Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels

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1   The contractile effect of neuropeptide Y (NPY) was tested on isolated segments of basilar artery, central ear artery, gastro-epiploic artery and vein, and femoral artery and vein from the rabbit. At 30 nM NPY did not evoke vasoconstriction; at 300 nM NPY evoked a weak and variable response.

2   NPY greatly potentiated the response of the gastro-epiploic and femoral arteries to noradrenaline without affecting the maximum response. As tested on the gastro-epiploic artery NPY was effective at concentrations of 1 nM and higher. As tested on the femoral artery the potentiating effect of 30 nM NPY on noradrenaline-evoked contractions was apparent immediately and 30 min after the application of NPY, but not after one hour.

3   NPY (30 nM) potentiated the contractile response to noradrenaline and histamine but not to 5-hydroxytryptamine or high K^+. The response to histamine was augmented in both arteries and veins, whereas the response to noadrenaline was enhanced in arteries but not in veins. NPY failed to potentiate the prostaglandin $F_{2\alpha}$-evoked contraction except in the gastro-epiploic vein.

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide, isolated from porcine brain (Tatemoto 1982; Tatemoto *et al.*, 1982). Immunocytochemistry has revealed a widespread occurrence of NPY-immunoreactive nerve fibres not only in the brain but also in the peripheral tissues. Particularly spectacular is the rich supply of NPY-immunoreactive nerve fibres around blood vessels (Lundberg *et al.*, 1982; Edvinsson *et al.*, 1983; Ekblad *et al.*, 1984a,b). There is considerable evidence that NPY coexists with noradrenaline in postganglionic sympathetic nerve fibres. NPY *per se* has been reported to cause vasoconstriction (Lundberg & Tatemoto 1982; Edvinsson *et al.*, 1983; Ekblad *et al.*, 1984a) and to reduce myocardial perfusion with inhibition of the force of contraction of the isolated perfused heart of the rabbit (Allen *et al.*, 1983). Furthermore, Ekblad *et al.* (1984a) have reported a potentiating effect of NPY via a postsynaptic mechanism on both electrically and drug-induced arterial contractions. The present study is an attempt to evaluate and to characterize the specificity of the effects of NPY on contractions evoked by a number of agents on isolated blood vessels, both arteries and veins, from the rabbit.

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Methods

Adult rabbits of either sex were killed by a blow on the neck and exsanguinated. The brain and the desired blood vessels (see Table 1) were rapidly removed and placed in ice-cold Krebs solution (for composition, see below). Care was taken to avoid stretching or other types of injury to the vessels. Segments, 2–3 mm long, were mounted on two L-shaped metal holders (0.2 mm in diameter), one of which was connected to a force displacement transducer and a Grass polygraph for continuous recording of the isometric tension. The position of the other holder could be changed by means of a movable unit allowing fine adjustment of the vascular tension by varying the distance between the metal prongs.

The mounted specimens were immersed in temperature-controlled (37 °C) tissue baths (2 or 4 ml in volume) containing a modified Krebs solution of the following composition (mM): NaCl 133, $NaHCO_3$ 16.3, KCl 4.7, $MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8. The solution was aerated with 7% CO_2 in O_2 giving a pH of 7.2–7.3. The arteries were given an initial tension of 5 mN and the veins 2.5 mN. This resulted in spontaneous relaxations which were compensated for by adjustment of the movable unit in order to maintain tensions of 4 mN and 2 mN, respectively.

