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Inhibition of sympathetic vasoconstriction in pigs *in vivo* by the neuropeptide Y-Y_1 receptor antagonist BIBP 3226

[1]Jan M. Lundberg & Agnes Modin

Division of Pharmacology, Department of Physiology and Pharmacology, Karolinska Institute, S-171 77 Stockholm, Sweden

1 Recently, a potent non-peptide antagonist of neuropeptide Y (NPY)-Y_1 receptors has been developed. In this study, the selectivity of this compound, BIBP 3226, as a functional Y_1 receptor antagonist, and the possible role of endogenous NPY in sympathetic vasoconstriction in different vascular beds have been investigated in anaesthetized pigs.

2 BIBP 3226 specifically displaced [^{125}I]-NPY binding with an IC_{50} value of 7 nM in membranes of pig renal arteries, which also were responsive to a Y_1 receptor agonist, but had only minor effects in the pig spleen (IC_{50} 55 μM), where instead [^{125}I]-NPY binding was markedly inhibited by a Y_2 receptor agonist. IC_{50} values in the same nM range for BIBP 3226 were also observed in rat and bovine cortex and dog spleen.

3 In anaesthetized control pigs *in vivo* BIBP 3226 (1 and 3 mg kg^{-1}) markedly inhibited the vasoconstrictor effects of the Y_1 receptor agonist [Leu^{31}, Pro^{34}] NPY(1-36), without influencing the responses to the Y_2 receptor agonist N-acetyl [Leu^{28}, Leu^{31}] NPY(24-36), or to noradrenaline, phenylephrine, α,β-methylene adenosine triphosphate or angiotensin II.

4 High frequency stimulation of the sympathetic trunk in control pigs caused a biphasic vasoconstrictor response in nasal mucosa, hind limb and skin: there was an immediate, peak response, followed by a long-lasting vasoconstriction. BIBP 3226 (1 and 3 mg kg^{-1}) reduced the second phase by about 50% but had no effect on the peak response. In the spleen, kidney and mesenteric circulation (which lack the protracted response) BIBP 3226 was likewise without effect on the maximal vasoconstriction, and did not influence noradrenaline overflow from spleen and kidney.

5 The corresponding S-enantiomer BIBP 3435 had only marginal influence on [^{125}I]-NPY binding (μM range) and did not inhibit the vasoconstrictor effects of any of the agonists used, including the Y_1 receptor peptide agonist. Furthermore, BIBP 3435 did not affect the response to sympathetic nerve stimulation. Both BIBP 3435 and BIBP 3226 caused a slight transient decrease in mean arterial blood pressure (by about 5 and 15 mmHg at 1 mg kg^{-1} and 3 mg kg^{-1}, respectively), accompanied by splenic and mesenteric vasodilatation, suggesting that this effect was unrelated to Y_1 receptor blockade.

6 The peptide YY (PYY)- and NPY-evoked vasoconstriction in the kidney of reserpine-treated pigs was markedly reduced (by 95%) by BIBP 3226 while the vasoconstrictor effect in the spleen was attenuated by only 20%. BIBP 3226 did not influence stimulation-evoked NPY release. The vasoconstrictor response in reserpine-treated pigs to single impulse stimulation, which is observed only in nasal mucosa and hind limb, was unchanged regarding maximal amplitude and the integrated effect was only moderately reduced (by about 25%) in the presence of BIBP 3226 (1 mg kg^{-1}). BIBP 3226 (1 mg kg^{-1}) markedly reduced (by 55-70%) the long-lasting vascular response (total integrated blood flow reduction) evoked by sympathetic nerve stimulation at high frequency (40 impulses at 20 Hz) in spleen, kidney, nasal mucosa and hind limb. Furthermore, the maximal amplitude of the vasoconstriction was reduced mainly in the kidney (by 60%) and also in the spleen (by 40%).

7 It is concluded that BIBP 3226 can act as a selective Y_1 receptor antagonist in the pig. Endogenous NPY via Y_1 receptor activation may play a role in evoking the long-lasting vasoconstriction seen in nasal mucosa, hind limb and skin after high frequency stimulation of sympathetic nerves in control pigs. Furthermore, NPY via Y_1 receptor mechanisms seems to be of major importance for the long-lasting component of the reserpine resistant sympathetic vasoconstriction in many vascular beds, and for the maximal vasoconstrictor response in the kidney. Circulating NPY and PYY induce splenic vasoconstriction via Y_2-receptors in contrast to neuronally released NPY which mainly activates Y_1 receptors.

Keywords: Y_1 receptor; Y_2 receptor; BIBP 3226; BIBP 3435; sympathetic vasoconstriction; reserpine; non-adrenergic

Introduction

Neuropeptide Y (NPY) was isolated from porcine brain (Tatemoto, 1982) and was shown to be localized to sympathetic perivascular nerves containing noradrenaline (NA) (Lundberg *et al.*, 1982). Furthermore, NPY was shown to be coreleased with NA, especially upon strong reflex sympathetic activation (Lundberg *et al.*, 1985). Since NPY was found to possess potent long-lasting vasoconstrictor activity (Lundberg & Tatemoto, 1982) and to exert prejunctional inhibitory effects on NA release (Lundberg *et al.*, 1982; Lundberg & Stjärne, 1984), great efforts have been made to clarify the possible role of this peptide in sympathetic neurotransmission (see Lundberg *et al.*, 1989a; 1990). The similarity between the slowly developing long-lasting vascular response evoked by exogenous administration of NPY and the response evoked by sympathetic nerve stimulation in the presence of adrenoceptor blockade further

[1] Author for correspondence.

suggested that the peptide is a non-adrenergic sympathetic transmitter (Lundberg & Tatemoto, 1982). However, *in vivo* studies with adrenoceptor blocking drugs can be associated with the risk of incomplete receptor blockade, especially considering the great diversity of adrenoceptor subtypes (Ruffolo *et al.*, 1991). Therefore, in the absence of NPY-receptor antagonists, another strategy was used to investigate non-adrenergic sympathetic vascular control. This approach included administration of reserpine, a substance known to deplete monoamines like NA from the nerve terminals through interference with the mechanism for granular storage (Carlsson, 1965). Reserpine also induced increased sympathetic nerve impulse activity (Pernow *et al.*, 1988b) resulting in a reduction of the tissue content of both NPY and NA due to prolonged and enhanced release in excess of peptide resupply (see Lundberg *et al.*, 1990). However, the combination of reserpine administration and inhibition of nerve impulse traffic, by e.g. transection of the sympathetic nerves, created a situation with markedly reduced tissue NA-levels but maintained levels of NPY. The long-lasting vasoconstriction evoked by electrical sympathetic nerve stimulation in this experimental *in vivo* model showed close similarity to the response evoked by exogenous NPY administration in a variety of species and vascular beds (see Lundberg *et al.*, 1989a; 1990). Furthermore, this response was associated with enhanced outflow of NPY-like immunoreactivity (-LI), suggesting increased release due to lack of α_2-adrenoceptor-mediated autoinhibition because of NA depletion (Lundberg *et al.*, 1986; 1989b; Pernow & Lundberg, 1989a). Furthermore, the NPY concentrations in the splenic venous effluent upon sympathetic nerve stimulation after reserpine treatment correlate well with the functional response (Lundberg *et al.*, 1989a). This endogenously released NPY reaches levels (Lundberg *et al.*, 1989b) at which exogenous NPY evokes vasoconstriction (Rudehill *et al.*, 1987) and the responses have a similar, characteristically long duration (Lundberg *et al.*, 1989a). However, in the absence of an NPY-antagonist, unequivocal evidence for NPY being a mediator of reserpine resistant vasoconstriction *in vivo* could not be obtained.

The presence of several different NPY receptors have been suggested but mainly two types have been more closely characterized: the Y_1 receptor, which causes vasoconstriction, and the Y_2 receptor, which inhibits NA release (Wahlestedt *et al.*, 1986; Sheikh *et al.*, 1989). However, Y_1 receptor activation can inhibit sympathetic transmitter release in rabbit vas deferens (Doods & Krause 1991). As for adrenoceptors, both types of NPY receptors can be postjunctional and mediate vasoconstriction (Lundberg *et al.*, 1988; Modin *et al.*, 1991). Various vascular beds differ with regard to the relative occurrence of Y_1 and Y_2-mediated vasoconstriction. Thus, Y_1 activation causes an increase in blood pressure (Modin *et al.*, 1991; Grundemar *et al.*, 1992) due to general vasoconstriction, whereas Y_2 agonists have especially prominent vasoconstrictor effects in the pig spleen (Lundberg *et al.*, 1988; Modin *et al.*, 1991; Modin 1994). In accord with this, Y_2 agonists like NPY (13–36) have profound effects on [^{125}I]-NPY binding in the pig spleen (Lundberg *et al.*, 1988). The Y_1 receptor has been cloned (Eva *et al.*, 1990; Herzog *et al.*, 1992; Larhammar *et al.*, 1992), but the sequence of the Y_2 receptor protein remains to be elucidated. NPY receptor desensitization has been shown to reduce non-adrenergic sympathetic vasoconstriction evoked by high frequency stimulation *in vitro* (Morris, 1991; Morris & Sabesan, 1994) and *in vivo* (Öhlén *et al.*, 1990) suggesting involvement of endogenous NPY.

