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The neuropeptide Y (NPY) Y_1 receptor antagonist BIBP 3226: equal effects on vascular responses to exogenous and endogenous NPY in the pig *in vivo*

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1 The antagonistic effects of the neuropeptide Y (NPY) Y_1 receptor antagonist BIBP 3226 on equally prominent vascular responses evoked by sympathetic nerve stimulation and exogenous NPY were compared during different intravenous (i.v.) infusions of the antagonist (0.19–190 nmol kg^{-1} min^{-1}, for 30 min).

2 High frequency sympathetic nerve stimulation in reserpine-treated pigs *in vivo* evoked non-adrenergic vasoconstrictor responses in kidney and hind limb, in the latter followed by a long-lasting phase of decreased blood flow. The vascular response in the kidney and the long-lasting phase in hind limb resembled the effects of exogenous NPY administered i.v. (kidney) and intraarterially (i.a.) (in the hind limb, which only responded to higher NPY doses).

3 Plasma levels of BIBP 3226 reached a plateau within 20 min and the inhibitory effects on vascular responses were studied during the last ten minutes of infusion. The elimination of BIBP 3226 from plasma was found to fit a two-compartment model with an α-phase of 2.0 ± 0.2 min and a β-phase of 20.1 ± 0.9 min.

4 Significant inhibition of presumably Y_1 receptor-mediated vascular responses evoked by both endogenous and exogenous NPY in kidney and hind limb was seen even during low-dose infusion of BIBP 3226 (1.9 nmol kg^{-1} min^{-1}), when plasma levels of the antagonist reached 59 ± 8 nM. The maximum inhibitory effects of BIBP 3226 were seen during the highest-dose infusion (190 nmol kg^{-1} min^{-1}), when the long-lasting vasoconstrictor responses in hind limb to sympathetic nerve stimulation and exogenous NPY administration (i.a.) were abolished. Simultaneously, the vascular responses in kidney to exogenous NPY were inhibited by 89% and to sympathetic nerve stimulation by 60%.

5 It is concluded that BIBP 3226 has a short half-life in plasma and should preferably be given by i.v. infusions to maintain blockade and avoid non-specific effects. Furthermore, BIBP 3226 dose-dependently and with similar potency antagonizes vascular responses to exogenous and endogenous NPY both in the kidney and hind limb of the reserpine-treated pig *in vivo*. Thus, inhibition of vascular responses to exogenous NPY may be a good indicator of the dose of this antagonist needed to inhibit sympathetic Y_1 receptor-transmission.

Keywords: BIBP 3226; NPY Y_1 receptor antagonist; endogenous and exogenous NPY

Introduction

Neuropeptide Y (NPY) is colocalized with noradrenaline (NA) in sympathetic nerve terminals (Lundberg *et al*., 1982) and coreleased with NA, especially upon strong activation (Lundberg *et al*., 1985). There seem to be at least two NPY receptor subtypes involved in sympathetic vascular control. The NPY Y_1 receptor subtype is mainly postjunctional, mediating vasoconstriction (Modin *et al*., 1991; Grundemar *et al*., 1992), but can also be prejunctional as for example in rabbit vas deferens, inhibiting sympathetic transmitter release (Doods & Krause, 1991). Conversely, the NPY Y_2 receptor subtype is thought to be preferentially prejunctional and to inhibit NA release (Wahlestedt *et al*., 1986; Sheikh *et al*., 1989), but postjunctional Y_2 receptors seem to mediate vasoconstrictor responses in pig spleen (Lundberg *et al*., 1988; Modin *et al*., 1991). The development of non-peptide NPY Y_1 receptor antagonists, BIBP 3226 ((**R**)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-argininamide) (Rudolf *et al*., 1994), SR 120107A and SR 120819A (Serradeil-Le Gal *et al*., 1994; 1995), has made it possible to establish finally that Y_1 receptors play a role in sympathetic vasoconstriction. Hence, both SR 120107A and BIBP 3226 strongly attenuate the non-adrenergic contraction of the guinea-pig caval vein, *in vitro*, evoked by high frequency stimulation of perivascular sympathetic nerves (Malmström & Lundberg, 1995a,b). It has now also been demonstrated, by use of BIBP 3226 and SR 120107A, that endogenous NPY, released by high frequency sympathetic nerve stimulation, acts on the Y_1 receptor to mediate non-adrenergic vasoconstriction in several vascular beds in the reserpine-treated pig *in vivo* (Lundberg & Modin, 1995; Malmström *et al*., 1996). The role of NPY in sympathetic vascular control in the control pig, in which NA levels are normal, is less obvious, probably because the release of NPY is restricted due to inhibition of neuronal NPY secretion mediated by NA acting on prejunctional α_2-adrenoceptors (Lundberg & Modin, 1995; Malmström & Lundberg, 1996; see Lundberg, 1996). In this study we have used the reserpine-treated pig model to study reserpine-resistant sympathetic vascular responses in two different vascular beds. The kidney and hind limb respond to sympathetic nerve activation with short and long-lasting vasoconstriction, respectively. These vascular responses mimic the responses evoked by administration of exogenous NPY in the two respective vascular beds.

Previous studies with BIBP 3226, given as bolus injections, revealed some non-specific effects such as hypotension and splenic vasodilatation, although enantiomer selectivity demonstrated that the antagonistic actions observed were exerted on Y_1 receptor-mediated effects. For this reason, in a previous study the inhibitory effects of BIBP 3226 were examined up to

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30 min post injection (Lundberg & Modin, 1995). However, it is not clear how this elapsed time affected the results since the pharmacokinetics of this agent in the pig remained to be established. Furthermore, it is important to titrate BIBP 3226 to plasma levels which exert Y_1 receptor blockade but do not evoke non-specific actions. Possibly, such drawbacks may be avoided by using continuous stepwise infusions of the drug. The aim of this study was to compare the potency of different doses of BIBP 3226, given as i.v. infusions, at antagonizing the vascular responses presumably evoked by endogenous NPY, released from sympathetic nerves, and exogenous NPY. The doses of NPY were adjusted to give vascular responses similar to those elicited by sympathetic nerve activation. Furthermore, NPY had to be given i.a. in hind limb, as NPY given i.v. in moderate doses does not evoke any responses in this vascular bed. In addition, we aimed to correlate the antagonistic action of BIBP 3226 with its circulating plasma levels.

Methods

In vivo *study*

This study was approved by the local ethics committee for animal research.

