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Neuropeptide Y in rat detrusor and its effect on nerve-mediated and acetylcholine-evoked contractions

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1 Immunohistochemical and isolated organ bath techniques were used to detect the presence of neuropeptide Y (NPY) in the rat urinary bladder and to determine its effect on tone, spontaneous activity and contractile responses of the detrusor muscle to electrical field stimulation, acetylcholine and α,β-methylene ATP (α,β-MeATP).

2 A very rich presence of NPY-immunoreactive nerve fibres was found mainly within the bundles of detrusor muscle cells. Chronic treatment with 6-hydroxydopamine did not affect the density of NPY-positive nerve fibres.

3 NPY (>1 nM) enhanced the force and frequency of spontaneous contractions and generated a rise in the resting tone of the detrusor. These effects of NPY on the tone and the spontaneous activity remained unaffected by atropine (3 μM), indomethacin (10 μM) and aspirin (100 μM) but were abolished by Ca^{2+}-withdrawal from the bathing medium.

4 The enhancing effects of NPY on the spontaneous contractions and the resting tone were not prevented by the induction of purinoceptor desensitization.

5 NPY (1–250 nM) potentiated electrical field stimulation (EFS, 1–64 Hz, 0.1 ms pulses duration, 10s train duration)-evoked, tetrodotoxin (0.5 μM)-sensitive contractions. The atropine (3 μM)-resistant component of EFS-evoked contractions was also potentiated by NPY. By contrast, the nifedipine (1 μM)-resistant but atropine-sensitive component of EFS-evoked contraction was inhibited by NPY.

6 NPY (250 nM) did not affect acetylcholine-evoked contractions, but potentiated α,β-MeATP-evoked contractions.

7 It is concluded that NPY-innervation of rat urinary bladder is largely confined to the detrusor muscle and is abundant and mainly non-adrenergic. It is further concluded that the enhancing effect of NPY on detrusor spontaneous activity and tone is caused by Ca^{2+} influx through nifedipine-sensitive Ca^{2+} channels and is not mediated through acetylcholine or cyclo-oxygenase-sensitive eicosanoids or ATP.

8 The results are consistent with the hypothesis that intrinsic NPY in the rat detrusor innervation contributes to the motor transmission in two ways: by promoting non-cholinergic motor transmission and by inhibiting prejunctionally the cholinergic transmission.

Keywords: Detrusor muscle; urinary bladder; neuropeptide Y; cholinergic neurotransmission; non-cholinergic neurotransmission; nifedipine

Introduction

Neuropeptide Y (NPY), a 36 amino acid peptide is present in the autonomic nerve supply of many organs including the urinary bladder of man and rat (Gu *et al.*, 1984; Mattiasson *et al.*, 1985). NPY-containing nerve fibres originate mainly in the non-adrenergic cell bodies of the pelvic ganglia and are richly distributed in the detrusor muscle (Mattiasson *et al.*, 1985). The physiological role of NPY-containing fibres in the detrusor and the potential pharmacological significance due to their presence is not known. The present investigation was undertaken with the object of firstly confirming its presence in rat detrusor and secondly to determine its effect on detrusor tone, contractility and spontaneous mechanical activity. A preliminary account of some of the results reported here has been presented at a meeting of the physiological Society (Iravani & Zar, 1988).

Methods

All experiments were performed on isolated strip preparations of rat detrusor prepared from the urinary bladder of adult male Wistar rats (200–250 g). The strips were prepared according to the method previously described by Zar *et al.* (1990). Rats were killed by concussion and decapitation. The lower abdomen was opened and the bladder was exposed. The bladder was held at its apex, slightly stretched and the investing layers of serosal coat, connective tissue and accompanying blood vessels were cut away as close as possible from the outer surface of the bladder wall. The bladder was excised by a cut above the trigone; any residual urine was absorbed on filter paper and the bladder was washed in a Petri dish with several changes of Krebs-Henseleit solution. The bladder was then opened by two lateral incisions and unfolded to give a rectangular sheet of tissue approximately 15 mm long and 6 mm wide. The unfolded tissue was laid on Krebs-soaked filter paper and the mucosal layer was carefully separated and removed. Strips of bladder, 10–15 mm long and 2 mm wide were then cut with the aid of a pair of fine scissors.

Detrusor strips were either processed for detection of NPY-immunoreactivity or set up in an organ bath for investigation of the effect of exogenously applied NPY on the spontaneous contractions or contractions evoked by electrical pulses and by acetylcholine (ACh).

Immunohistochemistry

For immunohistochemical detection of NPY-containing nerve fibres, small pieces of the detrusor strip (not larger

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than 2 × 2 × 2 mm) were cleaned of connective tissue. The strips were fixed by immersion in 0.01 M phosphate buffered saline (PBS) containing 0.4% *p*-benzoquinone for 2 h at 4°C. Following washing in several changes of PBS containing 10% sucrose for 24 h, strips were suspended in Tissue-Tec (Miles) and frozen in liquid nitrogen prior to sectioning. Cryostat sections (10 μm thick) were mounted onto poly-L-lysine coated slides and were processed for indirect immunofluorescence staining according to standard techniques (Polak & Van Noorden, 1986). First layer antibody was rabbit anti-NPY IgG (Peninsula Europe Ltd.) and it was used at a dilution of 1:400 with PBS containing 0.1% bovine serum albumin (BSA) and 0.01% NaN_3, incubated for 16 h at room temperature. After washing with PBS (3 × 5 min) the second layer antibody, goat anti-rabbit IgG, conjugated with fluorescein isothiocyanate (FITC) was applied at a dilution of 1:50 in PBS containing 0.1% BSA and 0.01% NaN_3 for 2 h at room temperature. Slide mounted sections were further washed in PBS (3 × 5 min) and coverslipped with phosphate buffered glycerol (Sigma) mountant. For control experiments the primary antibody was pre-absorbed with excess (10 μg) synthetic porcine NPY, before being applied as the first layer. The sections were visualised under a Leitz Ortholux II microscope with excitation filter KP 490, and TK 510 dichroic mirror and a barrier filter K 515. Photomicrographs were taken with Kodak T-MAX p3200.

