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ACTIONS OF NEUROPEPTIDE Y ON INNERVATED AND DENERVATED RAT TAIL ARTERIES

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SUMMARY

1. Neuropeptide Y caused a dose-dependent contraction and depolarization of the smooth muscle of the rat tail artery.

2. 30 nM-neuropeptide Y increased the contraction caused by either nerve-released noradrenaline or smooth muscle action potentials.

3. 30 nM-neuropeptide Y did not change the amplitude or rate of rise of the smooth muscle action potential. It did not change the amplitude of small excitatory junction potentials, suggesting that it did not affect neurotransmitter release.

4. 30 nM-neuropeptide Y increased the contraction caused by exogenous noradrenaline, 5-hydroxytryptamine and K in concentrations that gave submaximal contractions. It did not affect the response to higher concentrations that gave maximal or near-maximal contractions.

INTRODUCTION

Neuropeptide Y is a polypeptide comprising thirty-six amino acids, which was first isolated from brain tissue (Tatemoto, Carlquist & Mutt, 1982) and has since been identified by immunohistochemical methods in many sympathetic nerve fibres (Uddman, Ekblad, Edvinsson, Håkanson & Sundler, 1985; Morris, Gibbins, Furness, Costa & Murphy, 1985). It is co-released with noradrenaline by the adrenal medulla (Lundberg, Fried, Pernow & Theodorsson-Norheim, 1986) and is found in high levels in the plasma of humans with phaeochromocytoma. There has been no direct measurement of its release by nerves, but there is evidence that it mediates some of the long-lasting effects of sympathetic nerves supplying the heart (Potter, 1985) and possibly the rabbit ear artery (Glover, 1985).

Neuropeptide Y has been found in all perivascular sympathetic nerves examined so far (Morris *et al.* 1985), but its effects on arterial smooth muscle vary considerably from one artery to another. In some arteries neuropeptide Y is a potent vasoconstrictor (cat pial artery: Edvinsson, Emson, McCulloch, Tatemoto & Uddman, 1984*b*; cat salivary gland arteries; Lundberg & Tatemoto, 1982; guinea-pig uterine artery; Morris *et al.* 1985; rat femoral artery: Lundberg, Pernow, Tatemoto & Dahlof, 1985) but in others it has been found to cause contraction only at high concentrations (various rabbit arteries: Edvinsson, Ekblad, Håkanson & Wahlestedt, 1984*a*; rabbit ear artery; Glover, 1985).

In all the arteries examined low concentrations of neuropeptide Y increased the contractile effect of exogenous noradrenaline or perivascular nerve stimulation.

There have been conflicting reports of the effect of neuropeptide Y on the release of noradrenaline from sympathetic nerves. Ekblad, Edvinsson, Wahlestadt, Uddman, Håkanson & Sundler (1984) reported that neuropeptide Y did not alter the release of noradrenaline from nerves on the rabbit gastro-epiploic artery, but experiments using the rat portal vein (Dahlof, Dahlof, Tatemoto & Lundberg, 1985), rat femoral artery (Lundberg *et al.* 1985), and rat vas deferens (Lundberg & Stjarne, 1984) all showed that neuropeptide Y reduced noradrenaline release from nerves.

The experiments described below were performed on the rat tail artery, using techniques that permitted the simultaneous recording of smooth muscle membrane potential and contraction (Neild & Kotecha, 1985). The aim was to investigate the effects of neuropeptide Y on the smooth muscle membrane potential, excitatory junction potentials and action potentials.

METHODS

Wistar rats of either sex and weighing 230–300 g were killed by stunning and decapitation. The central tail artery was dissected out and a piece 2–2·2 mm long was taken from a region 80–90 mm from the base of the tail. The piece of artery was mounted on an apparatus similar to that described by Mulvany & Halpern (1977) and Neild & Kotecha (1985) that permitted the simultaneous recording of smooth muscle force in the circumferential direction and smooth muscle membrane potential (constructed by Polyplan, Glen Waverley, Victoria, Australia). The artery was held on a pair of tightly stretched wires attached to plastic supports, one of which was connected to an isometric force transducer.

