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In vivo receptor characterization of neuropeptide Y-induced effects in consecutive vascular sections of cat skeletal muscle

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1 It has been suggested that the vasoconstrictor response to neuropeptide Y (NPY) is located in the microvessels and that it increases with reduced vessel diameter. The aim of the present study was to analyse quantitatively, on the cat gastrocnemius muscle preparation *in vivo*, the effects of NPY on total regional vascular resistance (R_T) and its distribution to large-bore arterial resistance vessels (>25 μm; $R_{a,prox}$), small arterioles (<25 μm; $R_{a,micro}$) and the veins (R_v). Associated effects on capillary pressure ($P_{c,v}$) and fluid exchange were also studied.

2 Close-arterially infused NPY (1–32 μg kg^{-1} min^{-1}) caused a dose-dependent, slowly developing vasoconstriction in all three vascular sections, yet with a preferential action in the small arterioles. At 32 μg kg^{-1} min^{-1}, NPY raised R_T by 133±22%, $R_{a,prox}$ by 94±15%, $R_{a,micro}$ by 277±104% and R_v by 81±11%. However, the veins (ED_{50} = 3.9±1.2 μg kg^{-1} min^{-1}) were more sensitive to NPY than both large-bore arterial vessels (ED_{50} = 7.7±1.6) and small arterioles (ED_{50} = 7.0±1.4). NPY decreased $P_{c,v}$ due to an increase in the pre-to post-capillary resistance ratio.

3 Close-arterial infusions of Pro^{34}NPY and peptide YY evoked vasoconstrictor responses which did not differ from the response to NPY. In contrast, the Y_2-preferring C-terminal fragments: Ac-[Leu^{28}, Leu^{31}]-NPY(24–36) and NPY(13–36) were without effect in the muscle vascular bed. The selective NPY Y_1 receptor antagonist BIBP3226 (100 μg kg^{-1} min^{-1}, i.a.) abolished the vascular response to NPY.

4 The present findings indicate that the vasoconstrictor response to NPY in skeletal muscle is preferentially located in the small arterioles and mediated via the Y_1 receptor and, further, that Y_2 and Y_3 receptors do not play a significant role in the vasoconstrictor response to NPY in cat skeletal muscle. BIBP3226 was found to be an effective NPY antagonist *in vivo* and to lack agonist activity.

Keywords: Arteriole; BIBP3226; microcirculation; neuropeptide Y; vein; Y_1 receptor

Introduction

The sympathetic nervous system is of fundamental importance for the regulation of the peripheral circulation. The 36 amino acid peptide neuropeptide Y (NPY) is co-stored with the classical transmitter noradrenaline in sympathetic nerves supplying the cardiovascular system (Lundberg *et al*., 1982; Edvinsson *et al*., 1987). Perivascular NPY-containing fibres are more numerous around arteries and arterioles compared to veins, and as the diameter of the muscular arteries decreases, the density of the NPY innervation increases (Sundler *et al*., 1993).

Despite a strong vasopressor effect *in vivo*, NPY causes only weak constriction of the relatively large blood vessels which has been studied *in vitro* (Grundemar & Håkanson, 1994). Thus, it has been suggested that the vasoconstrictor effect of NPY is preferentially located in the microcirculation and that it may increase with reduced vessel diameter (Owen, 1993). To examine this hypothesis we analysed the effects of NPY in cat skeletal muscle, by use of an experimental *in vivo*-technique (Mellander *et al*., 1987a, b; Björnberg *et al*., 1988) which permitted continuous and simultaneous recordings of vascular resistance in the whole vascular bed and its consecutive sections, large bore arterial resistance vessels (>25 μm), small arterioles (<25 μm) and the veins.

The previous absence of specific NPY receptor antagonists has made receptor characterization difficult. However, by using agonists such as modified NPY analogues, at least three receptor types have been suggested: Y_1, Y_2 and Y_3 (for review see Wahlestedt & Reis, 1993). Among these the Y_1-receptor and the Y_2-receptor have been cloned (Larhammar *et al*., 1992; Herzog *et al*., 1992; Rose *et al*., 1995). Bioactivity and binding studies indicate that the vascular NPY receptor is mainly of the Y_1 type (Sheikh *et al*., 1991; Grundemar *et al*., 1992; Erlinge *et al*., 1993), but there is also evidence for the existence of Y_2-mediated vasoconstriction, e.g. in the splenic vascular bed in the pig (Modin *et al*., 1991). All three receptor types are activated by NPY. The truncated peptides NPY(13–36) and Ac-[Leu^{28}, Leu^{31}]-NPY(24–36) (Potter *et al*., 1994), are Y_2-prefering agonists which have weak or no effects on Y_1 and Y_3 (Wahlestedt & Reis, 1993). Conversely, Pro^{34}NPY activates Y_1 and Y_3 with weak effects on Y_2. Peptide YY (PYY) activates Y_1 and Y_2 with weak effects on Y_3 (Wahlestedt & Reis, 1993).

