Potentiation by neuropeptide Y of vasoconstriction in rat resistance arteries

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1 The effects of neuropeptide Y (NPY) on resistance arteries were investigated on 3rd generation mesenteric arterioles of the rat.

2 Contractions were elicited by noradrenaline (NA), 5-hydroxytryptamine (5-HT), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), depolarization (KCl substituted for NaCl) and by the calcium agonist Bay K 8644, in the absence and in the presence of NPY (100 nM), a concentration which by itself did not induce vasoconstriction.

3 NPY produced a leftward shift of the concentration-response curves to the agonists and to KCl, without any alteration of maximal contractions.

4 NPY also potentiated contractions elicited by addition of $CaCl_2$ to KCl-depolarized vessels, but its effect on calcium-induced contractions decreased with increasing KCl concentrations (from 20 to 100 mM).

5 Calcium-induced contractions were inhibited by the calcium channel blocker nitrendipine, both in the presence and absence of NPY (100 nM). NPY increased slightly (but significantly) the sensitivity to nitrendipine (the apparent K_B increased from 2.9×10^{-10} M to 1.6×10^{-10} M).

6 The KCl concentration necessary for the maximal effect of Bay K 8644 was decreased in the presence of NPY, and the sensitivity to the calcium channel agonist was increased.

7 Elevating the KCl concentration in the bath from 5 to 20 mM (which gives the same displacement to the left of the KCl concentration-effect curve seen in the presence of NPY) induced a parallel leftward shift of NA and 5-HT concentration-response curves. This shift was identical to the one induced by NPY on 5-HT-evoked contractions, but it was significantly smaller ($P < 0.001$) than the shift of the NA concentration-response curve observed in the presence of NPY. In the latter case, NPY enhanced more markedly the contractions induced by low NA concentrations (between 10^{-9} and 3×10^{-8} M) than those induced by high concentrations (up to 3×10^{-7} M), thus giving a shallow concentration-response curve.

8 The results strongly suggest that NPY partially depolarizes the arterioles and induces an increase in calcium entry through voltage-dependent channels, thus enhancing contractions elicited by agonists or by KCl-depolarization. In addition, they support the view that another mechanism also plays a part in the potentiation by NPY of the effects of low concentrations of NA.

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide which is widely distributed throughout the sympathetic nervous system (Tatemoto *et al.*, 1982; Lundberg & Tatemoto, 1982; Lundberg *et al.*, 1982). It coexists with noradrenaline in many sympathetic neurones supplying the cardiovascular system, and it is released in the circulation upon sympathetic stimulation (see reviews, O'Donohue *et al.*, 1985,

Allen & Bloom, 1986; Gray & Morley, 1986, Edvinsson *et al.*, 1987; Lundberg *et al.*, 1987). Recently, the coexistence of NPY with a certain sub-population of 5-hydroxytryptamine (5-HT)-containing intracardiac neurones was discovered (Hassal & Burnstock, 1987).

Intravenous perfusion of NPY in rats produces an increase in blood pressure as a result of enhanced peripheral resistance (Dählof *et al.*, 1985; Zukowska *et al.*, 1986). In this species as in others, NPY

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enhances *in vitro* contraction of arterial smooth muscle produced by various agonists, including noradrenaline (NA), and by stimulation of sympathetic nerve endings (Edvinsson *et al.*, 1984a,b; Edvinsson 1985a,b; Ekblad *et al.*, 1984; Neild, 1987; Pernow *et al.*, 1987). However, the direct contractile effect of the peptide varies markedly with the anatomical origin of the vessel: NPY is a potent vasoconstrictor of cerebral (Edvinsson *et al.*, 1983; 1984b; Edvinsson, 1985a,b; Hanko *et al.*, 1986; Thorpe *et al.*, 1987) and skeletal muscle arteries (Pernow *et al.*, 1987), but has a weak effect on other peripheral arteries (Edvinsson *et al.*, 1983; Glover, 1985; Pernow *et al.*, 1987; Thorpe *et al.*, 1987).

In order to elucidate further the role of this neuropeptide, the present work was aimed at the interaction of NPY with NA and other vasoconstrictor agents on rat resistance arteries.