Surgical preparation Pigs of either sex (19–27 kg) were premedicated with ketamine (20 mg kg^{-1}, i.m.) and atropine (0.02 mg kg^{-1},i.m.) and anaesthetized with sodium pentobarbitone (20 mg kg^{-1}, i.v.). After skeletal muscle relaxation had been induced (pancuronium, 0.5 mg kg^{-1}, i.v.), the animals were intubated and ventilated by a respirator (Servo ventilator 900, Siemens-Elema, Sweden). The depth of anaesthesia was checked by pinching the interdigital skin before administration of pancuronium. The retroperitoneal space was reached via a flank incision below the left costal margin, where the postganglionic sympathetic nerves to the left kidney and the sympathetic lumbar chains of both sides (level L3–L4) were exposed and sectioned. The incision was closed and before extubation, reserpine (1 mg kg^{-1}, i.v.) was administered. The following day, the pigs were re-anaesthetized (see above) and ventilated by the respirator via a tracheal tube. A catheter was inserted into the right brachial vein for infusion of drugs to maintain anaesthesia (pentobarbitone, 8 mg kg^{-1} h^{-1}), skeletal muscle relaxation (pancuronium (0.5 mg kg^{-1} h^{-1}), fluid balance (sodium chloride 154 mM and glucose 28 mM, 2 ml min^{-1}) and to prevent intravascular coagulation (heparin 250 iu kg^{-1} h^{-1}). Another catheter, connected to a Statham P23 AC pressure transducer, was inserted into the right brachial artery for measurement of mean arterial blood pressure. Heart rate was recorded by a tachograph unit triggered by the blood pressure. A catheter was also placed into the left brachial artery, for collection of systemic arterial blood. Ultrasonic flow probes (2RB) were placed around the splenic artery, the left renal artery and the femoral arteries of both sides, to measure local blood flows. The flow probes were connected to Transonic flowmeters (T202, Transonic Instruments, Ithaca, NY, U.S.A.). Electrodes were placed on the distal ends of the cut left and right lumbar sympathetic chain (supplying respective hind limb) and the left renal periarterial nerves, for electrical stimulation. The saphenous arteries of left and right hind limb were cannulated with a catheter in a retrograde direction, for local i.a. infusion of drugs. The abdomen was then closed, thereafter the pigs were allowed to stabilize for one hour before the experiments were commenced.

Experimental procedures Atropine (0.5 mg kg^{-1}, i.v.) was administered every fourth hour to prevent any cholinergic vasodilator response evoked by lumbar sympathetic stimulation in hind limb (Modin *et al*., 1993b). Electrical stimulation of the left renal periarterial sympathetic nerves, and the left and right lumbar sympathetic chain was performed in series by a Grass stimulator. The electrical stimulation was delivered as two high frequency bursts of 20 Hz for 1 s at a 10 s interval (5 ms, 25 V). This brief stimulation was chosen to avoid spontaneous decline of NPY release (Modin *et al*., 1993a). These nerve stimulations were followed by an i.v. bolus injection of NPY (70–160 pmol kg^{-1}), the dose of which was adjusted to evoke renal vasoconstriction to an extent similar to that evoked by the control renal sympathetic nerve stimulation. This was followed by i.a. bolus injections of NPY (0.6–1.4 nmol) into the right and left saphenous artery. The dose of i.a. NPY was adjusted to give roughly the same vasoconstrictor effect as lumbar sympathetic chain stimulation in the corresponding vessel. Four consecutive infusions of BIBP 3226 at increasing doses between 190 pmol kg^{-1} min^{-1} and 190 nmol kg^{-1} min^{-1} (equal to between 0.1 and 100 μg kg^{-1} min^{-1}) were performed at 1 h intervals. The infusions were given for 30 min and the nerve stimulation and NPY injection were then repeated between the 20th and 30th minute of each infusion. Thereafter, a recovery period of 90 min followed, after which the nerve stimulation and exogenous NPY injection were performed once again. Systemic arterial blood samples were collected before and at the 20th, 25th and 30th min of each infusion for determination of plasma levels of BIBP 3226. Systemic arterial blood samples were also collected 1, 2, 5, 10, 15, 30 and 60 min after cessation of the last infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1} for 30 min), to determine the half-life in plasma of the antagonist. One separate group of pigs served as control. The same experimental procedure as above was performed in these pigs, except that no antagonist was infused, in order to assure that the vascular responses were reproducible and not susceptible to any spontaneous decline.

Determination of BIBP 3226 levels in plasma

Blood samples were collected in ice-cold tubes containing EDTA (final concentration 10 mM), centrifuged 10 min (+4°C), then the plasma was pipetted off and stored at −20°C. The plasma concentrations of BIBP 3226 were determined in 200 μl of plasma by high-performance liquid chromatography (h.p.l.c.) with fluorescence detection (Supelcosil ABZ+column), following ion-pair extraction with a solidphase column (Isolut C18). The detection limit in this assay was 50 nM.

Calculations

All vascular responses are expressed as minimum remaining vascular conductance, calculated as blood flow divided by mean arterial blood pressure (see Stark, 1968), as percentage of basal vascular conductance (obtained before the vascular response). We have also used the time it takes for the vascular bed in question to regain 75% of its basal blood flow (before the vascular response) to give an indication of the duration of vasoconstrictor effects. The pharmacokinetic data for BIBP 3226 were calculated with the WinNonlin computer programme (SCI, Lexington, KY, U.S.A.). Data in the text are given as means ± s.e.mean, and statistical significance was calculated by multiple analysis of variance (ANOVA) followed by the *post*-test of Tukey. Linear correlation was assessed by use of the Pearson correlation test.

Drugs

Ketamine (Parke-Davis, CA, U.S.A.), sodium pentobarbitone (NordVacc, Sweden), atropine (premedication) and sodium heparin (KabiVitrum, Sweden), pancuronium bromide (Organon, The Netherlands), reserpine and atropine chloride (Sigma, St. Louis, MO, U.S.A.), NPY (Auspep, Australia). BIBP 3226 ((**R**)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-argininamide) was synthetized by Albany Molecular Research Inc. (Albany, U.S.A.).

Results

Effects of BIBP 3226 per se

The three lower doses of BIBP 3226 (0.19 – 19 nmol kg^{-1} min^{-1} for 30 min) did not have any cardiovascular effects *per se*. The last infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1} for 30 min) was accompanied by a slight (non significant) increase in splenic blood flow (19%) and a transient fall in mean arterial blood pressure (5 mmHg).