A pair of platinum wires were positioned one on either side of the artery and used to stimulate the perivascular nerves. The artery was superfused with a physiological saline solution containing (mM) Na, 145; K, 5; Ca, 2·5; Mg, 2; Cl, 134; HCO_3, 25; H_2PO_4, 1; dextrose 11. The solution was equilibrated with 95% O_2/5% CO_2 and warmed so that the temperature at the artery was 32 °C.

Membrane potential was recorded using glass micro-electrodes filled with 2 M-KCl and having resistances in the range 80–120 MΩ.

To denervate the tail artery rats were anaesthetized with pentobarbitone (Nembutal, 70 mg/kg I.P.) and the four major nerve trunks at the base of the tail were sectioned (Sittiracha, 1985). The animal was allowed to recover and was used for an experiment 10–25 days after the operation. Pieces of artery immediately adjacent to the piece used for the experiment were treated with glyoxylic acid to demonstrate catecholamine-containing nerves, but none were detected when all four nerves had been cut.

Cumulative dose–response data were analysed using a modification of the procedure of Nakashima, Angus & Johnston (1982). A modified logistic curve of the form

$$Y = Y_{max}/\{1+\exp(a+bX)\}^c$$

was fitted to the data using a Simplex optimization procedure (Cooper, 1981). Y is the force developed by the arterial smooth muscle, X is the logarithm of the agonist concentration, and a, b and c are constants which are determined by the Simplex algorithm to give the best least-squares fit. This equation generates a sigmoid curve that differs from the normal logistic curve in that it is not always symmetrical about its mid-point. The curve was then used to interpolate to obtain values for the ED_{10}, ED_{30}, ED_{50}, ED_{70} and ED_{90} for each experiment. The effective dose (ED) values from several arteries were averaged and used to compare the responses of arteries to the various treatments.

Drugs used were neuropeptide Y (porcine, synthetic, Sigma), noradrenaline bitartrate (Sigma), 5-hydroxytryptamine creatinine sulphate complex (5-HT; Sigma), benextramine tetrahydrochloride (Sigma), prazosin hydrochloride (Pfizer) and phentolamine mesylate (Regitine, Ciba).

In preliminary experiments it was found that the biological activity of neuropeptide Y solutions

declined rapidly with time. This appeared to be due to binding of neuropeptide Y to the wall of its container, and occurred with containers of glass, polypropylene or Teflon. The decline of neuropeptide Y activity was greatly slowed if the container was first coated by rinsing with a solution of bovine serum albumen (1 mg/ml, Fraction V, Commonwealth Serum Laboratories). When this procedure was followed neuropeptide Y solutions retained their full biological activity for at least 30 min.

In all experiments the rate of perivascular nerve stimulation was kept as low as possible to minimize any neuropeptide Y release from the nerves. In most experiments the stimulus rate was 1/min. When other workers have demonstrated effects that might have been due to nerve-released neuropeptide Y much higher rates of stimulation (at least 2 Hz for 1 min) were required (Glover, 1985; Potter, 1985).

Results are expressed as means ±S.E. of mean, with n equal to the number of arteries used. Statistical significance was assessed with Student's t test for paired or unpaired comparisons as appropriate.

RESULTS

Both normal and denervated arteries were exposed to cumulative concentrations of noradrenaline and the contractile force recorded. Denervation reduced the ED_{50} for noradrenaline from $1{\cdot}91 \pm 0{\cdot}23$ μM ($n = 7$) to 251 ± 65 nM ($n = 7$). These results indicate that the denervated arteries had developed supersensitivity to noradrenaline, as reported by previous workers (Abel, Urquilla, Goto, Westfall, Robinson & Fleming, 1981; Nasseri, Barakeh, Abel & Minneman, 1985) and confirmed that the arteries were denervated.

Denervation did not change the membrane potential of the arterial smooth muscle. The membrane potential in normal arteries was $-60{\cdot}1 \pm 0{\cdot}52$ mV ($n = 27$) and in denervated arteries was $-58{\cdot}1 \pm 0{\cdot}70$ mV ($n = 17$).

Smooth muscle contraction and depolarization by neuropeptide Y

Neuropeptide Y caused contraction and depolarization of the smooth muscle of the rat tail artery. The threshold concentration for contraction was 10–30 nM. Both the contraction and the depolarization were slow in onset compared to the actions of other constrictor agents such as noradrenaline or raised extracellular K concentration, reaching a peak 5–10 min after the start of exposure to neuropeptide Y. The effects of 30 nM-neuropeptide Y applied for 30 min are shown in Fig. 1. After 13 min a freshly made neuropeptide Y solution was substituted; this caused only a slight retardation of the decline of the contraction, showing that the decline was not simply due to loss of neuropeptide Y from the solution onto the surface of its container.

