 min) was added and a second set of responses was obtained. The degree of potentiation (noradrenergic responses) and inhibition (NANC responses) by NPY and L-NOARG was calculated for each frequency of stimulation in relation to their own control response.

Effect of neuropeptide Y on tritium overflow

The tissues were placed in a nylon net and immersed for 30 min in 10 ml of Krebs bicarbonate solution at 37°C continuously gassed with 95% O_2 , 5% CO_2 (stabilization period).

Thereafter, they were incubated for 60 min in 1 ml oxygenated Krebs solution at 37°C containing ($\pm$)-[^3H]-NA (2 $\mu\text{Ci ml}^{-1}$, 0.2 μM , sp. act. 12.8 Ci mmol^{-1}). The anococcygeus muscles were transferred into a superfusion chamber with two parallel platinum electrodes, 0.5 cm apart, connected to a stimulator (Cibertec model CS9, modified to supply the adequate current strength) for field electrical stimulation (200 mA, 0.3 ms, 2–4 Hz, for 1 min). The muscles were superfused at a rate of 2 ml min^{-1} with oxygenated Krebs solution at 37°C for 100 min during which the basal level of tritium efflux reached a steady state. The superfusate was collected in vials (10 in total) at 30 s intervals. These vials were distributed in the following manner: two before stimulation to determine the basal level of tritium efflux, two during and six after the stimulation, covering the period needed to recover the basal levels of tritium efflux. Ready-Protein (Beckman) was added to the vials and the radioactivity measured in a scintillation counter (Beckman LS 2800).

Two or three electrical stimulation periods (S_1 , S_2 and S_3) were applied to the tissues at 30 min intervals. To determine the effect of NPY on tritium release, 0.1 μM , and in some experiments 0.3 μM , of the peptide was added to the superfusate 8 min before S_2 and S_3 respectively. Cocaine (10 μM) and normetanephrine (10 μM) were added to the superfusion fluid after the incubation period to block neuronal and extraneuronal uptake of [^3H]-NA. To ensure the ability of our system to detect changes in overflow during stimulation, in a few experiments, tetrodotoxin (TTX, 1 μM) was added to the superfusate 15 min before S_2 .

The stimulation-evoked tritium release was calculated by subtraction of basal tritium release from the electrical stimulated evoked release. Thereafter, the ratios S_2/S_1 and S_3/S_1 were calculated in order to eliminate differences between tissues. These ratios were not significantly different. The effects of NPY on stimulated efflux were analysed by calculating their effects on these ratios.

Drugs

The following drugs were used: L-arginine (Sigma), cocaine hydrochloride (Depósito de estupefacientes, Ministerio de Sanidad y Consumo), desmethylinipramine hydrochloride (Sigma), noradrenaline hydrochloride (Sigma), prazosin hydrochloride (Fides), L-N^G-nitroarginine (Carl Biochem), normetanephrine hydrochloride (Sigma), neuropeptide Y (Cambridge Research Biochemical), propranolol hydrochloride (Sigma), sodium nitroprusside (Sigma), tetrodotoxin (Sigma), tyramine hydrochloride (Fluka).

All drugs were dissolved in sterile saline with the exception of NPY which was dissolved in BSA (0.1% w/v), aliquoted and stored at -70°C in silicone coated tubes.

Analysis of results

Results are expressed as mean $\pm$ s.e. pD_2 values for NA were calculated as the negative logarithm of the EC_{50} , i.e. the concentration that produced 50% of its maximal effect. Data were analysed by a paired or unpaired Student's t test.

Results

Effect of neuropeptide Y on motor responses induced by electrical stimulation, noradrenaline (NA) or tyramine

As shown in Figure 1, field stimulation of the rat anococcygeus muscle induced a frequency-dependent contraction. These contractions were markedly potentiated by 0.1 μM (Figure 1b) and 0.3 μM (Figure 1c) NPY. The vehicle used to dissolve the peptide was without effect on the electrically-stimulated contractions (Figure 1a). The potentiation by NPY of electrically-induced contractions was both frequency-

