British Journal of Pharmacology (1995) 116, 2729-2737

Regional involvement of an endothelium-derived contractile factor in the vasoactive actions of neuropeptide Y in bovine isolated retinal arteries

[1]Dolores Prieto, Ulf Simonsen & *Niels C. Berg Nyborg

Departamento de Fisiología, Facultad de Veterinaria, Universidad Complutense, 28040-Madrid, Spain and *Department of Pharmacology, University of Aarhus, DK-8000 Aarhus C, Denmark

1 *In vitro* experiments in a microvascular myograph were designed in order to investigate the effects of human neuropeptide Y (NPY), its receptor subtype and the mechanisms underlying NPY actions in bovine isolated retinal proximal (PRA) and distal (DRA) arteries.

2 A single concentration of NPY (10 nM) induced a prompt and reproducible contraction which reached a plateau within 1-4 min, after which the response returned to baseline over the next 2-10 min. Cumulative addition of NPY induced concentration-dependent contractions of bovine retinal arteries, with an EC_{50}[M] of 1.7 nM and a maximal response equal to $54 \pm 8\%$ of E_{max} (absolute maximal contractile levels of vessels) and not different from that obtained by a single addition of the peptide. There were no significant differences in either sensitivity or maximal response to NPY between PRA and DRA.

3 Porcine NPY and the selective Y_1-receptor agonist, [Pro^{34}]NPY, also induced concentration-dependent contractions of the retinal arteries with a potency and maximal response not significantly different from those of human NPY; in contrast, the selective Y_2-receptor agonist, NPY(13-36), caused only a 5% contraction at the highest concentration used.

4 Removal of extracellular Ca^{2+} or pretreatment with the 1,4-dihydropyridine Ca^{2+}-channel blocker, nifedipine (1 μM), reduced the contractile response of 10 nM NPY to $18.4 \pm 3.3\%$ ($n=6$) and $18.6 \pm 3.9\%$ ($n=6$), respectively, of the controls.

5 Mechanical removal of the endothelium depressed the maximal contraction elicited by NPY in PRA but did not affect either sensitivity or maximal response to the peptide in DRA. In endothelium-intact arteries, blockade of the cyclo-oxygenase pathway with 3 μM indomethacin increased resting tension in both PRA and DRA and significantly inhibited sensitivity and maximal contraction to NPY of PRA and DRA, respectively. The thromboxane A_2 (TXA_2)/prostaglandin H_2 (PGH_2) receptor antagonist, SQ30741, reduced both sensitivity and maximal contraction to NPY in PRA but not in DRA.

6 In endothelium-denuded PRA, indomethacin but not SQ30741 significantly reduced NPY maximal response and induced a marked increase in resting tension suggesting a basal release of a vasodilator prostanoid from smooth muscle cells.

7 Superoxide dismutase (SOD) (150 u ml^{-1}) reduced the maximal contraction to NPY in PRA. Inhibition of the nitric oxide (NO) synthase with N^G-nitro-L-arginine (L-NOARG) (30 μM), enhanced sensitivity and maximal contraction to NPY in both PRA and DRA. In the presence of L-NOARG, SOD did not further inhibit NPY responses in PRA.

8 NPY (10 nM) induced a 2.9 fold leftwards shift of the noradrenaline concentration-response curves in PRA and increased maximal response by $50 \pm 16\%$. Neither 1 nor 10 nM NPY affected noradrenaline responses in DRA. [Pro^{34}]NPY (10 nM), but not NPY(13-36), mimicked the potentiating effect of NPY on noradrenaline responses in PRA.

9 TXA_2 analogue, U46619, at 10 nM elicited 3.6 fold leftwards shift of the noradrenaline concentration-responses curves in PRA and increased the maximal contraction by $32 \pm 3\%$, whereas in the presence of 1 μM SQ30741, 10 nM NPY did not potentiate noradrenaline responses.

10 The present results indicate that NPY may play a role in the regulation of retinal blood flow through both a direct contractile action, independent of the vessel size and a potentiation of the responses induced by noradrenaline in the proximal part of the retinal circulation, both effects being mediated by Y_1 receptors. NPY promotes Ca^{2+} influx through voltage-dependent Ca^{2+} channels and stimulates the synthesis of contractile prostanoids in PRA and DRA, although only in PRA does the peptide trigger the release of an endothelium-derived contractile factor which facilitates the contraction and also seems to account for the potentiating effect of NPY.

Keywords: Neuropeptide Y: Y_1-receptor; calcium; endothelium-derived contractile factor; thromboxane A_2; superoxide anion; noradrenaline; potentiation; bovine retinal artery

Introduction

The 36-aminoacid peptide neuropeptide Y (NPY) (Tatemoto *et al.*, 1982) coexists with noradrenaline in a population of sympathetic neurones, especially those diverted to the cardiovascular system (Edvinsson *et al.*, 1987). Systemic administration of exogenous NPY induces powerful pressor actions, which are resistant to α- and β-adrenoceptor blockade, but reversed by Ca^{2+}-antagonists (Mabe *et al.*, 1985). At the sympathetic neuroeffector junction, NPY may exert three effects: (i) a direct postjunctional receptor-mediated vasoconstriction, (ii) potentiation of several vasoconstrictor-induced

[1]Author for correspondence at present address: Institute of Pharmacology, University of Aarhus, DK-8000 Aarhus C, Denmark.

responses, and (iii) prejunctional inhibition of noradrenaline release manifested in vas deferens, heart and certain blood vessels (Tatemoto *et al.*, 1982; Edvinsson *et al.*, 1987; Wahlestedt & Reiss, 1993).

In the eye, NPY-immunoreactive fibres have been localized in the anterior uvea, iris dilator, ciliary body and posterior uvea (Zhang *et al.*, 1984; Stone *et al.*, 1986). Some of these fibres are sympathetic and originate from cell bodies in the superior cervical ganglion; however, in the choroid, where NPY-fibres seem to envelop the extensive choroid vasculature, numerous fibres persist after sympathectomy indicating a non-sympathetic origin (Zhang *et al.*, 1984). In the rabbit eye, sympathetic nerve stimulation at high frequencies causes a reduction in choroidal blood flow, which is partially resistant to α-adrenoceptor blockade (Granstam & Nilsson, 1990). Intravenous infusion of NPY in this species induced dose-dependent increase in the uveal vascular resistance, the retinal blood flow being, however, unaffected (Nilsson, 1987).

There is an apparent lack of innervation of intraocular branches of the central retinal artery (Alm & Bill, 1987; Ye *et al.*, 1990). This has led to the hypothesis that local neurones, especially peptidergic amacrine cells in the inner plexiform layer of the retina, might constitute a local reflex system in the eye, regulating retinal blood flow (Ye *et al.*, 1990). In order for such a system to be operational, it is necessary that the vascular smooth muscle in the retinal vasculature possesses receptors for these transmitters. We have recently demonstrated that calcitonin gene-related peptide (CGRP), which is contained in peripheral sensory nerves and amacrine cells in the retina, has a powerful vasodilator action in the bovine retinal circulation *in vitro* (Prieto *et al.*, 1991a). Thus a crucial link between amacrine cells and the vascular smooth muscle may be present *in vivo*.

Since the role of NPY in the retinal vasculature is not yet known, the aim of the present study was to investigate the effects of the peptide, which is present in some amacrine cells in the retina (Stone *et al.*, 1987), on bovine isolated retinal resistance arteries. We have characterized the subtype of NPY-receptor responsible for the direct contractile response and further studied the role of the Ca^{2+}-ion and the vascular endothelium for this contraction. The possible regulatory function of NPY on responses induced by specific receptor stimulation (noradrenaline) was also assessed, since functional α_1-adrenoceptors are present in these arteries (Nielsen & Nyborg, 1989).