The contraction and depolarization caused by neuropeptide Y were concentration-dependent. A 5 min exposure of the tissue to neuropeptide Y gave a peak contraction of the same amplitude as a continuous exposure, and so 5 min applications were used to estimate the contractile force produced by different concentrations. The results from two arteries, one normal and one denervated, are shown in Fig. 2. The contractile force has been expressed as a percentage of the maximum produced by noradrenaline in order to allow comparison between arteries of different sizes. Because of the limited amount of neuropeptide Y available the highest concentration used was 1 μM. This produced the largest responses, but they were well below the maximum contraction that the arteries could develop; higher concentrations of neuropeptide Y might have given larger contractions. The depolarization produced

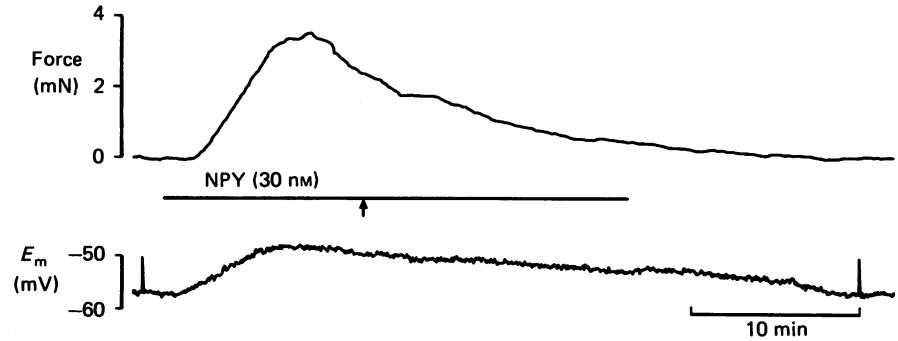

Fig. 1. Simultaneous recording of smooth muscle contractile force and membrane potential (E_m) changes in response to 30 nM-neuropeptide Y applied for 30 min. The time of application of neuropeptide Y is indicated by the bar below the force record. The preparation was continuously superfused; at the time marked by the arrow freshly made neuropeptide Y solution was substituted. The transient depolarizations at the beginning and end of the membrane potential record are e.j.p.s. NPY, neuropeptide Y in this and subsequent Figures.

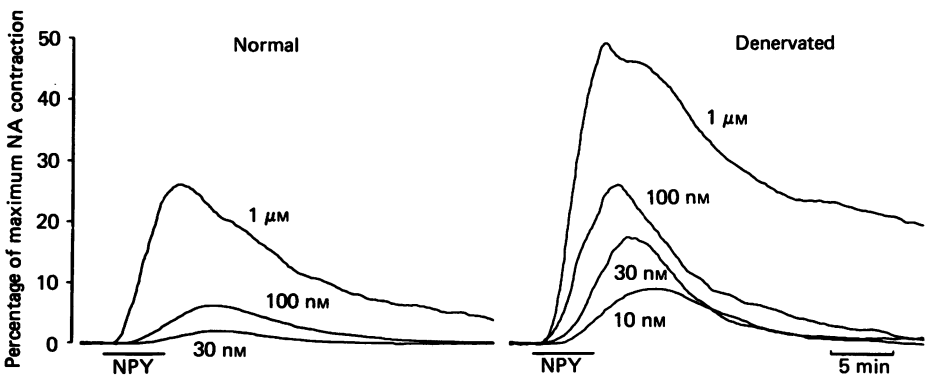

Fig. 2. Records of contractile force generated by normal and denervated arteries in response to a 5 min exposure to various concentrations of neuropeptide Y. The records were digitized by computer and the amplitudes normalized to the peak force produced by noradrenaline (NA).