Molecular cloning has recently revealed new members of the NPY-receptor family. A Y_4 receptor with high affinity for pancreatic polypeptide (PP) and PYY (Lundell *et al*., 1995), has been cloned and also a receptor involved in NPY-induced food intake, Y_5 (Gerald *et al*., 1996). A murine receptor with pharmacology resembling the Y_1-receptor seems to be expressed mainly in the brain (Weinberg *et al*., 1996).

Recently BIBP3226, a selective and highly potent non-peptide NPY Y_1 receptor antagonist, has been developed (Rudolf *et al*., 1994). *In vivo*, BIBP3226 antagonizes the NPY-induced increase in blood pressure with no effect on noradrenaline-, endothelin-, vasopressin- or angiotensin-induced pressor responses. BIBP3226 also antagonized the effects of NPY in vascular Y_1 receptor models such as the perfused rat kidney, mesentery or rabbit ear preparation (Doods *et al*., 1995). The subtype specificity has also been demonstrated by binding studies in different species (Wieland *et al*., 1995).

In the present study, the aim was to provide a precise and quantitative definition of the site of action of NPY along the vascular bed from artery to vein under *in vivo* conditions, and to characterize the receptor(s) which mediate the vascular ef-

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fects of this agonist. For this purpose, the selective antagonist BIBP3226 and modified NPY analogues were used. Skeletal muscle seems to be a well suited model organ to study the vascular effects of NPY, since it is by far the largest tissue in the body, and vascular effects in this organ will therefore be of great overall haemodynamic importance.

Methods

Skeletal muscle preparation and recordings

The study was performed on young adult male cats (mean body wt 4.1 kg). The animals were premedicated with atropine (50 μg kg^{-1} body wt) and anaesthetized with α-chloralose (50 mg kg^{-1} body wt, later supplemented by 20 mg kg^{-1} body wt), both drugs being applied through a cubital vein catheter inserted under local anaesthesia. After anaesthesia, a tracheal cannula was inserted to facilitate spontaneous respiration. Body temperature was monitored continuously and stayed within normal limits (38 ± 0.5°C) during the experiments. Expiratory P_{CO_2} was measured continuously (Eliza CO_2 analyzer, Gambro Engström, Bromma, Sweden), and arterial P_{O_2} and pH at intervals (ABL 330; Radiometer, Copenhagen, Denmark). When necessary, artificial ventilation (Servo ventilator 900B; Siemens-Elema, Stockholm, Sweden) was used and a trometamol/bicarbonate buffer solution (Tribonat; Kabi Pharmacia, Stockholm, Sweden) was infused i.v.

Observations were made on the sympathectomized lower leg muscles of the right hindlimb, a preparation described in detail previously (Mellander *et al*., 1987a, b; Björnberg *et al*., 1988). In brief, the muscle region was auto-perfused *in situ* via an arterial shunt placed between the femoral and popliteal artery. The venous outflow was diverted via a shunt to the right jugular vein. Regional blood flow was recorded continuously with a differential pressure flowmeter (Grände & Borgström, 1978) inserted in the arterial shunt. The muscle preparation with intact arterial and venous supply was placed in a plethysmograph to permit volumetric recordings of net transcapillary fluid flux with a gravimetric volume recorder connected to a Grass FT 10C transducer. Mean arterial inflow pressure (P_A) and venous outflow pressure (P_V) were recorded from T-tubes close to the popliteal artery and vein. P_V was set at the normal level of about 7 mmHg which in the control situation established an isovolumetric state (Starling fluid equilibrium) in the muscle preparation.

This technique provides reliable continuous recordings of the following circulatory variables: arterial, arteriolar, capillary and venous pressures, regional blood flow, transcapillary fluid exchange, and of resistances in the whole muscle vascular bed (R_T) and in the following consecutive vascular sections: large-bore arterial resistance vessels (>25 μm; $R_{a,prox}$), small arterioles (<25 μm; $R_{a,micro}$), and the veins (R_v). Arteriolar pressure ($P_{arteriole}$) and capillary pressure towards the venous capillary end ($P_{c,v}$) were recorded via fine catheters inserted into the sural artery and vein, respectively, as previously described in detail (Mellander *et al*., 1987a, b; Björnberg *et al*., 1988).