Methods

Dissection and mounting

Eyes from cows were obtained at the local slaughterhouse and transported to the laboratory in ice-cold physiological saline solution (PSS) of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $CaCl_2$ 1.5, $MgSO_4$ 1.18, KH_2PO_4 1.17, EDTA 0.026 and glucose 11. Segments, 1–2 mm in length, of the central retinal artery close to the optic papilla (proximal retinal artery, PRA) and from peripheral branches of retinal arteries (distal retinal artery, DRA) were dissected as previously described (Nyborg *et al.*, 1990). The arterial segments were mounted as ring preparations on a double myograph (JP Trading, Denmark) which allowed direct determination of the vessel isometric tension while the internal circumference was controlled (Mulvany & Nyborg, 1980). The vessels were equilibrated in PSS oxygenated with 5% CO_2 in O_2, pH 7.4 at 37°C during 30 min and then stretched to their optimal lumen diameter for active tension development, l_0, corresponding to $0.9 \times l_{100}$, where l_{100} is the diameter the vessels would have *in situ* if subjected to a passive transmural pressure of 13.3 kPa (100 mmHg) (Nyborg *et al.*, 1990). The contractile ability of the vessels was then tested by repetitive stimulation with K-PSS (equivalent to PSS but with NaCl exchanged with KCl on an equimolar basis), until reproducible responses were recorded. The maximal contractile capacity of the vessels, E_{max}, was determined by activating them with 10 μM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and 10 μM 5-hydroxytryptamine (5-HT) in K-PSS (Nyborg *et al.*, 1990). The presence of myogenic tone was evaluated at the end of each experiment by relaxing the arteries in either Ca^{2+}-free PSS (similar to PSS except that $CaCl_2$ was replaced by 0.1 mM EGTA) or 0.1 mM papaverine.

Experimental procedure

The direct contractile effect of human NPY on retinal arteries was tested either by stimulation with a single dose of the peptide or in cumulative concentration-response experiments. In order to test the reproducibility of the response to NPY, a single dose of NPY (10 nM) was added to the organ bath twice, 30 min between each stimulation. The vessels were thoroughly washed in drug-free PSS between each stimulation.

The receptor involved in the NPY-induced contraction was characterized by determining the agonist potency ratio of selective agonists, using [Pro^{34}]NPY for Y_1- and NPY(13-36) for Y_2-receptor activation (Wahlestedt & Reiss, 1993). Furthermore, in order to investigate the receptor requirements for a specific aminoacid sequence in the NPY molecule, we compared the contractile effect of human and porcine NPY, which differ by one amino acid in position 17, where leucine is substituted for methionine.

The role of extracellular calcium for the NPY-induced contraction was tested by stimulating the arteries with 10 nM NPY, thoroughly washing them in drug-free PSS for 15 min followed by an incubation in Ca^{2+}-free PSS for 15 min before NPY was added again. In another set of experiments vessels were incubated with 1 μM nifedipine 20 min before they were contracted again with 10 nM NPY.

In order to assess the role of the endothelium in NPY-induced contractions in retinal resistance arteries, experiments were performed on paired arterial segments, one of which served as a control while in the other one the endothelial layer was mechanically removed, by first setting the vessels to an internal diameter shorter than l_0 and then rubbing the intimal surface with either a human hair or a 40 μm wire, and gently pushing the hair/wire backwards and forwards. After this procedure, the internal lumen diameter of the arteries was reset to l_0 and they were allowed to equilibrate for 20–30 min. Successful removal of the endothelium was verified by the lack of relaxation to 10 μM acetylcholine (ACh) (Benedito *et al.*, 1991).

In another set of experiments, the effects of indomethacin (1 μM), the thromboxane A_2 (TXA_2)/prostaglandin H_2 (PGH_2) antagonist, SQ30741 (1 μM) (Ogletree & Allen, 1992), superoxide dismutase (SOD, 150 u ml^{-1}) and N^G-nitro-L-arginine (L-NOARG, 30 μM) were tested on NPY contractions of retinal arteries. Control concentration-response curves were run in parallel. The effect of indomethacin and SQ30741 were also examined in endothelium-denuded segments of PRA.

The possible regulatory effect of NPY on the response to other vasoactive agents was studied on cumulative concentration-response curves to noradrenaline. After a first noradrenaline control curve, the vessels were washed in drug-free PSS for 30 min and NPY (1 and 10 nM) was added. Once the response to NPY had stabilized (10–15 min), the noradrenaline curve was repeated. The potentiating effect of the selective NPY-agonists was also tested using similar concentrations and experimental protocol as with NPY. In addition, the effect of threshold concentrations of the thromboxane-mimetic, U46619, on noradrenaline responses and also the effect of the TXA_2/PGH_2 antagonist, SQ30741 on the potentiation induced by NPY on noradrenaline responses were investigated.

Drugs

Drugs used were: human and porcine NPY, NPY(13-36) fragment (Sigma, U.S.A), [Pro^{34}]NPY, a generous gift from

Prof. T.W. Schwartz, Rigshospitalet, Copenhagen (produced by NOVO, Denmark), acetylcholine HCl (ACh) (Merck, Germany), thromboxane antagonist, SQ30741 ([1S-[1α,2α(5Z),3α,4α]]-7[[[[[(oxaheptyl)amino]acetyl]amino]methyl] -7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid), a generous gift from Dr M. Ogletree, Bristol Mayer Squibs, Princeton NJ, U.S.A., prostaglandin $F_{2\alpha}$ (Dinoprost, UpJohn, Belgium), EGTA (ethylene glycol-*bis* (β-amino ethyl ether)-*N*, *N*, *N'*, *N'*,-tetra-acetic acid), indomethacin, 5-hydroxytryptamine creatinine sulphate complex (5-HT), N^G-nitro-L-arginine, (−)-noradrenaline and the thromboxane-mimetic, U46619 (11α, 9α-epoxymethano-PGH_2, Sigma, U.S.A.). The peptides were dissolved in water in albumin-coated polypropylene tubes in concentrations of 100 μM and stored at −70°C until use. Stock solutions of indomethacin, U46619 and SQ30741 were prepared in ethanol and further diluted in water. All other drugs were dissolved in water and added in volumes not exceeding 0.3% of the tissue bath (10 ml) to reach the required concentration.

Analysis of results and statistics

Vessel responses are expressed either as active tension, Nm^{-1} (Newton per metre of vessel wall), calculated as the increase in wall force above resting level divided by twice the vessel segment length, or as a percentage of the maximal response of the vessel, E_{max}. In the experiments examining the effect of NPY on noradrenaline contractions, noradrenaline-induced responses are given as a percentage of the maximal response of the control concentration-response curve. The residual contraction to NPY after 10–15 min was subtracted from those evoked by noradrenaline in these experiments, following the procedure earlier described for rat coronary arteries (Prieto *et al.*, 1991b).

Sensitivities to the agonists are given as pD_2 values, where $pD_2 = -\log EC_{50}[M]$ and $EC_{50}[M]$ is the concentration of agonist required to produce half-maximal contraction. The $EC_{50}[M]$ was determined by fitting the vessel responses to the logistic 'Hill-equation', $R/R_{max} = A[M]^n/(A[M]^n + EC_{50}[M]^n)$, where R is the vessel response, R_{max} maximal vessel response to A, A[M] is the concentration of A and n is a curve fitting parameter, 'Hill-coefficient', using the GraphPAD InPlot version 4.0 (GraphPAD Software, San Diego, CA, U.S.A.) nonlinear curve fitting programme for personal computers.

Results are expressed as means ± standard error mean (s.e.mean). Differences between values were analyzed with Student's *t* test for paired or unpaired comparisons where appropriate. A probability less than 0.05 was considered significant for both tests.

Results

Contractile responses to NPY and NPY-receptor agonists

In bovine isolated small arteries, a single concentration of NPY (10 nM) elicited a transient fast onset contractile response, that reached a plateau within 2–4 min, followed by a decline in tension back to the basal resting level or above over the next 4–10 min (Figure 1a). Added in a cumulative way, NPY (10 pM–30 nM) induced concentration-dependent contractions in the arteries with a threshold concentration around 30–300 pM (Figure 1b and Figure 2). The contraction obtained with 30 nM NPY was not significantly different from that obtained in a cumulative way, the average responses being 0.79 ± 0.08 ($n = 14$) and 0.71 ± 0.12 Nm^{-1} ($n = 6$) corresponding to 57 ± 4% ($n = 14$) and 54 ± 8% ($n = 6$) of E_{max}, when stimulating with a single concentration and cumulatively, respectively. The average pD_2-value for human NPY was 8.77 ± 0.08 ($n = 6$), corresponding to an $EC_{50}[M]$ concentration of 1.7 nM.

The contraction elicited by 10 nM NPY could be repeated, being 0.66 ± 0.15 ($n = 8$) and 0.62 ± 0.16 ($n = 8$) in a first and second stimulation, respectively, corresponding to 51 ± 9% and 46 ± 10% of E_{max} (1.25 ± 0.10 Nm^{-1}).