TABLE 1. Contractile force and depolarization (depol.) caused by different concentrations of neuropeptide Y (NPY) in normal and denervated rat tail arteries

	Normal		Denervated	
[NPY] (nM)	Peak force (% max.)	Peak depol. (mV)	Peak force (% max.)	Peak depol. (mV)
10	0 (9)	0 (8)	8·9±0·64 (3)	—
30	1·94±0·29 (11)	8·0±0·82 (9)	19·4±1·6 (9)	7·7±1·8 (7)
100	5·74±1·34 (4)	9·1±1·43 (3)	22·4±1·47 (4)	6 (1)
300	24·6 (1)	—	44·9 (1)	—
1000	23·1±2·2 (4)	—	48·0±2·24 (4)	—

Contractile force is expressed as a percentage of the maximum produced by exogenously applied noradrenaline. *n* in parentheses.

by neuropeptide Y was more variable than the contraction, and could not be measured reliably at the higher concentrations of neuropeptide Y because strong contractions often dislodged the micro-electrode. Most of the measurements were made using 30 nM-neuropeptide Y, which produced a depolarization of up to 16 mV. There was a weak but significant correlation between the amplitude of the depolarization and the contraction ($r = 0{\cdot}73$, d.f. = 9). The peak force and depolarization produced by various concentrations of neuropeptide Y is summarized in Table 1.

The denervated arteries produced more force than the normal arteries exposed to the same concentration of neuropeptide Y, showing that supersensitivity to neuropeptide Y had been developed. The depolarization caused by neuropeptide Y was not significantly different in normal and denervated arteries.

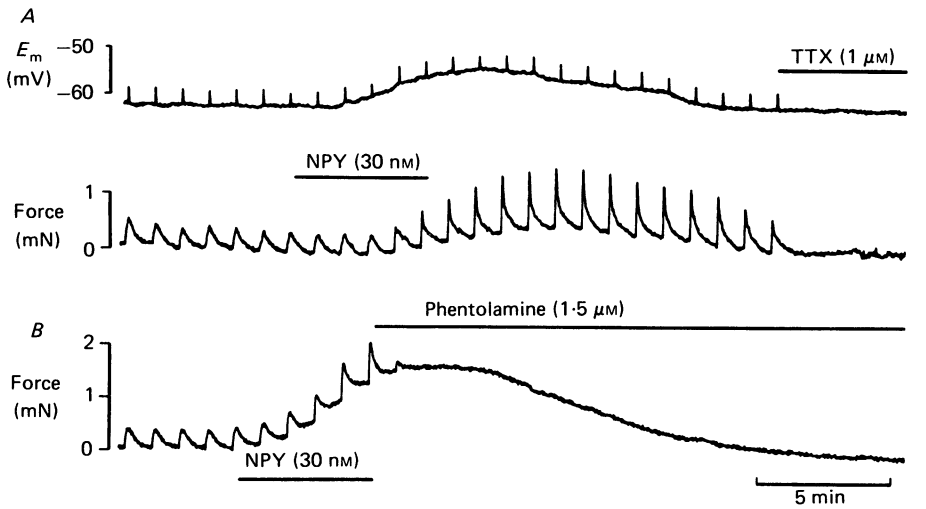

Fig. 3. Responses to single nerve stimuli (0·1 ms duration) given at 1 min intervals. *A*, the stimuli evoked e.j.p.s and small transient contractions, both of which were abolished by tetrodotoxin (TTX). Neuropeptide Y (30 nM for 5 min) increased the amplitude of the contractions but did not affect the e.j.p.s. *B*, in a similar experiment the contractions were abolished by the α-adrenoceptor antagonist phentolamine.

Effects of neuropeptide Y on responses to nerve stimulation

Neuropeptide Y (30 nM) increased the contractile force produced by nerve stimulation. It has been shown previously that in the rat tail artery the contraction following nerve stimulation can be caused by both the release of noradrenaline onto α-adrenoceptors and action potentials in the smooth muscle (Neild & Kotecha, 1985). Low-voltage stimuli did not produce muscle action potentials, and any contraction was due to α-adrenoceptor activation. Fig. 3 shows records from two experiments in which low-voltage single stimuli were used. In Fig. 3*A* the stimulus evoked an excitatory junction potential (e.j.p.) that was too small to produce an action potential in the smooth muscle, and a small contraction. Exposure to neuropeptide Y for 5 min caused a slow contraction and a depolarization, and increased the amplitude of the contractile response to nerve stimulation. The e.j.p. amplitude was unchanged.