Recently, a potent and selective non-peptide Y_1 receptor antagonist BIBP 3226 ((**R**)- N^2-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl]-argininamide) was developed (Rudolf *et al.*, 1994). In the present study, we have assessed the selectivity of BIBP 3226 as a functional *in vivo* Y_1 receptor antagonist, and investigated the possible role of endogenous NPY in sympathetic vasoconstriction in different vascular beds of control and reserpine-treated pigs. The corresponding S-enantiomer BIBP 3435 which is not active on the Y_1 receptors (Rudolf *et al.*, 1994) was used as control.

Parts of these results have been communicated in a preliminary form (Modin & Lundberg, 1995; Lundberg & Modin 1995).

Methods

Receptor binding studies

All tissues (except for bovine cortex which was obtained at a local abattoir) were collected from animals killed by an overdose of pentobarbitone. Tissue from spleen, intraparenchymal renal arteries and hypothalamus of the pig as well as dog spleen and rat and bovine frontal cortex was placed in 10 volumes of ice cold sucrose (0.3 M) containing 5 mM HEPES at a pH of 7.4. The tissues was homogenized with a Polytron and then centrifuged for 10 min at $1000 \times g$. The supernatant was centrifuged at $1000 \times g$ for 30 min. The resulting pellet was resuspended in 0.9% NaCl solution containing 5 mM HEPES, pH 7.4, to a tissue concentration of 1 g ml^{-1} and stored frozen. The protein concentration was measured using a Bio Rad Protein assay kit. For the binding studies, increasing concentrations of porcine NPY (1–36), the Y_2 agonists NPY (13–36) (Wahlestedt *et al.*, 1986) or N-acetyl-[Leu^{28}, Leu^{31}]-NPY(24–36) (Potter *et al.*, 1994), the Y_1 agonist [Leu^{31}, Pro^{34}]NPY(1–36) (Fuhlendorff *et al.*, 1990), the Y_1 antagonist, BIBP 3226 or its corresponding S-enantiomer BIBP 3435 were added to 30 µl of membranes (corresponding to about 100 µg protein) in Krebs solution at pH 7.4 of the following composition (in mM): NaCl 137, KCl 2.68, $MgCl_2$ 2.05 $CaCl_2$ 1.80 and HEPES 20. Non-specific binding was estimated by adding cold NPY (1–36) to a final concentration of 0.1 µM. [^{125}I] Bolton-Hunter labelled porcine NPY (1–36) (Amersham Dagenham, U.K. 30,000–40,000 c.p.m. for 30 µl membrane preparation) was then added. The specific activity of the tracer was determined by radioimmunoassay and corrected for decay with time. After incubation for 60 min at room temperature (+22°C), the bound and the free fractions were separated by centrifugation at $10,000 \times g$ for 1.5 min. The supernatant was aspirated, the pellet was washed with Krebs solution and further centrifuged. The supernatant was aspirated again and the pellet resuspended in 700 µl distilled H_2O. The radioactivity in the supernatant and in the pellet was counted in an LKB gamma counter.

In vivo *experiments*

Control pigs The experiments were approved by the local ethics committee for animal research. Pigs of either sex (18–22 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} as i.v. bolus followed by i.v. infusion of 8 mg kg^{-1} h^{-1}). Pancuronium (0.5 mg kg^{-1} h^{-1}) was given i.v. for skeletal muscle relaxation and the pigs were then tracheotomized and artificially ventilated by a respirator. The anaesthesia was checked by pinching the interdigital skin before administration of pancuronium. Blood gas analyses (pH, P_{O_2} and P_{CO_2}) were performed at regular intervals and the composition of the breathing gas was corrected when necessary. Catheters were inserted into the left femoral vein for infusion of drugs and into the left femoral artery for measurement of mean arterial blood pressure (MABP) and heart rate (HR). A brachial artery was cannulated for blood sampling. A catheter was also placed in the left renal vein or splenic vein for collection of venous effluent. For measurement of local blood flow ultrasonic flow probes (2 RB, Transonic Inc., NY, U.S.A.) were placed around the right femoral artery, left internal maxillary artery (supplying nasal mucosa), main splenic artery, left renal artery and a peripheral mesenteric artery (supplying small intestine). The flow probes were con-

nected to Transonic flow meters and blood flow in the various organs (as well as MABP and HR) was recorded on Grass Polygraphs. For estimations of superficial cutaneous blood flow, a laser Doppler flow meter (LDF) probe (Periflux PF2B, Perimed, Sweden) was placed on the skin of the left side of the snout. The LDF signal was also recorded on the Grass Polygraph. The pigs were given heparin (200 i.u. kg^{-1} h^{-1}) and fluid replacement (NaCl 154 mM and glucose 25 g l^{-1}, 2 ml min^{-1}) i.v. throughout the experiment. For electrical stimulation, electrodes were placed on the distal end of the cut, left lumbar sympathetic chain (L3-L4 level) supplying the hind leg, left cervical sympathetic trunk supplying nasal mucosa and skin of the snout, splenic, renal and mesenteric periarterial nerves. Stimulations were performed using 2 high frequency bursts at 20 Hz for 1 s (5 ms, 25 V) with a 10 s interval (total 40 impulses). After a recovery period of 60 min following completion of surgery saline (vehicle for BIBP 3435 and 3226) was given, nerve stimulations were performed in the different organs followed by i.v. injections of [Leu^{31}, Pro^{34}]NPY(1–36) (0.5 nmol kg^{-1}), N-acetyl[Leu^{28}, Leu^{31}]NPY(24–36) (2 nmol kg^{-1}), NA (15 nmol kg^{-1}) and angiotensin II (AII) (10 pmol kg^{-1}) at 5 min intervals. Doses were chosen to yield comparable changes in vascular conductance in the spleen. After a total of 30 min, BIBP 3435 (1 mg kg^{-1}) was given i.v. for 2 min and 5 min later another series of stimulations and i.v. injections was repeated. Subsequently, BIBP 3226 (1 mg kg^{-1} and 3 mg kg^{-1}) were given and the same protocol was repeated each time. In three experiments [Leu^{31}, Pro^{34}]NPY was given 30 min after BIBP 3226 (3 mg kg^{-1}) followed by 3 mg kg^{-1} of BIBP 3435. Since the above given agonists mainly affected splenic and renal blood flow, in a final series of experiments ($n=4$), two general vasoconstrictors phenylephrine (5 nmol kg^{-1}) or α,β-methylene adenosine triphosphate (20 nmol kg^{-1}) were given i.v. before and after BIBP 3435 and BIBP 3226 (1 mg kg^{-1}). For comparison, sympathetic stimulation with single impulses and 2 bursts at 20 Hz were also initially performed as above. In one final experiment NPY (228 pmol kg^{-1}) was given i.v. before and after BIBP 3226 (1 mg kg^{-1}).

Reserpine-treated pigs In the reserpine experiments, anaesthesia was induced as above. Via a flank incision below the left costal margin the retroperitoneal space was reached and the left major splanchnic nerve, the postganglionic sympathetic nerves to the left kidney and the left sympathetic lumbar chain (at level L3–L4) were exposed and sectioned. In the neck the left cervical sympathetic trunk was exposed and cut. The incisions were closed and before the anaesthesia was terminated, reserpine (1 mg kg^{-1}) was administered i.v. The following day, the pigs were re-anaesthetized and surgically prepared as described above. The blood flow of the splenic artery, the left renal artery and the left femoral artery as well as the blood flow of the internal maxillary artery supplying nasal mucosa was measured by ultrasonic flowprobes placed around the respective vessels. Bipolar platinum electrodes were placed around the distal branches of the cut postganglionic sympathetic nerves accompanying the splenic and renal arteries, the lumbar sympathetic chain and the cervical sympathetic trunk. Finally, a catheter was placed in the main splenic vein via a side branch, which allowed local venous blood sampling, and an additional catheter was placed in the right brachial artery for collection of systemic arterial blood. For further details of the preparations see Pernow & Lundberg (1989b), Modin *et al.* (1993b,c) and Lacroix *et al.* (1988). The abdomen was closed and the pigs were allowed to recover for 1 h before the experiments were started.