The experiments were carried out on rat isolated mesenteric arterioles. In these vessels, NA induces a partial depolarization of smooth muscle cells (Mulvany *et al.*, 1982) and allows extracellular calcium to produce contraction through a calcium channel blocker-sensitive mechanism (Julou & Freslon, 1986). The arterioles were thus stimulated either by NA, other agonists or KCl-depolarization. 5-HT and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were used to test the specificity of the potentiating effect of NPY or NA-induced vasoconstriction. Since the effects of NPY are reduced by calcium channel blockers (Edvinsson *et al.* 1983; Edvinsson, 1985a,b; Dählof *et al.*, 1985; Mabe *et al.*, 1985; 1987; Wahlestedt *et al.*, 1985; Pernow *et al.*, 1987), the interactions between NPY and two dihydropyridines (an antagonist and an agonist) were also investigated. The results show that NPY markedly enhances contractile responses of rat resistance arterioles to various agonists, especially those elicited by low concentrations of NA. They strongly suggest that these effects are partially produced through slight depolarization of the smooth muscle cells.

Part of this work has been presented to a joint meeting of the French, Belgium and Swiss Pharmacological Societies (Andriantsitohaina & Stoclet, 1988).

Methods

Arterial preparation and mounting

In all experiments, third generation branches of the superior mesenteric artery were dissected from 11–13 week old female Wistar rats bred in our institute. Rats were anaesthetized with pentobarbitone sodium ($60\,mg\,kg^{-1}$, i.p.). Arterial segments (length = 2 mm, internal diameter = $100\,\mu m$) were prepared and mounted on a previously described myograph

(Mulvany & Halpern, 1977; Julou & Freslon, 1986). Briefly, two tungsten wires ($30\,\mu m$ diameter) were inserted through the lumen of the vessel. Mechanical activity was recorded isometrically by a force transducer (DSG BE4, Kistler-Morse), connected to one of the two tungsten wires, the other being attached to a support carried by a micromanipulator (MR 50, Micro-Contrôle).

After mounting, vessels were equilibrated for 1 h in a physiological salt solution (PSS) (mM: NaCl 119, KCl 4.7, KH_2PO_4 0.4, $NaHCO_3$ 14.9, $MgSO_4$ 1.17, $CaCl_2$ 2.5, glucose 5.5) kept at 37°C and continuously gassed with 95% O_2 plus 5% CO_2 (pH 7.4). The resting tension of the vascular preparation was adjusted to about 200 mg. Following equilibration, the contractile capacity was assayed by exposing the arterial segment either to $10\,\mu M$ NA or to an isotonic 124 mM KCl solution prepared by substituting NaCl in the normal PSS with an equimolar amount of KCl. The concentrations used have previously been shown to elicit maximum contractions as determined from concentration-response curves for KCl and NA (Mulvany *et al.*, 1982). The presence of functional endothelium was controlled in all preparations by the ability of acetylcholine ($10^{-6}\,M$) to induce more than 50% relaxation of NA ($10^{-5}\,M$)-precontracted vessels.

Effects of NPY on NA, 5-HT and $PGF_{2\alpha}$-induced contractile responses

Sixty min after testing the contractile capacity of the vessels by exposure to NA $10\,\mu M$, responses to cumulative additions of noradrenaline (NA), 5-hydroxytryptamine (5-HT) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were elicited and concentration-response curves constructed. After a washout period of 45 min, NPY (100 nM) was applied 5 min before repeating cumulative additions of the agonist. During experiments with NA, $3\,\mu M$ of cocaine was present in the bath to inhibit neuronal uptake. In control experiments, two concentration-response curves obtained by two successive cumulative additions of the agonist were not different from each other in the absence of NPY, and the first concentration-response curve was taken as control.

Effects of NPY on calcium-induced contractile responses of KCl-exposed vessels and on the effects of dihydropyridines

After testing the response of the vessels to PSS containing KCl 124 mM and $CaCl_2$ 2.5 mM, the bathing solution was replaced by a 'calcium-free' depolarizing medium (Ca_0-KCl PSS), containing no added $CaCl_2$ and the indicated concentrations of KCl substituted for equimolar amounts of NaCl (20, 30, 40, 60 and 100 mM). Each preparation was exposed to

one KCl concentration only. Phentolamine ($1\,\mu M$) was added to the Ca_0-KCl PSS in order to minimize the effects of noradrenaline release following neuronal depolarization. After a 45 min washout period, cumulative additions of $CaCl_2$ (10^{-5} to 10^{-2} M) were repeated 3 or 4 times, separated by 45 min washout periods, and consecutive concentration-response curves constructed. Control experiments had shown that the second, the third and fourth curves were not significantly different in the absence of other treatment. The second curve was thus taken as control. When NPY (100 nM) was used, it was added 5 min before eliciting the cumulative concentration-response curve.

The calcium channel blocker nitrendipine was added 20 min before the cumulative addition of $CaCl_2$ on vessels exposed to Ca_0-KCl (40 mM) PSS.