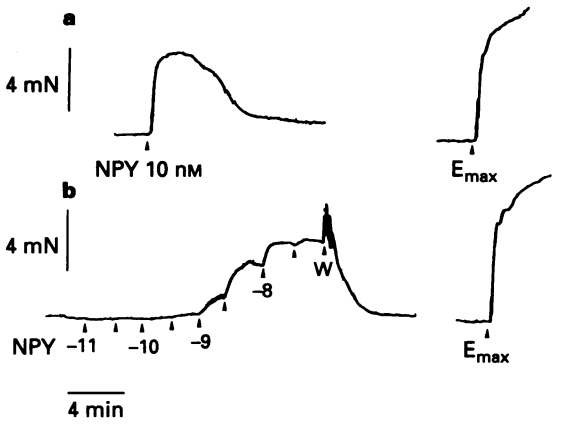

Figure 1 Isometric tracings showing the effects of (a) single (1 nM) and (b) cumulative addition of human NPY in bovine isolated retinal arteries. Effective lumen diameter, l_0, was 260 μm (a) and 235 μm (b). Vertical scale shows force (mN) and horizontal scale shows time (min). Arrows indicate the point at which each drug addition was made and numbers in (b) show the cumulative concentration of NPY (log M). E_{max} is the maximal contractile level of the vessels, achieved by activation with 10 μM $PGF_{2\alpha}$ and 10 μM 5-HT in K-PSS.

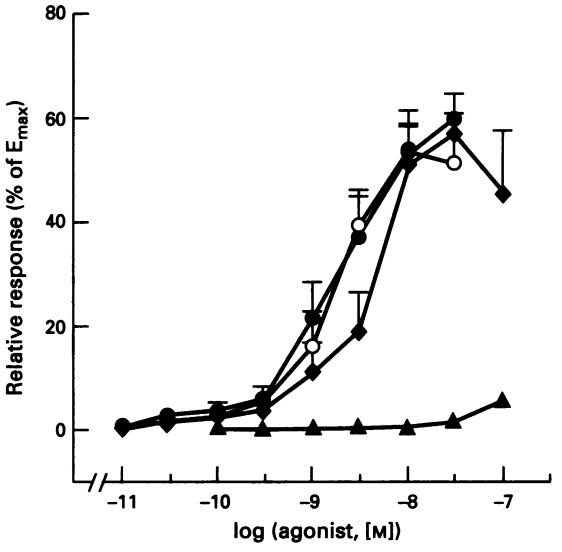

Figure 2 Contractile effects of human NPY (○), human [Pro^{34}]NPY (Y_1-selective) (◆), porcine NPY (●), and human NPY(13-36) (Y_2-selective) (▲) on bovine isolated retinal small arteries. Responses are shown as percentage of the maximal contractile response of each vessel, E_{max}. Points represent mean of 6–8 vessels with ± s.e.mean where this value exceeds the size of the symbol.

There were no significant differences in either the relative maximal contraction or the sensitivity to NPY between PRA ($l_0 = 248 \pm 3$ μm, $n = 24$) and DRA ($l_0 = 194 \pm 4$ μm, $n = 23$). The maximal responses to NPY were 1.11 ± 0.10 Nm^{-1} ($n = 24$) and 0.68 ± 0.05 Nm^{-1} ($n = 23$) for PRA and DRA, respectively, corresponding to 60 ± 2% and 60 ± 3% of E_{max} (1.83 ± 0.10 Nm^{-1}, $n = 24$ and 1.13 ± 0.08 Nm^{-1}, $n = 23$, respectively). The sensitivities to the peptide, expressed in terms of pD_2 were 8.82 ± 0.06 ($n = 24$) and 8.89 ± 0.06 ($n = 23$) for PRA and DRA, respectively.

The selective Y_1 agonist, human [Pro^{34}]NPY induced a concentration-dependent contraction of similar magnitude, 58 ± 9% of E_{max} ($n = 8$), as NPY but with a slightly but not significantly lower potency, $pD_2 = 8.48 \pm 0.14$ ($n = 8$), than human NPY. The Y_2-selective NPY agonist, NPY(13-36), caused only a weak contraction, 5 ± 1% of E_{max} ($n = 8$), at 100 nM. Porcine NPY also elicited concentration-dependent contractions with a potency, pD_2-value = 8.73 ± 0.12 ($n = 6$), and

maximal contraction, 59±5% of E_{max}, not significantly different from those of human NPY (Figure 2). Neither NPY nor NPY-receptor agonists elicited a significant relaxant response in retinal arteries submaximally precontracted with $PGF_{2\alpha}$ ($n=12$). Unless otherwise stated, the rest of the experiments were carried out with human NPY, since no differences were found between the responses to human and porcine NPY.

Mechanism of NPY-induced contraction

Both removal of extracellular Ca^{2+} and the 1,4-dihydropyridine channel blocker, nifedipine, affected NPY responses similarly in PRA and DRA. Incubation of the arteries with Ca^{2+}-free PSS containing 0.1 mM EGTA reduced the contraction induced by 10 nM (0.53±0.13 Nm^{-1}, $n=6$) to 18.6±3.9% of control ($P<0.001$) (Figure 3a). In the presence of 1 μM nifedipine, the response elicited by 10 nM NPY (0.78±0.12 Nm^{-1}, $n=6$) was also decreased to 18.4±3.3% of control ($P<0.001$) (Figure 3b).

Mechanical removal of the endothelium did not affect the basal resting tension of either PRA or DRA, but abolished and even converted into contractions the relaxations elicited by 10 μM ACh. In PRA and DRA with intact endothelium, the relaxant responses elicited by ACh were 37.9±8% ($n=7$) and 40.9±3% ($n=7$) of the 10 μM $PGF_{2\alpha}$-induced contraction, respectively. Endothelial cell removal did not change the contractile response to NPY of DRA, but it depressed the maximal contraction to the peptide in PRA (Figure 4a,b; Table 1). By contrast, both the sensitivity and relative maximal response to noradrenaline were enhanced in endothelium-denuded PRA compared to control arteries. In rings of PRA with intact endothelium, pD_2 and maximal response to noradrenaline were 6.17±0.07 ($n=5$) and 62.6±3.7% of E_{max}, respectively, whereas in endothelium-denuded arteries theses values were 6.71±0.14 ($n=5$) ($P<0.01$) and 78.9±2.0% of E_{max} ($n=5$) ($P<0.01$), respectively.

Blockade of the cyclo-oxygenase pathway by pretreatment with 3 μM indomethacin, increased the resting tension of PRA

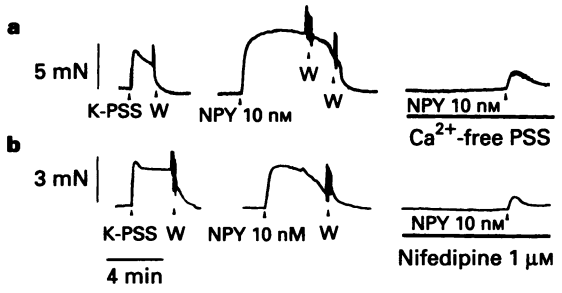

Figure 3 Effects of (a) incubation in a Ca^{2+}-free medium and (b) treatment with nifedipine on the contraction elicited by a single dose of NPY (10 nM) on bovine resistance arteries. The preparations were kept in PSS with $CaCl_2$ exchanged by 0.1 mM EGTA (a) or pretreated with nifedipine (1 μM) (b) for 20 min, before a new addition of the peptide. Effective lumen diameter, l_0, was 203 μm (a) and 156 μm (b). K-PSS: contractile response to 120 mM K^+-PSS. W: washing. Vertical scale indicates force (mN) and horizontal scale indicates time (min).

