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Review

Pharmacology of neuropeptide Y receptor antagonists Focus on cardiovascular functions

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Abstract

Neuropeptide Y is one of the most abundant mammalian neuropeptides identified to date. The possible actions of neuropeptide Y, that is co-localized and released with noradrenaline, as a sympathetic co-transmitter has attracted much attention during the last decade. In recent years, several non-peptide antagonists with high subtype selectivity for neuropeptide Y receptors have been introduced. With them, the status of neuropeptide Y as a sympathetic transmitter has been established, and so have profound cardiovascular effects mediated by neuropeptide Y Y_1 and Y_2 receptors. Significant release of neuropeptide Y occurs especially upon stronger sympathetic activation, and recent data suggest that the importance of neuropeptide Y seems enhanced in stress-related cardiovascular disorders. The true significance of neuropeptide Y has thus started to unfold, owing to the presence of the first generation of selective neuropeptide Y receptor antagonists. This review concerns the pharmacology of these agents, what we have learnt from them, and might find out in the future. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Noradrenaline is considered the primary transmitter of the sympathetic nervous system, intimately regulating cardiovascular functions. In recent years, increasing evidence has suggested that sympathetic co-transmitters, ATP and neuropeptide Y, may be involved in vascular control (for reviews, see Burnstock, 1986; Malmström, 1997). The extent to which ATP is involved has not been fully elucidated, due to a lack of specific and potent antagonists. The role of neuropeptide Y has, however, begun to be unravelled because of the recent introduction of much anticipated specific pharmacological tools. Subtype-selective antagonists soon followed the cloning of receptor subtypes for neuropeptide Y. Since the launch of these antagonists, evidence has been presented for the involvement of endogenous, neuronally released, neuropeptide Y in sympathetic vasoconstrictor responses and regulation of transmitter release, and furthermore, for the participation of neuropeptide Y Y_1 and Y_2 receptors in such effects. Thus, the possibility that neuropeptide Y may act as a sympathetic transmitter has been proven, the full significance of which is yet to be shown. More than a handful of antagonists selective for one neuropeptide Y receptor subtype exist today, more or less well characterized, that can trigger investigations into the questions surrounding the true role of neuropeptide Y in sympathetic cardiovascular control. This review will focus on peripheral actions and pharmacology of the first generation of selective non-peptide neuropeptide Y receptor antagonists.

2. Neuropeptide Y

Neuropeptide Y, a 36-amino-acid residue peptide isolated from porcine brain (Tatemoto et al., 1982), is one of the most abundant neuropeptides in both peripheral and central nervous systems (Lundberg et al., 1982b; Gray and Morely, 1986). Neuropeptide Y belongs to the pancreatic polypeptide family of peptides, peptide YY and pancreatic polypeptide being the other members found mainly in intestinal endocrine and pancreatic islet cells, respectively (Larsson et al., 1974; Lundberg et al., 1982a). In the central nervous system, neuropeptide Y is purportedly involved, e.g., in the promotion of feeding, in anxiolysis, and in modulating the

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release of a variety of hormones (Turton et al., 1997). In the peripheral sympathetic nervous system, neuropeptide Y, stored and released together with noradrenaline (Lundberg et al., 1982b), mediates several cardiovascular effects including vasoconstriction and modulation of transmitter release (Malmström and Lundberg, 1997a).

Over the years, evidence has accumulated to support the concept of neuropeptide Y as co-transmitter in the central and sympathetic nervous systems, although definitive pharmacological evidence for this was long lacking due to the absence of selective antagonists. In addition, as a consequence, the physiological and pathophysiological roles of neuropeptide Y had also been difficult to establish. However, in 1990, the first receptor for neuropeptide Y was cloned and it was soon to be followed by others (Eva et al., 1990; see Michel et al., 1998). In 1994, the first non-peptide antagonist, selective for a neuropeptide Y receptor subtype (Y_1) was introduced (Rudolf et al., 1994), and there followed several more, for this and other neuropeptide Y receptor subtypes. It has since been possible, e.g., to prove that neuropeptide Y is a sympathetic transmitter (see Malmström, 1997), and furthermore, to pinpoint receptor subtypes mediating the effects of neuropeptide Y.

3. Neuropeptide Y receptor subtypes

The effects of neuropeptide Y and related peptides are mediated via several subtypes of G protein-coupled receptors. The initial subdivision of neuropeptide Y receptors originated from pharmacological evidence obtained using bioassay systems. It was shown that pre- and postjunctional neuropeptide Y receptors possessed different ligand structure requirements for activation. Thus, a C-terminal fragment, peptide YY-(13–36), evoked prejunctional inhibition of nerve-evoked contraction but did not produce any vasoconstriction. In contrast, the entire neuropeptide Y (and peptide YY) molecule was required to elicit vascular contraction. Hence, a division of neuropeptide Y receptors into postjunctional (Y_1) and prejunctional (Y_2) subtypes was proposed (Wahlestedt et al., 1986). However, later studies revealed that the opposite situation might occur (Doods and Krause, 1991; Modin et al., 1991), e.g., in pig spleen (postjunctional Y_2 receptor) and in rabbit vas deferens (prejunctional Y_1 receptor).

Today, five distinct neuropeptide Y receptors have been cloned (see Michel et al., 1998), although it seem that mainly neuropeptide Y Y_1 and Y_2 receptors are involved in peripheral effects (sympathetic vascular control) and these will, therefore, be focused upon. In 1990, a receptor, originally classified as an orphan receptor (Eva et al., 1990), was cloned from rat and was later found to encode the neuropeptide Y Y_1 receptor (see Michel et al., 1998). There is great homology between neuropeptide Y Y_1 receptors expressed in different species and the same applies to the cloned neuropeptide Y Y_2 receptors. In contrast, neuropeptide Y Y_1 and Y_2 receptors are only about 31% identical. Thus, whereas the homology within the neuropeptide Y receptor family is very low, the respective neuropeptide Y receptor subtype can be highly conserved between species (see Larhammar, 1997). Both neuropeptide Y Y_1 and Y_2 receptors are functionally coupled to Ca^{2+} mobilization and inhibition of stimulated adenylate cyclase (see Michel et al., 1998). As mentioned above, characteristic for the neuropeptide Y Y_1 receptor is a demand for the integrity of the N-terminal part of the peptide. Upon elimination (or substitution) of one or a few amino-acid residues, creating C-terminal fragments, e.g., neuropeptide Y-(3–36) and neuropeptide Y-(13–36), etc., the ligand loses its affinity for this receptor. In contrast, substitutions in the C-terminal part of the peptide, e.g., [Pro^{34}]neuropeptide Y, can be introduced without loss of potency. The characteristic ligand rank order of potency for the neuropeptide Y Y_1 receptor is thus: neuropeptide Y ≥ peptide YY ≥ [Pro^{34}]substituted analogue ≫ C-terminal fragment > pancreatic polypeptide. In contrast, C-terminal fragments are potent ligands at the neuropeptide Y Y_2 receptor, whereas ligands modified in the C-terminus are not. An order of potency of neuropeptide Y ≥ peptide YY ≥ C-terminal fragment ≫ [Pro^{34}]substituted analogue>pancreatic polypeptide is thus characteristic for the neuropeptide Y Y_2 receptor. It was reported that in several model systems, e.g., rat adrenals, colon, and brainstem (central inhibitory cardiovascular effect), peptide YY was considerably less active than neuropeptide Y. These receptor sites are called "putative Y_3 receptors", although this receptor remains to be cloned. The principal feature of the cloned neuropeptide Y Y_4 receptor is its very high affinity for pancreatic polypeptides of the same species. Although expressed in several peripheral tissues, the function(s) of this receptor remains to be elucidated. In addition, the cloned neuropeptide Y Y_5 receptor is found mainly in the central nervous system, possesses affinity for a broad spectrum of ligands, and may be involved in food intake, whereas the cloned neuropeptide Y y_6 receptor may, at least in primates, correlate with a non-functional transcript (see Michel et al., 1998).