Sympathetic nerve stimulation

High frequency stimulation (two 1 s bursts at 20 Hz) of the renal and lumbar sympathetic nerves evoked rapid vasoconstriction in the renal and femoral vascular beds, respectively (Figures 1 and 2). In the kidney this vasoconstriction was short-lasting and followed by a slight hyperaemia (Figure 1), whereas in the hind limb the initial effect was followed by a slowly declining long-lasting vasoconstriction (Figure 2). Vascular conductance was maximally reduced to 60% of basal level in the kidney and 51% of basal level in the hind limb upon this nerve stimulation. The time to recovery of 75% of basal femoral blood flow was 5 min after cessation of the stimulation. The vascular response in kidney to sympathetic nerve stimulation was gradually attenuated in the presence of increasing doses of BIBP 3226 (Figure 1), and the effect reached significance at 1.9 nmol kg^{-1} min^{-1} (Figure 3). The greatest inhibitory effects were seen during the highest dose of BIBP 3226 (190 nmol kg^{-1} min^{-1}) when renal vascular conductance was only reduced to 84% of basal upon high frequency sympathetic nerve stimulation (Figure 3). The rapid phase of the vascular response in hind limb was not affected at the lower doses of BIBP 3226, whereas during the infusion of 19 and 190 nmol kg^{-1} min^{-1}, vascular conductance was at most reduced to 60% of basal and 65% of basal, respectively, upon high frequency nerve stimulation (Figure 4). The duration of the long-lasting phase of vasoconstriction in hind limb decreased gradually in the presence of increasing doses of BIBP 3226 (Figure 2). Hence, in the presence of BIBP 3226, the time needed to regain 75% of basal vascular blood flow after cessation of nerve stimulation became progressively shorter, with a significant effect at 190 pmol kg^{-1} min^{-1}. Indeed, this long-lasting vasoconstrictor phase was completely abolished at 190 nmol kg^{-1} min^{-1} (Figure 4). Ninety minutes after the last infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1}) these vascular responses had partially recovered (Figures 1 and 2). Thus, upon high frequency sympathetic nerve stimulation, vascular conductance was at most reduced to 77% of basal and 63% of basal in kidney (Figure 3) and hind limb (Figure 4), respectively, and the time needed to regain 75% of basal vascular blood flow was 29 s in hind limb (Figure 4).

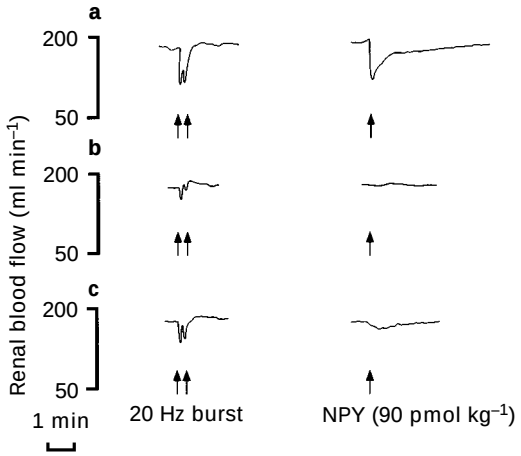

Figure 1 Original recordings of the renal arterial blood flow in reserpine-treated pigs. Vascular responses upon high frequency stimulation of renal sympathetic nerves (two 1 s bursts at 20 Hz at 10 s intervals) and to exogenous NPY (90 pmol kg^{-1}), given i.v., are shown (a) before (control) and (b) at the end of a 30 min infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1}) and (c) 90 min later (recovery).

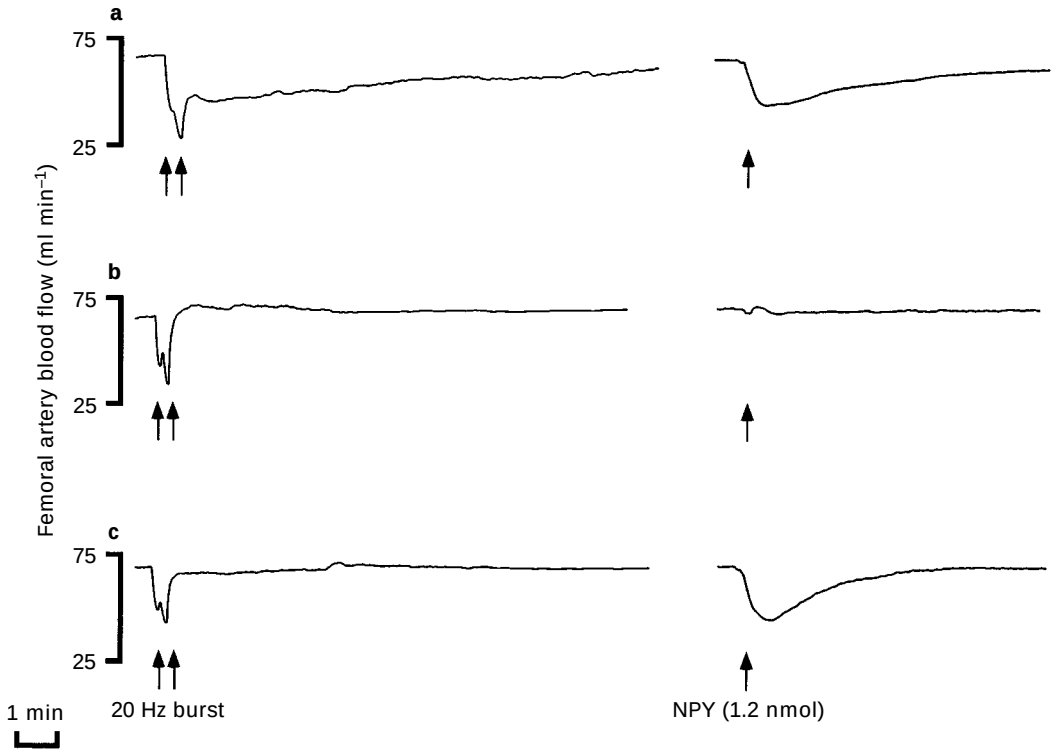

Figure 2 Original recordings of the arterial blood flow of hind limb in reserpine-treated pigs. Vascular responses upon high frequency stimulation of lumbar sympathetic nerves (two 1 s bursts at 20 Hz at 10 s intervals) and to exogenous NPY (1.2 nmol), given i.a., are shown (a) before (control) and (b) at the end of a 30 min infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1}) and (c) 90 min later (recovery).

In the control group, renal sympathetic nerve stimulation evoked vasoconstriction with a reduction of vascular conductance to 70% of basal (Figure 3). This vascular response was the same for the five following stimulations (Figure 3). Lumbar sympathetic nerve stimulation evoked rapid vasoconstrictor responses in the femoral artery (vascular conductance was reduced to 49% of basal in this group) followed by a long-lasting phase of reduced blood flow (the time needed to regain 75% of basal blood flow was 225 s) (Figure 4). The long-lasting phase of vasoconstriction was not significantly altered for the following five stimulations (Figure 4), whereas the initial rapid vasoconstrictor peak was slightly attenuated upon repeated stimulation (Figure 4).