After completion of surgery and instrumentation, the animals were left to equilibrate for at least 1 h so as to permit recovery of intrinsic myogenic vascular tone to a stable and normal (Maspers *et al*., 1990b) level before the start of the experimental interventions.

Drugs

By using a precision infusion pump (Harvard Apparatus, model 11), drugs were administered close-arterially to the muscle region via slow (0.01–0.2 ml min^{-1}) infusions in the shunt between the femoral and the popliteal artery. Each drug dose was infused until a clear-cut steady state vascular response had developed (usually after about 3 min) or, when no response was observed, for at least 5 min. The tip of the infusion needle was placed in the retrograde direction to facilitate mixing of the drugs in the blood stream. Control experiments in which isotonic saline was infused at the described rates demonstrated that infusion artifacts were negligible.

The drugs used were Ac-[Leu^{28}, Leu^{31}]-NPY(24–36) (Auspep, Parkville, Australia), BIBP3226 ((*R*)-N2-(diphenacetyl)-N-[4-hydroxyphenyl)methyl]-D-argininamide, a generous gift from Dr H. Doods and Dr K. Rudolf, Dr. Karl Thomae GmbH, Germany), NPY (Auspep), NPY(13–36) (Auspep), Pro^{34}NPY (a kind gift from Prof. Thue Schwartz, Copenhagen), PYY (Auspep). All drugs were dissolved in isotonic saline. Time data for the drug effects described in Results are corrected for dead space delay (15 s) in the tubing.

Calculations and experimental protocol

All observations were obtained on the skeletal muscle vascular bed after acute sympathectomy accomplished by severing the sciatic nerve. Total and segmental vascular resistances in the muscle preparation were derived as follows from the recorded regional blood flow (Q) and the relevant driving pressures obtained from the four pressure signals, arterial inflow pressure (P_A), pressure in arterioles of a size of about 25 μm ($P_{arteriole}$), capillary pressure towards the venous end of the capillaries ($P_{c,v}$) and the venous outflow pressure (P_V): $R_T = (P_A - P_V)/Q$; $R_{a,prox} = (P_A - P_{arteriole})/Q$; $R_{a,micro} = (P_{arteriole} - P_{c,v})/Q$; $R_v = (P_{c,v} - P_V)/Q$. Thus, the method implies that R_T always equals the sum of $R_{a,prox} + R_{a,micro} + R_v$. These vascular resistances and all other parameters were continuously recorded on line by a computer programme (AcqKnowledge for the Macintosh; Biopac Systems Inc., Goleta, CA, U.S.A.). Resistances are expressed in peripheral resistance units (pru; mmHg ml^{-1} min 100g tissue).

The changes in the pre- to postcapillary resistance ratio in response to the infused drugs, influencing the hydrostatic capillary pressure ($P_{c,v}$) and net transcapillary fluid exchange, was calculated as $(R_{a,prox} + R_{a,micro})/R_v$.

The sensitivity of the studied vascular regions to NPY was evaluated as the dose (μg kg^{-1} min^{-1}) of NPY eliciting half of the maximum increase in resistance (ED_{50}). ED_{50} values were calculated for each experiment.

Data are expressed as mean values $\pm$ s.e.mean. Percentage values for resistance changes were calculated for each individual experiment and then averaged. Comparisons of data groups were performed by analysis of variance (ANOVA) followed by Bonferroni's test with a significance level of 5%, or by using paired Student's *t* test, differences being considered significant at P values <0.05.

Results

Vascular response to NPY

General pattern of response NPY was infused close-arterially to the acutely sympathectomized muscle preparation in increasing doses (1, 2, 4, 8, 16, 32 μg kg^{-1} muscle tissue min^{-1}), to determine the dose-response characteristics and to establish the vascular smooth muscle effector sensitivity along the vascular bed. The pattern of vascular response to NPY (8 μg kg^{-1} min^{-1}, i.a.) is illustrated by the original tracings in Figure 1. As can be seen, NPY elicited a clear-cut vasoconstrictor response which reached an approximate steady state about 2.5 min after the start of the infusion, and disappeared slowly within about 5 min after cessation of the infusion. In the steady state, NPY increased overall regional vascular resistance (R_T) from 17.6 to 30.7 pru (+70%), in turn explained by constrictions in all three consecutive sections of the vascular bed ($R_{a,prox}$, $R_{a,micro}$ and R_v). This constrictor response was well maintained when the NPY infusion was continued for 5 min. At none of the tested doses was a vasodilator response to NPY observed.