4. Neuropeptide Y receptor antagonists

4.1. The predecessors

Attempts at pharmacological characterization of neuropeptide Y receptor subtypes, as well as at establishing the status of neuropeptide Y as a transmitter, were long hampered by the lack of selective antagonists. Either random screening or a rational mimetic strategy, using the neuropeptide of interest or fragments/derivatives thereof as the initial lead molecule, may serve as approach in the search for low molecular weight neuropeptide receptor antagonists. The latter approach yielded the first selective non-peptide neuropeptide Y Y_1 receptor antagonist, BIBP3226, (*R*)-N^2-

(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-arginina-mide (Rudolf et al., 1994). However, this was preceded by several peptide and non-peptide antagonists, the use of which was hampered by drawbacks such as low affinity and potency, lack of specificity and irreversibility. It was shown, e.g., that small peptide fragments of neuropeptide Y could act as (weak) antagonists at neuropeptide Y receptors (see Balasubramaniam, 1997). Among these, the synthetic hexapeptide, BRC672 (corresponding to residues 22–27 of neuropeptide Y), was reported to antagonize the hypertensive effect of neuropeptide Y in rats (Tseng et al., 1994). Moreover, high doses of BRC672 per se caused hypotension in anaesthetized rats as well as in conscious spontaneously hypertensive rats (Tseng et al., 1994). In parallel, the dimeric nonapeptide, 1229U91 (a.k.a. GR231118 and GW1229), one of a series of cyclic peptides, able to antagonize neuropeptide Y-evoked (Y_1 receptor-mediated) vascular effects in, e.g., isolated rat kidney and anaesthetized rats (Daniels et al., 1995), also caused a fall in blood pressure in conscious spontaneously hypertensive rats (Doods et al., 1995). However, this hypotensive effect could be antagonized by the histamine H_1 receptor antagonist, mepyramine, suggesting an indirect depressor action via histamine liberation from mast cells (Doods et al., 1995). Neuropeptide Y, and C-terminal fragments of the peptide, at high doses, may also cause the release of histamine from mast cells in both humans and rats (Grundemar and Håkanson, 1991; Mousli et al., 1994), which is the cause of the

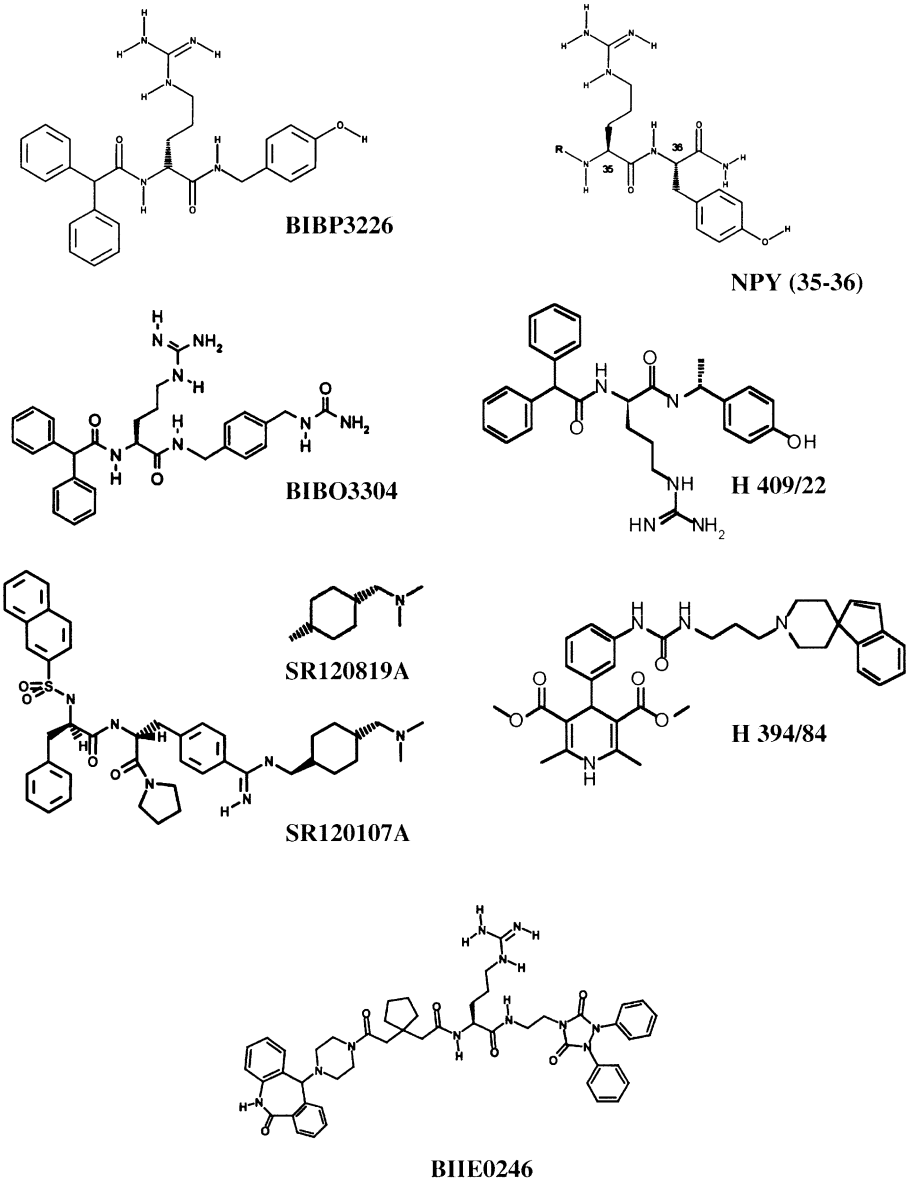

Fig. 1. Chemical structures of selective neuropeptide Y Y_1 and Y_2 receptor antagonists. The C-terminal part of neuropeptide Y, neuropeptide Y(35–36), is shown for comparison.