6-Hydroxydopamine pretreatment

Four animals were pretreated with 6-hydroxydopamine (6-OHDA). Four doses of 50 mg kg^{-1} were injected i.p. on days 1, 2, 4 and 6. The animals were killed one week after the last injection.

Organ bath study

The detrusor strip preparation was suspended in a 1 ml organ bath between built-in vertical platinum electrodes, at a resting tension of 0.5 g force, at 37°C in Krebs-Henseleit solution (composition mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11) gassed with 95% O_2 and 5% CO_2. The resting tension was kept constant at 0.5 g force throughout the experiment by appropriate adjustments whenever needed (except after application of drugs to the organ bath). An equilibration period of at least 30 min was allowed before starting each experiment; during this period the preparation was repeatedly washed with Krebs-Henseleit solution. For recording tension of the detrusor muscle, the preparation was connected to an isometric transducer and the changes in the tension were recorded on either a potentiometric chart recorder or a storage oscilloscope.

Spontaneous rhythmic activity of the detrusor strip was assessed by calculating the amplitude and frequency of spontaneous contractions. The amplitude was estimated by adding together the tension of individual contractions occurring during a period of 1 min, and dividing this sum by the number of contractions during this period. The value for the frequency of spontaneous activity (rate min^{-1}) was given by the total number of contractions occurring during this period.

Desensitization to the contractile action of α-β-methylene ATP (α,β-MeATP) was obatined by exposing the detrusor preparation, pretreated with indomethacin (10 μM) to α,β-MeATP (10 μM) and repeating this dose twice at 10 min intervals without washing out the drug present in the bath from preceding doses. Exposure to the initial application of α,β-MeATP caused a large contraction which subsided fully in about 5 min and subsequent applications of the drug did not evoke any contractile response.

When desired, the detrusor preparation was contracted either directly by addition of ACh or indirectly by electrical field stimulation (EFS) delivered through a Grass S-88 electronic stimulator and 2 platinum electrodes lying on either side of the muscle in the organ bath. In preliminary experiments, it was established that trains of pulses at 1–64 Hz and pulse duration of 0.1 ms at supramaximal voltage (80–90 V) produced motor responses which were fully abolished by tetrodotoxin (TTX) 0.5 μM; such responses were therefore deemed to be fully neurogenic. Further experimental details are provided in the appropriate results section.

Drugs

The drugs used and their sources were: acetylcholine chloride, acetyl salicylic acid (aspirin), atropine sulphate, 6-hydroxydopamine hydrochloride, indomethacin and α,β-methylene ATP (Sigma); tetrodotoxin (Sankyo); nifedipine (Bayer); neuropeptide Y (Cambridge Research Biochemicals). Solutions of drugs were made fresh on the day of their use except tetrodotoxin which was stored in form of stock solution, frozen at −20°C. All drugs were dissolved in distilled water except indomethacin and nifedipine which were dissolved in absolute alcohol to make solutions of 10^{-2} M and 10^{-3} M strengths respectively. NPY was first dissolved in distilled water containing 200 μg ml^{-1} bovine serum albumin (Sigma) aliquoted out into smaller volumes and then freeze dried and stored at −20°C until the day of experiment, when the aliquot was reconstituted with distilled water. All experiments involving the use of nifedipine were carried out in a laboratory illuminated solely with sodium light.

Statistics

All values are expressed as mean ± s.e. mean and the differences between means were calculated by Student's *t* test. Values of $P<0.05$ were considered as statistically significant.

Results

Immunohistochemistry

A very rich presence of NPY-immunoreactive nerve fibres were detected in the sections of the urinary bladder. Most of these fibres were seen within the bundles of detrusor muscle cells (Figure 1) and gave distinct impression of being varicose. Sub-epithelial layers of the bladder was virtually devoid of NPY-immunoreactive fibres and only an occasional NPY-immunoreactive fibre could be seen in this layer (Figure 1). 6-OHDA pretreatment had no apparent effect on the intensity of NPY-immunofluoresence or the number of NPY-positive nerve fibres, in the detrusor.

Organ bath studies

Spontaneous activity Isolated strips of rat detrusor exhibited spontaneous rhythmic activity. The rate and amplitude of the spontaneous activity varied from one preparation to another and even in different strips from the same bladder. Concentrations of NPY in excess of 1 nM produced an increase in both the rate and the amplitude of spontaneous contractions. The facilitating effect of NPY (100 nM) on spontaneous contractions is shown in a typical experiment in Figure 2a, and the results from 5 such experiments are summarised in Figure 2b. The effect of NPY was more marked on the amplitude compared to the frequency of contraction. Thus during the final 60 s of a 10 min exposure to NPY (100 nM), compared with the control values for 60 s immediately before NPY exposure the amplitude was increased by 220 ± 42% (mean ± s.e. mean; $n = 5$; $P<0.05$) while the rate increased by 37 ± 15% (mean ± s.e. mean; $n = 5$; $P<0.05$).