Tetrodotoxin (1 μM) abolished both the e.j.p. and the contractile response, showing that they had been due to stimulation of nerves. Fig. 3*B* shows the recording of contractile force from a similar experiment, but in this case a high concentration of the α-adrenoceptor antagonist phentolamine was added. This blocked the contractile response, suggesting that it had been due to nerve-released noradrenaline acting on α-receptors. The amplitude of the e.j.p. (not shown) was not affected by phentolamine.

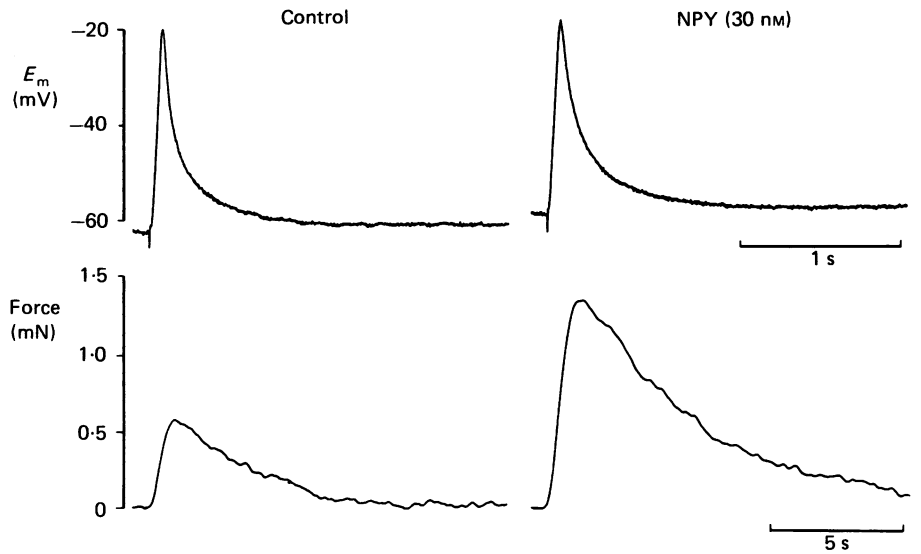

Fig. 4. Action potentials and the subsequent transient contractions following a single high-voltage nerve stimulus after blocking α-adrenoceptors with benextramine (10 μM for 20 min). 30 nM-neuropeptide Y increased the contraction but did not affect the action potential.

When the α-adrenoceptors were blocked by exposing the artery to prazosin (200 nM) or the irreversible antagonist benextramine (10 μM for 20 min, Melchiorre, 1981), nerve stimuli produced contractions only when there was an action potential in the smooth muscle. These contractions were also increased by 30 nM-neuropeptide Y. The amplitude and rate of rise of the action potentials was not changed (Fig. 4).

Effect of neuropeptide Y on e.j.p.s

The amplitude of the e.j.p. in response to a constant stimulus was measured as an indicator of the amount of neurotransmitter released. If the conductance change caused by the neurotransmitter is constant and brief compared to the membrane time constant then the e.j.p. amplitude will be proportional to the difference between the membrane potential and the reversal potential for the e.j.p. (Ginsborg, 1973). The conductance change is brief in other arteries (Hirst & Neild, 1978), and the reversal potential for the e.j.p. is close to 0 mV (Finkel, Hirst & van Helden, 1984).

The effect of neuropeptide Y on the e.j.p. depended on the initial amplitude, but did not suggest that there was any change in neurotransmitter release. Fig. 5 shows records from one experiment in which a continuous recording or membrane potential was maintained throughout the experiment. Prazosin (200 mM) was present to block α-adrenoceptors and reduce the size of the contraction caused by the stimuli; prazosin does not affect the amplitude of the e.j.p. (Neild & Zelcer, 1982). Initially the stimulus voltage was adjusted to give an e.j.p. 8 mV in amplitude (Fig. 5*A*). Neuropeptide Y