biphasic blood pressure response to high i.v. doses of neuropeptide Y in rats (Shen et al., 1991). This phenomenon was attributed to the basic structures within the peptide (Mousli et al., 1994), since there is a linear correlation between the number of positive charges within the neuropeptide Y fragment and the ability to cause mast cell degranulation (via a non-specific process leading to G protein activation). This is also well applicable for 1229U91 that possesses four basic arginine residues within its structure (Daniels et al., 1995). Apart from these non-specific effects, 1229U91 showed no neuropeptide Y receptor selectivity. Thus, in addition to neuropeptide Y Y_1 receptor antagonism (Daniels et al., 1995; Doods et al., 1995), 1229U91 possesses potent agonistic effects on neuropeptide Y Y_4 (Parker et al., 1998; Schober et al., 1998; Dumont and Quirion, 2000) and Y_6 (Parker et al., 1998) receptors, partial neuropeptide Y Y_1 receptor agonism (Doods et al., 1995), and weak agonistic properties on the neuropeptide Y Y_5 receptor (Parker et al., 1998; Dumont et al., 2000b). A template-assembled synthetic peptide based on four C-terminal neuropeptide Y fragments, T_4-[neuropeptide Y(33–36)]$_4$, was shown to competitively antagonize neuropeptide Y Y_2 receptor effects in vitro (Grouzmann et al., 1997). The affinity for the neuropeptide Y Y_2 receptor in vitro was moderate (IC_{50} 60–70 nM) and was approximately 100-fold less for the Y_1 receptor. Despite this, T_4-[neuropeptide Y(33–36)]$_4$ displaced neuropeptide Y binding from a neuropeptide Y Y_2 receptor cell line with less potency than did the neuropeptide Y Y_1 receptor agonist [Leu^{31},Pro^{34}]neuropeptide Y (Grouzmann et al., 1997), and the compound has not been substantially further evaluated either in vitro or in vivo.

Apart from peptide antagonists, several non-peptide compounds also reportedly antagonized peripheral effects of neuropeptide Y. However, the usefulness of, e.g., D-*myo*-inositol-1,2,6-trisphosphate (a.k.a. α-trinositol, pp56) (Edvinsson et al., 1990), an isomer of the second messenger substance, D-*myo*-inositol-1,4,5-trisphosphate, Benextramine (Doughty et al., 1990), an irreversible α-adrenoceptor antagonist, and HE90481 (Michel and Motulsky, 1990), a guanidine-based histamine receptor antagonist, was limited due to non-specific and non-competitive actions, low potency or other major drawbacks (see Balasubramaniam, 1997).

4.2. The selective non-peptide neuropeptide Y receptor antagonists

Several studies had shown that the C-terminal-located amino acids of neuropeptide Y, especially Arg^{35} and Tyr^{36}, are of major importance for the interaction with neuropeptide Y Y_1 and Y_2 receptors (Beck-Sickinger et al., 1994; Beck-Sickinger and Jung, 1995). Based on these observations, a number of low molecular weight analogues mimicking the C-terminal part of the neuropeptide Y molecule were synthesized (Fig. 1) and optimized with respect to neuropeptide Y Y_1 receptor affinity. This led to the discovery of the first selective non-peptide neuropeptide Y Y_1 receptor antagonists, BIBP3226 (Fig. 1) (Rudolf et al., 1994), SR120107A, 1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*trans*-cyclohexylmethyl]amidino]phenyl]propionyl]-pyrrolidine (*R*,*R*) stereoisomer (Fig. 1), and SR120819A, 1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*cis*-cyclohexylmethyl]amidino]phenyl]propionyl]-pyrrolidine (*R*,*R*) stereoisomer (Fig. 1) (Serradeil-Le Gal et al., 1994, 1995). Common requirements for binding to neuropeptide Y receptors include a pharmacophore involving a hydrophobic region together with a strong basic center (constituted by a guanidino group in BIBP3226 and a benzamidin group in the SR compounds; Fig. 1). These features are also quite applicable to the non-selective low-affinity compounds Benextramine and HE90481 (see above). The first selective non-peptide neuropeptide Y Y_1 receptor antagonists have been followed by others, among which some are structurally related to BIBP3226 (see below). Recently, the first non-peptide antagonist selective for the neuropeptide Y Y_2 receptor was described (Doods et al., 1999). Pharmacological characteristics of non-peptide subtype-selective neuropeptide Y receptor antagonists (see Table 1), the cardiovascular actions of which have been investigated, are discussed below.

Table 1
Pharmacological properties of non-peptide antagonists selective for either neuropeptide Y Y_1 or Y_2 receptors (see text for references)

Antagonists	Affinity, IC_{50} (nM)	In vitro pA_2	In vivo ID_{50}	Comment
Y_1-selective				
BIBP3226	3–7	7–8.5	190 nmol/kg or 7 nmol/kg/min	Short duration i. BIBP3435
H 409/22	2–16	8.2	5 nmol/kg/min	Tested in man i. H 510/45
BIBO3304	0.4–0.7	9	n.d.	i. BIBO3457
SR120107A	11–80	7	n.d.	Long-lasting + orally active
SR120819A	11–22	7.2	n.d.	Long-lasting + orally active
H 394/84	30	n.d.	12–41 nmol/kg/min	Long-lasting
Y_2-selective				
BIIE0246	3–15	8.1–8.6	2–3 nmol/kg	Short duration

n.d.: not determined; i.: inactive enantiomer.

4.3. BIBP3226 and related compounds

4.3.1. BIBP3226

The first selective non-peptide neuropeptide Y Y_1 receptor antagonist, BIBP3226, is also the most thoroughly studied. BIBP3226 possesses high affinity (IC_{50} values in between 3 and 7 nM) for neuropeptide Y Y_1 receptors in several species, including the human, pig, dog, and rat (Rudolf et al., 1994; Lundberg and Modin, 1995; Wieland et al., 1995). In contrast, BIBP3226 has virtually no affinity (IC_{50} values >10 μM) for neuropeptide Y Y_2 and Y_4 receptors, "putative Y_3 receptors", as well as for a variety of over 60 other receptors types (Rudolf et al., 1994, 1997; Lundberg and Modin, 1995; Wieland et al., 1995; Doods et al., 1996). A recent report indicates, however, that BIBP3226 possesses low affinity for, and may act as a weak antagonist on receptors for neuropeptide FF, an endogenous modulator of opioid functions (Mollereau et al., 2001). This property should be considered in future studies of this and related compounds. Competitive antagonism was shown by the effect of BIBP3226 on neuropeptide Y-evoked release of intracellular calcium in the human neuroblastoma SK-N-MC cell line, and the compound has no agonistic actions (Rudolf et al., 1994; Wieland et al., 1995). The *S*-enantiomer (to the *R*-configured BIBP3226), BIBP3435, is devoid of affinity for the neuropeptide Y Y_1 and other neuropeptide Y receptors (Lundberg and Modin, 1995; Wieland et al., 1995), indicating the stereospecificity expected for receptor-mediated actions. BIBP3435 is thus suitable for use as a control substance, to exclude non-specific actions of BIBP3226. Tritiated BIBP3226 can be used as a highly selective radioligand for the neuropeptide Y Y_1 receptor (Entzeroth et al., 1995). For example, [^{3}H]-BIBP3226 displays selective binding in canine splenic membranes (a neuropeptide Y Y_1 receptor preparation), as this was competitively inhibited by cold BIBP3226 and SR120107A (IC_{50} values 11 to 24 nM) much more potently than by BIBP3435 (IC_{50} value >1 μM) (Malmström et al., 1998).