Effects of exogenous NPY

Intravenous administration of NPY (70–160 pmol kg^{-1}) evoked a dose-dependent elevation of mean arterial blood pressure (MABP) (on average 20 mmHg) and vasoconstriction in the spleen and kidney (Figure 1). Hence, vascular conductance in the spleen and kidney was reduced to 25% of basal and 60% of basal, respectively (Figures 3 and 5). The effects of NPY on MABP, renal and splenic vascular conductance were gradually attenuated in the presence of increasing doses of BIBP 3226 (Figures 1, 3 and 5). Significant inhibition of the NPY-evoked effects on MABP and renal vascular conductance was seen at 1.9 nmol kg^{-1} min^{-1} (Figures 3 and 5) and maximum inhibition was seen during the highest dose of BIBP 3226 (190 nmol kg^{-1} min^{-1}), when exogenous NPY evoked an elevation of MABP by only 5 mmHg (Figure 5) and a modest decrease of renal vascular conductance to 95% of basal (Figure 3). In contrast to the renal circulation, the NPY-evoked decrease in vascular conductance in the spleen was largely unaltered by the lower doses of BIBP 3226, whereas during the infusion of 190 nmol kg^{-1} min^{-1}, vascular conductance was reduced to 42% of basal upon i.v. administration of NPY (Figure 5).

In the control group, i.v. administered NPY (90–140 pmol kg^{-1}) increased MABP by 19 mmHg and evoked vasoconstriction in the spleen and kidney. Vascular conductance in the spleen and kidney was reduced to 44% and 59% of basal, respectively (Figures 3 and 5). The NPY-evoked effects on MABP and renal vascular conductance were largely unaltered upon repeated NPY administration (Figures 3 and 5). In contrast, the splenic vascular responses to NPY tended to be augmented upon repeated NPY administration (Figure 5).

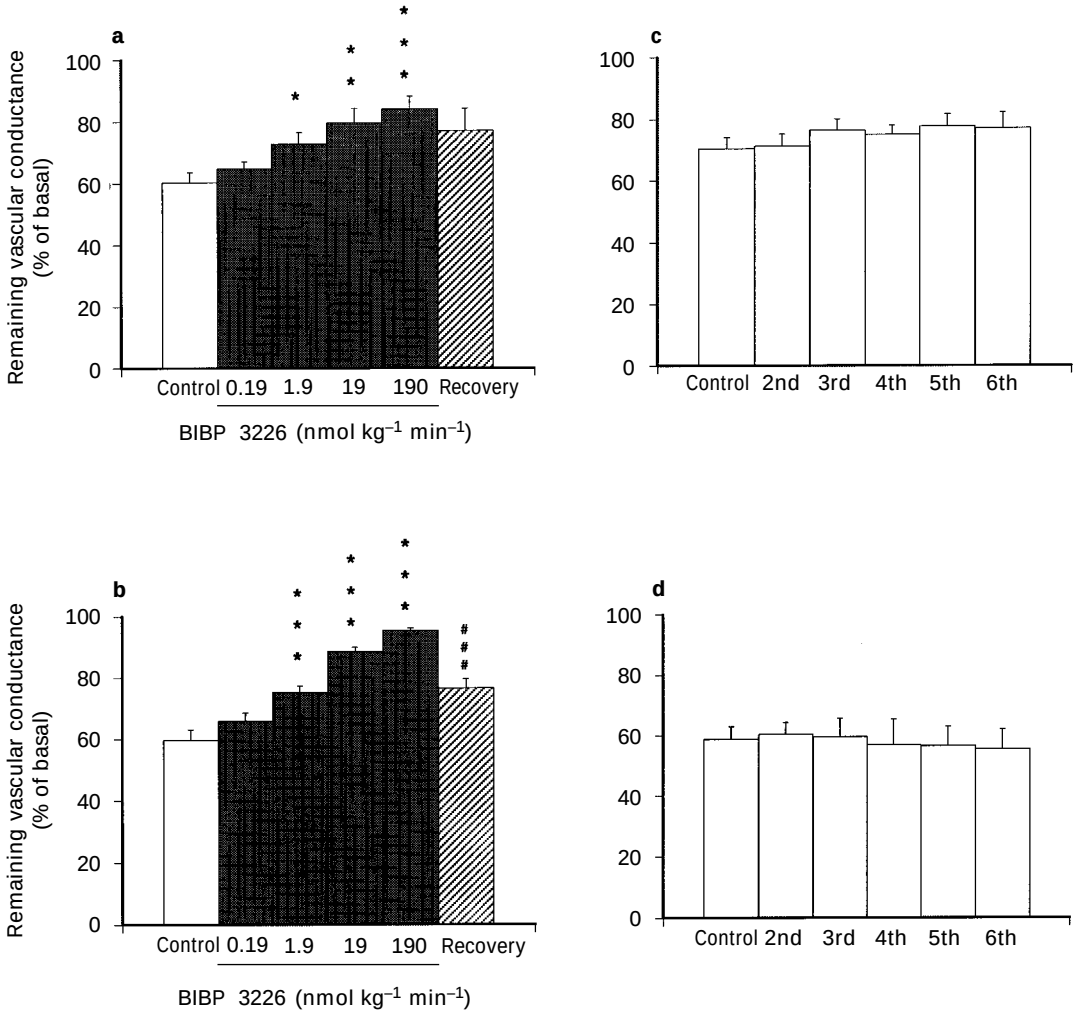

Figure 3 (a and b) Changes in renal vascular conductance upon (a) renal sympathetic nerve stimulation (two 1 s bursts of 20 Hz at 10 s intervals) and (b) i.v. NPY administration (70–160 pmol kg^{-1}, adjusted to match the control nerve response) in reserpine-treated pigs *in vivo*. The vascular responses are shown before and during consecutive 30 min infusions of BIBP 3226 (0.19–190 nmol kg^{-1} min^{-1}) and after a recovery period of 90 min. Data are given as means ± s.e.mean, $n = 7–10$. Significant differences compared to control are indicated $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Significant differences between BIBP 3226 (190 nmol kg^{-1} min^{-1}) and recovery are indicated $^{\#\#\#}P<0.001$. (c and d) Changes in renal vascular conductance upon (c) renal sympathetic nerve stimulation (same as above) and (d) i.v. NPY administration (90–140 pmol kg^{-1}, adjusted to match the control nerve response) in reserpine-treated pigs *in vivo*. Vascular responses are shown for control and for repeated nerve stimulation or NPY administration in the absence of antagonist. Data are given as means ± s.e.mean, $n = 4$. No significant differences from control were observed when this experimental procedure was repeated five times in the control series.

Local i.a. injection of NPY (0.6–1.4 nmol) into the saphenous artery elicited vasoconstriction in the hind limb. Thus, vascular conductance in the femoral artery was reduced to 52% of basal upon local NPY administration and 5 min was required for blood flow to recover 75% of basal after this response (Figures 2 and 6). In the presence of increasing doses of BIBP 3226 the vascular response in hind limb to exogenous NPY was gradually attenuated with regard to both the peak effect and duration (Figures 2 and 6). Significant attenuation of NPY-evoked responses in hind limb was seen during infusion of BIBP 3226 at 1.9 nmol kg^{-1} min^{-1}, and maximum inhibition of these responses was seen during infusion of the highest dose of BIBP 3226 (190 nmol kg^{-1} min^{-1}). At this dose of BIBP 3226, NPY only decreased vascular conductance to 97% of basal and just 7 s was required for blood flow to recover to 75% of basal after this response (Figure 6).