The experiment was initiated by administration of atropine (0.5 mg kg^{-1} i.v.) to prevent the cholinergic vasodilator response evoked in the hind limb by lumbar sympathetic stimulation in reserpine-treated animals (see Modin *et al.*, 1993b). Fifteen min later, electrical stimulation of the cervical sympathetic trunk, the lumbar sympathetic chain, the spleen and the kidney was performed in series by a Grass stimulator. The stimulation was given as single impulse or as two high frequency bursts of 20 Hz for 1 s (5 ms, 25 V) with an interval of 10 s (total 40 impulses). Brief stimulations were chosen to avoid spontaneous decline of NPY release (Modin *et al.*, 1993a). During the 20 Hz stimulation of the spleen, blood samples were collected from splenic vein and brachial artery before, at the end of, and 30 s and 2 min after the stimulation for measurements of plasma levels of NPY-LI and NA. The stimulations were followed by i.v. bolus injections of peptide YY (PYY) (114 pmol kg^{-1}) to establish the degree of blockade of vascular NPY receptors PYY was chosen as Y receptor agonist because it does not interfere with the NPY radioimmunoassay and therefore allows release studies (see Pernow & Lundberg, 1989a). All stimulations and the PYY-injection were repeated under control conditions (control I and II) and 5–15 min after i.v. administration of BIBP 3226 (1 mg kg^{-1}). Thereafter a recovery period of 2 h followed, and the stimulations and the PYY-injection were performed once again. In one separate reserpine-treated animal NPY (228 pmol kg^{-1}) was given as an i.v. bolus injection as well as BIBP 3435 (1 mg kg^{-1}), the S-enantiomer of BIBP 3226, which lacks activity at the Y_1 receptor (Rudolf *et al.*, 1994) and BIBP 3226 (1 mg kg^{-1}).

Determination of NPY-LI and NA in plasma

The blood samples were collected in prechilled tubes containing EDTA (final concentration 10 mM), centrifuged (10 min, +4°C) and the plasma was pipetted off and stored at −20°C. NPY-LI was determined with radioimmunoassay (using antibody N1) after ethanol extraction. The detection limit is 7.8 pmol l^{-1} and details of the method have been described

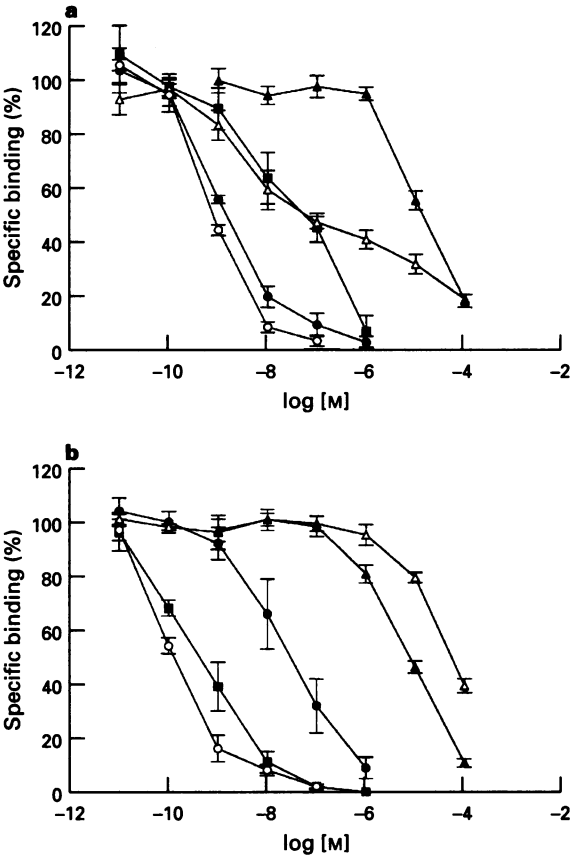

Figure 1 Inhibition of specific [^{125}I]-neuropeptide Y (NPY) binding to membranes from (a) rat cortex and (b) pig spleen by increasing concentrations of NPY (1–36) (○), the Y_1 agonist [Leu^{31}, Pro^{34}] NPY(1–36) (●), the Y_2 agonist NPY(13–36) (■), the Y_1 antagonist BIBP 3226 (△) or its S-enantiomer BIBP 3435 (▲). Data are given as means ± s.e.mean of 4–6 observations.

and evaluated by Theodorsson-Norheim *et al.* (1985). For characterization of NPY-LI in plasma from pig splenic venous effluent see Lundberg *et al.* (1989b). NA was extracted by perchloric acid, followed by alumina adsorption and assayed by cation exchange high performance liquid chromatography (h.p.l.c.) with electrochemical detection (see Pernow *et al.*, 1988a). The detection limit is 0.1 nmol l^{-1}.

Calculations

The concentration inhibiting 50% of the specific binding of [^{125}I]-NPY (1–36) is given as IC_{50} value for the different agents. The IC_{50} values for BIBP 3226 were calculated (from where the displacement curves levelled off) since less than 100% displacement was obtained at the highest concentration used. The vascular effects are expressed as changes in vascular conductance calculated as local arterial blood flow divided by MABP since MABP was changed by the various agonists (see Stark, 1968). To include aspects on duration of the vascular effects as well, the blood flow response from peak effect to basal value was integrated in some cases. Furthermore, the time required for half recovery to prestimulatory vascular conductance was also determined. For the LDF measurements, % change in signal is given. NA and NPY-LI overflow from the kidney or spleen as an indication of transmitter release was determined as the integrated area of the veno-arterial plasma concentration gradient multiplied by plasma flow. The haematocrit was determined after centrifugation of the blood samples. Data in the text are given as mean ± s.e.mean. Statistical analysis was performed by use of multiple analysis (ANOVA) followed by post-test of Tukey.

Drugs

All NPY-related peptides were from Auspep, Australia, AII from Peninsula, U.S.A., phenylephrine and α,β-methylene ATP from Sigma, U.S.A. and BIBP 3435 and 3226 from Karl Thomae, Germany. All substances were dissolved in saline.

Results

Receptor binding characterization

The specific binding of [^{125}I]-NPY incubated with membranes from pig tissues was 86% for spleen, 55% for renal arteries, 65% for hypothalamus, 56% for dog spleen and it was 69% for rat and bovine cortex. Characterization using selective NPY receptor peptide agonists revealed that pig spleen and hypothalamus contained mainly Y_2 sites while pig renal arteries, dog spleen and rat cortex bound the Y_1 agonist to the largest extent (Figure 1, Table 1). The IC_{50} value for N-acetyl[Leu^{28}, Leu^{31}]NPY(24–36) in pig spleen was somewhat higher than for NPY(13–36). BIBP 3226 had IC_{50} values between 3 and 27 nM in the tissues tested, except the pig spleen where the value was 5.5 μM. In contrast, the corresponding S-enantiomer BIBP 3435 only influenced [^{125}I]-NPY binding in pig spleen and rat cortex at 10^{-6} M or higher concentrations (Figure 1). The % displacement of [^{125}I]-NPY by BIBP 3226 at 10^{-7} M varied considerably between different tissues which may be related to the relative presence of Y_1/Y_2 receptors (Figure 1, Table 1).

Cardiovascular effects of BIBP 3226 and 3435 per se *in control pigs*

Intravenous administration of BIBP 3226 caused a slight but significant lowering of MABP (from basal values of 100 ± 3 mmHg by 7 ± 0.7 ($P<0.001$) and 13 ± 1 ($P<0.001$) mmHg at 1 and 3 mg kg^{-1}, respectively) without a consistent change in HR. Simultaneously, there was an increase in splenic vascular conductance by 27 ± 3% ($P<0.01$) and 62 ± 9% ($P<0.001$), suggesting vasodilatation. Similar effects were obtained with BIBP 3435, which lowered MABP by 5 ± 0.3 mmHg ($P<0.001$) and increased splenic vascular conductance by 23 ± 3% ($P<0.01$) at 1 mg kg^{-1}. These effects were transient and recovered within 5 min. BIBP 3435 3 mg kg^{-1} also transiently decreased MABP by 15 ± 1 mmHg and increased splenic vascular conductance by 75 ± 7% ($P<0.05$).

In the mesenteric artery the changes were comparable to those in the spleen while in the other vascular beds studied, the vascular conductance changes by BIBP 3226 were marginal or virtually absent (kidney). Administration of an equal volume of saline (vehicle) to that used for 3 mg kg^{-1} BIBP 3226 or 3435 slightly increased MABP (by 8 ± 0.7 mmHg, $P<0.001$) without affecting splenic vascular conductance.