Compiled data for the vascular response to NPY A detailed description and quantitative analysis of the vascular response to NPY is presented in Figure 2a ($n=7$ throughout). These infusions were all applied in a stable control situation when basal vascular tone in the muscle preparation, after sufficient equilibration, had reached a normal steady-state level. Overall vascular tone in the muscle preparation in this state corresponded to a total regional resistance (R_T) of 19.4 ± 1.6 pru, (Figure 2a; first column, total height of column), of which 12.1 ± 2.1 pru resided in the large-bore arterial resistance vessels ($R_{a,prox}$), 6.3 ± 1.2 pru in the small arterioles ($R_{a,micro}$) and 1.1 ± 0.1 pru in the veins (R_v). Blood flow in the control state was 5.4 ± 0.4 ml min^{-1} 100g^{-1} and $P_{c,v}$ was 12.1 ± 0.3 mmHg.

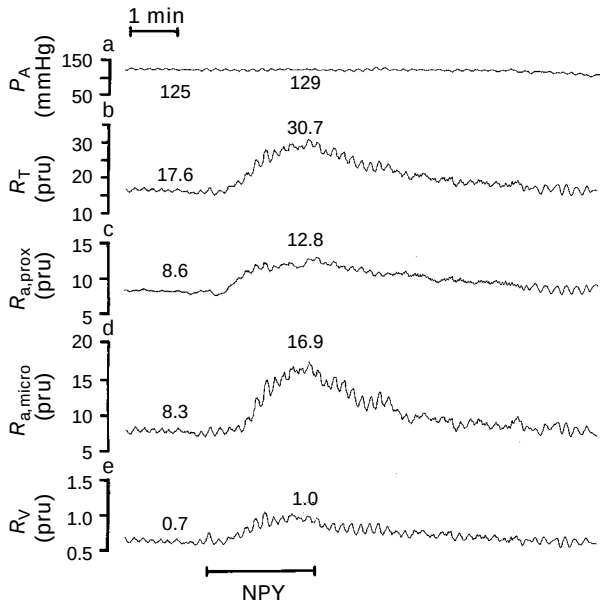

Figure 1 Pattern of vascular response in cat skeletal muscle to a close-arterial infusion of NPY ($8\,\mu g\,kg^{-1}\,min^{-1}$) on vascular resistance in (b) the whole vascular bed (R_T) and its consecutive sections, (c) large-bore arterial resistance vessels ($R_{a,prox}$), (d) small arterioles ($<25\,\mu m$; $R_{a,micro}$) and (e) the veins (R_v). (a) Shows the mean arterial inflow pressure (P_A). Note slowly developing and disappearing vasoconstriction.

NPY evoked a dose-dependent vasoconstriction which at the dose of 32 $\mu g\,kg^{-1}\,min^{-1}$ corresponded to a rise in R_T from the control value of 19.4 to 45.9 ± 7.3 pru ($+133\pm22\%$), in turn caused by an increase in $R_{a,prox}$ to 23.1 ± 4.4 pru ($+94\pm15\%$), in $R_{a,micro}$ to 21.0 ± 5.6 pru ($+277\pm104\%$) and in R_v to 1.8 ± 0.2 pru ($+81\pm11\%$). All these effects were statistically significant.

There was no difference in sensitivity to NPY between the large-bore arterial resistance vessels ($ED_{50}=7.7\pm1.6\,\mu g\,kg^{-1}\,min^{-1}$; $n=7$) and the small arterioles ($ED_{50}=7.0\pm1.4\,\mu g\,kg^{-1}\,min^{-1}$; $n=7$), but the veins ($ED_{50}=3.9\pm1.2\,\mu g\,kg^{-1}\,min^{-1}$; $n=7$) were more sensitive to NPY than both the large-bore arterial resistance vessels ($P<0.05$) and the small arterioles ($P<0.05$, Figure 3). In contrast, the amplitude of the vasoconstrictor response (% resistance increase) in the $R_{a,micro}$ section was (at doses $\geqslant4\,\mu g\,kg^{-1}\,min^{-1}$) significantly larger (ANOVA) than in the other sections (see Figure 3). It can be concluded that NPY is a potent vasoconstrictor in skeletal muscle *in vivo* with a slowly developing and long-lasting effect in all three studied consecutive vascular sections, yet with a preferential action in the small arterioles.