Ninety minutes after the last infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1}) these vascular responses to exogenous NPY had partially recovered (Figures 1 and 2). Thus, at this time, i.v. administered NPY evoked an elevation of MABP by 14 mmHg simultaneously with a decrease in renal and splenic vascular conductance to 77% and 31% of basal, respectively (Figures 3 and 5). Also, NPY given i.a. evoked a decrease in femoral arterial vascular conductance to 63% of basal, after which response it took 173 s for blood flow to regain 75% of the basal value (Figure 6).

In the control group, local i.a. administered NPY (0.7–1.2 nmol) decreased femoral arterial vascular conductance to 50% of basal. Recovery of blood flow to 75% of basal took 236 s after this vasoconstriction, and this time was not significantly altered upon repeated NPY administration (Figure 6). In contrast, the NPY-evoked peak vasoconstrictor response in hind limb tended to be augmented upon repeated NPY administration (Figure 6).

There was a linear correlation between the vascular responses to sympathetic nerve stimulation and exogenous NPY both in hind limb ($P<0.001$) and kidney ($P<0.01$). The effects of both stimuli (peak effect in the kidney and duration in the hind limb) were similarly and gradually antagonized by increasing doses of BIBP 3226 (Figure 7).

Plasma levels of BIBP 3226

No plasma levels of BIBP 3226 could be measured during the lowest infusion dose, as the detection limit in our assay was 50 nM. During the second and third infusion of BIBP 3226 (1.9 and 19 nmol kg^{-1} min^{-1}), when significant effects were observed on both vascular responses to exogenous NPY and sympathetic nerve stimulation, plasma levels reached 59 ± 8 nM and 690 ± 40 nM, respectively, and remained steady from the 20th to the 30th minute of infusion. During the final infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1}) plasma levels

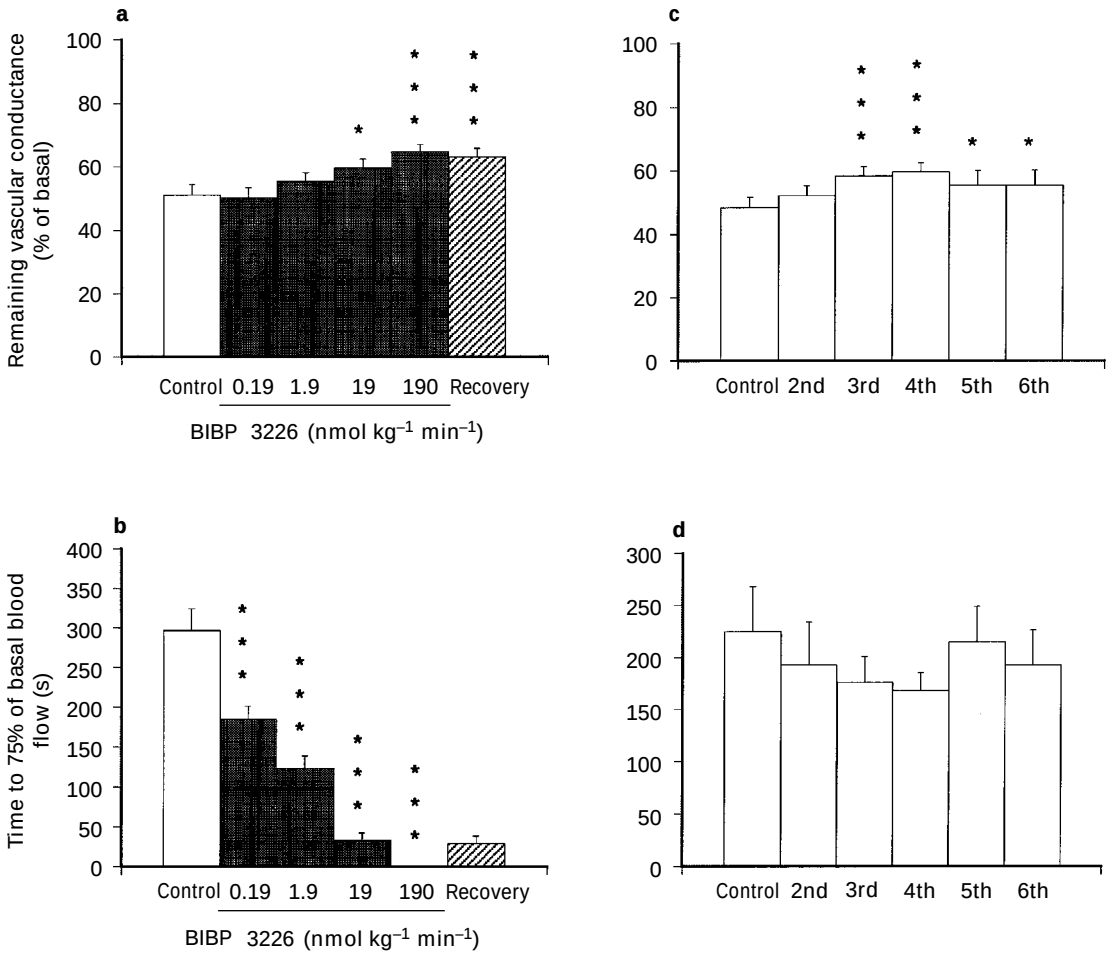

Figure 4 (a and b) Changes in (a) arterial vascular conductance of the hind limb upon lumbar sympathetic nerve stimulation (two 1 s bursts of 20 Hz at 10 s intervals) and (b) duration of these vascular responses (time required to regain 75% of basal vascular blood flow) in reserpine-treated pigs *in vivo*. The vascular responses are shown before and during consecutive 30 min infusions of BIBP 3226 (0.19–190 nmol kg^{-1} min^{-1}) and after a recovery period of 90 min. Data are given as means$\pm$s.e.mean, $n=14$. Significant differences compared to control are indicated $^{*}P<0.05$, $^{***}P<0.001$. (c and d) Changes in (c) arterial vascular conductance of the hind limb upon lumbar sympathetic nerve stimulation (two 1 s bursts of 20 Hz at 10 s intervals) and (d) duration of these vascular responses (time required to regain 75% of basal vascular blood flow) in the control group. Vascular responses are shown for control and for repeated nerve stimulation in the absence of antagonist. Data are given as means$\pm$s.e.mean, $n=7$. Significant differences compared to control are indicated, $^{*}P<0.05$, $^{***}P<0.001$.

were steady from the 20th to the 30th minute of infusion at 5400 ± 300 nM. After cessation of this infusion the plasma levels of BIBP 3226 dropped quickly to 3300 ± 400 nM (+2 min), 1800 ± 200 nM (+5 min), 800 ± 100 nM (+15 min), 420 ± 70 nM (+30 min) and 190 ± 30 nM (+60 min) (Figure 8). A two compartment model gave a good fit to the data resulting in a half-life of 2.0 ± 0.2 min and 20.1 ± 0.9 min of the α- and β-phase, respectively.