The effect of BIBP3226 has been investigated in several functional bioassays in vitro. For neuropeptide Y Y_1 receptors, e.g., neuropeptide Y-evoked inhibition of twitch responses in rabbit vas deferens (Doods et al., 1995), contractions of guinea pig vena cava (Malmström and Lundberg, 1995b) and of human cerebral arteries (Abounader et al., 1995), and elevation of perfusion pressure in the isolated rat kidney (Doods et al., 1995) were used. In these preparations, BIBP3226 showed potent and competitive neuropeptide Y Y_1 receptor antagonistic effects (pA_2 values 7.0 to 8.5). Contractions caused by endogenous neuropeptide Y released upon electrical field stimulation of perivascular sympathetic nerves, acting on neuropeptide Y Y_1 receptors, were likewise readily antagonized by BIBP3226 in, e.g., guinea pig vena cava (Fig. 2) and in rat and human mesenteric vessels (Malmström and Lundberg, 1995b; Racchi et al., 1997). The inactive enantiomer, BIBP3435, was devoid of antagonistic effects in guinea pig vena cava (Malmström and Lundberg, 1995b). BIBP3226 did not affect neuropeptide Y Y_2 and Y_4 receptor-mediated effects in rat vas deferens (Doods et al., 1995, 1996) and colon (Jacques et al., 1995; Pheng et al., 1999), dog saphenous vein (Pheng et al., 1997a), and rabbit ileum (Pheng et al., 1997b; Félétou et al., 1999). In addition, BIBP3226 does not affect contractions evoked by noradrenaline in guinea pig vena cava (Malmström and Lundberg, 1995b) and rat mesenteric artery (Doods et al., 1995), showing selectivity and specificity for its antagonistic actions in vitro.

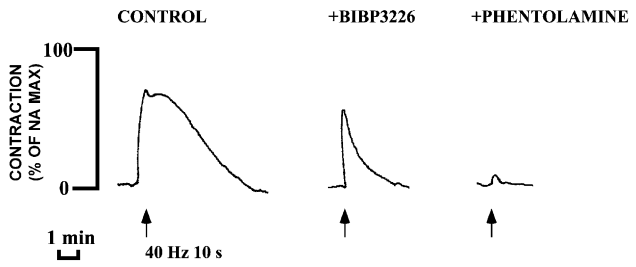

Fig. 2. Inhibitory effects of the neuropeptide Y Y_1 receptor antagonist, BIBP3226 (1 μM), and the α-adrenoceptor antagonist, phentolamine (1 μM), on the contraction evoked by electrical field stimulation (40 Hz, 10 s) in guinea pig vena cava in vitro. Note that BIBP3226 inhibits the long-lasting phase of contraction, whereas addition of phentolamine nearly abolished the initial rapid contraction. This supports co-transmission of noradrenaline and neuropeptide Y, acting as rapid and slow transmitters, respectively.

The pharmacological profile of BIBP3226 in vivo has been investigated in several vascular beds and species. For example, regarding neuropeptide Y Y_1 receptors, e.g., neuropeptide Y-evoked blood pressure elevation and renal vasoconstriction in pig and rat were used, whereas the neuropeptide Y Y_2 receptor was investigated in pig spleen (a vascular bed with a dual vascular neuropeptide Y receptor population, see below). BIBP3226, given as i.v. bolus doses, caused dose-dependent inhibition of the neuropeptide Y-induced pressor response in the pithed rat, the ID_{50} value for this inhibition being 0.1 mg/kg (Rudolf et al., 1994; Doods et al., 1995). This potency was identical to that observed in the anaesthetized pig (Malmström et al., 1997). Thus, BIBP3226, given as i.v. low-dose infusions, dose dependently inhibited the neuropeptide Y-evoked elevation of mean arterial pressure and vasoconstrictor responses evoked in hind limb and kidney, the ID_{50} value for this latter response being 7 nmol/kg/min equal to 3.7 μg/kg/min (since the infusion was given over 30 min, this corresponds to a total of 0.1 mg/kg). In the reserpine-treated pig in vivo, BIBP3226 dose dependently antagonized the non-adrenergic sympathetic vasoconstriction (mediated by endogenous neuropeptide Y acting on the neuropeptide Y Y_1 receptor, see below) evoked in kidney and hind limb (Fig. 3) (Malmström et al., 1997). Significant inhibitory effects of BIBP3226 were clear-cut even with a low dose (1 μg/kg/min) when plasma levels of the compound reached approximately 60 nM (Malmström et al., 1997). BIBP3226 antagonized vasoconstrictor responses to endogenous and

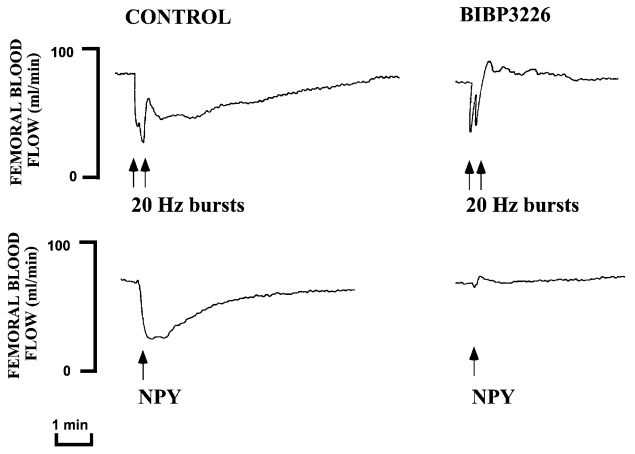

Fig. 3. Inhibitory effects of the neuropeptide Y Y_1 receptor antagonist, BIBP3226 (190 nmol/kg/min i.v.), on the vasoconstrictor responses in vivo to high-frequency sympathetic nerve stimulation (two 1-s bursts at 20 Hz at a 10-s interval) and exogenous neuropeptide Y (1.2 nmol, i.a.) in hind limb skeletal muscle of the reserpine-treated pig lacking noradrenaline. Note that BIBP3226 abolished the long-lasting phase of the non-adrenergic sympathetic vasoconstriction, which thus can be attributed to neuronally released neuropeptide Y acting on the neuropeptide Y Y_1 receptor.

exogenous neuropeptide Y with similar potency (Malmström et al., 1997). The inactive enantiomer, BIBP3435, did not affect vascular responses to either endogenous or exogenous neuropeptide Y in the pig (Lundberg and Modin, 1995).