Influence of BIBP 3226 and 3435 on exogenous agonists in control pigs

NPY evoked vasoconstriction in both the spleen (Figure 2) and kidney (Figure 6). The NPY effect was not influenced in the spleen but abolished in the kidney by BIBP 3226 (1 mg kg^{-1}) (Figures 2 and 6). The largest vascular changes for Y_1 and Y_2 agonists, NA or AII were seen in the spleen (Figure 2) and/or

Table 1 The concentration inhibiting 50% (IC_{50}) of the specific binding of [^{125}I]-neuropeptide Y (1–36) to membranes of various tissues

		NPY (1–36)	*NPY Leu^{31} Pro^{34}*	*NPY (13–36)*	*NPY (24–26) Leu^{28} Leu^{31}*	*BIBP 3226*	*BIBP 3435*
Pig spleen	IC_{50} (nM)	0.12	28	0.42	1.7	55,000	7800
	Displacement (%)	92	88	100	97		2
Pig renal arteries	IC_{50} (nM)	0.33	16	74		7	
	Displacement (%)	84	96	99		58	
Pig hypothalamus	IC_{50} (nM)	0.2	72	3.5		27	
	Displacement (%)	84	85	98		50	
Dog spleen	IC_{50} (nM)	0.65	5.5	36		3	
	Displacement (%)	90	92	87		67	
Rat cortex	IC_{50} (nM)	0.65	1.5	53		8	14000
	Displacement (%)	94	98	93		53	3
Bovine cortex	IC_{50} (nM)	0.13	4.2	14	9	10	
	Displacement (%)	87	90	90	88	26	

Since less than 100% displacement was obtained at the highest concentration values have been calculated from where the curves levelled off. % displacement is given for neuropeptide Y (NPY) (1–36) at 10^{-8} M, [Leu^{31}, Pro^{34}]NPY(1–36), NPY(13–36) and N-acetyl[Leu^{28}, Leu^{31}]NPY(24–36) at 10^{-6} M, BIBP 3226 and BIBP 3435 at 10^{-7} M. Data represent mean of 3–5 experiments.

kidney (Figures 2 and 3). In the other vascular beds, the effects were much smaller or biphasic (as for NA in the hind limb). Data on vascular conductance changes upon administration of these agonists are therefore only presented for kidney and spleen. BIBP 3435 (1 mg kg^{-1}) did not, compared to saline, influence MABP changes and local vascular effects of Y_1 or Y_2 agonists, NA or AII or the HR increase evoked by NA (Figure 3). BIBP 3226 dose-dependently inhibited the MABP increase and the vascular conductance decrease by the Y_1 agonist in both the kidney and spleen without influencing the effects of the Y_2 agonist, NA or AII (Figure 3). Separate experiments revealed that the most of the inhibitory effect of BIBP 3226 (3 mg kg^{-1}) on the Y_1 agonist response was still present after 30 min (i.e. the time necessary to carry out the experimental procedure, including sympathetic nerve stimulations followed by administration of agonists). In the second series of agonist experiments, phenylephrine and α,β-methylene ATP were shown to cause variable vasoconstriction in all vascular beds studied. Again the largest effects were observed in the spleen. Neither the maximal nor the integrated vasoconstrictor responses to phenylephrine or α,β-methylene ATP were changed in hind limb (Figure 9), nasal mucosa, skin or the other vascular beds after administration of BIBP 3226 (not shown).

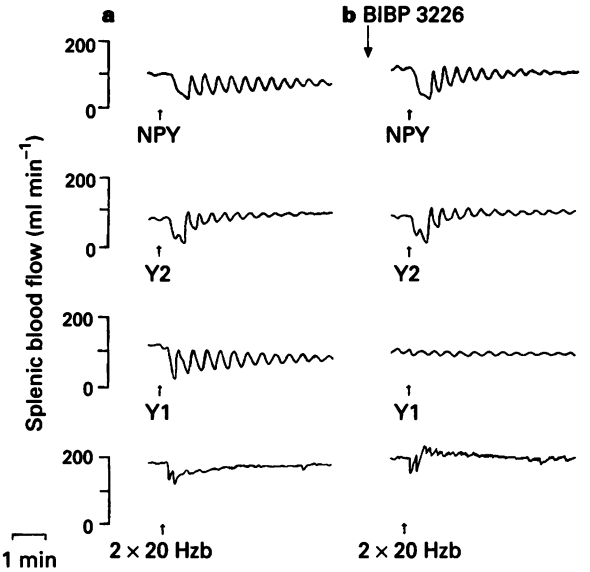

Figure 2 Recordings of arterial blood flow in pig spleen upon i.v. bolus administration (arrows) of neuropeptide Y (NPY, 0.228 nmol kg^{-1}) before (a) and after (b) administration of BIBP 3226 (1 mg kg^{-1}), the Y_2 agonist N-acetyl[Leu^{28}, Leu^{31}]NPY(24–36) (2 nmol kg^{-1}) and Y_1 agonist [Leu^{31}, Pro^{34}]NPY(1–36) (0.5 nmol kg^{-1}). Bottom recordings show response upon high frequency sympathetic nerve stimulation (two 1 s bursts at 20 Hz with 10 s interval, 2 × 20 Hzb) before and after BIBP 3226 (1 mg kg^{-1}) in a reserpine-treated pig. Time scale indicates 1 min.

Influence of BIBP 3226 and 3435 on the effect of sympathetic nerve stimulation in control pigs

Regional vascular conductances just before sympathetic nerve stimulations were not different after BIBP 3435 or 3226 compared to basal state (not shown). Significant vasoconstriction upon single pulse stimulation was only observed in hind limb, nasal mucosa and skin. Whereas the vasoconstrictor effects in kidney (Figure 6), spleen and mesenteric circulation following sympathetic stimulation at high frequency were short-lasting and followed by hyperaemia, the effects in the hind limb (Figure 9), nasal mucosa and skin only slowly recovered to baseline. The poststimulatory recovery was therefore analysed in the three latter vascular beds. At 1 mg kg^{-1} BIBP 3435 and 3226 did not influence the maximal vasoconstrictor response to single pulse (not shown) or high frequency sympathetic nerve stimulation in any vascular bed studied (Figure 4). At 3 mg kg^{-1} BIBP 3226 slightly reduced the maximal vascular

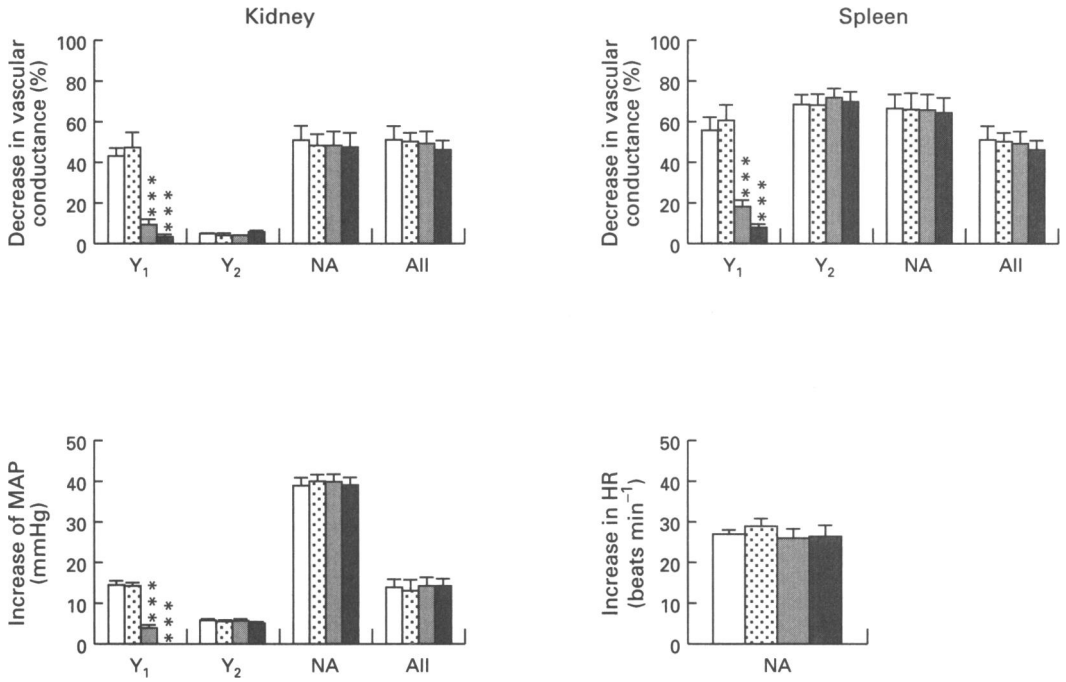

Figure 3 Summary of decrease in vascular conductance (%) in pig kidney and spleen as well as increase in mean arterial blood pressure (MABP, mmHg) upon i.v. administration of the Y_1 agonist [Leu^{31}, Pro^{34}]NPY(1–36) (0.5 nmol kg^{-1}), Y_2 agonist N-acetyl[Leu^{28}, Leu^{31}]NPY(24–36) (2 nmol kg^{-1}), noradrenaline (NA) (15 nmol kg^{-1}) and angiotensin II (AII) (10 pmol kg^{-1}) or increase in heart rate (HR, beats min^{-1}) in response to NA. The responses are observed in control condition (saline vehicle) (□), in the presence of BIBP 3435 (1 mg kg^{-1}) (▨) and BIBP 3226 (1 mg kg^{-1}) (■) and (3 mg kg^{-1}) (■). Data are given as mean ± s.e.mean ($n = 5$). Significant differences of the responses in the presence of BIBP 3435 compared to BIBP 3226 are illustrated, ***$P < 0.001$.

conductance decrease in response to high frequency stimulation in the hind limb and skin, although this was not consistent for the two subsequent bursts of impulses (Figure 4). Thus the immediate vasoconstrictor response was not much affected by either substance. However, there were clear differences in the way they affected the long-lasting vasoconstriction. BIBP 3435 (1 mg kg^{-1}) had no significant effect on the duration or on the integrated vasoconstrictor response to sympathetic stimulation, whereas BIBP 3226 (1 mg kg^{-1}) reduced both (Figure 5). These reductions were slightly greater after administration of BIBP 3226 at 3 mg kg^{-1} (Figure 5). NA overflow from the kidney or spleen upon sympathetic nerve stimulation was not influenced by BIBP 3435 (1 mg kg^{-1}) or BIBP 3226 (1 or 3 mg kg^{-1}, not shown).