Responses to cumulative additions of the calcium channel agonist Bay K 8644 (10^{-10} to 3×10^{-6} M) were elicited in PSS containing 2.5 mM $CaCl_2$ and the indicated concentrations of KCl. Cumulative responses were successively induced, with a washout period of 1 h between each, and gave concentration-response curves which were not significantly different from each other in the absence of other treatment. NPY was added to the bath 5 min before the 3rd cumulative addition of Bay K 8644.

Effect of a slight depolarization on NA- and 5-HT-induced contractile responses

Sixty min after testing the contractile capacity of the vessels by exposure to NA $10\,\mu M$, responses to cumulative additions into the bath of either NA or 5-HT were elicited. Then the PSS was replaced by a PSS containing KCl 20 mM. After a washout period of 45 min, a second concentration-response curve was elicited by cumulative additions of either NA or 5-HT.

Expression of results and statistical analysis

Sensitivities to agonists and to calcium were expressed as pD_2 values, where $pD_2 = -\log EC_{50}$, EC_{50} being the agonist or calcium concentration required to give a half-maximal contractile response. EC_{50} values were obtained by logit/log regression analysis. The potentiating effects of NPY and of depolarization were compared by calculating the shifts in pD_2 values in the absence and presence of either NPY or depolarization. The apparent affinity (equilibrium dissociation constant K_B) of nitrendipine was calculated by a computerized version of the method of Arunlakshana & Schild (1959).

All results are expressed as means $\pm$ s.e.mean of n experiments. The differences between the pD_2 values, obtained in the same artery rings, were tested for significance by use of Student's t test for paired obser-

vations. When the controls were run in parallel on different rings, the pD_2 values were compared by two-way analysis of variance, which was also used to compare the effects of different treatments studied in separate experiments.

Drugs

Noradrenaline bitartrate (Sigma) was dissolved in deionized water containing HCl 34 mM and Na_2SO_3 7.9 mM as a stock solution (10^{-2} M). A 14 mM stock solution of $PGF_{2\alpha}$ (Upjohn) was used and dilutions made daily in deionized water. 5-HT hydrochloride (Sigma), phentolamine hydrochloride (Ciba-Geigy) and cocaine hydrochloride (Cooperation Pharmaceutique Française) were prepared daily as 10 mM solutions in deionized water. Porcine NPY (Sigma) was dissolved in deionized water containing NaCl $9\,g\,l^{-1}$ and bovine serum albumin (Sigma) $1\,g\,l^{-1}$ as a stock solution of 10^{-4} M and subsequently kept frozen until used. Nitrendipine and Bay K 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl-pyridine-5-carboxylate, Bayer) were diluted in absolute ethanol as a stock solution of 1 mM and subsequently kept frozen until used. Nitrendipine and Bay K 8644 were kept in the dark and experiments using these drugs were performed in light-proof apparatus to minimize light-induced degradation.

Results

Effects of NPY on NA-, 5-HT- and $PGF_{2\alpha}$-induced contractile responses

When NPY was added in a cumulative fashion (10^{-9} to 10^{-6} M) to unstimulated isolated resistance vessels of the rat, no effect was observed at concentrations less than 10^{-7} M. Higher concentrations induced a weak contractile effect (less than 10% of that evoked by NA $10\,\mu M$; not shown).

As illustrated in Figure 1, NPY (10^{-7} M) produced significant leftward shifts of the NA, 5-HT and $PGF_{2\alpha}$ concentration-response curves, without any change in the maximal responses. The shift was parallel and comparable for 5-HT and $PGF_{2\alpha}$ with the pD_2 value being increased from 6.42 ± 0.04 to 6.68 ± 0.06 ($P < 0.001$) and from 4.87 ± 0.08 to 5.14 ± 0.06 ($P < 0.01$). The effect of NPY was more pronounced for NA compared to the other agonists ($P < 0.001$), with an increase of the pD_2 value from 6.61 ± 0.08 to 7.48 ± 0.12 ($P < 0.001$). In addition, the concentration-response curve for NA was more shallow in the presence of NPY, due to a greater enhancement of the response at low concentrations of NA.