BIBP3226 has a short half-life in plasma, its elimination fitting a two-compartment model with initial and terminal half-lives of 2 and 20 min, respectively, in the pig (Malmström et al., 1997). In parallel, the duration of action of BIBP3226 in vivo is rather short. Thus, in the anaesthetized pig and rat, some antagonistic effects persist 90 min after administration of BIBP3226 (Malmström et al., 1997; Modin et al., 1999), whereas a further 30 min later they do not (Lundberg and Modin, 1995). Selectivity (vs. the neuropeptide Y Y_2 receptor) in vivo was demonstrated as BIBP3226 antagonized the porcine renal and splenic vasoconstriction evoked by a neuropeptide Y Y_1 receptor agonist, whereas the splenic vascular response to a neuropeptide Y Y_2 receptor agonist was not affected (Lundberg and Modin, 1995). Furthermore, BIBP3226 only partially inhibits the vasoconstriction evoked in pig spleen by neuropeptide Y (Malmström et al., 1997), a vascular response mediated by both neuropeptide Y Y_1 and Y_2 receptors (see below). Specificity was demonstrated, as BIBP3226 did not affect vascular responses to, e.g., noradrenaline, phenylephrine, angiotensin II, α,β-methylene ATP, endothelin, and vasopressin in the rat (Doods et al., 1995; Modin et al., 1999) and pig (Lundberg and Modin, 1995; Malmström and Lundberg, 1997b). High bolus doses of BIBP3226 cause transient hypotensive effects in the pig, mainly due to splenic and mesenteric vasodilation (Lundberg and Modin, 1995). This is likely explained by a non-specific effect, as a similar phenomenon was observed with the inactive enantiomer, BIBP3435 (Lundberg and Modin, 1995). As discussed above, BIBP3226 structurally mimics the C-terminal part of neuropeptide Y (including a basic arginine-like moiety), high doses of which may cause mast cell degranulation (Grundemar and Håkanson, 1991; Mousli et al., 1994). Hence, these non-specific hypotensive effects of high bolus doses of BIBP3226 are likely due to histamine liberation from mast cells, but may be avoidable if the antagonist is given as a low-dose infusion (Malmström et al., 1997). This latter approach may also be more favorable considering the short duration of action of BIBP3226 in vivo.

Compounds structurally related to BIBP3226 include BIBO3304, (*R*)-*N*-[[4-(aminocarbonylaminomethyl)-phenyl]methyl]-N^2-(diphenylacetyl)-argininamide trifluoroacetate (Fig. 1) (Wieland et al., 1998), and H 409/22, (2*R*)-5-([amino(imino)methyl]amino)-2-[(2,2-diphenylacetyl)amino]-*N*-[(1*R*)-1-(4-hydroxyphenyl)ethyl]-pentanamide (Fig. 1) (Bergman et al., 1999).

4.3.2. H 409/22

H 409/22 differs from BIBP3226 by the presence of an additional (*R*)-methyl-group in the alpha position of the N-terminal benzamide moiety (Fig. 1). H 409/22 possesses high affinity (IC_{50} values 5–16 nM) for the neuropeptide Y Y_1 receptor, as shown in, e.g., rat brain cortex and human SK-N-MC cells (Bergman et al., 1999; Gedda et al., 1999). In SK-N-MC cells, H 409/22 displaced ^{125}I-peptide YY binding with an IC_{50} value (2.3 ± 0.4 nM) in a range similar to that for BIBP3226 (1.6 ± 0.3 nM). H 409/22 is devoid of affinity (IC_{50} values >10 μM) for neuropeptide Y Y_2, Y_4, and Y_5 receptors (Bergman et al., 1999; Gedda et al., 1999). Competitive antagonism (pA_2 value 8.2) was shown by the effect of H 409/22 on neuropeptide Y-evoked inhibition of forskolin-induced cAMP production in SK-N-MC cells. H 409/22 also competitively antagonized neuropeptide Y-induced metabolic stimulation (detected as extracellular acidification) of human neuropeptide Y Y_1 receptor-transfected CHO cells with similar potency (Gedda et al., 1999). Like the enantiomers, BIBP3226 and BIBP3435, the enantiomer to H 409/22, H 510/45, (2*S*)-5-([amino(imino)methyl]amino)-2-[(2,2-diphenylacetyl)amino]-*N*-[(1*S*)-1-(4-hydroxyphenyl)ethyl]-pentanamide, lacks affinity for neuropeptide Y Y_1 receptors (Gedda et al., 1999). Thus, H 510/45 showed no effect on the binding of either ^{125}I-peptide YY—or ^{3}H-H 409/22—to the neuropeptide Y Y_1 receptor in human SK-N-MC cells (IC_{50} values >10 μM, each). H 510/45 may therefore be suitable to serve as a control substance.

Although in vitro data from functional bioassays are lacking, the pharmacological profile of H 409/22 in vivo has been thoroughly investigated in several vascular beds and species, including the human. H 409/22 thus represents the first, and to date probably the only, neuropeptide Y Y_1 receptor antagonist tested in man. In the anaesthetized reserpine-treated pig in vivo, H 409/22 given as i.v. low-dose infusions dose dependently inhibited the neuropeptide Y-evoked elevation of mean arterial pressure and vasoconstrictor responses evoked in hind limb and kidney,

the ID_{50} value for this latter response being 5 nmol/kg/min (equal to 2.8 μg/kg/min) (Malmström et al., 2000). Hence, the potency is similar to, or slightly higher than that of BIBP3226. Furthermore, H 409/22 also exerted dose-dependent antagonism on non-adrenergic sympathetic vasoconstrictor responses (mediated by endogenous neuropeptide Y) evoked in kidney and hind limb of the reserpine-treated pig in vivo (Malmström et al., 2000). H 409/22 exerted equipotent antagonistic effects on responses to exogenous and endogenous neuropeptide Y, and significant inhibition was seen even at a low dose (1 μg/kg/min) when plasma levels of the compound were just below 80 nM (Malmström et al., 2000). In addition, H 409/22 dose dependently antagonized the neuropeptide Y-evoked elevation of blood pressure in anaesthetized guinea pigs, rats and dogs, renal vasoconstriction in rats and dogs, and coronary vasoconstriction in dogs (Nordlander et al., 1999; Abrahamsson, 2000). Finally, in healthy human subjects, H 409/22, at doses giving plasma levels between 2 and 4 μM, potently inhibited neuropeptide Y-evoked splanchnic and renal vasoconstriction (Ahlborg et al., 1999). The inactive enantiomer H 510/45 did not affect vascular responses to either endogenous or exogenous neuropeptide Y in the pig in vivo (Malmström et al., 2000).

The half-life of H 409/22 in plasma is short, its elimination fitting a two-compartment model with initial and terminal half-lives of 3 and 30 min, respectively, in the pig (Malmström et al., 2000). In parallel, the duration of action of H 409/22 in vivo is rather short. In the anaesthetized dog and rat, less than 50% of the antagonistic effects remain 50 min after administration of H 409/22 (Nordlander et al., 1999). In the anaesthetized pig in vivo, the remaining inhibition of neuropeptide Y-evoked renal vasoconstriction 90 min after administration of H 409/22 and BIBP3226 is 57% and 42%, respectively (Malmström et al., 1997, 2000). Thus, H 409/22 may have a slightly longer duration of action in vivo than BIBP3226 and this is in line with the marginal plasma half-life difference between the two compounds. Selectivity for the neuropeptide Y Y_1 receptor (vs. the neuropeptide Y Y_2 receptor) in vivo was demonstrated as H 409/22 antagonized the splenic vasoconstriction evoked by a neuropeptide Y Y_1 receptor agonist but not by a neuropeptide Y Y_2 receptor agonist, in the pig (Fig. 4) (Malmström, 2000; Malmström et al., 2000). Furthermore, H 409/22 only partially inhibits the neuropeptide Y Y_1 and Y_2 receptor-mediated vasoconstriction evoked in pig spleen by neuropeptide Y at a dose where there is complete inhibition of the neuropeptide Y Y_1 receptor-mediated renal vasoconstriction in response to neuropeptide Y (Malmström et al., 2000). In addition, neuropeptide Y Y_2 receptor-mediated inhibition of vagal function in anaesthetized guinea pigs and dogs is not affected by H 409/22 at doses that strongly inhibit neuropeptide Y Y_1 receptor-mediated vascular effects (Nordlander et al., 1999; Abrahamsson, 2000). Specificity was shown as H 409/22 did not affect vascular responses to, e.g., noradrenaline, α,β-methylene