NPY caused a dose-dependent fall in capillary pressure owing to an increase in the pre- to post-capillary resistance ratio. At the dose of 32 $\mu g\,kg^{-1}\,min^{-1}$, $P_{c,v}$ fell from the control value of 12.1 ± 0.3 to 10.9 ± 0.4 mmHg ($P<0.05$, $n=7$) with a consequent transcapillary fluid absorption.

NPY receptor characterization

Vascular effects of Pro^{34}NPY, PYY, NPY(13–36) and Ac-[Leu^{28}, Leu^{31}]-NPY(24–36) The modified NPY receptor

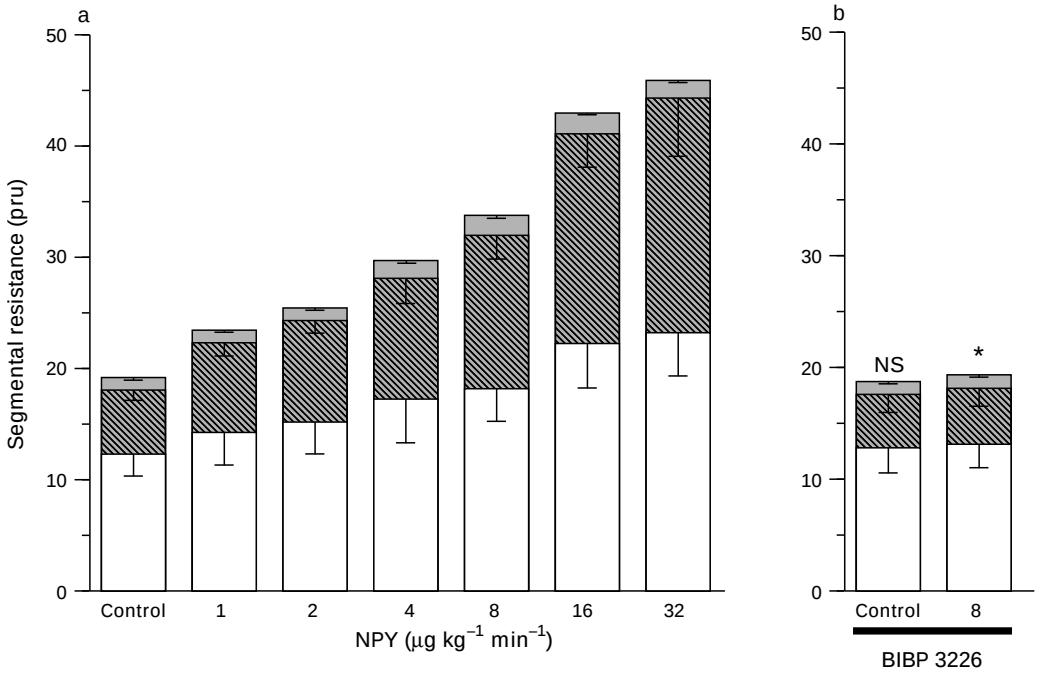

Figure 2 (a) Compiled data ($n=7$) for the vasoconstrictor response to close-arterially infused NPY on total regional vascular resistance (total height of the columns), and its segmental distribution to the $R_{a,prox}$ (open area), $R_{a,micro}$ (hatched area) and R_v (solid area) sections in cat skeletal muscle *in vivo*. Note preferential constrictor response in the $R_{a,micro}$ section. (b) Vascular response to NPY ($8\,\mu g\,kg^{-1}\,min^{-1}$, i.a.; $n=6$) in the presence of BIBP3226 ($100\,\mu g\,kg^{-1}\,min^{-1}$, i.a.). *$P<0.05$ compared to infusion of NPY ($8\,\mu g\,kg^{-1}\,min^{-1}$) in the absence of BIBP3226. NS = non significant compared to control in the absence of BIBP3226.