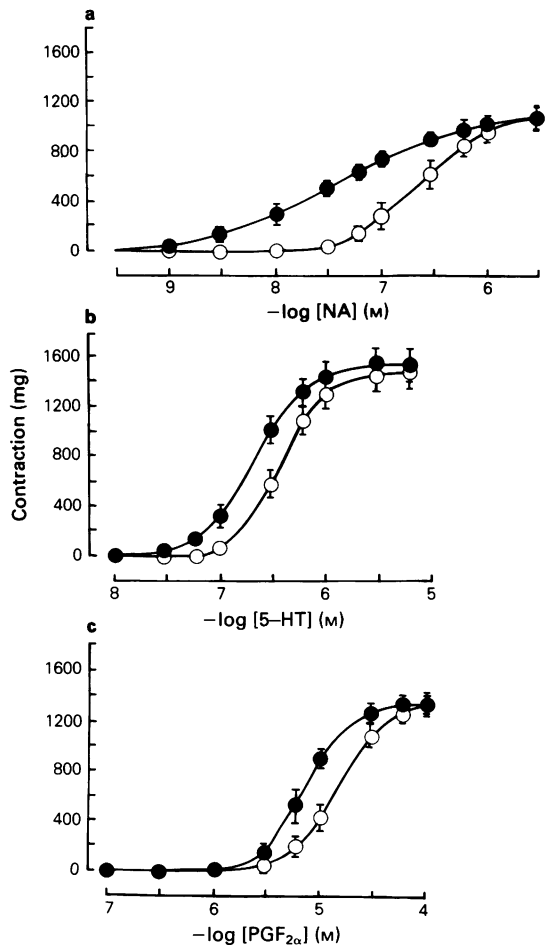The above results were obtained on arterioles in which the presence of functional endothelium was

Figure 1 Potentiation by neuropeptide Y (NPY) of contractions induced by different agonists on rat isolated rat mesenteric arterioles. Concentration-response curves in the absence of NPY (○) and in the presence (●) of NPY 100 nM for (a) noradrenaline (NA, $n = 7$), (b) 5-hydroxytryptamine (5-HT; $n = 6$) and (c) prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$; $n = 5$). Vertical lines indicate s.e.mean.

assessed by acetylcholine (10^{-6} M) producing more than a 50% relaxation of NA (10^{-5} M)-induced tension. However, on preparations in which the relaxant effect of acetylcholine was less (10–50%, about one third of the vessels), NPY was still able to potentiate the vasoconstriction induced by NA, 5-HT and $PGF_{2\alpha}$ (not shown).

Effects of NPY on calcium-induced contractile responses in KCl-exposed vessels

The calcium concentration-response curves obtained on arteries exposed to various KCl concentrations
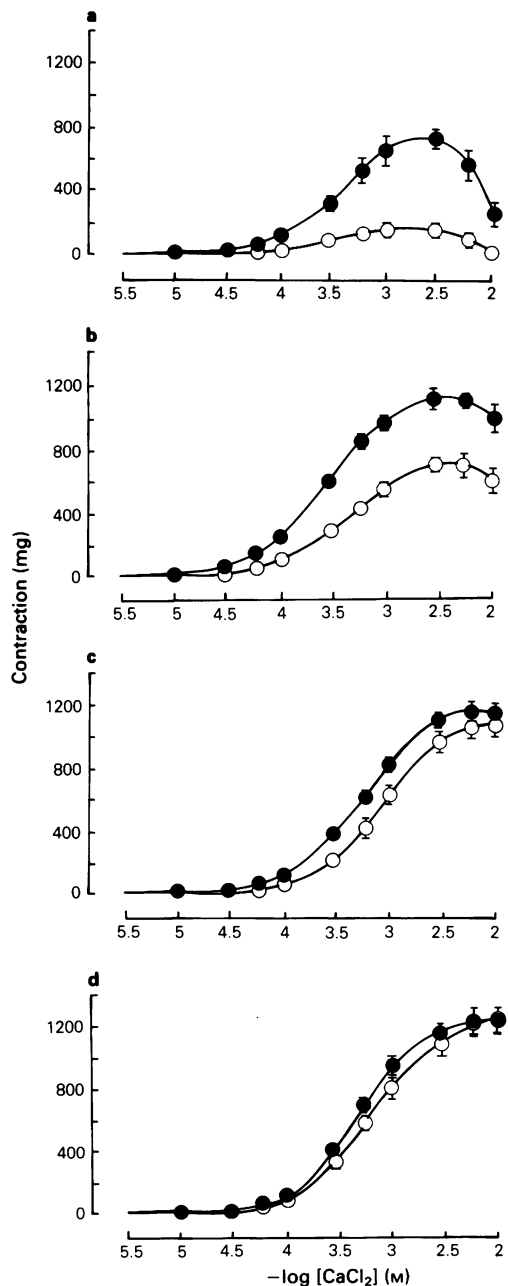

Figure 2 Potentiation by neuropeptide Y (NPY) of contractions induced by the addition of $CaCl_2$ to rat arteriole rings immersed in calcium-free depolarizing solutions: (a) KCl = 30 mM ($n = 8$), (b) KCl = 40 mM ($n = 23$), (c) KCl = 60 mM ($n = 8$) and (d) KCl = 100 mM ($n = 8$). Concentration-response curves in the absence (○) and in the presence (●) of NPY 100 mM. Vertical lines indicate s.e.mean.