Effects of exogenous NPY receptor agonists in reserpine-treated pigs

Intravenous administration of PYY (114 pmol kg^{-1}) and NPY (228 pmol kg^{-1}) evoked vasoconstriction in the spleen and the kidney together with an increase in blood pressure (17±3 mmHg for PYY) in control conditions. The vascular effects of i.v. PYY and NPY in hind limb and nasal mucosa, on the other hand, were marginal and therefore quantitative data are presented only for kidney and spleen. The maximal reduction of the vascular conductance by PYY was 48% and 78% in the kidney and the spleen, respectively (Figure 7). In the presence of BIBP 3226 (1 mg kg^{-1}), the PYY response in the kidney was markedly reduced both regarding peak effect

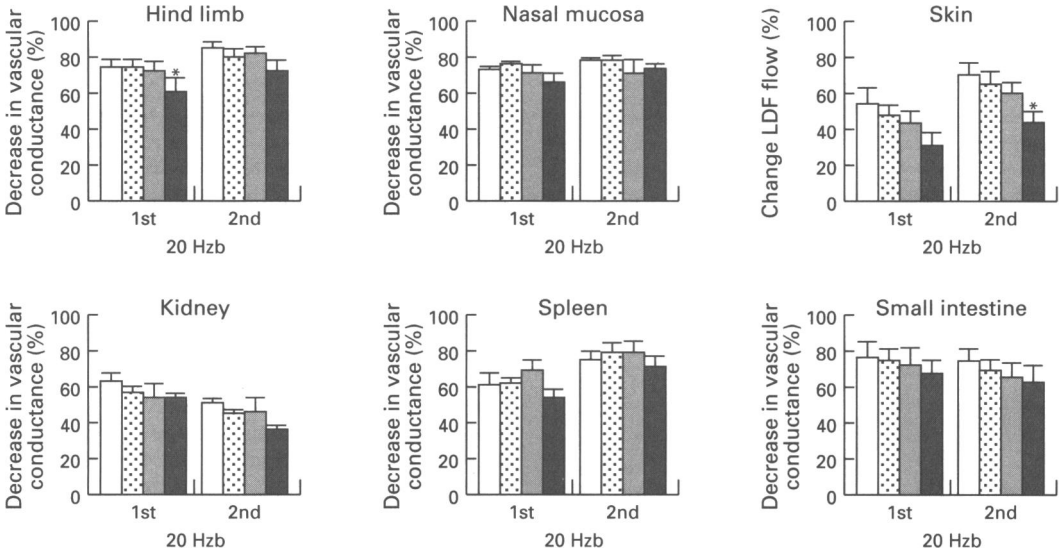

Figure 4 Summary of the maximal vasoconstrictor effects seen upon sympathetic nerve stimulation with two bursts (1st and 2nd) at 20 Hz for 1 s with 10 s interval (total 40 impulses) in different vascular beds of the pig in control condition (□) after administration of BIBP 3435 (1 mg kg^{-1}) (▨) or BIBP 3226 (1 mg kg^{-1}) (▩) or (3 mg kg^{-1}) (■). Responses in the skin are expressed as LDF units (%) while vascular conductance (% decrease) has been calculated for the other vascular beds. Data are given as mean±s.e.mean ($n=5-6$). Significant differences between the responses in the presence of BIBP 3435 and BIBP 3226 are illustrated, $^{*}P<0.05$.

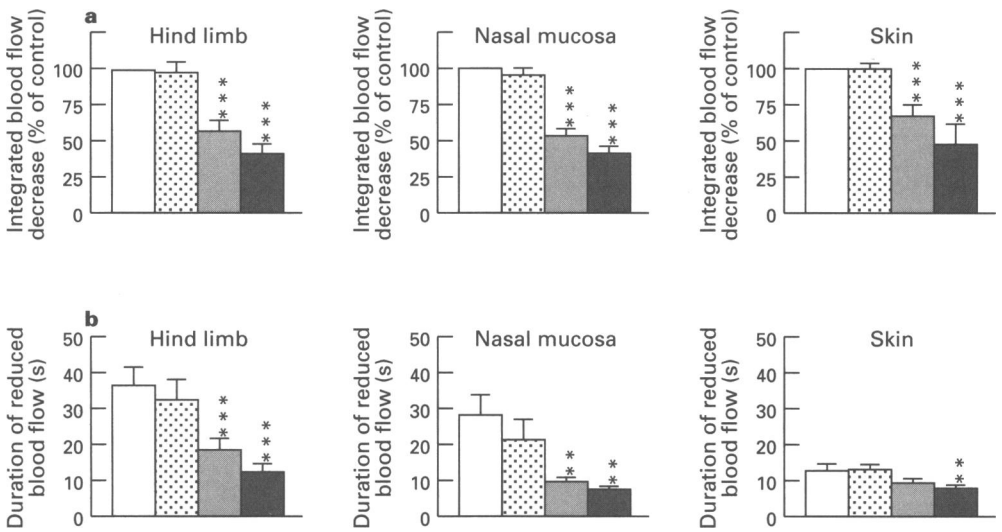

Figure 5 Summary of poststimulation vasoconstrictor events in hind limb, nasal mucosa and skin after sympathetic nerve stimulation in control conditions (□) after administration of BIBP 3435 (1 mg kg^{-1}) (▨) or BIBP 3226 (1 mg kg^{-1}) (▩) or (3 mg kg^{-1}) (■). Data are expressed as integrated area between maximal reduction to base line (a) and duration for half return of blood flow from maximal reduction to prestimulation value (b). For the integrated area, the control response was used as index (100%) and the other responses were related to this. Data are given as mean±s.e.mean ($n=6$). Significant differences between the responses in the presence of BIBP 3435 and BIBP 3226 are illustrated, $^{**}P<0.01$, $^{***}P<0.001$.

and duration, the integrated response to PYY was reduced by 95% (Figure 7). In the spleen a much smaller inhibition of the PYY vasoconstriction was observed (by 8% and 20% regarding peak effect on vascular conductance and integrated blood flow decrease in response to PYY, respectively). Both in the spleen and the kidney the responses to PYY had largely

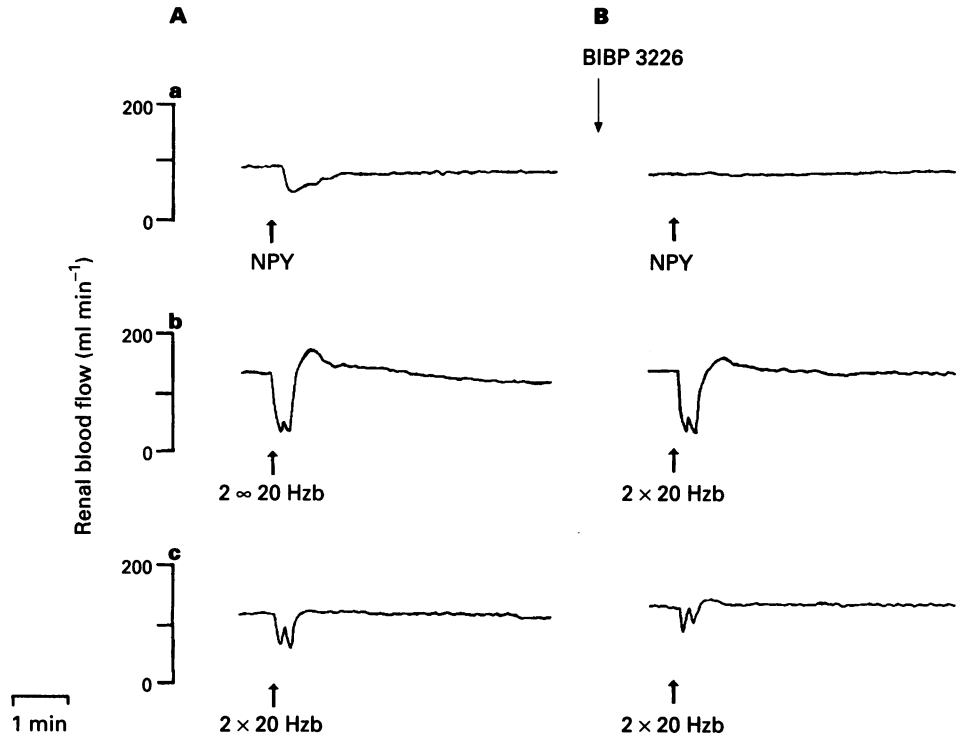

Figure 6 Recordings of arterial blood flow in pig kidney upon i.v. bolus administration (arrows) of neuropeptide Y (NPY, 228 pmol kg^{-1}, a) or upon high frequency sympathetic nerve stimulation (two 1 s bursts at 20 Hz with 10 s interval, 2 × 20 Hzb) in control (b) or reserpine-treated (c) pigs. The effects are compared before (A) and after (B) BIBP 3226 (1 mg kg^{-1}). Time scale is 1 min.