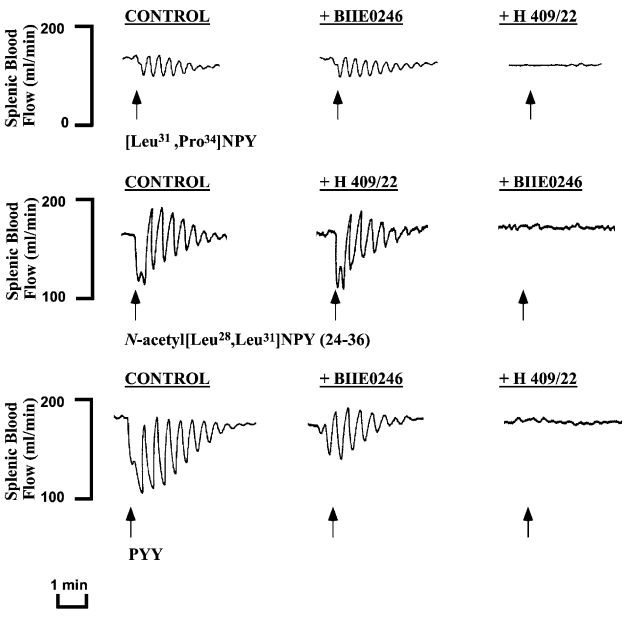

Fig. 4. Effects of H 409/22 (300 nmol/kg i.v.) and BIIE0246 (100 nmol/kg i.v.), antagonists selective for neuropeptide Y Y_1 and Y_2 receptors, respectively, on vasoconstrictor responses evoked in pig spleen by the neuropeptide Y Y_1 receptor agonist, [Leu^{31},Pro^{34}]neuropeptide Y, the neuropeptide Y Y_2 receptor agonist, *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y(24–36), and peptide YY (each 230 pmol/kg i.v.) in vivo. Note that the splenic vasoconstriction elicited by the neuropeptide Y Y_1 receptor agonist was unaffected by BIIE0246 but abolished by H 409/22, whereas the situation was the opposite for the response to the neuropeptide Y Y_2 receptor agonist. The splenic vasoconstrictor response to peptide YY was partially inhibited by BIIE0246 and then abolished after addition of H 409/22. Hence, evidence for the involvement of both neuropeptide Y Y_1 and Y_2 receptors in splenic vasoconstriction was presented.

ATP, angiotensin II, and phenylephrine in the anaesthetized pig (Malmström, 2000; Malmström et al., 2000), rat, and dog (Nordlander, personal communication). Similar to BIBP3226 (Malmström et al., 1997), the highest dose of H 409/22 (giving plasma levels over 7 μM) caused transient splenic vasodilation, albeit without any effect on blood pressure in the pig (Malmström et al., 2000). This is probably explainable by a non-specific effect as with BIBP3226 (see above). However, no such non-specific effects were observed with moderate doses of H 409/22 giving plasma levels of 0.9, 1.3, and 5.1 μM, respectively, in pig, rats, and dog (Nordlander et al., 1999; Malmström et al., 2000).

4.3.3. BIBO3304

The argininamide derivative, BIBO3304, is another antagonist from the same neuropeptide Y Y_1 receptor chemistry program as BIBP3226. BIBO3304 possesses very high affinity for the neuropeptide Y Y_1 receptor, as binding studies revealed affinity in the subnanomolar range (IC_{50} values between 0.4 and 0.7 nM) for human and rat neuropeptide Y Y_1 receptors (Wieland et al., 1998). The *S*-enantiomer (to the *R*-configured BIBO3304) BIBO3457 has in contrast low affinity (IC_{50} values >1 μM) for neuro-

peptide Y Y_1 and other neuropeptide Y receptors (Wieland et al., 1998). BIBO3304 lacks affinity (IC_{50} values >1 μM) for human and rat neuropeptide Y Y_2, Y_4, and Y_5 receptors, as well as for a variety of over 75 other receptor subtypes and enzyme systems (Wieland et al., 1998). Potent antagonism was shown by the effect of BIBO3304 on neuropeptide Y Y_1 receptor-mediated signal transduction in SK-N-MC cells where the neuropeptide Y-induced inhibition of cAMP synthesis was antagonized with a pKb of 9, and the compound was devoid of any agonistic action (Wieland et al., 1998).

BIBO3304 exerts potent antagonistic effects in the neuropeptide Y Y_1 receptor bioassay of rabbit saphenous vein (pA_2 value of 9), while it was inactive at the neuropeptide Y Y_2 receptor in rat vas deferens and the neuropeptide Y Y_4 receptor in rat colon and rabbit ileum (Félétou et al., 1999; Dumont et al., 2000a). In vivo, BIBO3304 antagonized the neuropeptide Y-evoked blood pressure elevation and renal vasoconstriction in the anaesthetized rat (Shin et al., 2000). Moreover, BIBO3304 seems equipotent to BIBP3226 for reducing the blood pressure elevation in response to neuropeptide Y in the anaesthetized cat (Capurro and Huidobro-Toro, 1999). In both anaesthetized rats and cats, administration of BIBO3304 caused transient hypotensive effects, even at the relatively low doses that were given to each species, and furthermore, in the cat, this effect was similar to that of BIBP3226 (Capurro and Huidobro-Toro, 1999; Shin et al., 2000). These hypotensive effects of BIBO3304 thus probably reflect the same non-specific actions (due to a basic arginine-like structure within the molecule) as discussed for C-terminal fragments of neuropeptide Y and BIBP3226 above.