In order to ascertain whether the potentiating effect of NPY on spontaneous activity was mediated through prostaglandins, some experiments were conducted in the presence of indomethacin, or aspirin. Indothemacin (10 μM) caused a

gradual inhibition of spontaneous activity almost to the point of extinction (Figure 3a). Addition of NPY (50–100 nM) led to an almost immediate appearance of spontaneous activity in the indomethacin-treated preparations, suggesting that prostaglandins were not the mediator of NPY effect on spontaneous activity. In 3 experiments, aspirin (100 μM), an irreversible cyclo-oxygenase inhibitor was substituted for indomethacin. The results with aspirin were indistinguishable from those using indomethacin. Aspirin (100 μM) was effective in abolishing the spontaneous activity but it did not prevent the initiation of spontaneous activity by NPY (100 nM).

We have also examined the effect of NPY on spontaneous activity after desensitization of the purinoceptors induced by α,β-MeATP. The experiments were performed in the presence of indomethacin (10 μM). It was invariably noted that the spontaneous activity, which had been abolished by indomethacin (10 μM), reappeared in a distinctly potentiated form after induction of purinoceptor desensitization by α,β-MeATP. Nevertheless, addition of NPY (100 nM) to the 'purinoceptor-desensitized' preparation induced a further increase in both amplitude and rate of spontaneous contractions (mean % increase ± s.e. mean: amplitude = 78 ± 12; rate = 25 ± 4; $n = 3$; $P < 0.05$).

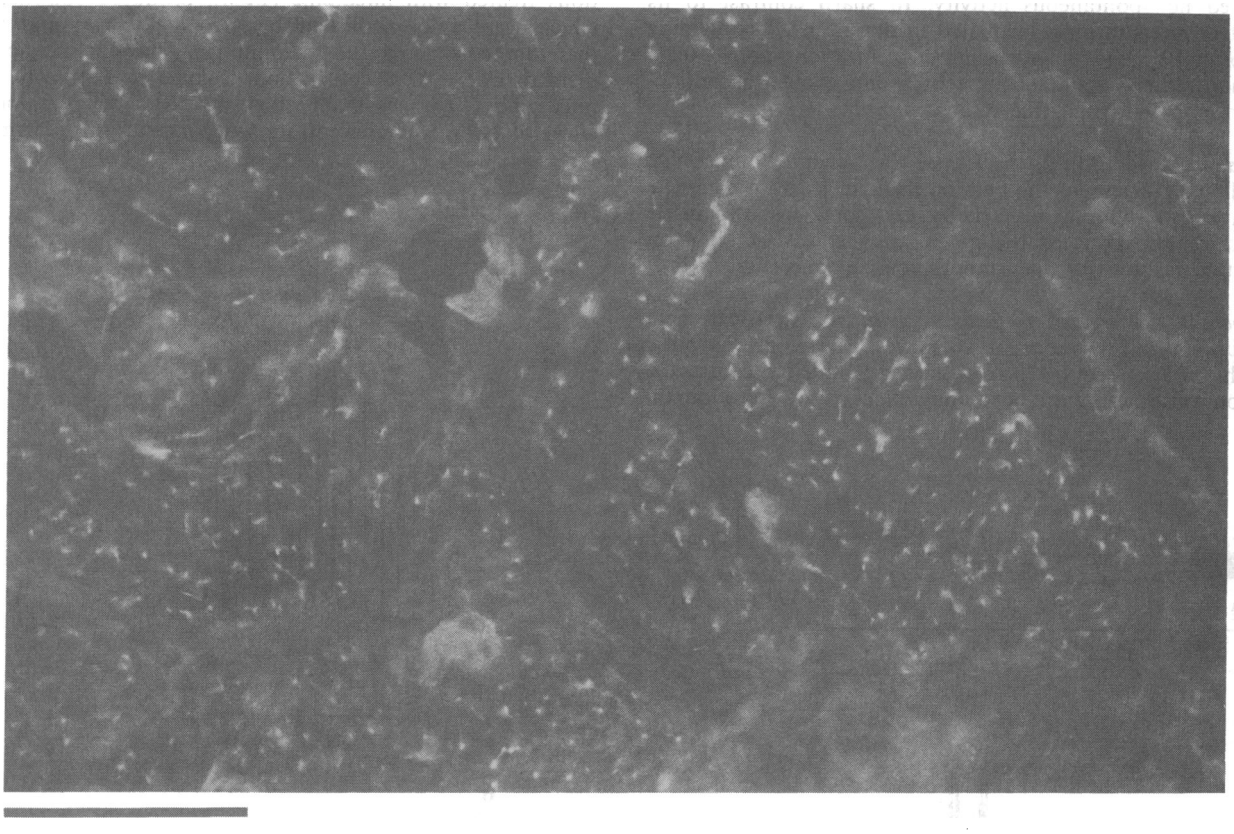

Figure 1 Neuropeptide Y (NPY) – immunofluorescence micrograph of the rat urinary bladder. NPY-immunoreactive nerve fibres and nerve endings are numerous and are mainly concentrated in the bundles of detrusor muscle cells. Scale bar = 50 μm.