After 90 min the contractile capacity of the specimens was examined by exposure to a K^+-rich solution containing (mM): KCl 137.7, $NaHCO_3$ 16.3,
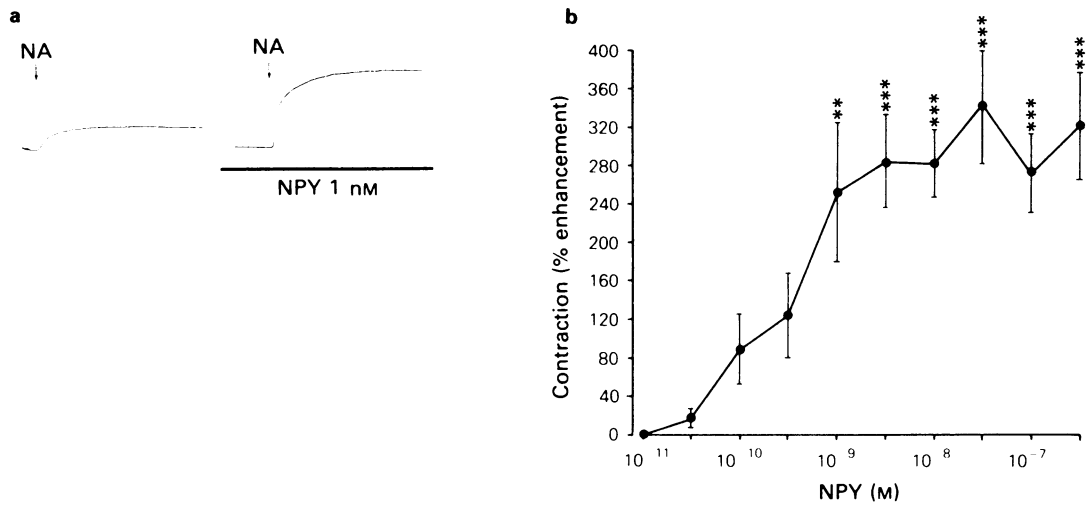
Figure 1 Tracings of the contractions of a rabbit gastro-epiploic artery after addition of noradrenaline (NA) 1 μM and 20 min later of the same concentration of NA in the presence of neuropeptide Y (NPY). The recordings (a) are from a typical experiment. Potentiation of the responses of the gastro-epiploic artery to 1 μM NA by different concentrations of NPY is shown in (b). The contractions were measured before and after addition of NPY to the bath, and the enhancement is expressed as the percentage of the response before addition of NPY. Mean values are shown with s.e.mean indicated by vertical lines. $n = 8-10$. Significance level: **$P < 0.01$; ***$P < 0.001$, using paired Student's t test.
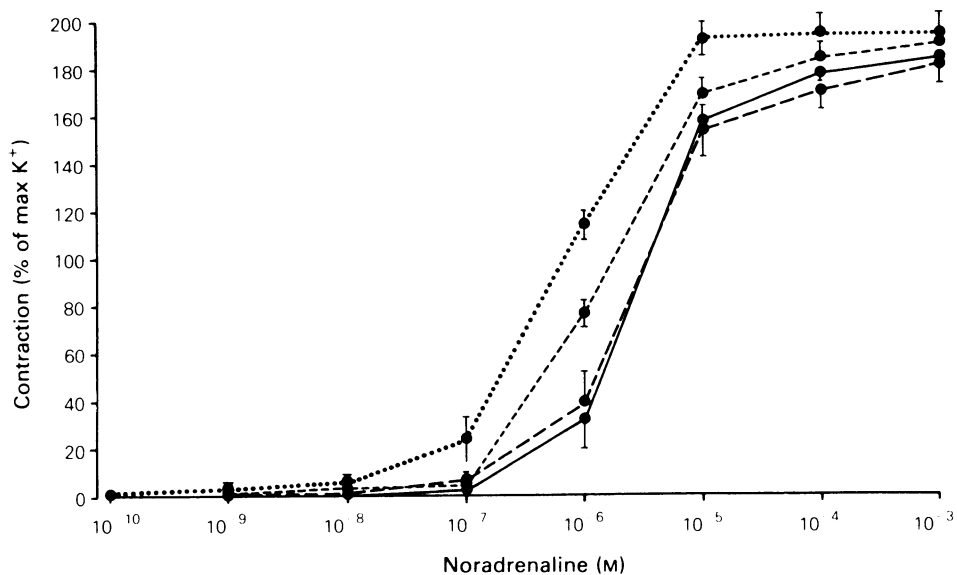