(20, 30, 40, 60 and 100 mM) are illustrated in Figure 2.

In Ca_0-KCl (20 mM) PSS, the addition of calcium alone did not induce a contraction. In vessels exposed to higher concentrations of KCl, addition of calcium elicited a concentration-dependent contractile response (Figure 2). When the concentration of $CaCl_2$ was greater than 3 mM in Ca_0-PSS containing 30 and 40 mM KCl, the developed tension decreased; calcium concentration-response curves were thus biphasic (Figure 2a,b). However, in Ca_0-KCl (60 mM and 100 mM)-exposed vessels, no relaxant effect of calcium was observed (Figure 2c,d).

When NPY was present in the bath, calcium elicited a weak contractile response in Ca_0-KCl(20 mM)-exposed vessels (not shown). In vessels exposed to Ca_0-KCl (30 mM) PSS, NPY potentiated markedly the maximal responses to further cumulative additions of calcium (Figure 2a). The calcium pD_2 values were not significantly different in the absence and in the presence of NPY (respectively, 3.47 ± 0.02 and 3.44 ± 0.02). In vessels exposed to Ca_0-KCl (40 mM) PSS, NPY induced a significant leftward shift of the concentration-response curve (Figure 2b). The pD_2 values, in the absence and in the presence of NPY, were, respectively, 3.34 ± 0.03 and 3.54 ± 0.02 ($P < 0.001$). In addition, NPY enhanced the maximal contractile response to calcium. NPY did not affect the relaxant effect of high calcium concentrations. In Ca_0-KCl (60 and 100 mM) PSS, the calcium concentration-response curves were significantly shifted to the left by NPY without an elevation of the maximal responses. The pD_2 values were increased in the presence of NPY from 3.10 ± 0.03 to 3.27 ± 0.02 (KCl 60 mM, $P < 0.01$) and from 3.18 ± 0.02 to 3.29 ± 0.02 (KCl 100 mM, $P < 0.001$).

The effect of NPY on contractions elicited by different concentrations of KCl in saline solution containing 3 mM $CaCl_2$ is shown in Figure 3. The KCl concentration-effect curve was shifted to the left in a parallel fashion by NPY. The EC_{50} values were, respectively: 37.7 ± 0.8 mM and 26.8 ± 0.7 mM ($P < 0.001$), in the absence and in the presence of NPY. The maximal response to KCl was not affected by NPY.

Influence of NPY on the effects of nitrendipine and Bay K 8644

The effects of nitrendipine on calcium-induced contractile responses elicited, in Ca_0-KCl (40 mM) PSS, in the absence and presence of NPY are illustrated in Figure 4. In both cases, nitrendipine induced a concentration-dependent shift to the right and a decrease in the maximum of the calcium concentration-response curves (except the lowest nitrendipine concentration in the absence of NPY).
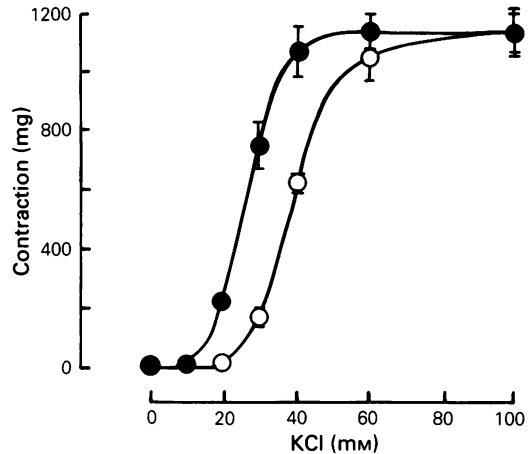