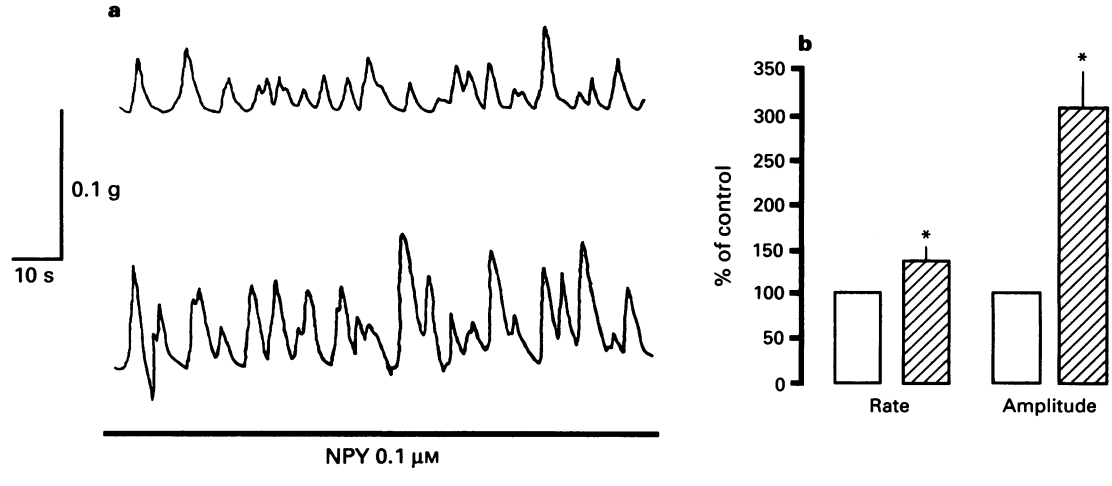

Figure 2 Effect of neuropeptide Y (NPY) on spontaneous activity of rat isolated detrusor muscle strip. (a) Record of spontaneous activity of a strip in the absence of NPY (upper panel) and after 10 min exposure to NPY 100 nM (lower panel). NPY potentiated both amplitude and frequency of spontaneous contractions. In (b) spontaneous activity is presented as histograms (open column = control; hatched column = NPY-treated) and quantified as % of control spontaneous activity prior to NPY exposure (mean ± s.e. mean; $*P < 0.05$; $n = 5$). The vertical scale shows tension in g force. The horizontal scale shows time.

Detrusor tone Addition of NPY to the bathing fluid caused a sustained rise in the smooth muscle tone accompanied with enhanced spontaneous activity. Elevation of tone by NPY was quantified by calculating the difference between stable resting tension before application of NPY and the maximum tension obtained after addition of NPY. Treatment with indomethacin (10 μM) alone (Figure 3b) or in combination with atropine (3 μM) did not prevent this effect of NPY. Tension generated by NPY 250 nM, in the presence and absence of indomethacin + atropine was respectively 0.23 ± 0.04 g force and 0.27 ± 0.05 g force (mean ± s.e. mean; $n = 5$, $P > 0.05$ Student's paired t test).

Absence of Ca^{2+} Detrusor incubated in Ca^{2+}-free Krebs, showed no spontaneous activity. In sharp contrast to its effect on preparations, incubated in normal Ca^{2+}-containing Krebs, NPY used in increasing concentrations upto 1.0 μM neither initiated spontaneous activity nor evoked a contractile response in Ca^{2+}-free Krebs.

Nifedipine Addition of the L-type Ca^{2+} channel antagonist, nifedipine (1.0 μM) to the bathing medium led to a complete cessation of spontaneous activity and some loss of muscle tone. It also abolished the potentiating effects of NPY on spontaneous activity and smooth muscle tone (Figure 3c).

Responses to electrical field stimulation Electrical field stimulation (EFS, trains of 20 pulses, 10 Hz, 0.1 ms pulse width, 30 V, 1 every 120 s) evoked reproducible contractile responses which could be readily blocked by tetrodotoxin (0.5 μM). NPY (0.25 μM) potentiated the contractile response to EFS. Tension generated by EFS was 1.6 ± 0.2 g force and 2.4 ± 0.4 g force (mean ± s.e. mean, $n = 7$, $P < 0.05$ Student's paired t test) respectively in the absence and presence of NPY. For studying the effect of NPY on cholinergic and non-cholinergic components of the contractile responses to EFS, each component was isolated by the use of selective blockers, atropine (3 μM) for blocking cholinergic component and nifedipine (1 μM) for blocking the non-cholinergic component (Iravani *et al.*, 1988; Bo & Burnstock, 1990; Zar *et al.*, 1990). The non-cholinergic component of the response to EFS, isolated by atropinisation was potentiated by NPY (Figure 4b). The degree of potentiation was not influenced by pulse frequency within the stimulus-trains. EFS of preparations treated with nifedipine (1 μM), produced contractions which were fully abolished by atropine (3 μM) and were therefore considered fully cholinergic. The cholinergic element in EFS-evoked contraction, isolated by treatment with nifedipine (1 μM), was inhibited by NPY. The inhibitory effect of NPY was concentration-dependent. An experiment

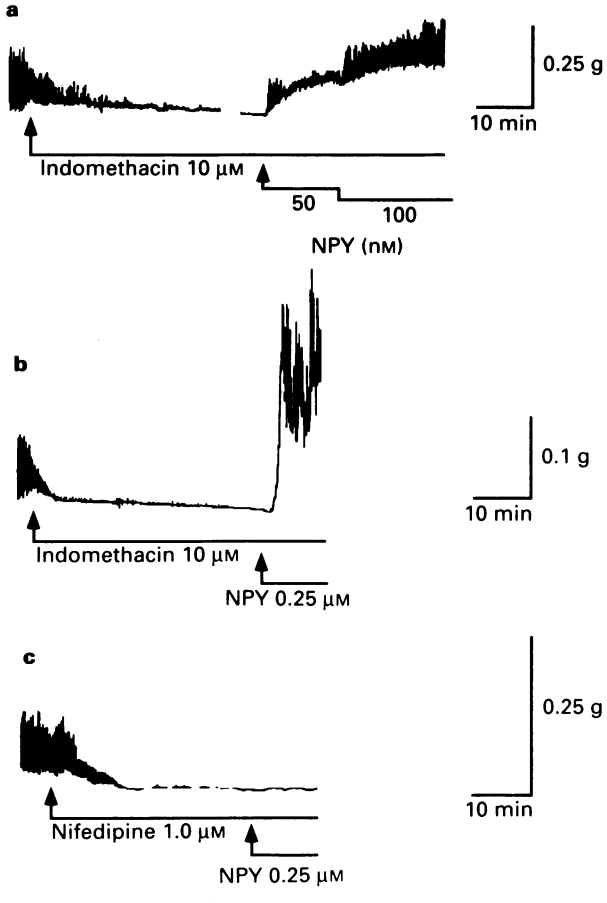

Figure 3 Rat isolated detrusor strip: The effect of indomethacin (a, b) and nifedipine (c) on the potentiation of detrusor muscle tone and spontaneous activity by neuropeptide Y (NPY). Note that although both indomethacin and nifedipine reduced or abolished spontaneous activity, only the latter prevented the effects of NPY on tone and spontaneous activity. The vertical scales show tension in g force. The horizontal scales show time.