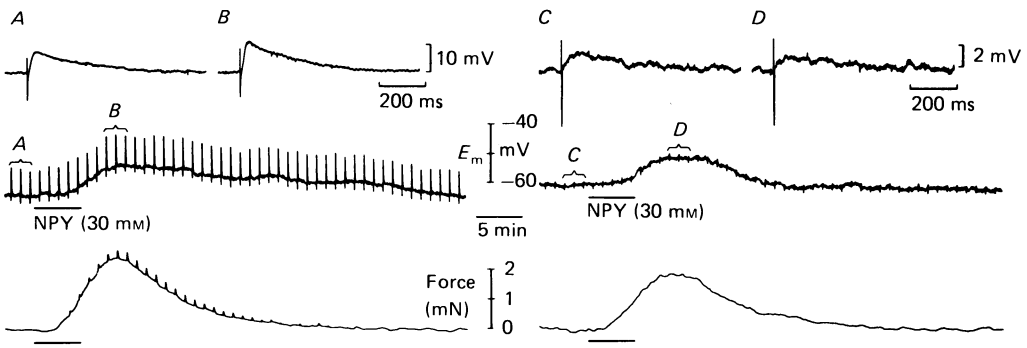

Fig. 5. Effect of 30 nM-neuropeptide Y on e.j.p.s evoked by single stimuli once per minute. α-adrenoceptors were blocked by prazosin (200 nM). Left, centre: membrane potential showing e.j.p.s. Groups of three e.j.p.s were digitized and averaged (*A* and *B*), showing that 30 nM-neuropeptide Y slightly increased their amplitude. The lower records shows contractile force. The stimuli caused transient contractions when the e.j.p. amplitude was increased, suggesting activation of voltage-dependent conductances. Right: responses from the same preparation but using a lower stimulus voltage to produce a smaller e.j.p. (*C* and *D*). Neuropeptide Y caused no change in e.j.p. amplitude and there were no contractions in response to the stimuli.

(30 nM) caused an 11 mV depolarization, and the amplitude of the e.j.p. increased (Fig. 5*B*). However, each stimulus also caused small transient contractions when neuropeptide Y was present, suggesting that voltage-dependent membrane processes were being activated. At the peak of the e.j.p. the membrane potential was −40 mV, and at this level inward Ca currents are activated in other arteries (Hirst, Silverberg & van Helden, 1986). The increase in e.j.p. amplitude could therefore have been due to a Ca current, and could not be taken as evidence of increased neurotransmitter release. To avoid activating voltage-dependent conductances the stimulus voltage was reduced so that the e.j.p. was smaller (Fig. 5*CD*). Neuropeptide Y did not change the amplitude of the small e.j.p., and it was concluded that it did not affect neurotransmitter release.

Effect of neuropeptide Y on the contraction caused by noradrenaline

Neuropeptide Y (30 nM) increased the contraction produced by low concentrations of noradrenaline (< 3 μM). Fig. 6 shows records from an experiment in which the contraction produced by 500 nM-noradrenaline was increased to three times its control amplitude after 15 min exposure to 30 nM-neuropeptide Y. The effect of neuropeptide Y was less when higher concentrations of noradrenaline were used and

the contractions were larger. 30 mM-neuropeptide Y did not change the amplitude of contractions caused by concentrations of noradrenaline greater than 3 μM, and did not change the maximum response.

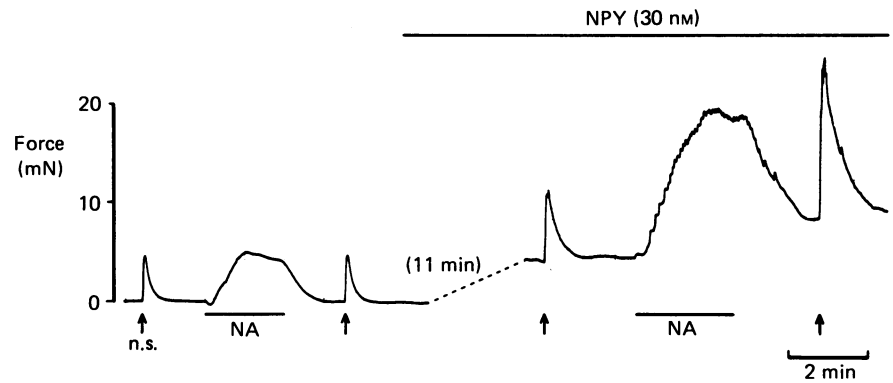

Fig. 6. Contractile force produced by brief trains of nerve stimulation (n.s. 8 stimuli at 8 Hz) and applications of 500 μM-noradrenaline (NA). The responses to both were increased by exposure to 30 nM-neuropeptide Y. The maximum contractile force that could be generated by this artery was 52 mN.