Figure 1 Frequency-response curves for the contractile responses of rat anococcygeus muscles induced by field stimulation (0.25–5 Hz, 0.5 ms, supramax V, 10 s every 3 min) in the absence (○) or presence (●) of (a) 0.1% BSA; (b) 0.1 μ M neuropeptide Y (NPY) and (c) 0.3 μ M NPY. Results are expressed as a percentage of the contraction induced by 5 Hz before treatment. Each point is the mean of 6–8 experiments. The vertical bars show s.e.mean. ** $P < 0.01$; * $P < 0.05$ by Student's *t* test for paired observations

and concentration-dependent being statistically significant at all the frequencies studied. The presence of prazosin (0.1 μ M) completely abolished the frequency-response curves in the presence of 0.3 μ M NPY (results not shown). In three experiments in which DMI (0.1 μ M) was present in the Krebs solution, the potentiation of field stimulation was the same in the presence or absence of NPY (results not shown). In contrast to the effects of NPY on responses to electrical stimulation, the peptide (0.1–0.3 μ M) produced a significant potentiation effect on low concentrations of exogenous NA (0.003–0.01 μ M) in a non-concentration-dependent manner (Figure 2a) without modifying the pD_2 values (control: 7.86 ± 0.46 ; 0.1 μ M NPY: 7.7 ± 0.34 ; 0.3 μ M NPY: 7.96 ± 0.37). Since lower concentrations of NPY (0.01–0.03 μ M) were without effect on NA-induced contractile responses (results not shown), a concentration-range of 0.1–0.3 μ M of

Figure 2 Concentration-response curve for the contractile effect of (a) noradrenaline and (b) tyramine in the absence (○) or presence of 0.1 μ M neuropeptide Y (NPY) (●) or 0.3 μ M NPY (□). Results are expressed as a percentage of the maximum contraction to NA. Each point is the mean of 6–8 experiments. The vertical bars show s.e.mean. ** $P < 0.01$, * $P < 0.05$ by Student's *t* test for unpaired observations.

NPY was selected to elucidate the differences on responses induced by NA exogenously applied or endogenously released. In addition, the responses induced by tyramine (1–300 μ M) were not affected by 0.1 and 0.3 μ M NPY (Figure 2b). The concentration of the peptide used (0.1 and 0.3 μ M) had only a slight contractile effect in some but not all preparations (0.1 μ M NPY: $6.1 \pm 0.34\%$, $n = 3$; 0.3 μ M NPY: $11.2 \pm 1.5\%$, $n = 5$; % relative to maximum contraction of NA).

Effect of neuropeptide Y on tritium overflow

Since NPY seemed to potentiate preferentially responses to nerve stimulation, we tested whether NPY could influence the release of NA. Electrical stimulation of the anococcygeus muscle with 2 and 4 Hz produced a rise in tritium release (Figure 3). The basal tritium efflux from anococcygeus muscle was not modified by NPY at concentrations of 0.1 and 0.3 μ M.

The electrical stimulation of the muscle produced a marked rise over the basal level in tritium release. At 2 Hz the tritium overflow in S_2 and S_3 was less than that observed in S_1 but the ratios S_2/S_1 and S_3/S_1 were not significantly different ($S_2/S_1 = 0.795 \pm 0.024$; $S_3/S_1 = 0.777 \pm 0.038$). However, at 4 Hz a small decrease in the release of tritium was observed in the third period of stimulation ($S_2/S_1 = 0.80 \pm 0.06$; $S_3/S_1 = 0.56 \pm 0.08$). The addition of NPY (0.1 and 0.3 μ M) to the superfusate 8 min before the electrical stimulation (2 Hz) was applied produced a slight increase in tritium overflow (Figure 3a) that did not reach a statistical significance. The release of tritium induced by stimulation at 4 Hz was also unaffected by 0.1 and 0.3 μ M NPY (Figure 3b).

Responses induced by 2 Hz (S_2/S_1 : 0.82 ± 0.14 , $n = 4$) were dramatically reduced by 1 μ M TTX (S_2/S_1 : 0.1 ± 0.06 , $n = 4$).

Figure 3 Effects of neuropeptide Y (NPY) on the overflow of tritium from the superfused rat anococcygeus muscle induced by electrical stimulation at (a) 2 Hz and (b) 4 Hz. The open columns represent control, the hatched columns in presence of 0.1 μ M NPY and the cross-hatched columns in presence of 0.3 μ M NPY. The number of experiments is indicated in parentheses above the columns; vertical bars show s.e.mean. The muscles had been loaded with [3 H]-NA (see Methods).