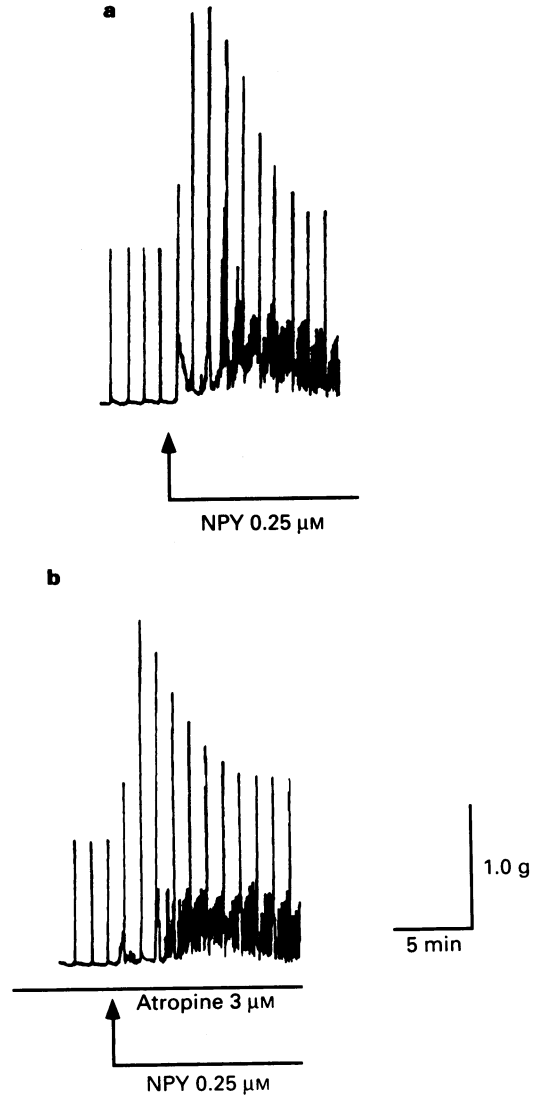

Figure 4 Rat isolated detrusor muscle strip: effect of neuropeptide Y (NPY) on contractions evoked by electrical field stimulation (EFS, train of 20 pulses, 0.1 ms pulses-duration, 2 Hz, 1 every min) in the absence (a) or presence (b) of atropine. In (b), the isolated preparation had been exposed to atropine, 3 μM for 30 min prior to the application of NPY. Note the potentiating effect of NPY on electrically-evoked contractions and its insensitivity to atropine. The vertical scale shows tension in g force. The horizontal scale shows time.

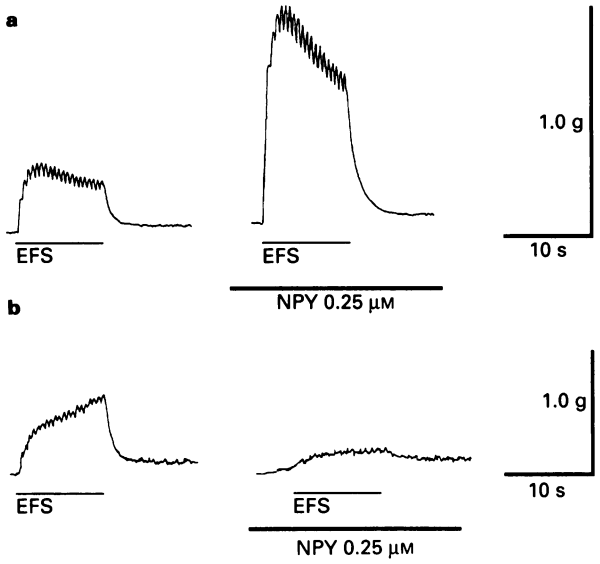

Figure 5 Rat isolated detrusor muscle strips: effect of neuropeptide Y (NPY) on contractions evoked by electrical field stimulation (EFS, train of 20 pulses at 2 Hz) and recorded on a fast time base using a storage oscilloscope. The contractions on the left in both (a) and (b) were evoked after 30 min exposure to atropine, 3 μM (a) or nifedipine, 1 μM (b) and represent stable, reproducible responses to EFS. Contractions on the right, in both panels were evoked by EFS after 5 min pretreatment with NPY (0.25 μM) in the continued presence of atropine (a) or nifedipine (b). Note the contrasting effects of NPY on electrically-evoked responses in the presence of atropine or nifedipine. The vertical scale shows tension in g force. The horizontal scale shows time.

showing the contrasting effects of NPY (0.25 μM), inhibitory on cholinergic and potentiating on non-cholinergic motor transmission is presented in Figure 5. The effect of NPY (0.25 μM) was also ascertained on cholinergic responses to different frequencies of EFS (10 s bursts of pulses at 1, 4, 8, 16, 32 and 64 Hz all at 0.1 ms pulse width). The cholinergic response to EFS, as earlier demonstrated (Zar *et al.*, 1990), was biphasic and both phases of the response were reduced by NPY (Figure 6a). The reduction was greatest at the lowest frequency (1 Hz) and declined with increasing stimulus frequency, being least at 64 Hz (Figure 7).