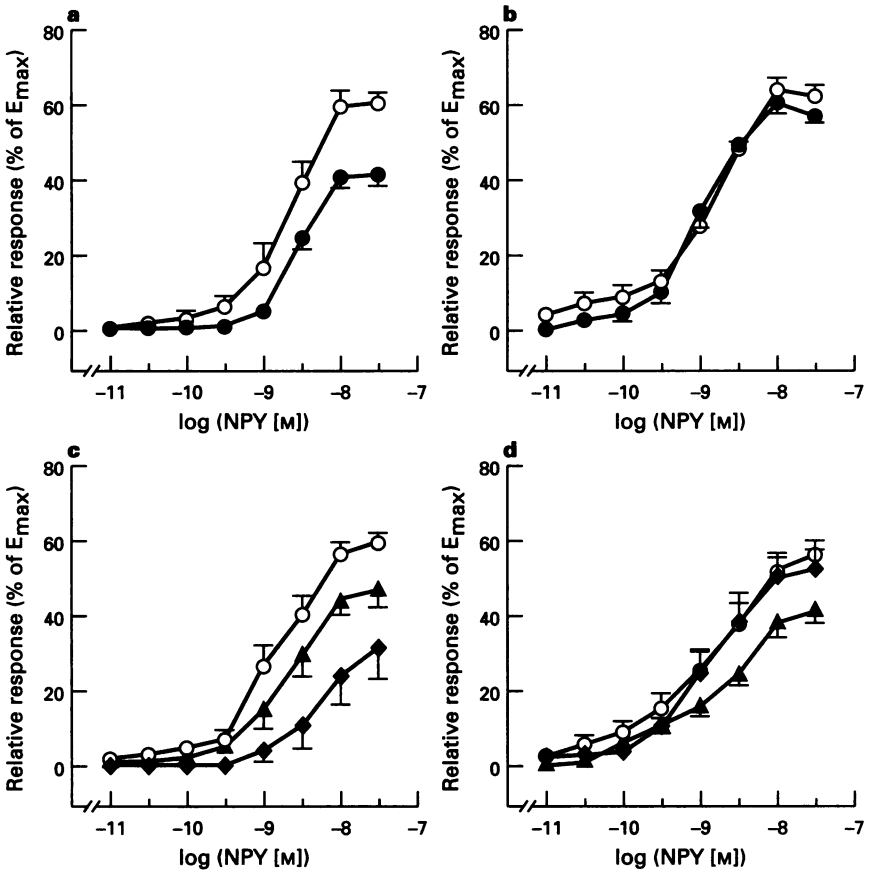

Figure 4 Effects of mechanical removal of the endothelium (a,b) and indomethacin and SQ30741 (c,d) on the concentration-dependent contractions induced by NPY in proximal (PRA) (a,c) and distal (DRA) (b,d) retinal arteries. Endothelium was mechanically removed by rubbing the intimal surface with either a 40 μm wire or a human hair. Arteries were pretreated with either 3 μM indomethacin or 1 μM SQ30741 for 30 min and the drugs were kept in the bath during the construction of the curves to NPY. Results are expressed as percentage of the maximal contraction of the vessel, E_{max}. Increases in basal tension induced by indomethacin were subtracted from those induced by NPY. Points represent mean of 5–13 vessels with s.e.mean. (○) Control experiments; (●) NPY concentration-response curves in endothelium-denuded arteries; (▲) in the presence of 3 μM indomethacin; (◆), in the presence 1 μM SQ30741.

and DRA by 6.6±2% ($n=7$) and 20.5±5% ($n=6$) of E_{max}, respectively, and significantly decreased sensitivity and maximal contraction to NPY in PRA and DRA, respectively (Figure 4c,d; Table 1). In contrast, the TXA_2/PGH_2 receptor antagonist, SQ30741 (1 μM), distinctly inhibited NPY-elicited responses only in PRA, reducing both sensitivity and maximal contraction to the peptide (Figure 4c; Table 1); however, incubation (30 min) with SQ30741 did not change contractions to NPY in DRA (Figure 4d; Table 1). At 1 μM, SQ30741 competitively inhibited the contractile response elicited by the thromboxane-mimetic, U46619, thus showing the specificity of the antagonist in blocking the TXA_2/PGH_2 receptor. pD_2 values and maximal responses to U46619 were 7.53±0.09 ($n=4$) and 1.70±0.07 Nm^{-1} in the absence, and 6.27±0.06 ($n=4$) ($P<0.01$) and 1.61±0.07 Nm^{-1} ($n=4$), in the presence of 1 μM SQ30741.

In endothelium-denuded PRA, contractions evoked by NPY were affected by treatment with indomethacin but not with SQ30741, and the former increased the basal resting tension of the arteries by 19.1±4% (0.24±0.05 Nm^{-1}) of E_{max}. pD_2 values and maximal contractions to NPY of PRA were 8.77±0.08 ($n=6$) and 0.73±0.13 Nm^{-1} ($n=6$) in the absence, and 8.89±0.07 ($n=6$) and 0.40±0.07 Nm^{-1} ($P<0.05$) ($n=6$), in the presence of indomethacin, respectively. On the other hand, pD_2 values and maximal contractions to NPY were 8.70±0.23 ($n=5$) and 0.57±0.13 Nm^{-1} ($n=5$) in endothelium-denuded control PRA, and 8.78±0.17 ($n=5$) and 0.73±0.05 Nm^{-1} ($n=5$) in endothelium-denuded PRA treated with 1 μM SQ30741.

Preincubation (10 min) of endothelium-intact PRA with SOD (150 u ml^{-1}), reduced the maximal response to NPY without significantly affecting the sensitivity to the peptide (Figure 5; Table 1). Blockade of NO synthase with 30 μM L-NOARG, increased the basal tension of both PRA and DRA by 7.8±3% ($n=5$) and 8.3±2% ($n=5$) of E_{max}, respectively, and enhanced the sensitivity and maximal contractions to NPY in both types of arteries (Figure 5; Table 1). Furthermore, SOD did not inhibit NPY responses of PRA in the presence of L-NOARG (Figure 5; Table 1).

Effect of NPY and NPY-receptor agonists on noradrenaline contractile responses

NPY (10 nM) caused a 2.9 fold leftward shift of the noradrenaline concentration-response curves in PRA, increasing the maximal contraction by 49.9±16% ($n=7$) (Figure 6a; Table 2). However, neither 1 nor 10 nM NPY significantly affected the contractile responses to noradrenaline in DRA (Figure 6b; Table 2). The selective Y_1-receptor agonist, [Pro^{34}]NPY (10 nM), but not the selective Y_2-receptor agonist, NPY (13-36) (10 nM), mimicked although with less potency, the potentiating effect of NPY on noradrenaline responses of PRA, and induced a 1.9 fold leftward shift of the control curves, increasing the maximal contraction to noradrenaline by 48.2±21 ($n=6$) (Table 2).

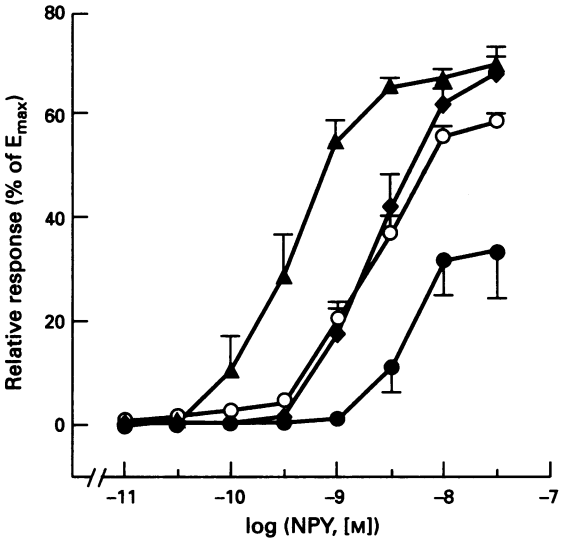

Figure 5 Effects of superoxide dismutase (SOD), N^G-nitro-L-arginine (L-NOARG) and SOD plus L-NOARG on concentration-response curves to NPY in proximal retinal arteries (PRA). Arteries were preincubated with SOD (150 u ml^{-1}) for 10 min and with L-NOARG (30 μM) for 30 min, both drugs being present throughout the experiments. Results are expressed as percentage of the maximal contraction of the vessel, E_{max}. Increases in basal tension induced by L-NOARG were subtracted from those induced by NPY. Points represent mean of 5–6 vessels with s.e.mean. (○) Control concentration-response curves; (●) in the presence of 150 u ml^{-1} SOD; (▲) in the presence of 30 μM L-NOARG; (◆) in the presence of SOD (150 u ml^{-1}) plus L-NOARG (30 μM).