Effect of neuropeptide Y on electrical stimulation and nitrovasodilator-induced relaxations

Field stimulation of the rat anococcygeus muscle elicited frequency-response NANC relaxations of guanethidine-induced tone (Figure 4a). NPY (0.1 μ M) produced a small but not significant increase in tone. However, the tone after 0.3 μ M NPY (6.8 ± 0.29 g) was significantly greater ($P < 0.05$ by paired Student's t test) from that observed in nontreated preparations (6.3 ± 0.3 g). The stimulation-induced relaxations were reduced by 0.1–0.3 μ M NPY at all the frequencies studied (Figure 4b, c). Nevertheless, differences observed at 1–5 Hz (0.1 μ M NPY) and 1 Hz (0.3 μ M NPY) did not reach statistical significance. The inhibitory effect of NPY on stimulation-induced NANC relaxations was concentration-dependent (Figure 4b, c).

Cumulative addition of SNP (0.01–10 μ M) produced concentration-related relaxations of guanethidine-induced tone (Figure 5). NPY (0.1 μ M) and (0.3 μ M) reduced the responses to low concentrations of SNP (0.01–1 μ M) without modifying the responses to higher concentrations of the nitrovasodilator (Figure 5c, d). The vehicle used to dissolve the peptide was without effect on SNP-induced relaxation (Figure 5a) of guanethidine-induced tone.

Effect of L-N^G-nitroarginine on motor responses in the absence and presence of neuropeptide

The contractile responses of the anococcygeus elicited by field stimulation (0.25–1 Hz) in the absence of guanethidine were potentiated ($P < 0.01$) by L-NOARG (30 μ M; Figure 6a). However, the potentiation observed with higher frequencies (2.5–5 Hz) of stimulation was not statistically significant. L-Arginine (300 μ M) failed to reverse the NPY-potentiated motor responses induced by electrical stimulation (results not

Figure 4 Frequency-response curves to field stimulation of NANC nerves at frequencies between 0.25 to 5 Hz for 10 s. Tone was induced by guanethidine (30 μ M). Control responses (○) and responses in the presence (●) of (a) 0.1% BSA; (b) 0.1 μ M neuropeptide Y (NPY) and (c) 0.3 μ M NPY. Results are calculated as a percentage of the tone existing before stimulation. Each point is the mean of 6 experiments. The vertical bars show s.e.mean. ** $P < 0.01$; * $P < 0.05$ by Student's t test for paired observations.

presented). Exposure to NPY (0.1 μ M) for 6 min did not modify the electrically-stimulated motor responses in presence of L-NOARG (30 μ M) at the lowest frequencies (0.25–0.5 Hz, Figure 6b). There was a trend to potentiate the responses obtained at higher frequencies (1–5 Hz) of stimulation that did not reach statistical significance (Figure 6b).

Comparative effects of neuropeptide Y and L-N^G-nitroarginine on NANC and noradrenergic responses

The degrees of inhibition and potentiation of stimulation (0.5–2.5 Hz)-induced noradrenergic and NANC responses by NPY (0.1–0.3 μ M) or L-NOARG (3–10 μ M) are shown in Figure 7.

A clear relationship between inhibition of NANC responses and potentiation of motor responses seems to exist for NPY (Figure 7a, c) but not for L-NOARG (Figure 7b, d) at 0.5 Hz. However, with a higher frequency of stimulation (2.5 Hz) the increase in potentiation induced by both com-

Figure 5 Concentration-response curve to sodium nitroprusside. Tone was induced by guanethidine ($30 \mu\text{M}$). Control responses (O) and responses in the presence (●) of (a) 0.1% BSA, (b) $0.1 \mu\text{M}$ neuropeptide Y (NPY) and (c) $0.3 \mu\text{M}$ NPY. Results are calculated as a percentage of the tone existing before adding the nitrovasodilator. Each point is the mean of 6 experiments. The vertical bars show s.e.mean. ** $P < 0.01$, * $P < 0.05$ by Student's *t* test for paired observations.

pounds was not parallel to the degree of inhibition achieved (Figure 7).

Discussion

This study, though indirect, suggests that the greater potentiation of field stimulated motor responses by NPY compared with those induced by NA in the rat anococcygeus muscle is mainly due to the blockade of NANC relaxation responses induced by electrical stimulation.