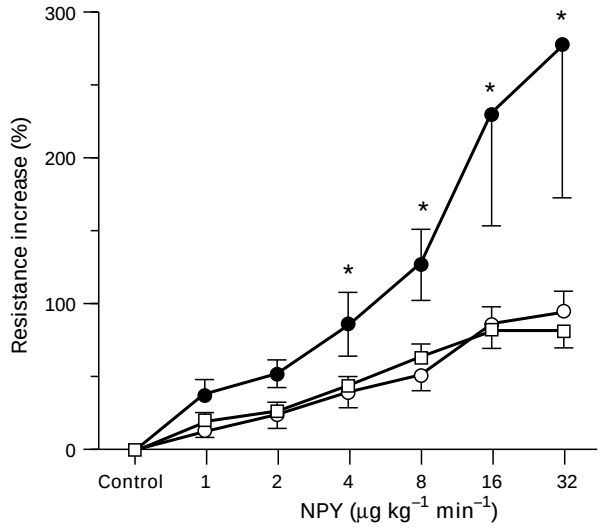

Figure 3 Compiled data ($n=7$ throughout) for the vasoconstrictor response to close-arterially infused NPY expressed as % resistance increase in the $R_{a,prox}$ (○), the $R_{a,micro}$ (●) and the R_v (□) sections in cat skeletal muscle *in vivo*. Note preferential constrictor response in the $R_{a,micro}$ section and higher sensitivity for R_v. $^*P<0.05$; $R_{a,micro}$ compared to both $R_{a,prox}$ and R_v.

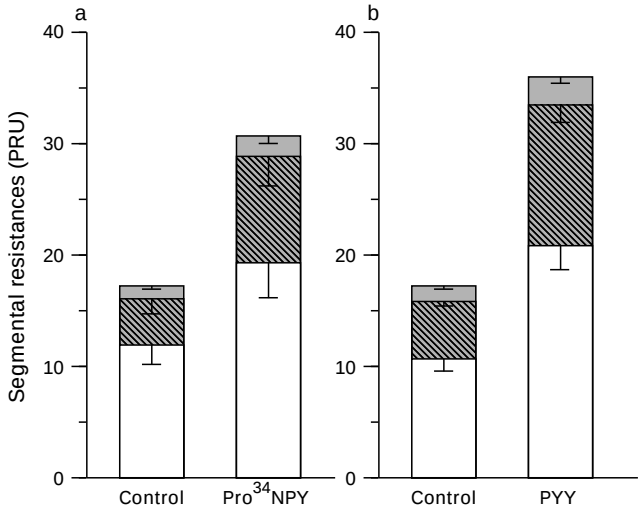

Figure 4 Compiled data for constrictor responses to $Pro^{34}NPY$ (a; $n=6$) and PYY (b; $n=6$) given at a dose of 8 μg kg^{-1} min^{-1}, i.a., on total regional vascular resistance (total height of the bars), and its segmental distribution to the $R_{a,prox}$ (open area), $R_{a,micro}$ (hatched area) and R_v (solid area) sections in cat skeletal muscle *in vivo*. The vascular responses to the two agonists were not significantly different.

agonists were all close-arterially infused at a dose of 8 μg kg^{-1} min^{-1}. $Pro^{34}NPY$ (Figure 4a; $n=6$) elicited a vasoconstrictor response which corresponded to a rise in R_T from the control value of 17.2 ± 2.3 pru to 30.5 ± 3.6 pru (+77%), in turn caused by an increase in $R_{a,prox}$ by 60%, in $R_{a,micro}$ by 132% and in R_v by 64%. PYY (Figure 4b; $n=7$), evoked a similar constriction which increased R_T from the control value of 17.2 ± 0.9 pru to 35.8 ± 3.0 pru (+110%), $R_{a,prox}$ by 93%, $R_{a,micro}$ by 153% and R_v by 79%. The vasoconstrictor responses to $Pro^{34}NPY$ and PYY were statistically significant and did not differ significantly from the contractile effect of NPY in any of the consecutive vascular sections studied. Neither NPY(13–36) ($n=6$) nor Ac-[Leu^{28}, Leu^{31}]-NPY(24–36) ($n=6$) elicited any significant effects in the muscle vascular bed.

Effects of selective NPY_1 receptor blockade on the vascular response to NPY To assess the ability of BIBP3226 to inhibit the vascular effects of NPY under *in vivo* conditions, BIBP3226 was infused i.a. to the muscle preparation at a dose of 100 μg kg^{-1} min^{-1} for 3 min, followed by a superimposed i.a. infusion of NPY at a dose of 8 μg kg^{-1} min^{-1} (Figure 2b). BIBP3226 had no significant effect on vascular tone in the control situation (cf. control columns in Figure 2a and b), indicating that BIBP3226 lacks agonist activity and that there is no significant basal endogenous release of NPY in sympathectomized skeletal muscle. However, BIBP3226 totally antagonised the constrictor effects of NPY in all three vascular sections (Figure 2b, $n=6$), which indicates that BIBP3226 is a potent NPY antagonist *in vivo* and, with the present knowledge of NPY receptor subtypes, that the vasoconstrictor response to NPY in the vascular bed of cat skeletal muscle is mediated solely by the Y_1 receptor.