Table 1 Effects of endothelial cell removal (-E), indomethacin (3 μM), SQ30741 (1 μM), superoxide dismutase (SOD) (150 u ml^{-1}) and N^G-nitro-L-arginine (L-NOAG) (30 μM) on sensitivity and absolute maximal contraction to neuropeptide Y (NPY) in proximal (PRA) and distal (DRA) retinal arteries

		Proximal retinal artery (PRA)			
	n	pD_2 (-logEC_{50})	T_{max} (Nm^{-1})	ΔBT (Nm^{-1})	l_0 (μm)
Control	35	8.72±0.06	1.07±0.06		251±3
-E	6	8.58±0.03	0.52±0.08[b]	–	258±8
Indomethacin	7	8.66±0.09[a]	0.84±0.14	0.11±0.03[a]	253±4
SQ30741	6	8.08±0.19[a]	0.65±0.18[a]	–	264±10
SOD	6	8.22±0.16	0.57±0.17[a]	–	252±7
L-NOARG	5	9.45±0.16[a]	1.53±0.20[a]	0.20±0.08[a]	249±11
SOD+L-NOARG	5	8.69±0.10	1.23±0.12	–	246±10
		Distal retinal artery (DRA)			
	n	pD_2 (-logEC_{50})	T_{max} (Nm^{-1})	ΔBT (Nm^{-1})	l_0 (μm)
Control	22	8.87±0.06	0.61±0.05	–	202±5
-E	6	8.95±0.11	0.49±0.06	–	199±5
Indomethacin	6	8.98±0.22	0.53±0.06[a]	0.25±0.07[b]	222±11
SQ30741	5	8.78±0.09	0.76±0.10	–	216±8
L-NOARG	5	9.50±0.09[b]	0.97±0.05[b]	0.11±0.03[a]	195±5

Values are mean±s.e.mean. n, number of vessels (one from each animal). EC_{50} is the concentration of agonist required to produce half-maximal contraction. T_{max} is the maximal absolute response obtained with NPY. ΔBT is the increase in basal tension after treatment. l_0 is the normalized internal diameter of the retinal arterial segment at which the experiments were performed.
Significantly different parameter compared to control tested by Student's t test: [a]$P<0.05$; [b]$P<0.01$.

Furthermore, a threshold concentration (10 nM) of the thromboxane-mimetic, U46619, which did not elicit significant contractions (0.08 ± 0.01 Nm^{-1}, $n=5$), induced a potent 3.6 fold leftward shift of the noradrenaline concentration-response curves in PRA, and increased the maximal contraction by $31.8 \pm 2.6\%$ ($n=5$) (Figure 6c). pD_2 values and absolute maximal contractions to noradrenaline were 6.29 ± 0.11 ($n=5$) and 1.32 ± 0.03 Nm^{-1} ($n=5$) in control curves, and 6.85 ± 0.17 ($n=5$) ($P<0.05$) and 1.74 ± 0.01 Nm^{-1} ($n=5$) ($P<0.01$) in the presence of 10 nM U46619. Preincubation (30 min) with 1 μM of the TXA_2/PHG_2 receptor antagonist, SQ30741, abolished the potentiating effect of 10 nM NPY on noradrenaline-elicited contractions of PRA (Figure 6c). pD_2 values and maximal responses to noradrenaline were 6.15 ± 0.09 ($n=5$) and 1.21 ± 0.20 Nm^{-1} ($n=5$) in control curves, and 6.06 ± 0.10 ($n=5$) and 1.19 ± 0.10 Nm^{-1} ($n=5$), after incubation with both SQ30741 plus subsequent addition of NPY.

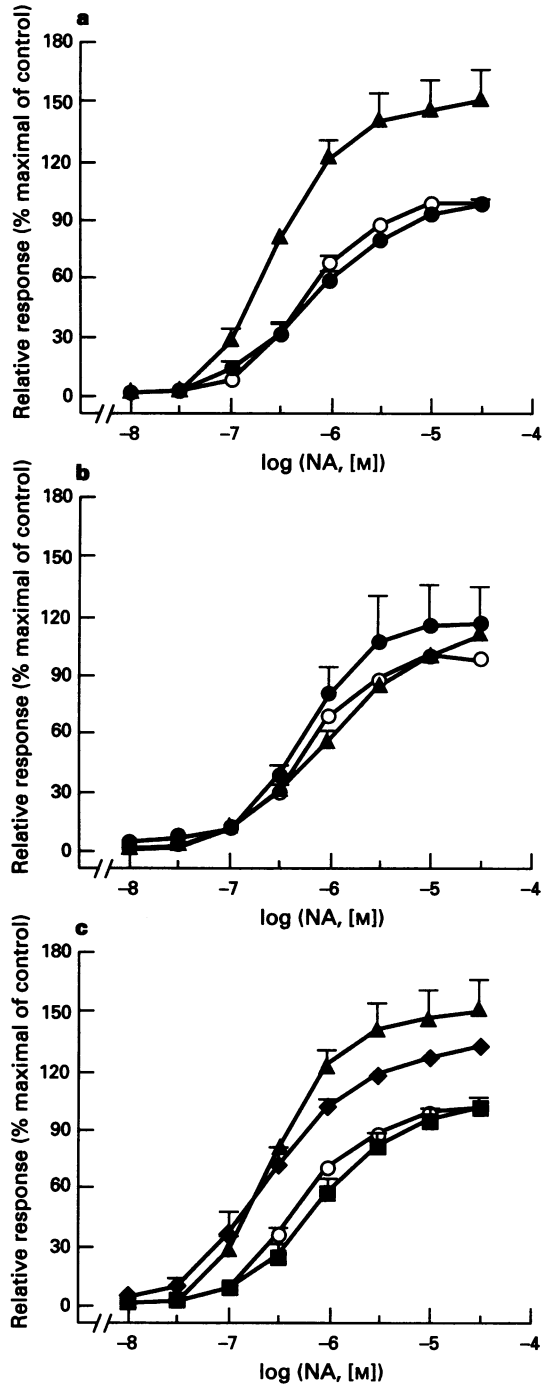

Figure 6 Potentiating effect of NPY on the contractions induced by noradrenaline (NA) in proximal (PRA) (a) and distal (DRA) (b) bovine retinal arteries. NPY (1 and 10 nM) was added 10–15 min before the construction of a second dose-response curve to NA. (c) Comparative effects of NPY, the thromboxane analogue, U46619, and SQ30741 plus NPY on NA contractile responses of PRA. Arteries were pretreated with either NPY (10 nM) or U46619 (10 nM) for 10–15 min and with SQ30741 (1 μM) for 30 min before a second concentration-response curve for NA was constructed. Results are expressed as percentage of the maximal response to NA in the control curve. Residual contractions to either NPY or U46619 after 10 min were subtracted from those elicited by NA. Points are mean of 5–18 vessels with s.e.mean. (○) Control NA concentration-response curves: (●) in the presence of 1 nM NPY; (▲) in the presence of 10 nM NPY; (◆) in the presence of 10 nM U46619; (■) in the presence of 10 nM NPY after 30 min preincubation with 1 μM SQ30741.

Discussion

The results of the present study demonstrate firstly that NPY may act on bovine retinal arteries *in vitro* through Y_1-receptors, causing both a direct contractile effect and a potentiation of the responses induced by noradrenaline. The contraction induced by NPY was transient, quick in onset and not sustained in most vessels. It was reversed by wash-out and could be repeated on at least a second stimulation without any significant loss of contractile effect. NPY-evoked vasoconstriction *in vitro* has been reported for several vascular beds (Edvinsson *et al.*, 1987; Pernow, 1988; 1989; Prieto *et al.*, 1991b; Owen, 1993; Tessel *et al.*, 1993), although the contraction elicited by the peptide in these vascular preparations was characterized by a slow onset and long duration, and it underwent rapid tachyphylaxis upon repeated exposure (Pernow, 1988; Prieto *et al.*, 1991b). Specifically, intravenous infusion of NPY induces a decrease in the rabbit uveal blood flow which is moderate at 2 min and maximal at 10 min (Granstam & Nilsson, 1990), in contrast to the contraction evoked by NPY in isolated retinal arteries. These distinct differences in the vasoconstrictor properties of NPY in the retinal circulation compared to other vascular beds has also been noted for other vasoactive peptides, such as endothelin-1 (Nyborg *et al.*, 1991) and CGRP (Prieto *et al.*, 1991a) both causing reproducible effects, which could be ascribed to either a different metabolism by endopeptidases or different signal transduction mechanisms coupling the peptide and its receptor, which would make these agents capable of adjusting the vascular tone and thus possibly also the autoregulatory capacity of the retinal blood flow (Alm & Bill, 1987).

Our results strongly indicate that NPY mediates its contractile effect in retinal resistance arteries through activation of Y_1-receptors, since the selective Y_1-agonist, [Pro^{34}]NPY (Potter *et al.*, 1991) has a similar effect to genuine NPY and the selective Y_2-agonist NPY(13-36) fragment was virtually inactive in eliciting contraction in the retinal arteries. The results are thus in agreement with the concept that the majority of postjunctional vascular NPY receptors belong to the Y_1-subtype (Wahlestedt & Reis, 1993), although some vessels have been reported to have a mixed population of postjunctional Y_1 and Y_2 receptors (Tessel *et al.*, 1993). The bovine NPY receptor does not appear to discriminate between human and porcine NPY, possibly because these two peptides only differ by one amino acid in position 17, where methionine is substituted with leucine in porcine NPY (Wahlestedt & Reiss, 1993).