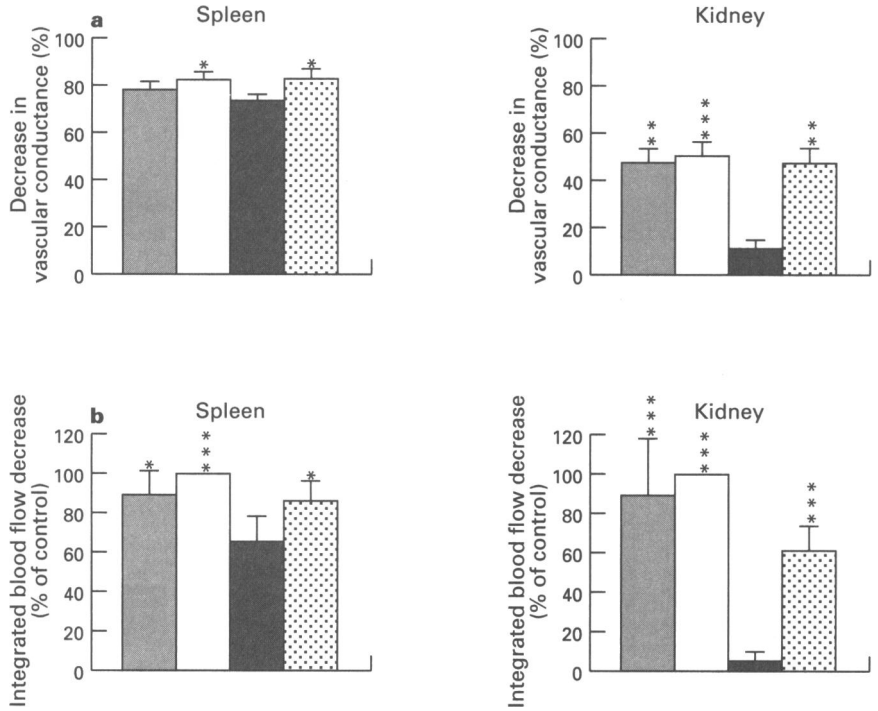

Figure 7 Maximal decrease in vascular conductance (a) and total integrated arterial blood flow change (b) in the spleen and kidney upon i.v. injection of peptide YY (PYY, 114 pmol kg^{-1}) in reserpine-treated pigs. The vascular response is shown twice under control conditions (control I (■) and II (□)), after administration of BIBP 3226 (1 mg kg^{-1} i.v.) (■) and 2 h later (recovery) (▨). For the total integrated area, the second control response was used as an index (100%) and the other responses were related to this. Data are given as means ± s.e.mean ($n = 5$). Statistically significant differences compared to BIBP 3226 are illustrated, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

returned after two hours of recovery (Figure 7). Principally similar inhibition of vasoconstrictor responses by BIBP 3226 i.e. mainly in the kidney but not in the spleen was observed in the single experiment with NPY.

Sympathetic nerve stimulation in reserpine-treated pigs

Basal vascular conductances were not influenced at 5 min after BIBP 3226 (1 mg kg^{-1}), although an initial transient increase (by about 20%) was observed especially in the spleen concomitant with a slight fall in blood pressure (by 10–15 mmHg). Electrical stimulation with single impulses reduced blood flow only in nasal mucosa and hind limb where vascular conductance was reduced by about 15%. These effects were reproducible and their maximal amplitude was not significantly influenced by BIBP 3226 (1 mg kg^{-1}) (not shown). When area under the curve for the vasoconstrictor responses was integrated, a slight reversible reduction of the single pulse-evoked responses was detected. This reduction amounted to about 20–25% in hind limb ($P<0.01$) and nasal mucosa ($P<0.05$), and was mainly due to a shorter duration of action. Nerve stimulation at high frequency (two 1 s bursts of 20 Hz) of the spleen, kidney, hind limb and nasal mucosa evoked clear-cut vasoconstriction in the respective vascular beds (Figures 2, 6 and 9). The maximal reductions in vascular conductance were 30–50% and especially long-lasting effects were observed in the hind limb (Figure 9) and nasal mucosa. The responses were reproducible: under control conditions, the effects of the second stimulation were virtually the same as those of the first (Figures 8 and 10). After BIBP 3226 (1 mg kg^{-1}) the maximal vasoconstriction, i.e. reduction of vascular conductance, especially upon renal nerve stimulation (Figures 6 and 8), and also to some extent in the spleen, was attenuated compared to the control condition (Figure 8). The response in the kidney was reduced at both bursts of impulses by BIBP 3226 although the reduction was largest at the second burst (by 60%) (Figure 8). Two hours after BIBP 3226-administration, the maximal vascular responses evoked by renal and splenic nerve stimulation were largely restored (Figure 8). Analysis of the total integrated blood flow change evoked by nerve stimulation also showed clear reproducible results in control conditions (Figure 8). However, after administration of BIBP 3226, the vasoconstrictor effects in response to stimulation were of much shorter duration (Figure 2) and the

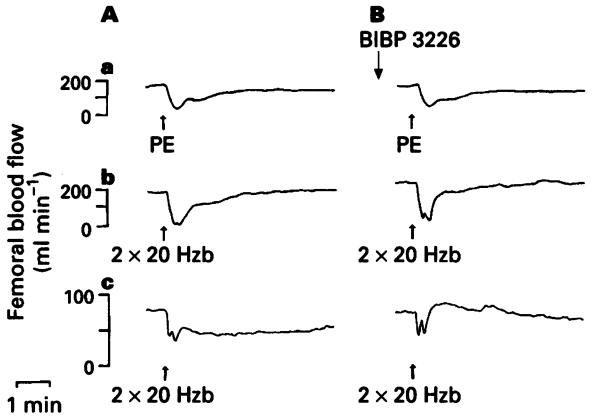

Figure 9 Recordings of arterial blood flow in the femoral artery upon i.v. injection of phenylephrine (PE) (5 nmol kg^{-1}) in a control pig (a) or upon electrical sympathetic nerve stimulation with two bursts of 20 Hz for 1 s at 10 s intervals (2 × 10 Hzb) in a control pig (b) and a reserpine-treated pig (c). Responses are compared before (A) and after (B) BIBP 3226 (1 mg kg^{-1} i.v.). Time scale indicates 1 min.

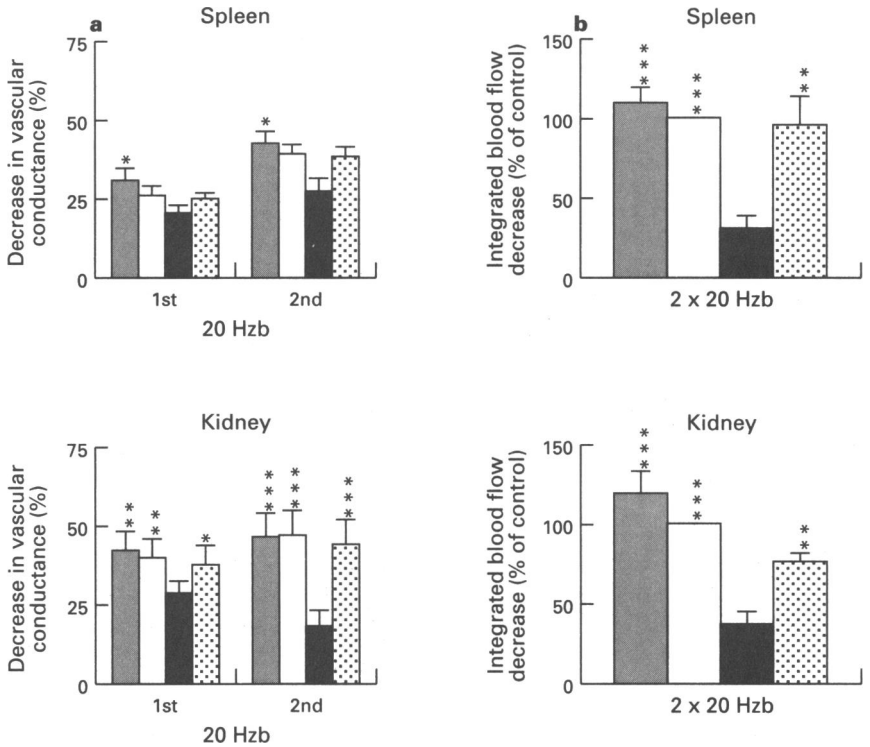

Figure 8 Maximal decrease in vascular conductance (a) or total integrated blood flow decrease (b) upon electrical sympathetic nerve stimulation of the spleen and kidney with two bursts of 20 Hz (2 × 20 Hzb) for 1 s at 10 s intervals (1st and 2nd stimulation) in reserpine-treated pigs. The vascular response is shown twice in the absence of BIBP 3226 (■, □) and after BIBP 3226 (1 mg kg^{-1}) (■) and 2 h later (recovery) (▨). For integrated blood flow decrease, the second stimulation (i.e. just before BIBP) was used as an index (100%) and the responses in the other situations were related to this. Data are given as means ± s.e.mean ($n=5$). Significant differences compared to BIBP 3226 are indicated, $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

integrated blood flow responses decreased markedly. In the spleen and kidney the integrated responses in the presence of BIBP 3226 were only about 30% of those observed at the previous control stimulation and after two hours of recovery, the responses had normalized (Figure 8).