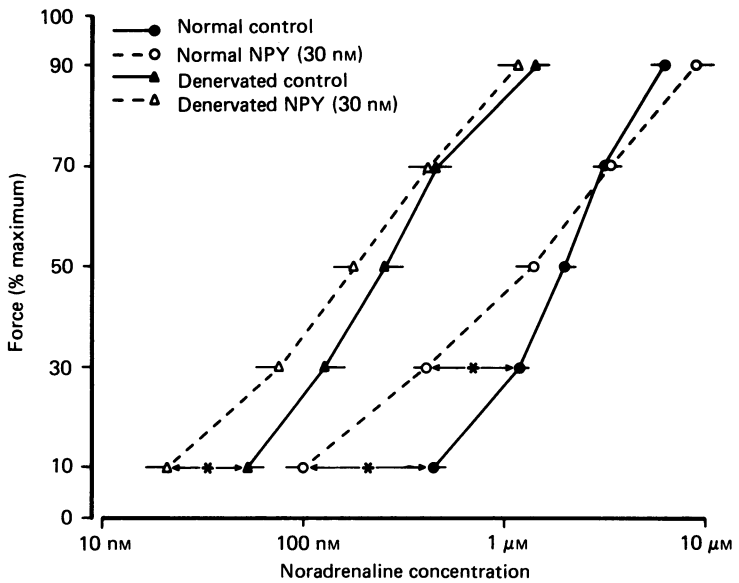

Fig. 7. Changes in the ED_{10}–ED_{90} values for noradrenaline caused by 30 nM-neuropeptide Y in normal and denervated arteries. Asterisks mark significant changes ($P < 0{\cdot}05$).

The contractile response to a range of noradrenaline concentrations from 10 nM to 100 μM were investigated using cumulative concentrations of noradrenaline. The values for ED_{10} to ED_{90} obtained are shown in Fig. 7. Neuropeptide Y significantly reduced the ED_{10} and ED_{30} values, but did not change those for the ED_{50}, ED_{70} or ED_{90}.

Neuropeptide Y had similar effects on the responses of denervated arteries to noradrenaline (Fig. 7), causing a significant decrease only in the value for ED_{10}.

Effects of neuropeptide Y on contraction caused by raised extracellular K

The arterial smooth muscle was made to contract by raising extracellular K concentration to 25, 40 and 100 mM. 200 nM-prazosin was present to abolish the effect of any noradrenaline released from the nerves by the K (Vanhoutte & Verbeuren, 1976). The amplitudes of the contractions caused by K were expressed as a percentage of the maximum contraction that could be produced by noradrenaline. The maximum contraction produced by K (100 mM) was 62% of the maximum response to noradrenaline.

TABLE 2. The effect of 30 nM-neuropeptide Y (NPY) on the contractile force caused by different extracellular K concentrations

	Contractile force (% of NA max.)	
K (mM)	Control	30 nM-NPY
25	3·1 ± 0·27	10·0 ± 0·65*
40	36·1 ± 1·8	42·6 ± 2·3*
100	62·4 ± 0·65	62·9 ± 0·52

Data from five arteries. The maximum contractile force that could be produced by noradrenaline (NA) was determined for each artery and the force produced by K was expressed as a percentage of that maximum. Force is given as mean ± S.E. of the mean. Values marked with an asterisk were significantly different from control values (paired t test, $P < 0·01$).

TABLE 3. The effect of 30 nM-neuropeptide Y (NPY) on the contractile force caused by 5-HT

	Contractile force (% of NA max.)	
5-HT (nM)	Control	30 nM-NPY
10	0·73 ± 0·31	5·1 ± 0·43*
100	35·9 ± 1·7	52·7 ± 1·6*
3000	99·7 ± 1·2	101·7 ± 1·2

Data from three arteries. The maximum contractile force that could be produced by noradrenaline (NA) was determined for each artery and the force produced by 5-HT was expressed as a percentage of that maximum. Force is given as mean ± S.E. of the mean. Values marked with an asterisk are significantly different from control values (paired t test, $P < 0·01$).

30 nM-neuropeptide Y increased the amplitude of the contractions caused by 25 and 40 mM-K, but did not change the response to 100 mM-K. The results are summarized in Table 2.