Acetylcholine- and α,β-MeATP-evoked contractions Submaximal contractions of the detrusor were evoked by ACh (10 μM) before and during exposure to NPY (0.5 μM). The presence of NPY had no obvious effect on the tension generated by ACh (Figure 8a). Identical results were obtained on repeating the experiment in the concurrent presence of nifedipine (1 μM) (Figure 8b).

Submaximal contractions of the detrusor were evoked by α,β-MeATP (1 μM; 45 s exposure-duration). The contractions were reproducible if 10 min or longer intervals were allowed between successive exposures. Pretreatment for 5 min with NPY (0.25 μM) potentiated the α,β-MeATP contraction by 110 ± 15% (mean ± s.e. mean; $n = 3$; $P < 0.05$).

Discussion

The present finding of a rich presence of NPY-containing fibres in rat detrusor confirms an earlier similar report by

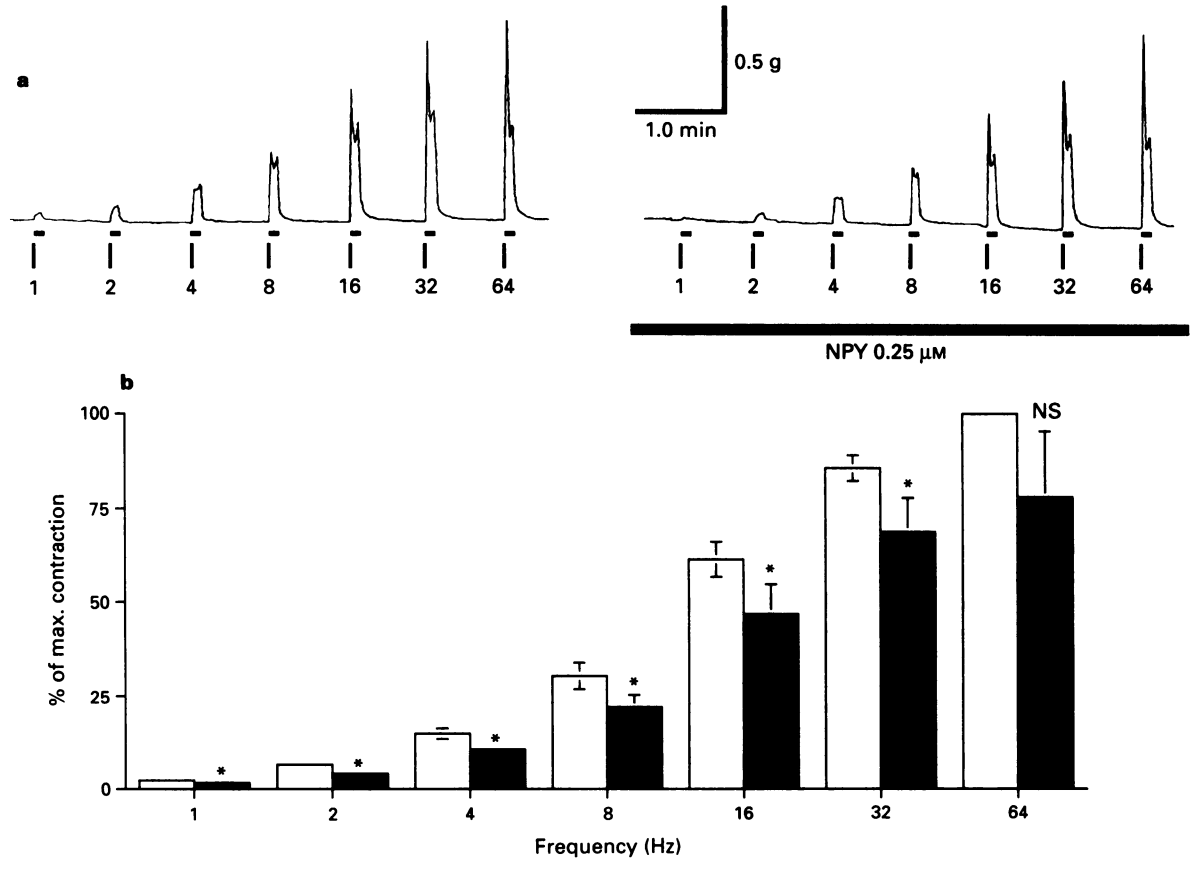

Figure 6 Rat isolated detrusor muscle: cholinergic contractile responses to different frequencies of electrical field stimulation (EFS, 1, 2, 4, 8, 16, 32 and 64 Hz; 10 s every min) in the absence (a: left panel; b: open columns) or presence of neuropeptide Y (NPY) 0.25 μM (a: right panel; b: solid columns). Bathing medium contained nifedipine (1 μM) throughout the duration of experiment. In (a), the actual contractile responses of a single experiment is shown. In (b), the contractions are expressed as a percentage of the maximal contractile response at 64 Hz in the absence of NPY (mean ± s.e. mean; $n = 7$). The response in the presence of NPY has been compared with its corresponding control response at each frequency and the level of significant difference from the control response has been expressed as $^*P < 0.05$; NS = not significant, Student's t test. The vertical scale shows tension in g force. The horizontal scale shows time.