Figure 2 Duration of the potentiation of the response of the rabbit femoral artery to various doses of noradrenaline by 30 nM neuropeptide Y (NPY). NPY was present in the bath for 2 (. . . .) 30 (----) or 60 (---) min before application of noradrenaline (NA). Controls (————) did not receive NPY. Means with s.e.mean. shown by vertical lines. $n = 6-8$.
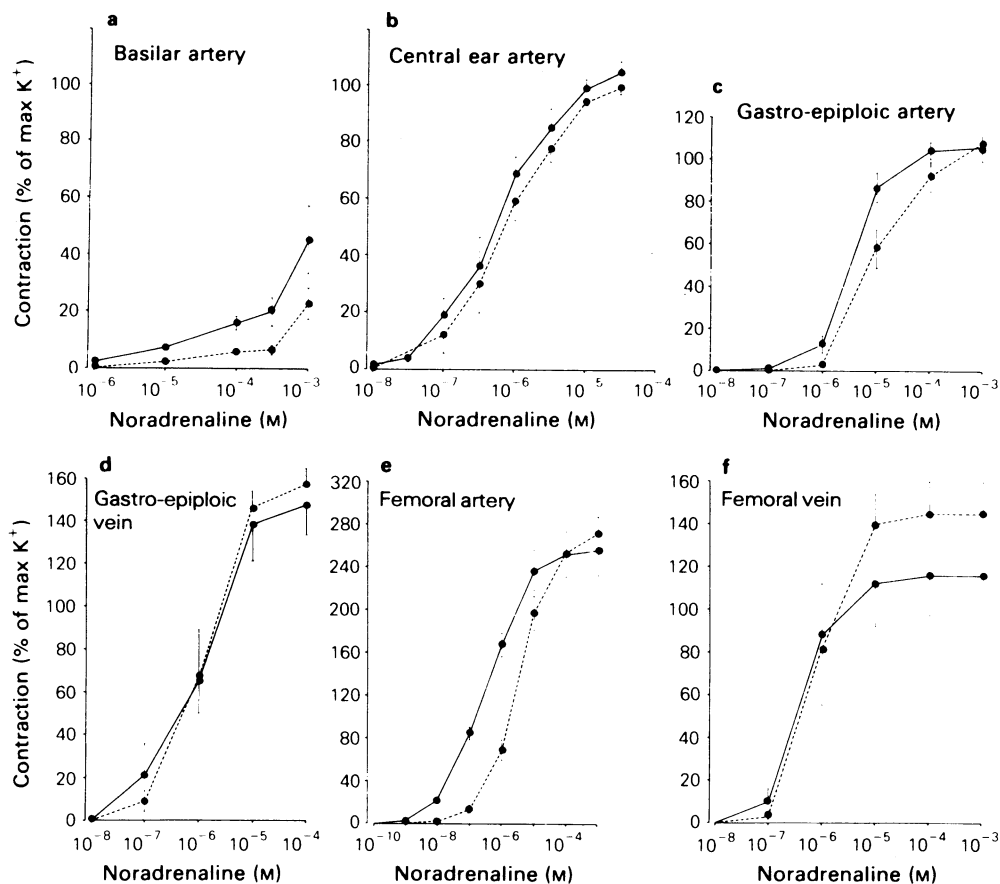
Figure 3 Concentration-response curves for noradrenaline in the absence (interrupted line) or presence (continuous line) of 30 nM neuropeptide Y (NPY): (a) rabbit basilar artery, (b) central ear artery, (c) gastro-epiploic artery, (d)gastro-epiploicvein, (e) femoral artery, and (f) femoral vein. The maximum contraction elicited by 137 nM K^+ was set as 100 and the effects of noradrenaline given as a percentage. Means are shown with s.e.mean given by vertical lines. The results in (a) could not be used for calculation of the EC_{50} values; the effect of NPY was statistically significant ($P<0.05$).