The maximal vasoconstrictor effects to nerve stimulation in the hind limb and nasal mucosa were not influenced by BIBP 3226 (Figures 9 and 10). In the hind limb and nasal mucosa the duration of the vasoconstrictor responses were much shorter (see Figure 9 for hind limb) and the integrated response after BIBP 3226 was only 25% and 45% of the second control stimulation, whereas the recovery was incomplete after 2 h (Figure 10). The S-enantiomer BIBP 3435 (1 mg kg^{-1}) did not influence the vascular effects of sympathetic nerve stimulation in any of the vascular beds studied, although a slight initial fall in MABP and transient increase in splenic vascular conductance similar to those evoked by BIBP 3226 were observed.

BIBP 3226 did not influence basal arterial plasma levels of NPY-LI (not shown). The integrated overflow of NPY-LI from the spleen upon the two 20 Hz bursts of stimulation was 6.1 ± 2 pmol in control conditions and could be reproduced. In the presence of BIBP 3226 the overflow was 6.0 ± 2 pmol. No splenic overflow of NA could be detected upon stimulation in these reserpine-treated animals, either before or after BIBP 3226.

Discussion

The present results show that BIBP 3226, a non-peptide Y_1 receptor antagonist (Rudolf *et al.*, 1994), reduces the duration of the vasoconstrictor response to sympathetic nerve stimulation, especially at high frequency, in both controls and reserpine-treated pigs *in vivo*. This finding is well in line with the idea of preferential release of NPY at high frequency stimulation (Lundberg *et al.*, 1989b) or strong reflex sympathetic activation (Lundberg *et al.*, 1985) as well as with the ability of exogenous NPY to mimic these usually long-lasting vascular effects (Lundberg *et al.*, 1986).

This study also confirms and extends the evidence (Rudolf *et al.*, 1994) that BIBP 3226 seems to be a selective inhibitor of Y_1 receptor mediated effects. In control pigs *in vivo* we showed that cardiovascular responses mediated by the Y_2 receptor agonists, NA, phenylephrine, α,β-methylene ATP or AII are uninfluenced by BIBP 3226. The presently obtained IC_{50} values for the displacement of [^{125}I]-NPY binding by BIBP 3226 (3–27 nM) are also comparable with the findings of Rudolf *et al.* (1994) and are similar in several species (pig, rat, cow, dog and man). The relative proportion of Y_1 receptors varies greatly in different tissues, and BIBP 3226 may therefore be used to elucidate the fraction of Y_1 receptor sites present. In agreement with data using partially selective peptide agonists, the results for BIBP 3226 clearly indicate that the dog spleen is a Y_1-receptor dominated organ while in the pig, Y_2 receptors are most abundant. [^{125}I]-NPY binding to pig splenic membranes was virtually unaffected by BIBP 3226 up to 10^{-6} M although in the pig spleen *in vivo* the Y_1 agonist caused powerful vasoconstriction which could be blocked by BIBP 3226. This functional observation clearly suggests that not only Y_2 but also Y_1 receptor mechanisms can mediate NPY-evoked vasoconstriction in the spleen in accord with earlier data obtained using peptide agonists (Modin *et al.*, 1991, see Modin, 1994). However, in homogenates of the whole pig spleen, Y_2 sites dominate, while Y_1 sites on vascular smooth muscle are likely to represent only a minor subpopulation of splenic NPY receptors. When NPY and PYY are given via the i.v. route, mainly non-Y_1, presumably Y_2 receptor mechanisms are activated in pig spleen in contrast to neuronally released NPY (see below). Although BIBP 3226 did not displace all [^{125}I]-NPY binding sites on membranes from intraparenchymal renal arteries, the Y_2 agonist only caused minor changes in renal vascular conductance, suggesting that the dominating NPY

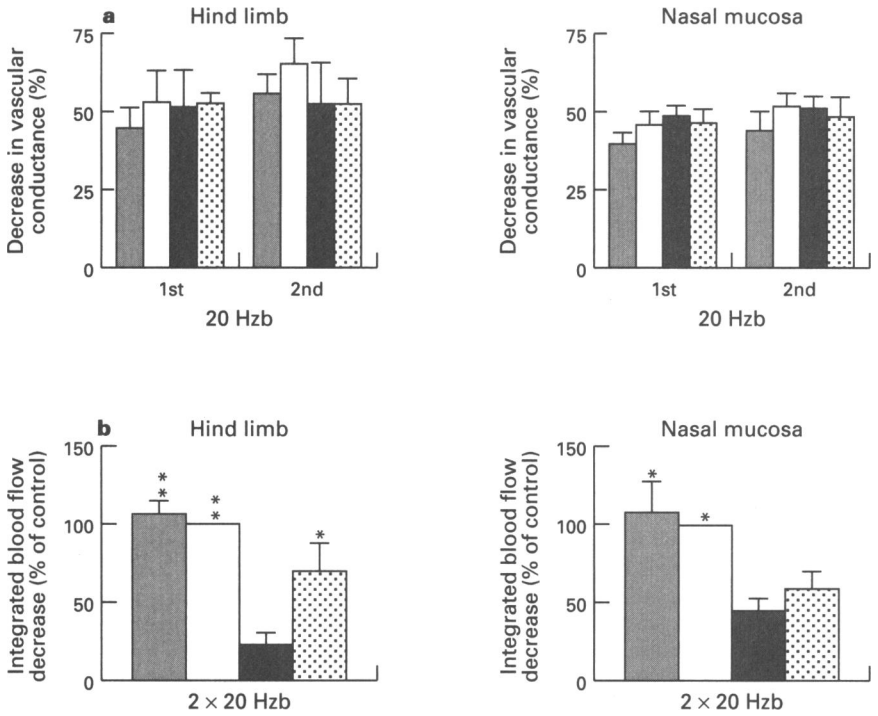

Figure 10 Maximal decrease in vascular conductance or total integrated blood flow decrease upon electrical sympathetic nerve stimulation of the hind limb and nasal mucosa with two bursts of 20 Hz (20 Hzb) for 1 s at 10 s intervals (1st and 2nd stimulation) in reserpine-treated pigs. (a) The vascular response is shown twice in the absence (■, □) and after administration of BIBP 3226 (1 mg kg^{-1}) (■) and 2 h (recovery later)(▨). (b) For the integrated blood flow decrease, the second stimulation (i.e. just before BIBP) was used as an index (100%) and the responses in the other situations were related to this. Data are given as means ± s.e.mean ($n = 5$). Significant differences compared to BIBP 3226 are indicated, $^*P < 0.05$, $^{**}P < 0.01$.

receptor regulating blood flow in small resistance vessels of the kidney is probably of the Y_1 subtype (see also Rudolf *et al.*, 1994). This is also supported by the observations showing differences in the ability of BIBP 3226 to block the effects of peptide YY, a non-selective NPY receptor agonist: BIBP 3226 markedly inhibited vasoconstriction in the kidney, but had only marginal effects in the spleen.

The transient moderate effects of BIBP 3226 *per se* on MABP and basal vascular conductance in spleen and mesenteric circulation, without major changes in HR, were also seen in response to BIBP 3435, the S-enantiomer, which only marginally displaces [^{125}I]-NPY binding in membranes with Y_1 receptors (see also Rudolf *et al.*, 1994). Therefore, this hypotensive effect is unlikely to involve Y_1 receptor mechanisms. Furthermore, since the non-Y_1 receptor related effect of BIBP 3226 was transient and basal vascular conductance had largely returned before nerve stimulations were performed or the exogenous Y_1 agonist given, it is likely that truly specific Y_1 antagonistic effects were observed. Furthermore, BIBP 3435 did not influence the vasoconstrictor responses to the Y_1 agonist. Since the effect of the exogenous Y_1 agonist was inhibited by BIBP 3226 even though this agent was without 'specific' effects on basal MABP, it seems unlikely that vascular Y_1 receptors contribute to basal blood pressure regulation to any major extent in the presently used anaesthetized pig model.

The vasoconstrictor responses to single pulse nerve stimulation occurring mainly in nasal mucosa and hind limb were not influenced by BIBP 3226 in control pigs and only marginally after reserpine; earlier data have also indicated that NPY release mainly occurs upon strong sympathetic activation, for example during heavy physical exercise (Lundberg *et al.*, 1985) or high frequency stimulation of sympathetic nerves (Lundberg *et al.*, 1989b). A critical issue is whether the dose of BIBP 3226 used was high enough to block not only the effects of the exogenous Y_1 agonists but also the response to endogenous NPY released locally, presumably in high concentrations and close to receptor sites. However, the non-specific effects of BIBP 3226, i.e. those not due to its effects at Y_1 receptors, especially at 3 mg kg^{-1} did restrict the usefulness of higher doses. Y_1 receptor activation can inhibit sympathetic transmitter release in rabbit vas deferens (Doods & Krause, 1991), while in most other cases including sympathetic perivascular nerves in pig spleen (Modin, 1994) and rat vas deferens (Wahlestedt *et al.*, 1986) Y_2 receptors mediate the prejunctional effects of NPY. These Y_2 receptors in rat vas deferens are not influenced by BIBP 3226 (Rudolf *et al.*, 1994). BIBP 3226 did not influence NA overflow from spleen or kidney upon sympathetic nerve stimulation in control pigs or NPY overflow from spleen of reserpine-treated pigs, suggesting that these tissues lack prejunctional Y_1 receptor mechanisms that are involved in regulation of transmitter release.