It has been demonstrated that subthreshold concentrations of NPY markedly enhanced the responses to NA and to electrical stimulation in different vascular preparations (Eklblad *et al.*, 1984; Lundberg *et al.*, 1985; Pernow *et al.*, 1986; Edvinsson *et al.*, 1989). Most of these studies seem to indicate that the potentiation of the electrical stimulation induced responses is due to a postjunctional effect, mainly via α_1 -adrenoceptors. Nevertheless, in other blood vessels, subthreshold concentrations of NPY enhanced the stimulation-induced responses, but failed to modify the responses to

Figure 6 Frequency-response curve for the contractile responses of the anococcygeus muscle to field stimulation (a) in presence of saline (O) or $30 \mu\text{M}$ L- N^G -nitroarginine (L-NOARG, ●), (b) in presence of $30 \mu\text{M}$ L-NOARG (□) or $30 \mu\text{M}$ L-NOARG plus $0.1 \mu\text{M}$ neuropeptide Y (■). Results are expressed as a percentage of the response obtained with the highest frequency of stimulation in the first curve. Each point is the mean of 6 experiments. The vertical bars show s.e.mean. ** $P < 0.01$; * $P < 0.05$ by Student's *t* test for paired (a) or unpaired (b) observations.

exogenous NA (Hanko *et al.*, 1986; Cheung, 1991). On rat vas deferens, a lack of effect of NPY on the responses to exogenous NA has also been demonstrated (Lundberg *et al.*, 1982).

Our results in the anococcygeus show that NPY, at concentrations that do not contract the muscle, clearly potentiate the contractile responses induced by electrical stimulation. However, the peptide only enhanced the responses of very low doses of NA without modifying the pD_2 values. It has recently been demonstrated that NPY neuromodulates co-transmission in the guinea-pig vas deferens by inhibiting the release of ATP and NA and that these effects predominate over the postjunctional enhancement (Ellis & Burnstock, 1990). In addition, in the guinea-pig saphenous artery, NPY potentiates neurally-induced contraction by a specific action on the purinergic response without modifying the noradrenergic contractile response (Cheung, 1991). Furthermore, DMI failed to modify the NPY potentiation of motor responses to electrical stimulation. These results are in good agreement with findings on rabbit femoral (Wahlestedt *et al.*, 1985) and rat tail arteries (Vu *et al.*, 1989) where cocaine did not influence the enhancement by NPY of transmural nerve stimulation responses, indicating that the mechanism responsible for the potentiation effect of NPY is other than a blockade of neuronal uptake.

The fact that NPY failed to modify the contractile responses to tyramine, a drug that releases NA by a mechanism different (Thoa *et al.*, 1975; Marin & Sanchez, 1980) from nerve stimulation, give further support to a hypothetical mechanism for the potentiation effect of NPY linked to nerve stimulation rather than a postjunctional enhancement of NA responses via α_1 -adrenoceptors.

In our experiments the possibility that NPY could facilitate the release of NA via a prejunctional mechanism could

Figure 7 Percentage inhibition of NANC responses (upper panel) and potentiation of motor responses (lower panel) to 0.5 and 2.5 Hz by neuropeptide Y (NPY, a,c) and L-N^G-nitroarginine (L-NOARG, c,d). Results are expressed, for each frequency, as a percentage of their own control response. Each point is the mean of 6 experiments. The vertical bars show s.e.mean. NPY (0.1 μ M) and L-NOARG (3 μ M) (open columns); NPY (0.3 μ M) and L-NOARG (10 μ M) (hatched columns).

be excluded because NPY failed to modify the basal as well as the stimulation-induced tritium release at 2 and 4 Hz. These results are consistent with those obtained on the rat tail artery (Vu *et al.*, 1989) where NPY produced a greater potentiation on nerve stimulation-induced responses than on NA-induced vasoconstriction without altering the release of tritium. Moreover, NPY decreases the release of tritium and the contractions of rat (Lundberg & Stjärne, 1984) and guinea-pig (Ellis & Burnstock, 1990) vas deferens. The fact that TTX, a known blocker of nerve activity, almost abolished the tritium overflow, indicated that our system is able to detect changes during electrical stimulation, results that correlate with previous studies in blood vessels (Balfagón & Marin, 1989).