Table 1 pD_2-values for various agonists and blood vessels in the absence and presence of neuropeptide Y (NPY, 30 nM)

Agonist	Vessel	pD_2 control	pD_2 NPY	Significance level
Noradrenaline	Central ear artery	6.12 ± 0.12	6.26 ± 0.15	NS
	Gastro-epiploic artery	5.14 ± 0.10	5.76 ± 0.07	$P<0.001$
	vein	5.33 ± 0.19	5.52 ± 0.20	NS
	Femoral artery	5.80 ± 0.07	6.56 ± 0.04	$P<0.001$
	vein	6.16 ± 0.08	6.20 ± 0.16	NS
Histamine	Basilar artery	5.72 ± 0.11	6.02 ± 0.06	$P<0.05$
	Central ear artery	5.79 ± 0.14	5.97 ± 0.09	NS
	Gastro-epiploic artery	5.22 ± 0.07	5.63 ± 0.07	$P<0.005$
	vein	5.25 ± 0.15	6.06 ± 0.30	$P<0.05$
	Femoral artery	5.68 ± 0.04	6.74 ± 0.11	$P<0.001$
	vein	5.56 ± 0.05	6.31 ± 0.03	$P<0.001$
Prostaglandin $F_{2\alpha}$	Gastro-epiploic artery	5.26 ± 0.24	5.73 ± 0.11	NS
	vein	4.76 ± 0.04	6.09 ± 0.12	$P<0.001$
	Femoral artery	5.31 ± 0.06	5.32 ± 0.08	NS
	vein	5.72 ± 0.33	5.83 ± 0.10	NS

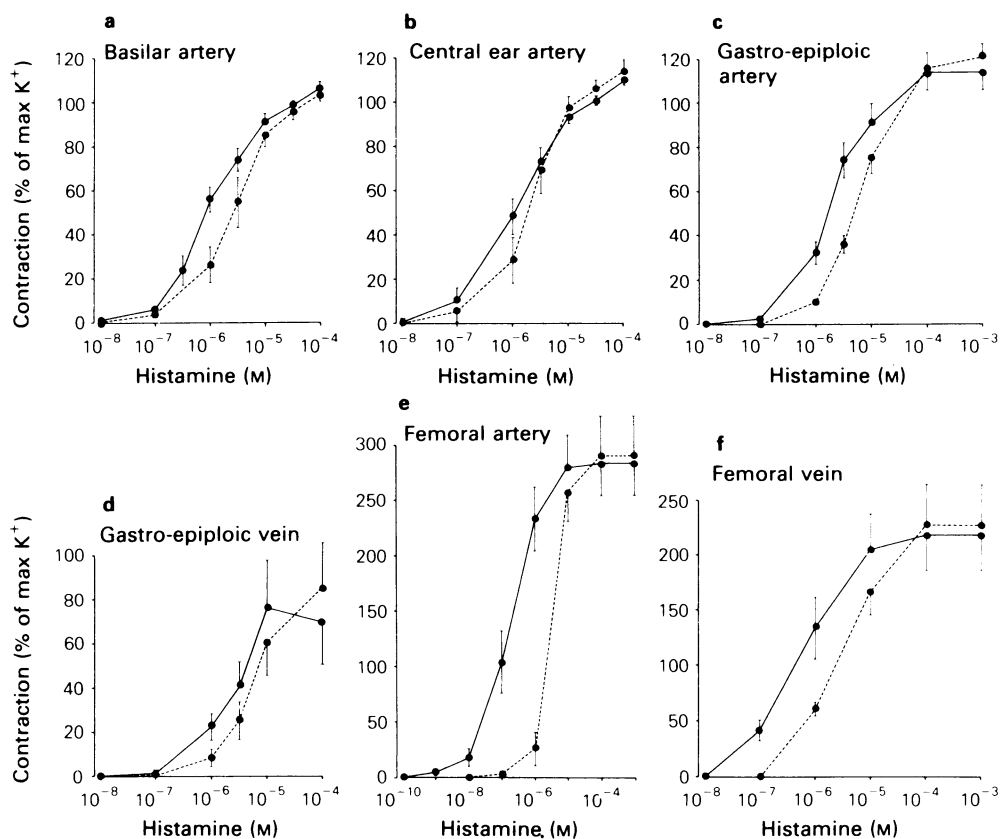Values are given as means $\pm$ s.e.mean, 6–10 experiments in each group. NS: not significant.

Figure 4 Concentration-response curves for histamine in the absence (interrupted line) or presence (continuous line) of 30 nM neuropeptide Y (NPY).) (a) Rabbit basilar artery, (b) central ear artery, (c) gastro-epiploic artery, (d) gastro-epiploic vein, (e) femoral artery and (f) femoral vein. The maximum contraction elicited by 137 mM K^+ was set as 100 and the effects of histamine given as a percentage. Means are shown with s.e.means indicated by vertical lines.