Effects of neuropeptide Y on contraction caused by 5-HT

5-HT caused contraction of the smooth muscle. The contractions were not affected by the α-adrenoceptor antagonists prazosin, phentolamine or benextramine, and were probably mediated by a specific 5-HT receptor (Cohen, Fuller & Wiley, 1981). The maximum force in response to 5-HT was the same as that produced by noradrenaline.

In three arteries neuropeptide Y increased the contraction produced by submaximal concentrations of 5-HT, but did not affect the response to a supramaximal concentration (3 μM). The results are summarized in Table 3.

DISCUSSION

The results presented above show that the threshold concentration of neuropeptide Y for contraction of the rat tail artery (10–30 nM) is similar to that for the rat femoral artery (Lundberg *et al.* 1985), the guinea-pig uterine artery (Morris *et al.* 1985) and cat pial arteries (Edvinsson *et al.* 1984*b*). In contrast, various arteries from the rabbit show no contraction to neuropeptide Y at concentrations below 300 nM (Edvinsson *et al.* 1984*a*; Glover, 1985). It is not clear why there are such large differences between arteries; it appears from the limited data available that rabbits may be particularly insensitive to the direct vasoconstrictor effects of neuropeptide Y. However, there are considerable differences in the responses of different arteries from the rabbit (Edvinsson *et al.* 1984*a*), and also from the guinea-pig (J. L. Morris, personal communication). The tachyphylaxis of the contractile response has not been reported previously, although Edvinsson *et al.* (1984*a*) noted that the potentiation of the response to noradrenaline declined with time.

The increased sensitivity of denervated arteries to the constrictor effects of neuropeptide Y was not unexpected, as denervation causes supersensitivity to a wide range of substances (Fleming, 1976). The potentiating effect of neuropeptide Y on noradrenaline contractions did not seem to be greatly altered by denervation, but no firm conclusions could be drawn because the change in shape of the dose–response relationship prevented a rigorous analysis of the data. At this stage the results from denervated arteries add no extra information on the mechanism by which neuropeptide Y acts.

The potentiating effect of neuropeptide Y on the constrictions caused by nerve stimulation or noradrenaline have been observed in all studies of the action of neuropeptide Y. It also potentiated contractions caused by histamine in some rabbit arteries (Edvinsson *et al.* 1984*a*), showing that this action was not confined to contractions caused by catecholamines. The results presented here show that on the rat tail artery neuropeptide Y potentiates the contractions to K, 5-HT and noradrenaline. The finding that neuropeptide Y caused depolarization of the arterial smooth muscle may partly explain its potentiating actions. A slight depolarization, caused by either raising the external K concentration (Casteels, Kitamura, Kuriyama & Suzuki, 1977; Mulvany, Nilson & Flatman, 1982) or by denervation (Abel *et al.* 1981) will cause increases in the sensitivity of arterial smooth muscle to a variety of constrictor agonists. It has also been found that neuropeptide Y inhibits cyclic AMP formation in arterial smooth muscle (Fredholm, Jansen & Edvinsson, 1985), and this might also lead to potentiation of contractions. It is interesting to note, however, that neuropeptide Y did not potentiate the constrictor effects of K, 5-HT or prostaglandin $F_{2\alpha}$ on various rabbit arteries (Edvinsson *et al.* 1984*a*), whereas this would have been expected if either of the two proposed mechanisms were applicable.

Neuropeptide Y did not change the amplitude of small e.j.p.s, suggesting that neuropeptide Y did not affect neurotransmitter release. Previous work on the release of [^{3}H]noradrenaline from sympathetic nerves has shown either a decreased (Lundberg & Stjarne, 1984; Lundberg *et al.* 1985; Dahlof *et al.* 1985) or unchanged (Ekblad *et al.* 1984) release in the presence of neuropeptide Y. The present experiments are not stricly comparable, as the e.j.p.s were evoked with single stimuli rather than the

long trains needed in previous experiments. E.j.p.s are probably a more accurate indicator of release as it would occur physiologically, and have the added advantage that there is no assumption about the nature of the neurotransmitter. Although there is good evidence that noradrenaline is the neurotransmitter in arteries (Neild & Zelcer, 1982), ATP has also been suggested (Farmer, 1985).

Neuropeptide Y clearly has a profound effect on the contractile activity of the rat tail artery, and if it is released from the nerves it is likely that it participates in the physiological control of artery diameter. The observations presented here indicate that its action is on the arterial smooth muscle, and that it does not affect neurotransmitter release.

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