Figure 3 Effect of neuropeptide Y (NPY) on contractions elicited by KCl on rat isolated mesenteric arterioles in saline solutions containing 3 mM $CaCl_2$ and increasing concentrations of KCl. Concentration-response curves in the absence (O) and in the presence (●) of NPY 100 nM. Vertical lines indicate s.e.mean.
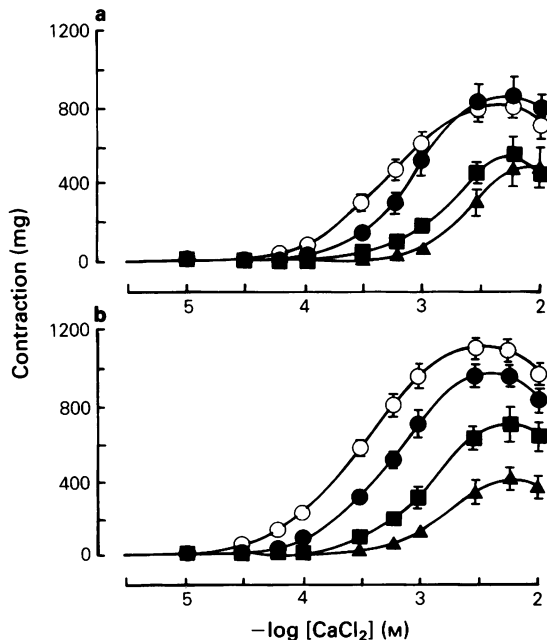

Figure 4 Inhibition by nitrendipine of contractions induced by $CaCl_2$ in KCl (40 mM) depolarized rat mesenteric arteriole rings: (a) in the absence and (b) in the presence of neuropeptide Y 100 nM. Control curves: (O), $n = 18$ (a) and 17 (b) and in the presence of nitrendipine at the following concentrations: 3×10^{-10} M (●), $n = 6$ (a) and 6 (b); 10^{-9} M (■), $n = 6$ (a) and 6 (b); 3×10^{-9} M (▲), $n = 6$ (a) and 5 (b). Vertical lines indicate s.e.mean.
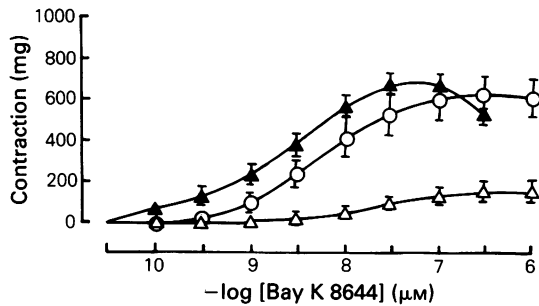
Figure 5 Effect of neuropeptide Y (NPY) on contractions elicited by Bay K 8644 on rat isolated mesenteric arterioles. Responses in KCl (15 mM) depolarizing solution in the absence ($\triangle$, $n = 3$) and in the presence ($\blacktriangle$, $n = 6$) of NPY 100 nM, and in KCl (20 mM) depolarizing solution in the absence of NPY ($\bigcirc$, $n = 6$). Vertical lines indicate s.e.mean.

When the data of the higher calcium concentrations, producing a relaxant response, were not taken into account, the results were in agreement with competitive inhibition. The apparent K_B values were then 2.9×10^{-10} M and 1.6×10^{-10} M in the absence and in the presence of NPY ($P < 0.001$), respectively, suggesting that the affinity of nitrendipine was increased in the presence of NPY.

The effect of NPY on contractions induced by Bay K 8644 on isolated mesenteric vessels is illustrated in Figure 5. Bay K 8644 did not induce any contractile response in normal PSS (not shown). In vessels exposed to KCl (15 mM) PSS, cumulative additions of Bay K 8644 (10^{-10} to 10^{-6} M) produced a weak contractile response (less than 15% of that evoked by NA 10 μM). In the presence of NPY (100 nM), Bay K 8644 induced a concentration-dependent contractile response on vessels exposed to KCl (15 mM) PSS. In KCl (20 mM) PSS exposed vessels, Bay K 8644 elicited concentration-dependent contractile responses. The maximal response did not differ significantly from that obtained on vessels exposed to KCl (15 mM) PSS with NPY. The pD$_2$ value of Bay K 8644 was significantly higher ($P < 0.01$) in the vessels exposed to KCl (15 mM) PSS and NPY (8.7 ± 0.16) as compared to vessels exposed to KCl (20 mM) PSS without NPY (8.13 ± 0.16).