Discussion

Vascular responses to endogenous and exogenous NPY

The present results showed that continuous i.v. infusion of the non-peptide NPY Y_1 receptor antagonist, BIBP 3226, is superior to bolus i.v. injections: by infusing, i.v., one can avoid non-specific effects (Lundberg & Modin, 1995) and control the duration of action. In the present study, BIBP 3226 seemed to be equally potent in antagonizing vasoconstrictor responses to endogenous NPY and exogenous NPY in both kidney and hind limb. This was demonstrated by comparing the ability of BIBP 3226, at different doses, to attenuate non-adrenergic vasoconstrictor responses evoked by high frequency stimulation of sympathetic nerves and equally prominent responses to exogenous NPY. It was of interest to compare not only the effects of BIBP 3226 on endogenous versus exogenous NPY but also on short-lasting versus long-lasting vascular responses to NPY. Hence, we chose to study the vascular beds of the kidney and hind limb. The non-adrenergic sympathetic vasoconstriction in kidney could be largely mimicked by the effects of exogenous NPY administered i.v., whereas the long-lasting phase of the non-adrenergic sympathetic vasoconstriction in hind limb was mimicked by exogenous NPY given i.a. These differences in functional responses might be due to the tissue-specific characteristics of the endothelium: NPY given i.v. evokes vasoconstriction e.g. in kidney and spleen, vascular beds which have a fenestrated endothelium, much more permeable than that of the vasculature in hind limb (Bennett *et al*., 1959). This difference in endothelium permeability could also explain why endogenous NPY, released from sympathetic nerves, mediates long-lasting vasoconstrictor responses in hind limb, but more short-lasting responses in the kidney. Interestingly, it has been demonstrated that overflow of NPY-like immunoreactivity (LI) evoked by sympathetic nerve stimulation in the hind limb is difficult to detect and is markedly delayed (Modin *et al*., 1993b) in comparison with, for example, the kidney. In fact, 'washout' of the peptide from the tissue could be one effective means of terminating responses to a vasoactive neurotransmitter like NPY, with a long half-life.

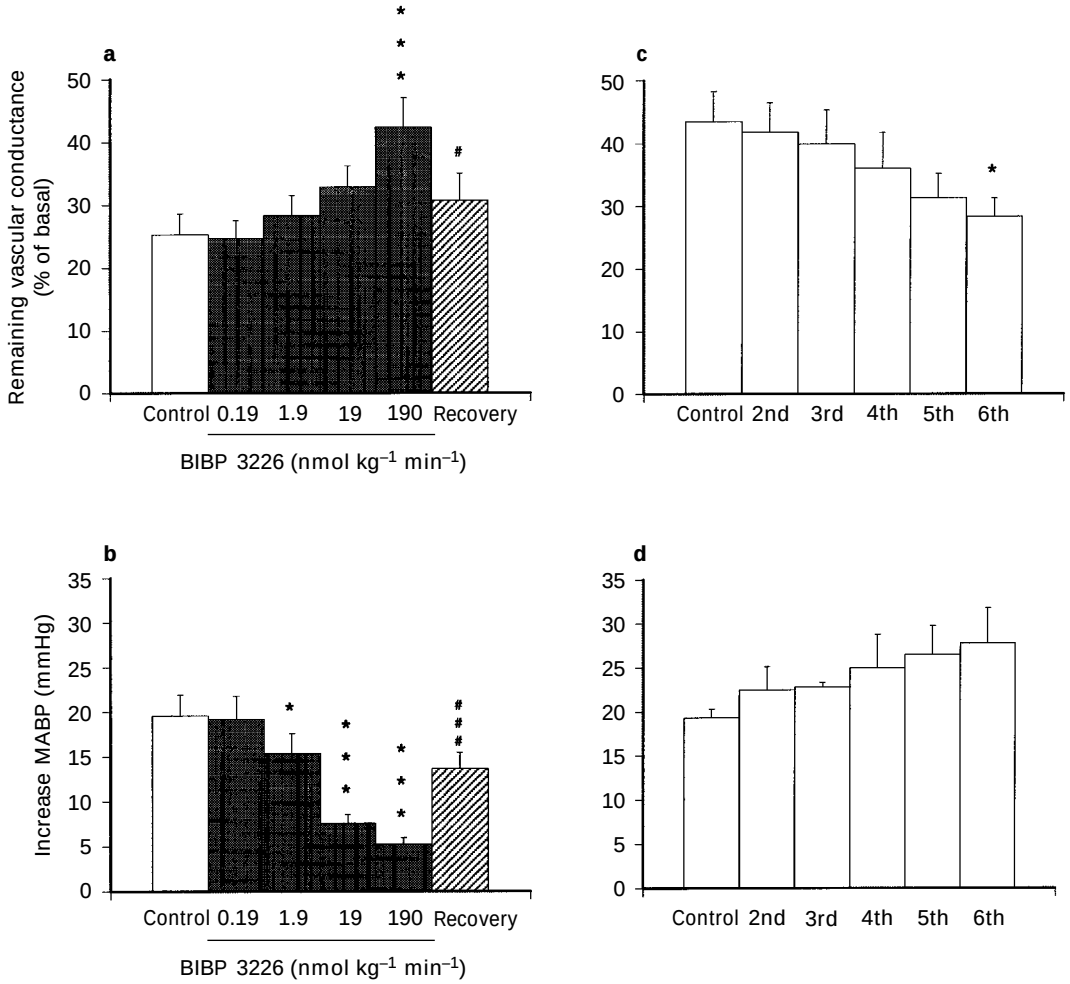

Figure 5 (a and b) Changes in (a) splenic vascular conductance and (b) mean arterial blood pressure (MABP) upon i.v. NPY administration (70–160 pmol kg^{-1}) in reserpine-treated pigs *in vivo*. The vascular responses are shown before and during consecutive 30 min infusions of BIBP 3226 (0.19–190 nmol kg^{-1} min^{-1}) and after a recovery period of 90 min. Data are given as means ± s.e.mean, $n = 7–10$. Significant differences compared to control are indicated, $^{*}P<0.05$, $^{***}P<0.001$. Significant differences between BIBP 3226 (190 nmol kg^{-1} min^{-1}) and recovery are indicated, $^{\#}P<0.05$, $^{\#\#\#}P<0.001$. (c and d) Changes in (c) splenic vascular conductance and (d) mean arterial blood pressure upon i.v. NPY administration (90–140 pmol kg^{-1}) in the control group. Vascular responses are shown for control and for repeated NPY administration in the absence of antagonist. Data are given as means ± s.e.mean, $n = 4$. Significant differences compared to control are indicated, $^{*}P<0.05$.