In many cell types, NPY receptor-stimulation alters free intracellular Ca^{2+} concentrations, by at least four distinct mechanisms: activation of Ca^{2+} influx through L-type channels and inhibition of Ca^{2+} influx through N-type channels, and mobilization of Ca^{2+} from intracellular stores secondary to activation of phospholipase C or independent of inositol phosphate (Michel, 1991). The present study demonstrates that NPY promotes influx of extracellular Ca^{2+} through voltage-dependent L-type calcium channels in bovine retinal resistance arteries, since removal of extracellular Ca^{2+} and the L-type channel blocker, nifedipine, reduced NPY contractions to a

Table 2 Effects of neuropeptide Y (NPY) and NPY-receptor agonists on sensitivity and absolute maximal contraction to noradrenaline (NA) in proximal (PRA) and distal (DRA) retinal arteries

		Proximal retinal artery (PRA)			
	n	pD_2 (-logEC$_{50}$)	T_{max} (Nm^{-1})	ΔpD_2	l_0 (μm)
Control NA	7	6.08±0.09	1.15±0.06		245±5
1 nM NPY	7	6.14±0.11	1.11±0.07	–	245±5
Control NA	8	6.21±0.05	0.98±0.18		243±7
10 nM NPY	8	6.80±0.05[b]	1.37±0.15[b]	0.44±0.07[a]	243±7
Control NA	5	6.21±0.05	0.69±0.08		242±3
10 nM [Pro^{34}]NPY	5	6.49±0.14[a]	0.95±0.06[a]	0.29±0.12[a]	242±3
Control NA	5	5.93±0.09	1.54±0.18		246±4
10 nM NPY(13–36)	5	5.96±0.04	1.46±0.17	–	246±4
		Distal retinal artery (DRA)			
	n	pD_2 (-logEC$_{50}$)	T_{max} (Nm^{-1})	ΔpD_2	l_0 (μm)
Control NA	5	6.29±0.07	0.53±0.09		198±13
1 nM NPY	5	6.25±0.13	0.58±0.11	–	198±13
Control NA	5	6.23±0.05	0.61±0.11		200±5
10 nM NPY	5	6.16±0.07	0.64±0.09	–	200±5

Values are mean±s.e.mean. *n*, number of vessels (one from each animal). EC_{50} is the concentration of agonists required to produce half-maximal contraction. T_{max} is the maximal absolute response obtained with NA. ΔpD_2 is the increase in sensitivity to NA after NPY or NPY-agonists treatment. l_0 is the normalized internal diameter of the retinal arterial segment at which the experiments were performed. Residual contractions to NPY and [Pro^{34}]NPY were subtracted from those to NA in the second concentration-response curve. Significantly different parameter compared to control tested by paired *t* test: [a]$P<0.05$; [b]$P<0.01$.

similar extent. In addition, NPY induced a small contraction in arteries kept in Ca^{2+}-free PSS which suggests that either some Ca^{2+} for the peptide contraction may be released from intracellular stores (Erdbrüger *et al.*, 1993) or NPY may sensitize the contractile apparatus to Ca^{2+} (Lobaugh & Blackshear, 1990). The greater extracellular Ca^{2+}-dependence for NPY contraction in retinal arteries compared to other vascular preparations (Mabe *et al.*, 1985; Pernow, 1988; Erdbrüger *et al.*, 1993) could be ascribed to the vessel size: vascular smooth muscle in resistance arteries is known to be highly dependent on the presence of extracellular Ca^{2+} for maintenance of agonist-induced contractions (Laher & Van Breemen, 1991).

The vascular endothelium has been reported either not to affect (Pernow, 1989) or to depress (Prieto *et al.*, 1991b) contractile responses elicited by NPY *in vitro*. One of the major findings of the present study, however, is that the endothelium differentially facilitates NPY contractions in bovine retinal vascular bed, since mechanical removal of endothelial cells depressed NPY responses in PRA but not in DRA. The endothelium-independence of NPY contractions in the more distal parts of the retinal vasculature is in agreement with that reported for small arteries of other vascular bed (Pernow, 1989; Prieto *et al.*, 1991b). A great deal of evidence is now available which demonstrates that in addition to mediating relaxations, endothelium can also facilitate contractile responses of vascular smooth muscle through the release of contractile factors (Rubanyi, 1988; Katusic *et al.*, 1988; Descombes *et al.*, 1993). Thus, high K^+ solutions, certain agonists, stretch and anoxia, can trigger the synthesis/release of endogenous vasoconstrictor mediators from the endothelium in several vascular beds. The possibility that the procedure of endothelial cell removal damaged the underlying vascular smooth muscle, thus reducing NPY contractions in PRA, can be excluded in the present experiments since, according to the criteria suggested by Rubanyi (1988): (a) in DRA from the same vascular bed, neither sensitivity nor maximal response to NPY were reduced by this procedure, (b) the sensitivity to NPY was not significantly affected in PRA, (c) contractile responses to other agonists such as noradrenaline, were enhanced rather than depressed by mechanical endothelial cell removal.

In addition, the present results strongly suggest a TXA_2-like substance as a possible vasoconstrictor mediator of the NPY-induced contraction in PRA, an observation which is supported by the distinct inhibitory effect of the TXA_2 antagonist, SQ30741, on the response to NPY in endothelium-intact PRA, and which confirms a recent *in vivo* study showing that TXA_2 mediates part of the constrictor effect of NPY in the dog coronary circulation (Martin *et al.*, 1992). Furthermore, the TXA_2/PGH_2 receptor antagonist, SQ30741, did not have any inhibitory effect on the contractile responses to NPY of endothelium-denuded PRA, thus suggesting that the vasoconstrictor prostanoid involved in NPY responses derives from the endothelium. Endothelial cells in culture can produce TXA_2, both spontaneously or after arachidonic acid stimulation (Buzzard *et al.*, 1993). In addition, contractions elicited by certain agonists in the basilar (Katusic *et al.*, 1988; Descombes *et al.*, 1993) and pulmonary artery (Altiere *et al.*, 1986; Buzzard *et al.*, 1993) involve endothelial TXA_2 production. Therefore, the present results provide evidence that NPY contractions in PRA are partly due to the release of TXA_2 from the vascular endothelium.

On the other hand, the present study demonstrates a vasoconstrictor effect of indomethacin which suggests a basal release of a vasodilator prostanoid, previously reported for other vascular beds with autoregulatory properties such as cerebral (Klaas & Wadsworth, 1989) and coronary arteries (Szwajkun *et al.*, 1991). This observation also confirms earlier *in vivo* studies showing that injection of indomethacin decreased retinal blood flow in rabbits (Bill, 1979). Although the endothelium is a known source of prostaglandins production, smooth muscle cells have been shown to synthesize amounts of prostacyclin equivalent to endothelial cells in culture (Baezinger *et al.*, 1979). According to this, removal of the endothelium greatly enhanced the constrictor effect of indomethacin in PRA, which indicates that this vasodilator prostanoid may be released from smooth muscle cells. In addition, indomethacin caused a significant inhibition of NPY contractions in both endothelium- denuded PRA and endothelium-intact DRA, NPY contractile effect not being affected by the TXA_2 antagonist, in either condition. This, along with the fact that endothelial cell removal did not affect NPY responses in DRA, suggests that the peptide triggers the synthesis/release of a contractile prostanoid other than TXA_2, from retinal vascular smooth muscle.

The inhibition of NPY contractions in PRA by SQ30741 can result from either TXA_2-receptor blockade, PGH_2-receptor blockade or both, since the prostaglandin endoper-

oxides, PGG_2 and PGH_2, the common precursors of prostaglandins and TXA_2, cause contraction of blood vessels via interaction with receptors shared with TXA_2 (Maiss *et al.*, 1985). In addition, the hydroperoxidase activity of PGH synthase is a very potent generator of superoxide anions (Kukreja *et al.*, 1986) and the concentration and capacity of this enzyme is several fold higher in endothelial cells than in smooth muscle (Smith, 1986). In the present study, the inhibitory effect of SOD, a superoxide scavenger, indicates that NPY responses in PRA are partly due to the generation of superoxide anions. These results are in agreement with those reported for canine large cerebral arteries, which have been used as a model where certain agonists induce endothelium-dependent contractions involving TXA_2, PGH_2 and superoxide anions under normal conditions (Katusic *et al.*, 1988; Cosentino *et al.*, 1994).