Effect of slight depolarization on NA- and 5-HT-induced contractile responses

The results given above suggest that NPY may slightly depolarize the arteries, since the concentration-effect curve for KCl was shifted to the left by about 10–15 mM KCl (Figure 3) in the presence of the peptide, and since the KCl concentration allowing Bay K 8644 to induce a contraction
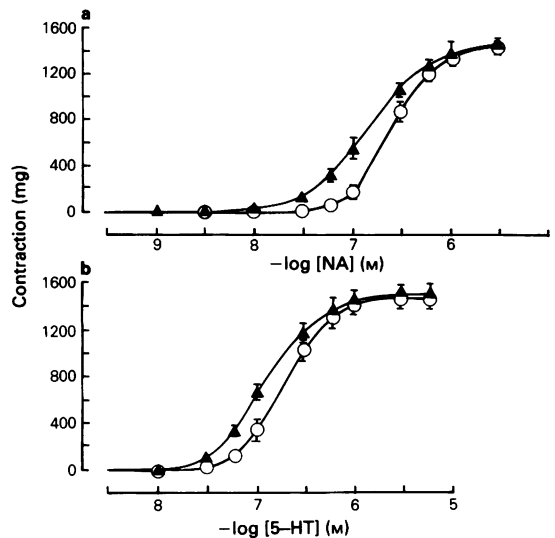

Figure 6 Effect of slight depolarization (KCl = 20 mM) on (a) noradrenaline (NA; $n = 6$)- and (b) 5-hydroxytryptamine (5-HT; $n = 6$)-induced contractile responses. ($\bigcirc$) Responses in normal PSS and ($\blacktriangle$) responses in KCl (20 mM) PSS. Vertical lines indicate s.e.mean.

was less in the presence than in the absence of NPY (Figure 5). To test this hypothesis, the effects of agonists were studied in depolarizing PSS containing 20 mM KCl and CaCl$_2$ 2.5 mM.

As shown in Figure 6, concentration-effect curves for NA and 5-HT were displaced to the left without any alteration of the maximum in the presence of 20 mM KCl. Potentiation of the effect of 5-HT was not significantly different from that shown to be induced by NPY (100 nM) (Figure 1b): the shift in pD$_2$ values was 0.20 ± 0.06 and 0.26 ± 0.03 for KCl 20 mM and NPY, respectively. The potentiation of the effect of NA induced by KCl 20 mM (pD$_2$ shift 0.25 ± 0.03) was comparable to the potentiation seen with 5-HT, but it was significantly less ($P < 0.001$) than the potentiation of NA (Figure 1a) induced by NPY (pD$_2$ shift 0.87 ± 0.11). In addition, the concentration-effect curve obtained in the presence of NPY was shallower than that obtained in KCl 20 mM PSS.

Discussion

The aim of this study was to characterize and to investigate the effects of NPY on rat resistance arteries. Rat mesenteric arterioles contribute to peripheral resistance (Nilsson, 1985; Mulvany *et al.*, 1987; Prewitt *et al.*, 1987), and these vessels have a

rich sympathetic innervation, with nerve endings containing NPY-like immunoreactivity (Furness et al., 1983; 1984). Moreover, NPY has important physiological effects at the sympathetic neuroeffector junction (Wahlestedt et al., 1986). Thus, mesenteric arterioles present a good experimental system to study the effects of NPY.

The results of this study show that NPY enhanced arterial smooth muscle contraction produced by different agonists and by KCl-depolarization at a concentration at which it had no effect by itself. The data are in agreement with those from in vivo experiments on pithed rats where infusion of NPY, at a dose which per se did not affect blood pressure, enhanced the vasopressor response to phenylephrine, NA and electrical stimulation of the sympathetic outflow (Dahlöf et al., 1985; Zukowaka et al., 1986; Evequoz et al., 1987). These results and the present findings strongly suggest that the in vivo effect of NPY on blood pressure is due to a direct vasoconstrictor effect on resistance arterioles.

Daly & Hielbe (1987) showed that NPY could promote the release of an endothelium-derived constricting factor in rabbit ear artery. Recently, a vasoconstrictor peptide produced by endothelial cells, endothelin, was discovered (Yanagisawa et al., 1988). Thus, the endothelium could mediate the potentiating effect of NPY. In contrast to the endothelium of large vessels, the endothelium of resistance arteries is difficult to remove without causing smooth muscle damage and a partial loss of contractile responses of the vessels. For this reason, the results presented here were obtained on mesenteric arterioles in which the presence of functional endothelium was shown by the ability of acetylcholine to produce relaxation. However, there was no obvious difference between these data and the results obtained on vessels in which the endothelial function was impaired, probably because the endothelium was damaged during the mounting procedure. This suggests that the role of the endothelium was not essential in the potentiating effect of NPY.

Inhibition of prejunctional release of NA has also been found in isolated preparations in the presence of NPY (Lundberg & Tatemoto, 1982; Dahlöf et al., 1985; Serfozo et al., 1986; Pernow et al., 1987), but this mechanism cannot explain the potentiating effect exerted by NPY on vasoconstrictor agonists, both in vitro and in vivo.