4.4. SR120107A and SR120819A

SR120107A and SR120819A were the first orally active selective neuropeptide Y Y_1 receptor antagonists described (Serradeil-Le Gal et al., 1994). The (*R*,*R*) *cis* molecule, SR120819A, is presumably the more active enantiomer, whereas SR120107A is the *trans* isomer of SR120819A (Fig. 1). SR120819A possesses a somewhat higher affinity (IC_{50} values between 11 and 22 nM) for the neuropeptide Y Y_1 receptors in rat and guinea pig cortex and human SK-N-MC cells than does SR120107A (IC_{50} values around 80 nM) (Serradeil-Le Gal et al., 1994, 1995). SR120107A, however, exhibits high affinity (IC_{50} value 11 nM) for the neuropeptide Y Y_1 receptor in dog spleen, and is devoid of affinity (IC_{50} value >10 μM) for the neuropeptide Y Y_2 receptor in pig spleen (Malmström et al., 1998). Neither of the compounds binds to human cortex neuropeptide Y Y_2 receptors at concentrations up to 10 μM, and SR120819A is inactive in 30 other typical binding assays (Serradeil-Le Gal et al., 1994, 1995). The two compounds exhibit similar, or marginally lower, in vitro affinity for the neuropeptide Y Y_1 receptor than does BIBP3226. Thus, BIBP3226 was slightly more effective than SR120107A (IC_{50} values 11 and 24 nM, respectively) to displace [^{3}H]-BIBP3226 binding in dog spleen (Malmström et al., 1998), and than SR120819A (IC_{50} values 10 and 27 nM, respectively) to displace ^{125}I-peptide YY binding in human adipocytes (Serradeil-Le Gal et al., 2000). SR120819A and BIBP3226, however, showed equal affinity for the neuropeptide Y Y_1 receptor in SK-N-MC cells (Serradeil-Le Gal, 1997). Potent antagonism (IC_{50} value 92 nM) was shown by SR120819A on the neuropeptide Y-induced inhibition of cAMP accumulation in SK-N-MC cells, in which preparation the compound was devoid of any agonistic action (Serradeil-Le Gal et al., 1995).

Functional in vitro studies have been performed, using the neuropeptide Y Y_1 receptor bioassays of rabbit vas deferens and guinea pig vena cava. In the rabbit vas deferens, SR120819A and SR120107A competitively antagonized neuropeptide Y-evoked inhibition of twitch responses with pA_2 values of 7.2 and 7, respectively (Serradeil-Le Gal et al., 1994, 1995). In the guinea pig vena cava, SR120107A seemed roughly equally potent to BIBP3226 for inhibition of contractions evoked by exogenous and endogenous (released by electrical field stimulation of perivascular sympathetic nerves) neuropeptide Y (Malmström and Lundberg, 1995a). Specificity was shown in the latter bioassay as contractions evoked by noradrenaline were not affected by SR120107A (Malmström and Lundberg, 1995a).

The pharmacological profiles in vivo of SR120107A and SR120819A have been investigated in pithed rats, anaesthetized guinea pigs, dogs, and pigs. In the pithed rat, SR120819A shifted to the right the dose-response curve for neuropeptide Y-evoked blood pressure elevation (estimated pA_2 value of 7.1) without affecting the maximal hypertensive effect (Serradeil-Le Gal, 1997). In anaesthetized guinea pigs, both SR120819A and SR120107A dose dependently inhibited the hypertensive effect of a neuropeptide Y Y_1 receptor agonist (Serradeil-Le Gal et al., 1994, 1995). In the anaesthetized pig, SR120107A exerted dose-dependent inhibition of peptide YY-evoked renal vasoconstriction (Malmström and Lundberg, 1996). In addition, SR120107A effectively antagonized vascular responses to endogenous neuropeptide Y, as in the reserpine-treated pig in vivo the compound inhibited non-adrenergic sympathetic vasoconstriction (mediated by endogenous neuropeptide Y acting on the neuropeptide Y Y_1 receptor, see below) evoked in kidney, spleen, hind limb, and nasal mucosa (Malmström et al., 1996). SR120107A and SR120819A both possess long duration of action in vivo. In the anaesthetized pig, up to 60% of the antagonistic effects of SR120107A remained 3 h after i.v. administration of the drug (Malmström et al., 1996). In the anaesthetized guinea pig, the antagonistic effects of SR120819A lasted for more than 3 h after i.v. administration (Serradeil-Le Gal et al., 1995). Both compounds are also effective after oral administration, and the antagonistic effects of SR120107A and SR120819A lasted for more than 3 and 4 h, respectively, in the anaesthetized guinea pig when administered per os

(Serradeil-Le Gal et al., 1994, 1995). Selectivity (vs. the neuropeptide Y Y_2 receptor) in vivo was demonstrated as SR120107A antagonized renal and splenic vasoconstriction evoked by a neuropeptide Y Y_1 receptor agonist, whereas splenic vasoconstriction elicited by a neuropeptide Y Y_2 receptor agonist was not affected in the pig (Malmström et al., 1998). Furthermore, while completely antagonizing the neuropeptide Y Y_1 receptor-mediated renal vasoconstriction, SR120107A only partially inhibited the neuropeptide Y Y_1 and Y_2 receptor-mediated splenic vasoconstriction elicited by neuropeptide Y (Malmström and Lundberg, 1996). Specificity was demonstrated as SR120107A did not affect vascular responses to phenylephrine, angiotensin II, or α,β-methylene ATP in the pig (Malmström and Lundberg, 1996; Malmström et al., 1996). As with BIBP3226 (Lundberg and Modin, 1995), high i.v. bolus doses of SR120107A caused transient splenic vasodilation accompanied by a slight fall in blood pressure in the pig (Malmström and Lundberg, 1996; Malmström et al., 1996). Since BIBP3226 and SR120107A share some of their structural characteristics, these hypotensive effects are likely explained by a non-specific effect as for BIBP3226 (see above).

4.5. H 394/84

A series of dihydropyridines that possess high affinity for neuropeptide Y Y_1 receptors was developed (Poindexter et al., 1996), among which one compound, H 394/84 (1,4-Dihydro-4-[3-[[[[3-[spiro(indene-4,1′-piperidin-1-yl)]propyl]amino]carbonyl] amino]phenyl]-2,6-dimethyl-3,5-pyridine dicarboxylic acid, dimethylester) (Fig. 1), has been thoroughly studied in vivo. Although as a class these types of dihydropyridines have significant affinity for α_1-adrenoceptors and Ca^{2+} channels, the compounds of this series possess much weaker affinities for these (Poindexter et al., 1996) and instead have high affinity (IC_{50} values below 10 nM) for the neuropeptide Y Y_1 receptor expressed in SK-N-MC cells (Poindexter et al., 1996), and have thus been considered selective neuropeptide Y Y_1 receptor antagonists. The binding profile of H 394/84 was studied further on neuropeptide Y receptor subtypes. High affinity was demonstrated for H 394/84 at the neuropeptide Y Y_1 receptor in rat cortex (IC_{50} value 30 nM). In contrast, H 394/84 is devoid of affinity (IC_{50} values >5–10 μM) for neuropeptide Y Y_2, Y_4, and Y_5 receptors as demonstrated in pig splenic membranes (Y_2) and human recombinant neuropeptide Y Y_4 and Y_5 receptor binding assays (K. Gedda, personal communication).