To study the selectivity of BIBP3226, noradrenaline was close-arterially infused (1 μg kg^{-1} min^{-1}; $n=6$) in the absence and presence of BIBP3226 (100 μg kg^{-1} min^{-1}, i.a.) In the control situation (absence of BIBP3226) noradrenaline evoked a strong vasoconstrictor response which increased R_T by 13.2 ± 0.5 pru from the control value of 14.8 ± 1.0 pru (+89%), with a significantly (ANOVA) stronger action on the small arterioles (+143%) than on the large-bore arterial vessels (+77%) and the veins (+44%). The vasoconstriction to noradrenaline in the presence of BIBP3226 increased R_T by 16.7 ± 3.7 pru from the control value of 17.2 ± 2.6 pru (+97%) and this response did not differ significantly from that observed in the absence of the antagonist. Taken together with results previous studies (Rudolf *et al*., 1994), this indicated that BIBP3226 is a selective antagonist of NPY *in vivo*.

Discussion

NPY is a 36 amino acid peptide co-stored and released together with noradrenaline from sympathetic nerves supplying the cardiovascular system. NPY constricts human blood vessels *in vitro* and *in vivo* and is one of the most potent vasoconstrictors known so far. Its abundant presence in sympathetic nerves supplying arteries and veins in all organs has stimulated an intense research activity.

NPY increases perfusion pressure in skeletal muscle with similar (Corder *et al*., 1986), or even higher potency than noradrenaline (Pernow *et al*., 1988a), but with less potency than endothelin and angiotensin II (Minkes *et al*., 1989). However, the effect of NPY on vascular resistance in different sections of the vascular bed has not been examined *in vivo*.

In the present study, close-arterially infused NPY caused a dose-dependent, long-lasting vasoconstriction in skeletal muscle affecting all three consecutive vascular sections studied, yet with a preferential site of action on the small arterioles. The relatively slow onset and the long duration of the constrictor response found after cessation of the infusion is in agreement with results from previous studies in canine skeletal muscle (Pernow *et al*., 1988a), indicating that NPY released during sympathetic nerve stimulation mediates a slowly developing and long-lasting increase in perfusion pressure (Pernow *et al*., 1988b).

A vasodilator effect of NPY has been demonstrated in studies on brain vessels in the cat (Kobari *et al*., 1993) and in human subcutaneous vessels (Lind *et al*., 1995). However, in cat skeletal muscle *in vivo* no vasodilator response was detected in any section of the vascular bed, either to NPY or to any of the modified NPY agonists tested.

Previous studies on the site of action along the vascular bed of NPY have been performed with the vital microscopy technique in haemodynamically less important tissues, i.e. the rat cremaster muscle (Joshua, 1991), the hamster cheek pouch (Kim *et al*., 1994) and the guinea-pig ear (Morris, 1994), in which no information of changes in vascular resistance could

be obtained. In the rat cremaster muscle (Joshua, 1991), the contractile effect of NPY was inversely related to arterial diameter, in agreement with the present findings, but with no effect on venules, in contrast to our finding of a prominent increase in venous resistance (maximum +81%) in response to NPY. This discrepancy in results could be explained by a preferential effect of NPY on larger veins, since in the present study we recorded total venous resistance. If so, this would be in contrast to the inverse relationship between the contractile effect and vessel diameter on the arterial side. In the hamster cheek pouch (Kim *et al*., 1994), the vasoconstriction to NPY was strongest in arterioles of a diameter of 30–39 μm, with somewhat less effect on smaller (10–29 μm) and larger (40–59 μm) arterioles. Veins were not examined. This is compatible with the present finding that the vasoconstrictor response to NPY is preferentially located in the small arterioles, and can explain the potent pressor effect of systemically administered NPY despite its poor contractile effects in the relatively large blood vessels hitherto studied *in vitro* (Grundemar & Håkanson, 1994). Species differences in the effect of NPY might exist, since in the guinea-pig ear (Morris, 1994) arterioles <40 μm were rather insensitive to NPY, whereas more proximal arteries constricted upon application of the agent.