The relaxation responses induced by electrical stimulation were decreased by NPY. The substance released by electrical stimulation of NANC nerves in the rat and mouse anococcygeus seems to be NO (Gillespie & Sheng, 1990; Gibson *et al.*, 1990). The endothelium derived relaxing factor (EDRF), a powerful smooth muscle relaxant is almost certainly NO

and its precursor is L-arginine (Palmer *et al.*, 1987). It has been clearly demonstrated that L-N^G-monomethyl arginine (L-NMMA), a competitive inhibitor of L-arginine that blocks the synthesis of NO, attenuates the NANC responses in the rat and mouse anococcygeus (Gillespie *et al.*, 1989; Gibson *et al.*, 1990) and increases the motor responses in rat anococcygeus and bovine retractor penis (Li & Rand, 1989; Liu *et al.*, 1990). In addition, L-NOARG a more potent inhibitor of EDRF (Moore *et al.*, 1990), completely inhibits the NANC responses and potentiates the motor responses of mouse anococcygeus (Gibson *et al.*, 1990). The effects of L-NMMA and L-NOARG on the relaxation and contraction of rat anococcygeus and bovine retractor penis are reversed by L-arginine (Liu *et al.*, 1991). We have observed an increase of stimulation induced motor responses in the rat anococcygeus by L-NOARG, results that are consistent with studies on mouse anococcygeus (Gibson *et al.*, 1990) and bovine retractor penis (Liu *et al.*, 1991) and that suggest that the potentiation of stimulation-induced motor responses in the rat anococcygeus results from the removal of NANC relaxations during electrical stimulation. Since NPY inhibits NANC responses and potentiates the motor responses induced by electrical stimulation, it is quite possible that the differences between stimulation-induced motor responses and those induced by exogenous NA in the presence of NPY could be explained by a reduction of NANC responses by the peptide. We have also tested whether the peptide was still able to potentiate the stimulation-induced motor responses in presence of L-NOARG. Results obtained with NPY in the absence or presence of L-NOARG were similar at all frequencies of stimulation. Nevertheless, at 1–5 Hz, NPY was still able to increase further the motor responses in the presence of L-NOARG though the effect did not reach statistical significance. These results suggest that an identical mechanism of action for the decrease of NANC responses in rat anococcygeus may not be shared by NPY and L-NOARG. To test this hypothesis we studied whether L-arginine could reverse the potentiation effect of NPY on motor responses. In our hands, L-arginine was unable to modify the NPY-induced potentiation of contractile responses, giving further support to the idea that the mechanism of action of the peptide is different from that of L-NOARG and suggesting that NPY is not acting prejunctionally, as an inhibitor of NO synthesis, but rather acts postjunctionally decreasing the NANC responses. If the latter is true, relaxations induced by a nitrovasodilator drug should also be reduced by NPY. In fact, relaxations induced by low concentrations of SNP were inhibited by the peptide, results that support the postjunctional effect of NPY. Nevertheless, when the degree of potentiation is compared to the degree of inhibition for NPY and L-NOARG, the relationship is not straightforward. At 0.5 Hz, the greater the inhibition of NANC responses by NPY the greater is the potentiation of motor responses but this parallelism is lost for L-NOARG (0.5 Hz) and for both compounds at a higher frequency (2.5 Hz) of stimulation. The degree of potentiation observed for L-NOARG and NPY at 2.5 Hz was similar but the decrease of the inhibitory responses produced by L-NOARG was more pronounced. The results obtained with the NO synthase inhibitor correlate with those observed in the mouse anococcygeus muscles, where a concentration of L-NOARG that almost abolished NANC responses has been seen to produce an increase of around 140% of the control response (Gibson *et al.*, 1990). Taken together, these results suggest that NPY at concentrations devoid of contractile effect preferentially potentiates the motor responses of the rat anococcygeus muscle probably as a consequence of the decrease of NANC relaxing responses. Nevertheless, since the effects on NANC responses are rather small, we cannot exclude the possibility that NPY could also act by increasing the release of a cotransmitter which does not contract, by itself, the anococcygeus muscle but potentiates the actions of NA. Moreover, it is also possible that in addition to the postsynaptic effect observed with SNP, the

peptide could also be acting presynaptically on the NANC nerves to inhibit the release mechanism. Further studies should be pursued in order to clarify the mechanism of action of NPY on rat anococcygeus muscle.

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