The preventative effect of SOD on vascular contractions can be due to either an inhibition of the direct contractile effect of superoxide anions on the vascular smooth muscle (Katusic & Vanhoutte, 1989) or a protection of the endothelium-derived relaxing factor, since superoxide anions are known to react with and to inactivate NO, thus promoting vasoconstriction (Gryglewski *et al.*, 1986). Contractions to NPY in both PRA and DRA were greatly enhanced in arteries with intact endothelium treated with L-NOARG, thus indicating that these contractions may be inhibited by a basal release of endothelial NO (Benedito *et al.*, 1991), as demonstrated for NPY contractions in rat large coronary arteries (Prieto *et al.*, 1991b). The apparent discrepancy between the enhancing effect of L-NOARG and the inhibitory or lack of effect of endothelial cell removal on NPY contractions of PRA and DRA, respectively, could be ascribed to the increased production of vasodilator prostanoids by the muscle in endothelium-denuded arteries, as indicated by the experiments with indomethacin, although a basal release of an endothelium-derived contractile factor, earlier proposed for this arterial bed (Benedito *et al.*, 1991), cannot be ruled out. On the other hand, the fact that in L-NOARG-treated PRA, contractions to NPY were less sensitive to the inhibitory effect of SOD suggests that superoxide anions may modulate NPY endothelium-dependent contractions via inactivation of NO. These results are similar to those reported for the calcium ionophore A23187 in canine basilar artery (Katusic *et al.*, 1988; Cosentino *et al.*, 1994), that translocates calcium into the endothelial cells thus activating both the cyclo-oxygenase and the L-arginine/NO pathway; the generation of superoxide anions would impair the balance between endothelial relaxing and contractile factors thus leading to constriction of the underlying smooth muscle.

On the other hand, the present study demonstrates that NPY enhances noradrenaline-induced responses in the proximal part of the retinal arterial system, results which agree with the view that the potentiation of noradrenaline-induced vasoconstriction by NPY is more pronounced in large arteries (Pernow, 1988; Prieto *et al.*, 1991b; Owen, 1993). In addition, the present results are consistent with recent reports demonstrating that NPY potentiates the vasoconstriction to noradrenaline through postsynaptic Y_1 receptors (Wahlestedt & Reiss, 1993). The mechanisms underlying the potentiating effect of NPY in arterial smooth muscle have been reported to be variable depending on the vascular bed. Thus, a large amount of evidence supports the theory that NPY may potentiate vasoconstriction by promoting Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels (Adriantsitohaina & Stoclet, 1988; Adriantsitohaina *et al.*, 1993; Xiong *et al.*, 1993). On the other hand, in porcine cultured aortic smooth muscle cells, NPY stimulates myosin light-chain kinase thus allowing sensitization of the contractile proteins (Lobaugh & Blackshear, 1990). As noted above, potentiation of noradrenaline responses by NPY occurs in PRA but not DRA, although the magnitude of NPY contractions was similar in both large and small bovine retinal arteries. The differences in the potentiating effect of NPY between proximal and distal parts of the retinal arterial tree could be related to the different mechanisms underlying NPY contractile responses in PRA and DRA, as demonstrated in the present study. Thus, the possibility that NPY amplifies the noradrenaline-induced contraction in PRA by either an increased calcium influx or an additive effect of the residual contraction to the peptide after 10 min on noradrenaline tone, seems unlikely, since NPY also induces calcium entry in DRA and there is also a residual contraction to the peptide in the smallest retinal peripheral branches, where the peptide does not potentiate noradrenaline responses. On the contrary, NPY triggers the synthesis/release of a TXA_2-like substance from the vascular endothelium only in PRA and this could account for the potentiating effect of the peptide, since TXA_2 has been shown to sensitize the contractile apparatus of vascular smooth muscle cells to calcium via a G protein (Crichton *et al.*, 1993). The present experiments show that threshold concentrations of U46619, not eliciting significant contraction, mimic the potentiating effect of NPY in PRA, and furthermore, that this amplifying effect of the peptide on noradrenaline responses is abolished by TXA_2/PGH_2 receptor blockade. Therefore, we propose an indirect potentiating mechanism of NPY in PRA through the release of endothelial TXA_2 or a TXA_2-like substance which would, in turn, increase the sensitivity of the contractile apparatus to calcium, thus enhancing noradrenaline contraction. The fact that NPY elicits potentiation of noradrenaline responses at a concentration of 10 nM, despite the TXA_2 antagonist having an inhibitory effect on the contractions to lower concentrations of the peptide, can be ascribed to the release not only of TXA_2, but probably also PGH_2 and superoxide anions upon NPY receptor stimulation, as indicated by the experiments with SOD.

In summary, the present experiments demonstrate that NPY, acting on postsynaptic Y_1-receptors, can regulate bovine retinal vascular tone through both a potent, direct contractile effect which involves cyclo-oxygenase activation, and a potentiation of the vasoconstriction induced by noradrenaline. Moreover, an endothelium-derived contractile factor seems to play an important role as mediator of the peptide vasoactive actions in the proximal part of the retinal circulation.

Manuel Perales and Francisco Puente are thanked for technical assistance and Dr I. Sáenz de Tejada for helpful discussions. Eyes were donated by Matadero Municipal de Madrid and Århus Offentlige Slagtehus. Supported by grants from Universidad Complutense de Madrid, grant No. PR 189/92-4174, the Danish Medical Research Council, grant No. 12-8717 and Øjenfonden. This study is part of the EURAD research programme.

References

ADRIANTSITOHAINA, R., BIAN, K., STOCLET & BUKOSKI, R.D. (1993). Neuropeptide Y increases force development through a mechanism that involves calcium entry in resistance arteries. *J. Vasc. Res.*, **30**, 309–314.

ADRIANTSITOHAINA, R. & STOCLET, J.C. (1988). Potentiation by neuropeptide Y of vasoconstriction in rat resistance arteries. *Br. J. Pharmacol.*, **95**, 419–428.

ALM, A. & BILL, A. (1987). Ocular circulation. In *Adler's Physiology of the Eye*, ed. Moses, R.A., pp. 183–203. St. Louis, U.S.A.: Mosby.

ALTIERE, R.J., KIRITSY-ROY, J.A. & CATRAVAS, J.D. (1986). Acetylcholine-induced contractions in isolated rabbit pulmonary artery: Role of thromboxane A_2. *J. Pharmacol. Exp. Ther.*, **236**, 535–541.

BAEZINGER, N.L., BECHERER, P.R. & MAJERUS, P.W. (1979). Characterization of prostacyclin synthesis in cultured human smooth muscle cells, venous endothelial cells and skin fibroblasts. *Cell*, **16**, 967–974.

BENEDITO, S., PRIETO, D., NIELSEN, P.J. & NYBORG, N.C.B. (1991). Role of endothelium in acetylcholine-induced relaxation and spontaneous tone of bovine isolated retinal small arteries. *Exp. Eye Res.*, **52**, 575–579.

BILL, A. (1979). Effects of indomethacin in conscious rabbits- a microsphere study. *Acta Physiol. Scand.*, **105**, 437–442.

BUZZARD, C.J., PFISTER, S.L. & CAMPBELL, W.B. (1993). Endothelium-dependent contractions in rabbit pulmonary artery are mediated by thromboxane A_2. *Circ. Res.*, **72**, 1023–1034.

COSENTINO, F., SILL, J.C. & KATUSIC, Z.S. (1994). Role of superoxide anions in the mediation of endothelium-dependent contractions. *Hypertension*, **23**, 229–235.

CRICHTON, C.A., TEMPLETON, A.G.B., MCGRATH, J.C. & SMITH, J.L. (1993). Thromboxane A_2 analogue, U-46619, potentiates calcium-activated force in human umbilical artery. *Am. J. Physiol.*, **264**, H1878–H1883.

DESCOMBES, J.-J., DEVYS, M., LAUBIE, M. & VERBEUREN, T.J. (1993). Endothelial thromboxane production plays a role in the contraction caused by 5-hydroxytryptamine in rat basilar arteries. *Eur. J. Pharmacol.*, **243**, 193–199.

EDVINSSON, L., HÅKANSON, R., WAHLESTEDT, C. & UDDMAN, R. (1987). Effects of neuropeptide Y on the cardiovascular system. *Trends Pharmacol. Sci.*, **8**, 231–232.