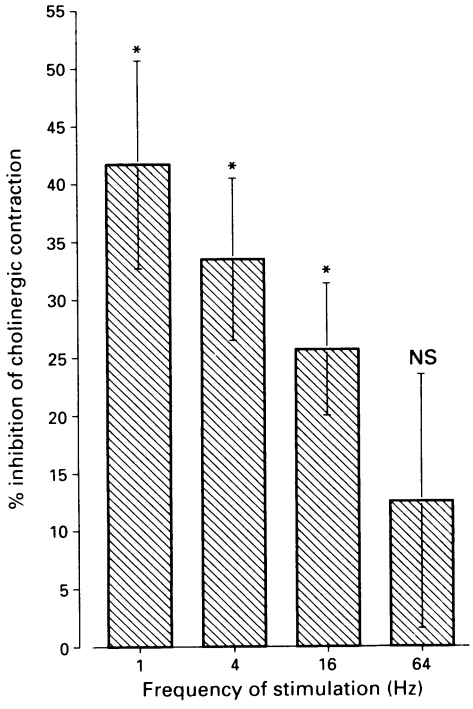

Figure 7 Rat isolated detrusor muscle preparation: histograms showing frequency-dependent inhibition of cholinergic responses to electrical field stimulation (EFS, 1, 2, 4, 16 and 64 Hz, 10 s, at 1 min intervals) by neuropeptide Y (NPY, 0.25 μM). Bathing fluid contained nifedipine (1 μM) throughout the duration of the experiment. Each histogram represents mean % inhibition of the response to EFS by NPY (0.25 μM) at a given frequency (indicated by the subscript) and the vertical bars indicate s.e. mean ($n = 7$). $^*P < 0.05$ vs. control; NS = not significant; Student's t test.

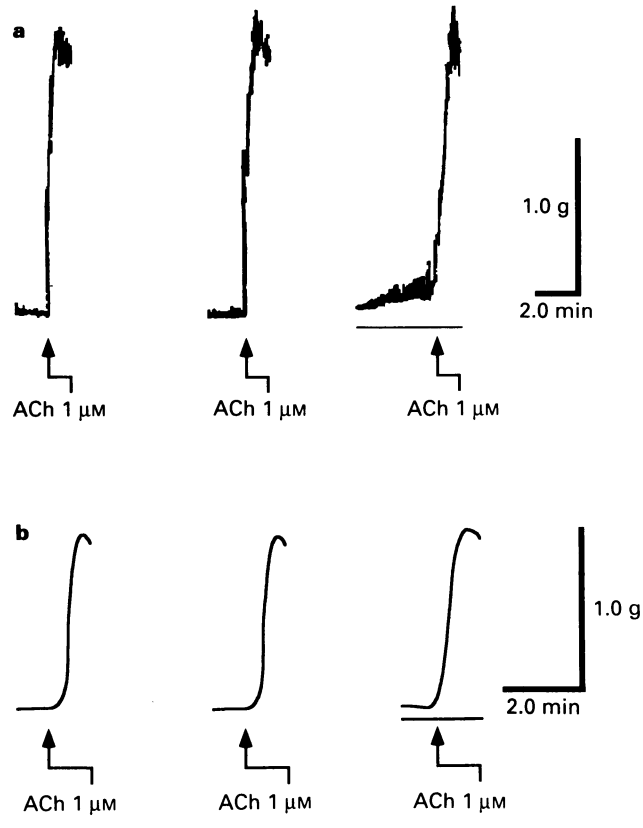

Figure 8 Effect of neuropeptide Y (NPY) on acetylcholine (ACh)-evoked contractions of rat isolated detrusor, in the absence (a) or in the presence (b) of nifedipine, 1 μM. The preparations were exposed to ACh, 1 μM (at arrows) for 1 min at 5 min interval and 4 min before the last response to ACh, NPY 0.25 μM (indicated by the thick line) was added to the bathing fluid. The vertical scale shows tension in g force. The horizontal scale shows time.

Mattiasson *et al.* (1985). In agreement with Mattiasson *et al.* (1985), we have also noticed no decrease in the number of NPY-immunoreactive fibres of rat detrusor after 6-OHDA treatment. Insensitivity of NPY-immunoreactive fibres to 6-OHDA implies that the vast majority of these fibres are not adrenergic. This conclusion is in keeping with the knowledge that in the rat bladder, the adrenergic nerve supply is concentrated in the trigone and the rest of the detrusor has a sparse adrenergic innervation (El-Badawi & Schenk, 1966; Alm & Elmer, 1975), since the combination of sparseness of adrenergic innervation and the abundance of NPY-innervation is obviously inconsistent with the presence of NA in all NPY-containing nerve fibres. The findings of Nagata *et al.* (1987) showing a failure of 6-OHDA treatment to reduce substantially the NPY content of the bladder also points towards a similar conclusion. It appears therefore that autonomic innervation of the rat detrusor is atypical, although not unique, from other peripheral organs, in having an NPY-innervation largely independent of adrenergic innervation. It remains to be established whether NPY is present in the detrusor nerve terminals on its own or in the company of some other neurotransmitters. In the mammalian detrusor, nerves containing ACh, ATP, substance P (SP), and vasoactive intestinal polypeptide (VIP) are known to be present. It is unlikely that NPY is co-localised with SP since complete disappearance of SP innervation by chronic capsaicin treatment has been reported to have no effect on NPY-innervation (Mattiasson *et al.*, 1985). Its possible co-localization with ACh, ATP or VIP remains to be investigated.

The presence of NPY-innervation in the detrusor raises the obvious question of its physiological role in this tissue. In our experiments NPY increased the level of spontaneous activity of the detrusor. In concentrations greater than 0.25 μM, it contracted the detrusor. We do not know whether the concentration of endogenously released NPY following its release reaches this level in the vicinity of its smooth muscle activating sites. Samson & Harris (1992) have calculated that a synaptic neurotransmitter concentration of 1000 μM is a reasonable estimate. If Samson & Harris's estimate of synaptic neurotransmitter concentration applies to NPY in the rat detrusor, our results indicate that NPY would be a powerful physiological contractile agent in this tissue.