Comparison of the antagonistic effects of BIBP 3226

This study demonstrated that there was a linear correlation between the antagonistic actions of BIBP 3226 on vascular responses evoked by exogenous and endogenous NPY, both in kidney and hind limb. Neuronally released NPY presumably activates a number of receptors that are located at some distance from the vascular lumen. In order to exert effects at these receptors, a circulating substance – be it NPY or BIBP 3226 – must pass from the lumen and reach the receptors in sufficient quantities. Because of the diffusion barriers, the plasma concentrations of an exogenously administered agent are substantially higher than those at its site of action. (This is probably of greater importance in skeletal muscle, with its relatively impermeable endothelium, than in vascular beds with fenestrated endothelium). BIBP 3226 is only one-eighth the size of NPY, and is thus likely to penetrate to the relevant receptors more readily than the peptide. The linear correlations observed in this study also suggest that this is the case. In accord, BIBP 3226 inhibited the nerve-stimulation response with equal efficacy in kidney and hind limb, while the kidney was much more responsive to circulating NPY. Interestingly, it has been shown in the pig spleen that low doses of circulating NPY seem to activate exclusively Y_2 receptors, whereas the contribution of Y_1 receptors to this vasoconstrictor effect becomes gradually larger upon increasing doses of NPY (Malmström & Lundberg, 1996). In the present study BIBP 3226, albeit only at the highest dose, attenuated some of the NPY-evoked vasoconstriction in the spleen, indicating the participation of Y_1 receptors in this response. The splenic vascular responses to exogenous NPY were augmented upon repeated administration in the control series. Thus, this could explain why BIBP 3226 was less potent in antagonizing part of the vasoconstriction evoked by NPY in the spleen than in the other vascular beds.

In contrast, neuronally released NPY seems to activate preferentially mainly Y_1 receptors in the pig spleen (Lundberg & Modin, 1995; Malmström *et al*., 1996). A similar difference in transmitter accessibility to receptor subtypes has, for a long time, been observed for vascular α-adrenoceptors. Thus, in several vascular beds it has been demonstrated that neuronally released NA seems to activate primarily the α_1-adrenoceptor, whereas circulating NA mediates vascular responses predominantly via the α_2-adrenoceptor (see Langer & Hicks, 1984). A difference in the anatomical distribution of these receptor subtypes might explain this discrepancy and it has been suggested that the α_2-adrenoceptor is predominantly located close to the intima in the inner layers of the media, while the α_1-

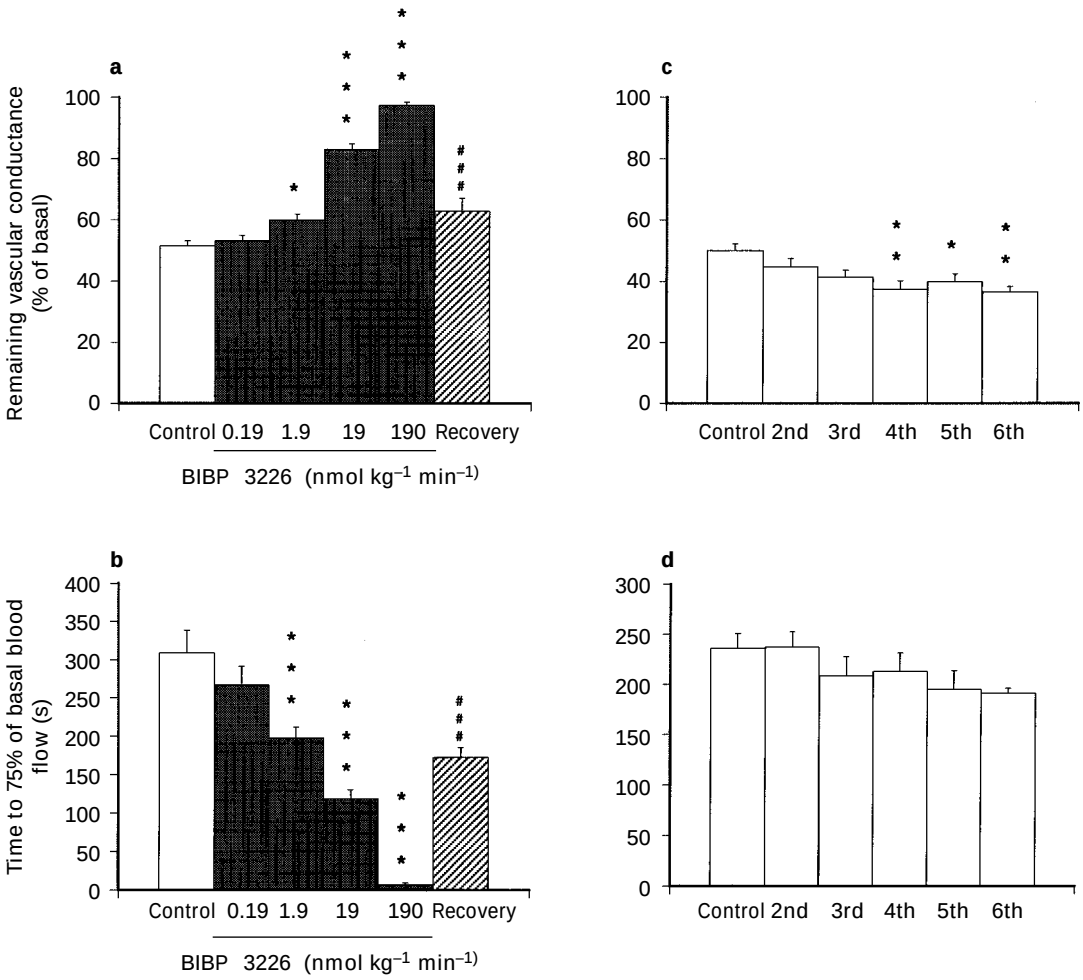

Figure 6 (a and b) Changes in (a) arterial vascular conductance of the hind limb upon i.a. administration of NPY (0.6 – 1.4 nmol, adjusted to match the control nerve response) and (b) duration of these vascular responses (time required to regain 75% of basal vascular blood flow) in reserpine-treated pigs *in vivo*. The vascular responses are shown before and during consecutive 30 min infusions of BIBP 3226 (0.19 – 190 nmol kg^{-1} min^{-1}) and after a recovery period of 90 min. Data are given as means ± s.e.mean, $n = 14$. Significant differences compared to control are indicated, $^{*}P < 0.05$, $^{***}P < 0.001$. Significant differences between BIBP 3226 (190 nmol kg^{-1} min^{-1}) and recovery are indicated, $^{\#\#\#}P < 0.001$. (c and d) Changes in (c) arterial vascular conductance of the hind limb upon i.a. administration of NPY (0.7 – 1.2 nmol, adjusted to match the control nerve response) and (d) duration of these vascular reponses (time required to regain 75% of basal vascular blood flow) in the control group. Vascular responses are shown for control and for repeated NPY administration in the absence of antagonist. Data are given as means ± s.e.mean, $n = 7$. Significant differences compared to control are indicated, $^{*}P < 0.05$, $^{**}P < 0.01$.