$MgCl_2$ 1.0, NaH_2PO_4 1.4, $CaCl_2$ 2.5 and glucose 7.8. These contractions served as internal standards and were set as 100% in the experiments used for construction of concentration-response curves. In one set of experiments a series of K^+ concentrations was used: 10, 20, 50 and 137.7 mM; Na^+ was used to replace K^+. The following vasoconstrictor agents were tested: noradrenaline, histamine, prostaglandin $F_{2\alpha}$ and 5-hydroxytryptamine. In each case, the concentration-response curve was obtained by adding the drug in a cumulative fashion.

Unless otherwise stated NPY was applied 2 min before the contractile agent. As controls, an equal number (6–10) of matched vascular segments were used; these received the contractile agent without prior exposure to NPY. Whenever possible pD_2 (i.e. the negative logarithm of the EC_{50} value) was calculated. The difference between the corresponding pD_2 values was tested for significance using Student's *t*

test for paired observations.

Drugs used were neuropeptide Y (NPY; Peninsula, Belmont, CA, USA), (−)-noradrenaline bitartrate (NA; Sigma, St. Louis, Mo., USA), histamine dihydrochloride (Sigma), prostaglandin $F_{2\alpha}$ (Amoglandin, Astra, Södertälje, Sweden) and 5-hydroxytryptamine creatinine sulphate (Sigma), dissolved in saline and administered in volumes of 10–30 µl to the tissue baths.

Results

Effect of neuropeptide Y (NPY)

NPY *per se* rarely caused contractions. None of the peripheral arteries studied responded to 30 nM NPY. A weak contraction of the gastro-epiploic artery was noted in 4 out of 6 experiments with 300 nM NPY. Less than 20% of the veins responded with a weak,

transient contraction to 30 nM NPY and less than 5% of the veins responded with a contraction that was about 1/10 of that evoked by high K^+.

Effect of neuropeptide Y on agonist-induced vasoconstriction

Noradrenaline (NA) NPY enhanced NA-evoked contractions in the nanomolar concentration range (Figure 1a). This was established using the gastro-epiploic artery and a fixed concentration of NA (1 μM) which resulted in reproducible contractile responses in the lower third of the linear portion of the concentration-response curve. The NPY-induced

enhancement was concentration-dependent (Figure 1b). The lowest concentration of NPY that caused a significant enhancement was 1 nM ($P < 0.01$).