ERDBRÜGER, W., VISCHER, P., BAUCH, H.-J. & MICHEL, M.C. (1993). Norepinephrine and neuropeptide Y increase intracellular calcium in cultured porcine aortic smooth muscle cells. *J. Cardiovasc. Pharmacol.*, **22**, 97–102.

GRANSTAM, E. & NILSSON, S.F.E. (1990). Non-adrenergic sympathetic vasoconstriction in the eye and some other facial tissues in the rabbit. *Eur. J. Pharmacol.*, **175**, 175–186.

GRYGLEWSKI, R.J., PALMER, R.M.J. & MONCADA, S. (1986). Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature*, **320**, 454–456.

KATUSIC, Z.S., SHEPHERD, J.J. & VANHOUTTE, P.M. (1988). Endothelium-dependent contractions to calcium-ionophore A23187, araquidonic acid and acetylcholine in canine basilar arteries. *Stroke*, **19**, 476–479.

KATUSIC, Z.S. & VANHOUTTE, P.M. (1989). Superoxide anion is an endothelium-derived contracting factor. *Am. J. Physiol.*, **257**, H33–H37.

KLAAS, M. & WADSWORTH, R. (1989). Contraction followed by relaxation in response to hypoxia in sheep isolated middle cerebral artery. (1989). *Eur. J. Pharmacol.*, **168**, 187–192.

KUKREJA, R.C., KONTOS, H.A., HESS, M.Z. & ELLIS, E.F. (1986). PGH synthase and lipoxygenase generate superoxide in the presence of NADH and NADPH. *Circ. Res.*, **59**, 612–619.

LAHER, I. & VAN BREEMEN, C. (1991). Regulation of calcium sensitivity in vascular smooth muscle. In *The Resistance Vasculature*. ed. Bevan, J.A., Halper, W. & Mulvany, M.J., pp. 305–317. Totowa, New Jersey: Humana Press.

LOBAUGH, L.A. & BLACKSHEAR, P.J. (1990). Neuropeptide Y stimulation of myosin light-chain phosphorylation in cultured aortic smooth muscle cells. *J. Biol. Chem.*, **265**, 18393–18399.

MABE, Y., TATEMOTO, K. & HUIDOBRO-TORO, P. (1985). Neuropeptide Y-induced pressor responses: Activation of a non-adrenergic mechanism, potentiation by reserpine and blockade by nifedipine. *Eur. J. Pharmacol.*, **116**, 33–39.

MAISS, D.E., SAUSSY, D.L., CHAIKOUNI, A., KOCHEL, P.J., KNAPP, D.R., HAMANAKA, N. & HALUSSHKA, P.V. (1985). Pharmacological characterization of human and canine thromboxane A_2/prostaglandin H_2 receptors in platelets. *J. Pharmacol. Exp. Ther.*, **223**, 408–424.

MARTIN, S.E., KUVIN, J.T., OFFENBACHER, S., ODLE, B.M. & PATTERSON, R.E. (1992). Neuropeptide Y and coronary vasoconstriction: role of thromboxane A_2. *Am. J. Physiol.*, **263**, H1045–H1053.

MICHEL, M.C. (1991). Receptors for neuropeptide Y: multiple subtypes and multiple second messengers. *Trends Pharmacol. Sci.*, **12**, 389–394.

MULVANY, M.J. & NYBORG, N.C.B. (1980). An increased sensitivity of mesenteric resistance vessels in young and adult spontaneously hypertensive rats. *Br. J. Pharmacol.*, **71**, 585–596.

NIELSEN, P.J. & NYBORG, N.C.B. (1989). Adrenergic responses in isolated bovine retinal resistance arteries. *Int. Opthalmol.*, **13**, 103–107.

NILSSON, S.F.E. (1987). Effects of NPY on ocular blood flow and local blood flow in some other tissues in the rabbit. *J. Physiol.*, **390**, 119P.

NYBORG, N.C.B., KORSGAARD, N. & NIELSEN, P.J. (1990). Active wall tension - length curve and morphology of isolated bovine retinal small arteries: important feature for pharmacodynamic studies. *Exp. Eye Res.*, **51**, 217–224.

NYBORG, N.C.B., PRIETO, D., BENEDITO, S. & NIELSEN, P.J. (1991). Endothelin-1-induced contraction of bovine retinal small arteries is reversible and abolished by nitrendipine. *Invest. Opthalmol. Vis. Sci.*, **32**, 27–31.

OGLETREE, M.L. & ALLEN, G.T. (1992). Interspecies differences in thromboxane receptors: studies with thromboxane receptor antagonists in rat and guinea-pig smooth muscles. *J. Pharmacol. Exp. Ther.*, **260**, 789–794.

OWEN, M.P. (1993). Similarities and differences in the postjunctional role of neuropeptide Y in the sympathetic vasomotor control of large vs. small arteries of rabbit renal and ear vasculature. *J. Pharmacol. Exp. Ther.*, **265**, 887–895.

PERNOW, J. (1988). Co-release and functional interactions of neuropeptide Y and noradrenaline in peripheral sympathetic vascular control. *Acta Physiol. Scand.*, **Suppl 568**, 1–56.

PERNOW, J. (1989). Actions of constrictor (NPY and endothelin) and dilator (substance P, CGRP and VIP) peptides on pig splenic and human skeletal muscle arteries: involvement of the endothelium. *Br. J. Pharmacol.*, **97**, 983–989.

POTTER, E.K., FUHLENDORFF, J. & SCHWARTZ, T.W. (1991). [Pro^{34}]neuropeptide Y selectively identifies postjunctional mediated actions of neuropeptide Y in vivo in rats and dogs. *Eur. J. Pharmacol.*, **193**, 15–19.

PRIETO, D., BENEDITO, S., NIELSEN, P.J. & NYBORG, N.C.B. (1991a). Calcitonin gene-related peptide is a potent vasodilator of bovine retinal arteries in vitro. *Exp. Eye Res.*, **53**, 399–405.

PRIETO, D., BENEDITO, S., SIMONSEN, U. & NYBORG, N.C.B. (1991b). Regional heterogeneity in the contractile and potentiating effects of neuropeptide Y in rat isolated coronary arteries: modulatory action of the endothelium. *Br. J. Pharmacol.*, **102**, 754–758.

RUBANYI, G.M. (1988). Endothelium-derived vasoconstrictor factors. In *Endothelial cell*, ed. Ryan, U.S., Vol. III, pp. 61–70, CRC Press, Boca Raton, Florida, U.S.A.

SMITH, W.L. (1986). Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. *Ann. Rev. Physiol.*, **48**, 251–262.

STONE, R.A., LATIES, A.M. & EMSON, P.C. (1986). Neuropeptide Y and the ocular innervation of rat, guinea-pig, cat and monkey. *Neuroscience*, **17**, 1207–1216.

SZWAJKUN, K., LAMPING, K.G. & DOLE, W.P. (1990). Role of endothelium-derived relaxing factor and prostaglandins in responses of coronary arteries in vivo. *Circ. Res.*, **66**, 1729–1737.

TATEMOTO, K., CARLQUIST, M. & MUTT, V. (1982). Neuropeptide Y - A novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature*, **296**, 659–660.

TESSEL, R.E., MILLER, D.W., MISSE, G.A., DONG, X. & DOUGHTY, M.B. (1993). Characterization of vascular postsynaptic neuropeptide Y reception function. 1. NPY-induced vasoconstriction in isolated rat femoral artery is mediated by both Y_1 and Y_2 receptors: evidence from benextramine protection studies. *J. Pharmacol. Exp. Ther.*, **265**, 172–177.

WAHLESTADT, C. & REISS, D.J. (1993). Neuropeptide Y-related peptides and their receptors- are the receptors potential therapeutic drug targets? *Annu. Rev. Pharmacol. Toxicol.*, **32**, 309–352.

XIONG, Z., BOLZON, B.J. & CHEUNG, D.W. (1993). Neuropeptide Y potentiates calcium-channel current in single vascular smooth muscle cells. *Pflügers Arch.*, **423**, 504–510.

YE, X., LATTIES, A.M. & STONE, R.A. (1990). Peptidergic innervation of the retinal vasculature and optic nerve head. *Invest. Ophthalmol. Vis. Sci.*, **31**, 1731–1737.

ZHANG, S.Q., TERENGHI, G., UNGER, W.G., ENNIS, K.W. & POLAK, J. (1984). Changes in substance P and neuropeptide Y-immunoreactive fibres in rat and guinea-pig irides following unilateral sympathectomy. *Exp. Eye Res.*, **39**, 365–372.

(Received December 20, 1994
Revised June 24, 1995
Accepted July 27, 1995)