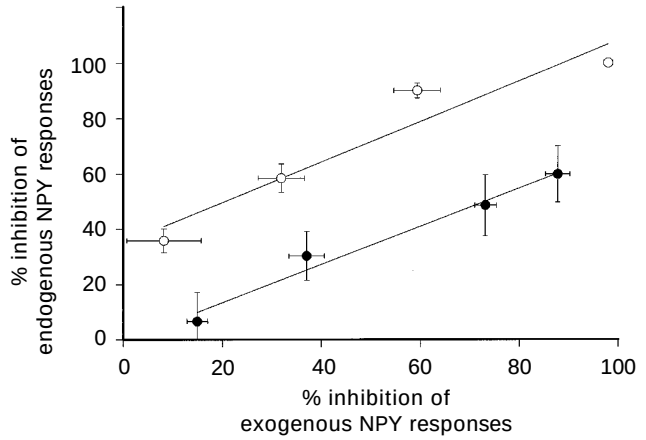

Figure 7 Correlation between the antagonistic effects of BIBP 3226 (0.19–190 nmol kg^{-1} min^{-1}) on vascular responses evoked in the kidney (●) and hind limb (○) by sympathetic nerve stimulation and exogenous NPY (calculated as % inhibition of control response). Data are given as means ± s.e.mean, $n = 7–13$.

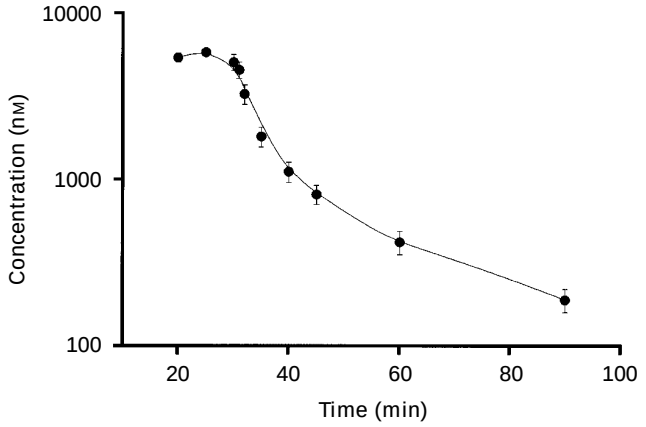

Figure 8 Plasma levels of BIBP 3226 plotted against time. The infusion of BIBP 3226 ended at the 30th minute. Data are given as means and vertical lines show s.e.mean, $n = 8$.

adrenoceptor is mainly located at the adventitial-medial border close to the sympathetic nerves (see Langer & Shepperson, 1982). However, in other sympathetic tissues the reverse location of adrenoceptors seems to occur (see Bao, 1993). In contrast, the present study suggests that there is a single NPY receptor subtype in the vascular beds of the kidney and hind limb of the pig: the Y_1 receptor. However, BIBP 3226 at the highest dose seemed somewhat more potent in antagonizing the renal vascular response to exogenous NPY than to sympathetic nerve stimulation. It is a matter of speculation whether the vasoconstrictor response to sympathetic nerve activation in the kidney, that remains in the presence of this highest dose of BIBP 3226, could be mediated by some other sympathetic transmitter (ATP?) or, less likely, by NPY acting on a receptor subtype other than Y_1.

The situation was the reverse in the hind limb, where BIBP 3226 seemed somewhat more potent in antagonizing the long-lasting vascular responses to sympathetic nerve stimulation than to exogenous NPY. However, this could be due to the fact that the dose of NPY administered i.a. was adjusted according to the maximal vasoconstrictor effect (the initial peak) evoked by sympathetic nerve stimulation. Our results indicate that this initial peak of sympathetic vasoconstriction may well be mediated by another rapidly acting sympathetic transmitter such as ATP, since it was only marginally affected by BIBP 3226. This initial rapid sympathetic vasoconstrictor response in hind limb was slightly attenuated upon repeated nerve stimulation, both in the control group and in the BIBP 3226-treated group, indicating a slight spontaneous fatigue of the response. The long-lasting phase of sympathetic vasoconstriction, which followed after this initial peak, only reached about 50–75% of this maximal amplitude of vasoconstriction. Hence, the responses to exogenous NPY were slightly oversized compared to those mediated by endogenous NPY.

Duration of actions of BIBP 3226

The results in this study are strengthened by the reproducible results seen in the control group as well as by the fact that a partial recovery of all vascular responses was seen after a period of 90 min (after completion of the last BIBP 3226 infusion). Most of the vascular responses seen after this recovery period corresponded in amplitude and duration to those seen during the second infusion of BIBP 3226 (1.9 nmol kg^{-1} min^{-1}). The exception was the vascular response in hind limb to sympathetic nerve stimulation, which corresponded to that seen during the third BIBP 3226 infusion (19 nmol kg^{-1} min^{-1}). Accordingly, 90 min after completion of the last infusion of BIBP 3226 (190 nmol kg^{-1} min^{-1}), some of the antagonistic actions remained. The plasma level of BIBP 3226 was 190 nM at 60 min after the last infusion. It is likely that the plasma level of BIBP 3226 at 120 min (or 90 min after completed infusion), according to the calculated half-life, would be in the range of that obtained during the second infusion of BIBP 3226 (the calculated value at 120 min was 63 nM and the level during the second infusion was 59 nM), which would also correspond to the moderate antagonistic actions evoked by BIBP 3226 at this time.

Conclusions

In this study we have demonstrated that BIBP 3226 has a short half-life in plasma (≈ 2 min). Therefore, we believe that studies with BIBP 3226 are most conclusive when performed during an infusion, especially when the antagonistic action is to be correlated with the dose. This type of administration also seems to produce fewer non-specific hypotensive effects than bolus injections. Furthermore, we conclude that BIBP 3226 dose-dependently and with the same order of potency antagonizes vascular responses both to exogenous and endogenous NPY in the pig *in vivo*. This finding may also facilitate future dosing of low molecular weight non-peptide Y_1 receptor antagonists in experiments on various conditions of sympathetic hyperactivity.

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