The observed site of action along the vascular bed of NPY is similar to that of circulating (Results) and neurally released (Maspers *et al*., 1990a) noradrenaline, but differs from other endogenous vasoconstrictors such as angiotensin II which preferentially constrict the large bore arterial resistance vessels (Ekelund, 1996) and endothelin-1 which causes a relatively stronger constriction (% resistance increase) on the venous than on the arterial side, at least in skeletal muscle (Ekelund *et al*., 1993). The segmental resistance changes to NPY, notably the changes in the ratio of pre- to postcapillary resistance, are functionally important also insofar as they influence hydrostatic capillary pressure and net transcapillary fluid exchange. Like most vasoconstrictors, with the notable exception of endothelin-1 (Ekelund *et al*., 1993), NPY caused an increase in the pre-/ postcapillary resistance ratio and a fall in capillary pressure, with a consequent net transcapillary fluid absorption. NPY released from sympathetic nerves could thus, like noradrenaline, via a decreased capillary pressure and venoconstriction, mobilise interstitial fluid to the circulation and increase venous return.

Although the strongest vasoconstrictor effect of NPY was observed in the small arterioles, the veins were significantly more sensitive to the agent. The veins responded earlier and reached their maximum response at lower concentrations of NPY. Similar observations have previously been made *in vitro* on human blood vessels (Erlinge, 1994), especially from skeletal muscle, in which the sensitivity of the veins to NPY was significantly higher ($pD_2 = 9.0 \pm 0.2$, $n = 8$) than that of the arteries (7.8 ± 0.2, $n = 11$, $P < 0.01$). Other investigators have found small or no effects of NPY in veins and venules from human and rabbit skeletal muscle (Pernow *et al*., 1987). The findings in the present study could indicate different receptor populations in the arteries and veins. However, the described results with the modified NPY agonists and BIBP3226 suggest similar receptors in arteries and veins. Thus, the higher sensitivity in the veins may reside in a non-receptor mechanism, such as the architecture of the vessel wall (e.g. short diffusion distance), which may increase the possibility for the blood-borne peptide to reach its receptors on the smooth muscle cell.

The identity of the NPY receptor type mediating the vasoconstriction was investigated with a range of agonists and the selective Y_1 receptor antagonist BIBP3226. The vascular responses to Pro^{34}NPY and to PYY did not differ from the constrictor effect of NPY, whereas neither of the two Y_2 preferring C-terminal fragments NPY(13–36) and Ac-[Leu^{28}, Leu^{31}]-NPY(24–36) had any significant contractile effects. These results, taken together, suggest that the vascular response to NPY is most likely mediated via the Y_1 receptor and indicate that Y_2 and Y_3 receptors do not play a significant role in the vasoconstrictor response in cat skeletal muscle.

The Y_4 receptor is not activated by NPY (Lundell *et al*., 1995) and the Y_5 could have been activated by the C-terminal fragments (Gerald *et al*., 1996). The murine receptor with pharmacology resembling the Y_1 receptor cannot be excluded by the agonist studies (Weinberg *et al*., 1996). However, none of these recently cloned receptors are antagonized by BIBP3226, thus it is unlikely that they are involved in the vascular effects of NPY.

In vivo, the newly developed selective NPY receptor antagonist BIBP3226 inhibits the NPY-induced increase in blood pressure with no effect on noradrenaline-, endothelin-, vasopressin- or angiotensin-induced pressor responses (Rudolf *et al*., 1994). With BIBP3266, Lundberg & Modin (1995) demonstrated a 50% reduction of the long-lasting vasoconstrictor response to sympathetic nerve stimulation in the pig hindlimb. In the present study, BIBP3226 abolished the vasoconstrictor response to NPY in all three vascular sections but did not affect the vasoconstriction to noradrenaline. These results indicate that BIBP3226 is a potent NPY antagonist in cat skeletal muscle *in vivo*, and, taken together with previous data (Rudolf *et al*., 1994; Lundberg & Modin, 1995), that BIBP3226 is selective for NPY receptors *in vivo*. With the present knowledge of NPY receptor subtypes, the observations with BIBP3226 further support our conclusion that all the vasoconstrictor effects of NPY in cat skeletal muscle are mediated by the Y_1 receptor.

In conclusion, the present data indicate that circulating NPY causes a dose-dependent, slowly developing vasoconstrictor response which is mediated solely via the Y_1 receptor and is preferentially located in the small arterioles. This site of action along the vascular bed is similar to that of neurally released and circulating noradrenaline. Hence, NPY released from sympathetic nerves may increase peripheral resistance, increase venous return and mobilise interstitial fluid to the circulation. An important role for NPY in states of circulatory stress with increased sympathetic drive, e.g. hypovolemic shock or even hypertension, is possible.

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