Our results are also in accordance with those of different studies showing that NPY enhances contractile responses produced by NA and other vasoconstrictor agonists on several vascular smooth muscles isolated from different mammalian species (Edvinsson et al., 1984a,b; 1985; Ekblad et al., 1984; Wahlestedt et al., 1985; 1986; Neild, 1987; Pernow et al., 1987). The displacement to the left of the

concentration-effect curves for agonists in the presence of NPY could be due to increased affinity for their respective receptors. However, this possible explanation does not hold for the NPY-induced leftward shifts observed with KCl and calcium. The maximal contractions induced by all tested vasoconstrictor agents were not different from each other, and the addition of two vasoconstrictor agents (e.g. KCl and agonist, or maximally active concentrations of 2 agonists, not shown) did not produce any increase in the maximal contraction. Thus, in the presence of NPY, the maximal contractile response of the vessels was reached in response to the different vasoconstrictor agents, and the displacement to the left of the concentration-effect curves could be due to an enhancement of contraction, allowing the maximal effect to be reached at a lower concentration. NPY could perhaps produce an increase in tension, too small to be detected in the absence of other vasoconstrictor agents, but increasing the effect of these agents at low concentrations. However, this is not the case for calcium-induced contractions (see Figure 2).

A close association between the degree of depolarization of smooth muscle cells and their sensitivity to various agonists has been suggested by Fleming (1980). Mulvany et al. (1982) also demonstrated that depolarization increased the sensitivity of rat mesenteric arterioles to NA. The present findings provide strong evidence that NPY induced a partial depolarization of these vessels and this mechanism may participate in the enhancement of vasoconstriction induced by agonists and KCl. The following observations support the hypothesis of partial depolarization: (a) the KCl concentration-response curve was displaced to the left by about 10–15 mM in the presence of NPY; (b) an equivalent increase of the KCl concentration in the PSS enhanced the effects of NA and 5-HT; (c) the threshold depolarizing concentration of KCl, for which contractions with the calcium agonist Bay K 8644 were observed (an effect which requires sufficient activation of voltage-dependent calcium channels: Schramm et al., 1983; Hess et al., 1984), was decreased by NPY; (d) NPY slightly but significantly increased the apparent affinity of the calcium antagonist nitrendipine and the sensitivity of the calcium agonist Bay K 8644. Assuming that the affinity of the calcium channels for the dihydropyridines is higher in the activated than in the resting state (Lee & Tsien, 1983; Sanguinetti & Kass, 1984), it is possible that NPY induced a moderate depolarization, which did not produce a contraction per se, but partially activated voltage-dependent dihydropyridine-sensitive calcium channels. Thus, slight depolarization would enhance the vasoconstrictor effects of agonists, of external calcium and of Bay K 8644, by promoting calcium

influx through voltage-operated channels. It might also increase the affinity of nitrendipine.

However, the results presented here show a difference between NA and the other agonists with respect to potentiation by NPY. While depolarization produced by increasing the PSS KCl concentration to 20 mM potentiated the effect of 5-HT to the same extent as did NPY, the presence of 20 mM KCl was not sufficient to reproduce the NPY-dependent, leftward shift of the NA concentration-effect curve. The NA curve was displaced to a greater extent and was more shallow in the presence of NPY in normal PSS than in KCl (20 mM) PSS. Hence, mechanisms in addition to NPY-induced depolarization seem necessary to explain the potentiating effect of the peptide on the vasoconstriction induced by NA. It has been demonstrated that NPY inhibits adenylate cyclase activity and cyclic AMP formation in different tissues including blood vessels (Fredholm *et al.*, 1985; Häggblad & Fredholm, 1987; Kassis *et al.*, 1987; Petrenko *et al.*, 1987; Lundberg *et al.*, 1988). It has also been shown that NPY enhanced α_1-adrenoceptor-induced accumulation of inositol triphosphate in the rat vas deferens (Häggblad &

Fredholm, 1987). The latter mechanism could explain the critical role of calcium from intracellular stores that has been observed under the effect of NPY in the rabbit femoral artery (Wahlestedt *et al.*, 1985). The existence of these mechanisms and their physiological role remain to be established in resistance vessels.

In conclusion, the results presented here show that NPY enhances smooth muscle contraction induced not only by the sympathetic neuromediator NA, but also by other agonists in rat resistance vessels. They provide evidence that these effects of NPY are partially accounted for by partial depolarization and subsequent calcium influx through voltage-dependent calcium channels. In addition, they suggest that another mechanism is also involved in the potentiation of the vasoconstrictor effect of NA by NPY, a mechanism apparently less important for the action of 5-HT and $PGF_{2\alpha}$.

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