In one set of experiments the duration of the potentiating effect of NPY was tested on the femoral artery by adding NPY (30 nM) to the bath, after which NA was applied cumulatively 2, 30 or 60 min later (Figure 2). Control segments run in parallel did not receive NPY. After 2 min in the bath, NPY caused a marked potentiation of NA contractions. After 30 min the potentiation of the NA response was reduced; the pD_2 value was 6.15 ± 0.07 compared with 6.47 ± 0.09, 2 min after addition of NPY (means $\pm$ s.e.mean; $P < 0.05$). After 60 min NPY no
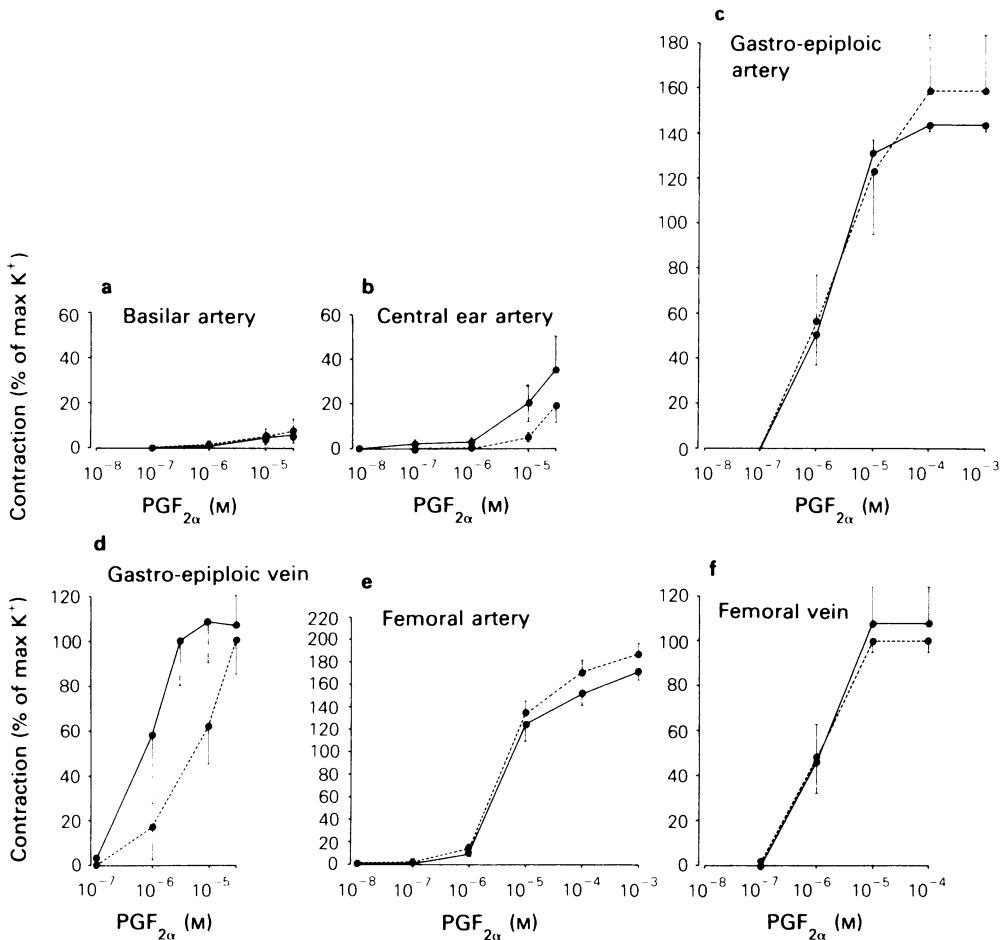

Figure 5 Concentration-response curves for prostaglandin $F_{2\alpha}$ in the absence (interrupted line) or presence (continuous line) of 30 nM neuropeptide Y (NPY). (a) Rabbit basilar artery, (b) central ear artery, (c) gastroepiploic artery, (d) gastro-epiploic vein, (e) femoral artery and (f) femoral vein. The maximum contraction elicited by 137 mM K^+ was set as 100 and the effects of prostaglandin $F_{2\alpha}$ given as a percentage. Means are shown with s.e.means indicated by vertical lines.
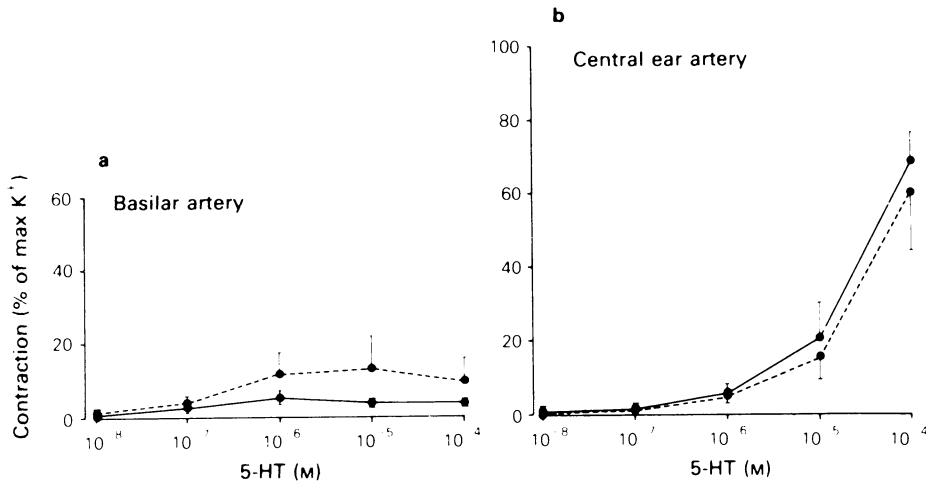
Figure 6 Concentration-response curves for 5-hydroxytryptamine (5-HT) in the absence (interrupted line) or presence (continuous line) of neuropeptide Y (NPY) (a) Rabbit basilar artery and (b) central ear artery. The maximum contraction elicited by 137 mM K^+ was set as 100 and the effects of 5-hydroxytryptamine given as a percentage. Means are shown with s.e.means indicated by vertical lines.

longer seemed to be effective; the pD_2 value was 5.85 ± 0.14, i.e. not different from that in control specimens, 5.71 ± 0.06 (means ± s.e.mean) (Figure 2).