A large animal like the pig provides a unique opportunity to compare in parallel the responses to exogenous agonists or sympathetic nerve stimulation in different vascular beds, although it must be borne in mind that sympathetic nerves contain some fibres (cholinergic or sensory) that mediate vasodilatation and that the degree of autoregulation of blood flow varies among the organs studied. There are striking differences regarding both the vascular responsiveness to sympathetic stimulation at single impulses and the duration of the vasoconstrictor effects upon high frequency stimulation, as illustrated in the present study. Thus, whereas hind limb, nasal mucosa and skin respond strongly to sympathetic stimulation, with long-lasting vasoconstrict effects, the vasoconstriction in the spleen, kidney and mesenteric circulation is short-lasting and followed by hyperaemia. In the splenic circulation, there is also a very characteristic oscillatory change in blood flow that follows a vasoconstrictor event, regardless of the stimuli used.

Intravenously administered NPY or Y_1 agonists have a slowly developing, long-lasting vasoconstrictor effect which, however, varies markedly in magnitude between different vascular beds (Rudehill *et al.*, 1987). Being a comparatively large peptide with 36 amino acid residues, NPY is likely to have limited ability to pass over the endothelial barrier of the vasculature. In the spleen and kidney, which responded well to the i.v. administration of the Y_1 agonist, the capillary endothelium is fenestrated and therefore more permeable also for peptides the size of NPY. In contrast, in skeletal muscle, nasal mucosa and skin, higher plasma NPY concentrations are necessary before vasoconstriction is observed (Rudehill *et al.*, 1987); usually, i.a. administration is required (Lacroix *et al.*, 1988; Modin *et al.*, 1993). Conversely, it is likely that NPY passage from release sites in the outer portion of the vascular wall into the venous effluent is more optimal in spleen and kidney: NPY overflow in these organs is relatively rapid and is much more easily detected (Lundberg *et al.*, 1989b; Pernow & Lundberg 1989b) than NPY overflow in nasal mucosa (Lacroix *et al.*, 1989) and hind limb (Pernow *et al.*, 1988a; Modin *et al.*, 1993b). In fact, diffusion into the venous effluent or lymph after release may represent one important inactivation mechanism for NPY since this peptide has a comparatively long half-life, 5 min (see Rudehill *et al.*, 1987). The difference in the time required for NPY removal after release may contribute to the very different duration of sympathetic vasoconstriction in different vascular beds in both control and reserpine-treated pigs. In accord with the previous discussion on long-lasting constrictor effects of Y_1 agonists it was found that the vasoconstrictor response to high frequency sympathetic nerve stimulation was reduced, mainly in duration, in hind limb, nasal mucosa and skin, three vascular beds with characteristic long-lasting vasoconstrictor effects without poststimulation hyperaemia.

In contrast to the poststimulation event, the maximal vasoconstrictor responses were largely unaffected by Y_1-receptor blockade in all vascular beds, implying that other co-transmitter mechanisms, like adrenoceptor activation by NA and/or purinoceptor (P_{2x}) stimulation by ATP (von Kügelgen & Starke, 1985; Burnstock, 1988; Bao, 1993), were of major importance for the peak effects. It is of interest that the α_1-receptor agonist phenylephrine and the P_{2x} receptor agonist α,β-methylene ATP caused vasoconstriction in all vascular beds studied. However, the peak effects or the integrated responses to these two agonists were not influenced by BIBP 3226 (or BIBP 3435). This suggests that the reduction of the poststimulation vasoconstriction response upon sympathetic nerve stimulation in hind limb, nasal mucosa and skin by the Y_1 antagonist BIBP 3226 was not caused by a non-specific effect on adrenoceptor- or P_{2x}-mediated responses. The peak vasoconstriction response to sympathetic nerve stimulation in the hind limb and skin was slightly reduced by the high dose of BIBP 3226 (3 mg kg^{-1}). Providing that this is a specific effect, it could possibly be due to inhibition of the Y_1-mediated vasoconstrictor effects of endogenous NPY, or to removal of the enhancing effects of endogenous NPY on NA-evoked vasoconstriction (Ekblad *et al.*, 1984).

In comparative studies using animals depleted of NA due to reserpine treatment the peak vasoconstrictor response in nasal mucosa and hind limb was largely uninfluenced by BIBP 3226 (1 mg kg^{-1}), suggesting other mediator mechanisms. The maximal vasoconstrictor effects in response to sympathetic nerve stimulation were reduced by BIBP 3226, most strongly in the kidney and to some extent in the spleen, that is, in the two vascular beds that were most sensitive to the effects of intravenously given exogenous PYY (and NPY). It should be emphasized that NPY (and PYY) given i.a. in higher doses causes vasoconstriction in both skeletal muscle and nasal mucosa, presumably mainly via Y_1 receptor mechanisms (Modin *et al.*, 1991; 1993b). In this context it is of interest that the non-adrenergic sympathetic vasoconstriction (defined as resistant to adrenoceptor blocking agents or NA depletion by reserpine) in nasal mucosa and hind limb of large animals like the pig, cat or dog is often characterized by a long duration; this is also a characteristic feature for the Y_1 receptor-mediated response (see Lundberg *et al.*, 1990). In the spleen of reserpine-treated pigs, the long-lasting phase of the vasoconstriction to nerve stimulation was abolished by BIBP 3226 suggesting that

neuronally released NPY activates Y_1-receptors in contrast to circulating PYY and NPY. Since a powerful Y_2 receptor-mediated vasoconstriction is also present, especially in the spleen, we cannot exclude that Y_2 receptors also play a role in the sympathetic vasoconstriction response until specific Y_2 receptor antagonists become available, although the remaining rapid, short-lasting vasoconstrictor effect to nerve stimulation after BIBP 3226 is not mimicked by the Y_2 agonist (see Figure 2).

It should be emphasized that NPY release is enhanced after reserpine treatment. This in turn probably means that the contribution of Y_1 receptor mechanisms is exaggerated compared to their importance in the control situation. However, this may also mean that BIBP 3226, being a competitive antagonist, can more easily block the effects of NPY released in 'normal' amounts. It is therefore of interest that also in controls, the duration of the sympathetic vasoconstriction in hind limb and nasal mucosa was reduced by BIBP 3226 but not by BIBP 3435. Even at single impulse stimulation, when autoinhibition by α_2-adrenoceptor mechanisms is not likely to be operating, only slight if any NPY release probably occurs, as indicated by the fact that the durations of the reserpine-resistant vasoconstrictor responses in hind limb and nasal mucosa were only slightly reduced by BIBP 3226.

Although it cannot be excluded that BIBP 3226 does not completely block the effects of neuronally released NPY, the lack of effect on the maximal vasoconstriction suggests the presence of other receptor mechanisms or sympathetic transmitters in these vascular beds. No NA release was detected upon nerve stimulation, and reserpine in the dosage used in this study is known to cause a marked (>90%) depletion of tissue NA in the pig (see Lundberg *et al.*, 1990). The presumably non-adrenergic vascular effects of sympathetic stimulation in reserpine-treated pigs in the presence of a Y_1 receptor antagonist may therefore be caused by some rapidly acting and quickly metabolized transmitter, like ATP, especially at low frequency stimulation (von Kügelgen & Starke, 1985; Bulloch & McGrath, 1988). Rapid, presumably ATP-mediated nerve stimulation-evoked contractions of isolated blood vessels *in vitro* thus remain after reserpine (Muramatsu, 1987). Since the release of ATP is normally inhibited by endogenous NA acting on prejunctional α_2-adrenoceptors (Ramme *et al.*, 1987) also the purine release upon high frequency burst stimulation is likely to be increased after reserpine (in contrast to single pulse stimulations). Unfortunately, *in vivo* desensitization with the P_{2x} receptor agonist α,β-methylene ATP has not yielded conclusive evidence for ATP being a sympathetic non-adrenergic transmitter in pig nasal mucosa or spleen (Lundberg *et al*, 1989c), even at single pulse stimulation (Lacroix *et al.*, 1989). Experiments using selective purinoceptor antagonists, both in control and in reserpine-treated pigs, will be necessary to solve this issue.

It is concluded that the data obtained using BIBP 3226 and its S-enantiomer BIBP 3435, which is largely without effects on NPY receptors, suggest that Y_1 receptor mechanisms activated by endogenously released NPY are crucial for the long-lasting component of the vasoconstriction upon high frequency stimulation seen in several vascular beds of reserpine-treated pigs. Furthermore, especially in the kidney, the maximal amplitude of the sympathetic vasoconstrictor response after reserpine also depends on Y_1 receptor mechanisms. NPY may also have a role in the long-lasting vasoconstrictor responses in control pigs.

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