It is now generally agreed that the detrusor of the rat, in common with many other mammalian species, has a dual motor transmission system comprising a cholinergic and a non-cholinergic component (Ambache & Zar, 1970; Burnstock *et al.*, 1972; Brading & Williams, 1990). In the present investigation, nifedipine a blocker of L-type Ca^{2+} channels abolished the contractile action of NPY as well as its potentiating action on spontaneous activity. It indicates that NPY, presumably through the induction of some membrane depolarization, activated voltage-sensitive Ca^{2+} channels and evoked contraction and potentiation of spontaneous activity through Ca^{2+} influx. This interpretation is supported by the loss of the NPY effect in Ca^{2+}-free medium. Nifedipine has been shown to block preferentially the non-cholinergic component of motor transmission in rat detrusor (Iravani *et al.*, 1988; Bo & Burnstock, 1990; Zar *et al.*, 1990). It has been observed that the contractions of guinea-pig and rat detrusor by ATP are also dependent upon the presence of extracellular Ca^{2+} (Iacovou *et al.*, 1988) and are antagonized by nifedipine (Katsuragi *et al.*, 1990; Bo & Burnstock, 1990). Evidently, both ATP and NPY share the property of acting through a nifedipine-sensitive, Ca^{2+}-transferring mechanism to evoke

their effects on the detrusor and it is, therefore, not surprising that NPY potentiated the contractions evoked by stimulation of purinoceptors. There is already strong evidence for ATP as the non-cholinergic motor transmitter in the rodent detrusor (Brown *et al.*, 1979; Kasakov & Burnstock, 1983; Hoyle & Burnstock, 1985; Fujii, 1988; Brading & Mostwin, 1989; Brading & Williams, 1990). On the other hand the possibility that ATP might not be the sole non-cholinergic motor transmitter is supported by partial persistence of tetrodotoxin (TTX)-sensitive contractile response after atropinisation and ATP-desensitization (Choo & Mitchelson, 1980; Luheshi & Zar, 1990; Creed *et al.*, 1991; Maggi, 1991; Parija *et al.*, 1991). The results also show that exogenous NPY potentiated the non-cholinergic motor transmission. The potentiation of non-cholinergic motor transmission by NPY appeared to be selective since it did not affect ACh-evoked contractions. Our observations that NPY contracts detrusor, potentiates non-cholinergic motor transmission and is present in abundance in the nerve-supply to the detrusor together with the finding that its effect is mediated by Ca^{2+} influx through nifedipine-sensitive channels, as is the effect of non-cholinergic motor transmitter(s), lead us to propose that NPY serves as a motor transmitter in addition to ATP in the rat detrusor. This proposal also explains why nifedipine, unlike purinoceptor-desensitization, invariably and readily blocks completely the non-cholinergic motor transmission since both ATP and NPY evoke contractions by activating voltage-operated Ca^{2+}-channels.

NPY exerted an inhibitory influence on cholinergic motor transmission. This effect of NPY is evidently prejunctional since ACh-evoked contractions were not inhibited by NPY. Prejunctional inhibition of neurotransmitter release by NPY is a very well documented phenomenon (Wahlestedt *et al.*, 1986; Edvinsson *et al.*, 1987; Westfall *et al.*, 1990; Michel, 1991) and its inhibitory effect on the cholinergic motor transmission in rat detrusor provides another example of this action.

The effect of NPY on spontaneous mechanical activity also deserves some discussion. Isolated detrusor strips from mammalian species, so far investigated, all exhibit a varying degree of spontaneous mechanical contractions (Sibley, 1984; Brading, 1987). The spontaneous activity is not inhibited by exposure to atropine, TTX and hexamethonium or by purinoceptor desensitization, giving rise to the suspicion that it is likely to be myogenic and not nerve-mediated. In our experiments, the spontaneous mechanical activity was abolished by nifedipine or withdrawal of Ca^{2+} from the bathing medium, suggesting that entry of extracellular Ca^{2+} across the cell membrane through voltage-sensitive Ca^{2+} channels is needed. NPY potentiated the frequency and amplitude of spontaneous activity through a prostaglandin- and acetylcholine-independent mechanism. The failure of NPY to initiate spontaneous activity after nifedipine-exposure or in Ca^{2+}-free medium makes it tempting to speculate that spontaneous release of intrinsic NPY from nerves may be involved in the genesis of spontaneous mechanical activity. In our experiments, the potentiation of spontaneous activity by NPY was not prevented by purinoceptor-desensitization suggesting that the effect of NPY on spontaneous activity is not mediated by ATP. An enhancing effect of α,β-MeATP on spontaneous activity in rat detrusor has been known (Luheshi & Zar, 1990). This effect of α,β-MeATP is resistant to purinoceptor-desensitization and treatment with atropine and indomethacin (Luheshi & Zar, 1990). The potentiation of spontaneous activity by NPY, also through a mechanism independent of purinoceptor-desensitization raises the possibility that the enhancing effect of α,β-MeATP on spontaneous activity might be mediated through the release of endogenous NPY.

In conclusion, the present investigation has demonstrated a rich presence of NPY-innervation in the rat urinary bladder. NPY-innervation is non-adrenergic and is almost exclusively localized within detrusor muscle bundles. The results are consistent with the proposal that NPY-containing innervation is involved in the motor transmission of the detrusor muscle, NPY acting as a non-cholinergic motor transmitter. The results also raise the possibility that spontaneous release of intrinsic NPY is responsible for spontaneous mechanical activity of the detrusor muscle.

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