The most marked potentiation of NA-evoked contractions was observed in the femoral artery, but the gastro-epiploic and basilar arteries were also sensitive to NPY (Figure 3, Table 1). NPY did not enhance NA-evoked contractions in any of the veins studied.

Histamine-evoked contractions were greatly potentiated by NPY (Figure 4, Table 1). This was apparent in both arteries and veins, i.e. in all vessels studied except the central ear artery.

Prostaglandin $F_{2\alpha}$-evoked contractions were not affected by NPY (Figure 5, Table 1) with the exception of the gastro-epiploic vein (Figure 5d).

5-Hydroxytryptamine Only the basilar and the central ear arteries responded with contraction to 5-hydroxytryptamine (Figure 6). NPY did not potentiate the response.

Potassium ions Contractions evoked by K^+ in concentrations ranging from 10 to 137.7 mM were not affected by pretreatment with 30 nM NPY (not illustrated).

Discussion

The present study shows that NPY, itself, has a weak vasoconstrictor effect, but a marked ability to enhance the vasoconstriction produced by other agents, notably NA and histamine. The potentiating effect of NPY was apparent in nanomolar concentrations, far lower than those necessary to evoke a contractile effect of NPY alone, and seemed to be fairly long-lasting, the effect was still present after 30 min but not after 60 min. With the knowledge that NPY coexists with NA in postsynaptic sympathetic nerve endings, it is tempting to speculate that NPY cooperates with NA. In fact, Agnati *et al.* (1983) have suggested that α-adrenoceptors may be 'unmasked' by NPY, thereby increasing the number of available α-adrenoceptor sites. Interestingly, in the present study, NPY enhanced the contractile response not only to NA, but also to histamine. The fact that the potentiating effect of NPY is not restricted to NA does not necessarily invalidate the belief that these findings are functionally relevant, since vasoconstrictor agents are thought to act via a limited repertoire of second messengers.

The specificity of the potentiating effect of NPY was evaluated by examining contractions evoked by a number of agents on different vascular segments. The vessels were chosen in order to cover a spectrum of vessels with different regional distribution and

physiological characteristics. The central ear artery, the femoral artery and the femoral vein are examples of large, well characterized vessels and the gastro-epiploic artery and vein represent peripheral vessels with a smaller diameter. The basilar artery was chosen because of the possibility that NPY is important for the cerebral circulation.

NA-evoked contractions were potentiated by NPY in all the arteries except the central ear artery, but not in the two veins. This may reflect a different mechanism of action of NA and/or NPY on arteries and veins. However, NPY was found to potentiate histamine-evoked contractions of arteries as well as veins. It is notable also that the vasoconstriction evoked by NPY alone was more marked with veins than with arteries (Ekblad *et al.*, 1984).

Except for the gastro-epiploic vein, prostaglandin $F_{2\alpha}$-evoked contractions were not affected by NPY.

There is no obvious explanation for this discrepancy between different blood vessels.

Contractions evoked by high K^+ are thought to be caused by a cell membrane depolarization, which activates 'voltage-operated Ca^{2+}-channels', distinct from 'receptor-operated Ca^{2+}-channels' (Bolton, 1979). Since contractions evoked by high K^+ were unaffected by NPY it seems that if Ca^{2+} channels are involved, only the latter type is influenced by NPY.

The present data suggest that NPY is potent in enhancing the vasoconstrictor effect of NA and histamine; this could be established from studies of several different blood vessels from the rabbit. However, all blood vessels did not display increased sensitivity to NA or histamine in the presence of NPY. The mechanism by which NPY exerts its potentiating effect is as yet unknown.

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