The pharmacological profile of H 394/84 in vivo has been investigated in the anaesthetized rat and pig. In the anaesthetized rat, H 394/84 exerted dose-dependent inhibition of neuropeptide Y-evoked renal vasoconstriction with an ID_{50} value of 41 nmol/kg/min (equal to 25 μg/kg/min). Significant inhibitory effects were already seen at a dose of 5 nmol/kg/min (Malmström et al., 2001a). In the anaesthetized reserpine-treated pig, H 394/84, given as i.v. low-dose infusions, dose dependently inhibited the neuropeptide Y-evoked elevation of mean arterial pressure and vasoconstrictor responses evoked in hind limb and kidney (Fig. 5), the ID_{50} value for this latter response being 12 nmol/kg/min (Malmström et al., 2001a). The potency was thus similar to or slightly lower than that of BIBP3226 and H 409/22. Furthermore, H 394/84 also exerted dose-dependent antagonism on non-adrenergic sympathetic vasoconstrictor responses (mediated by endogenous neuropeptide Y) evoked in kidney (Fig. 5) and hind limb of the reserpine-treated pig in vivo (Malmström et al., 2001a). H 394/84 exerted equipotent antagonistic effects on responses to exogenous and endogenous neuropeptide Y in the same vascular bed, but had over 5-fold more potent antagonistic effects in the kidney than in the hind limb. The ID_{50} value of 19 nmol/kg/min for inhibition of renal vasoconstriction evoked by endogenous neuropeptide Y thus correlates well with that calculated for the response to exogenous neuropeptide Y above. In the hind limb, the ID_{50} values for inhibition of vasoconstriction evoked by exogenous and endogenous neuropeptide Y were 67 and 65 nmol/kg/min, respectively. This phenomenon has not been reported for other neuropeptide Y Y_1 receptor antagonists, e.g., BIBP3226 and H 409/22, being equally potent in these two vascular beds (Malmström et al., 1997, 2000). There is a difference between these two vascular beds concerning endothelial permeability. Hence, the vessels of skeletal muscle do not, in contrast to those in the kidney, possess a fenestrated endothelium (Bennett et al., 1959) and, because of this, could be less permeable to larger molecules.

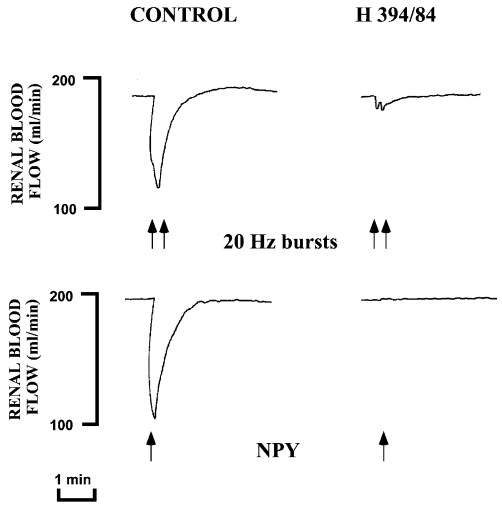

Fig. 5. Inhibitory effects of the neuropeptide Y Y_1 receptor antagonist, H 394/84 (100 nmol/kg/min i.v.), on the renal vasoconstrictor responses in vivo to high-frequency sympathetic nerve stimulation (two 1-s bursts at 20 Hz at a 10-s interval) and exogenous neuropeptide Y (230 pmol/kg i.v.) in the reserpine-treated pig lacking noradrenaline. Note that H 394/84 almost abolished the non-adrenergic sympathetic vasoconstriction evoked in kidney, which thus can be attributed neuronally released neuropeptide Y acting on the neuropeptide Y Y_1 receptor.

In line with this, the molecular weight of H 394/84 is higher than that of BIBP3226 and of H 409/22, and since H 394/84 is not structurally related to these compounds, there obviously may be other reasons for the difference in ability to reach the target receptor within the tissue.

The plasma half-life of H 394/84, as well as the duration of action, is decidedly longer than that of either H 409/22 or BIBP3226 (Malmström et al., 1997, 2000). The elimination of H 394/84 fits a two-compartment model with initial and terminal half-lives of approximately 3 and 50 min, respectively, and 70% of the antagonistic effect exerted on neuropeptide Y-evoked renal vasoconstriction persisted 90 min after administration of H 394/84 in the pig (Malmström et al., 2001a). Selectivity in vivo was demonstrated as H 394/84 did not affect the splenic vasoconstriction evoked by a neuropeptide Y Y_2 receptor agonist in the pig (Malmström et al., 2001a), and specificity was also shown, as H 394/84 did not affect vascular responses to α,β-methylene ATP and phenylephrine. In the anaesthetized rat, at moderate doses, H 394/84 did not affect the renal vasoconstrictor responses to angiotensin II and noradrenaline. However, at a dose 100-fold higher than that needed for significant neuropeptide Y Y_1 receptor antagonism, slight inhibitory effects were seen on the responses to angiotensin II and noradrenaline. Thus, whereas significant antagonism on neuropeptide Y Y_1 receptor-mediated responses were observed at doses between 1 nmol/kg/min (pig) and 5 nmol/kg/min (rat), significant inhibition of non-neuropeptide Y receptor-mediated responses was seen at 500 nmol/kg/min (rat). Hence, it seems that the specificity in vivo of H 394/84 for the neuropeptide Y Y_1 receptor is between 100- and 500-fold vs. other vascular receptors (Malmström et al., 2001a). H 394/84 belongs to a class of dihydropyridines known to have significant affinity for Ca^{2+} channels. Although reported to possess much weaker affinity for the Ca^{2+} channel (Poindexter et al., 1996), it cannot be excluded that, at a dose as high as that inhibiting non-neuropeptide Y receptor-mediated events in the rat, H 394/84 may exhibit some Ca^{2+} channel antagonistic properties. It was not possible to demonstrate any hypotensive effects in vivo that might have been anticipated had H 394/84 possessed any significant Ca^{2+} channel antagonistic properties, because of vehicle-related side-effects. Thus, the vehicles used for the highest dose of H 394/84 in the pig (10% ethanol) and rat (polyethylene glycol and dimethylacetamide) caused slight hyper- and hypotensive effects, respectively. Since these effects were similar in the presence or absence of H 394/84, it seems unlikely that the antagonist would exert substantial Ca^{2+} antagonist-related hypotensive effects per se (Malmström et al., 2001a).

4.6. BIIE0246

Recently, the first non-peptide antagonist selective for the neuropeptide Y Y_2 receptor subtype, BIIE0246, ((*S*)-N^2-[[1-[2-[4-[(*R*,*S*)-5,11-dihydro-6(6*H*)-oxodibenz[*b*,*e*]azepin-11-yl]-1-piperazinyl]-2-oxoethyl]cyclopentyl]acetyl]-*N*-[2-[1,2-dihydro-3,5(4*H*)-dioxo-1,2-diphenyl-3*H*-1,2,4-triazol-4-yl]ethyl]-argininamide) (Fig. 1) was presented (Doods et al., 1999). BIIE0246 possesses high affinity for the neuropeptide Y Y_2 receptor of several species. Affinity in the low nanomolar range (IC_{50} values in between 3 and 15 nM) was demonstrated for BIIE0246 at the neuropeptide Y Y_2 receptor expressed in the SMS-KAN neuroblastoma cell line, cloned rat and guinea pig neuropeptide Y Y_2 receptors, and at neuropeptide Y Y_2 receptor binding sites in rat brain and human frontal cortex (Doods et al., 1999; Berglund et al., 2000; Dumont et al., 2000b). Selectivity was shown, as no binding for BIIE0246 was detected at human, rat, and guinea pig neuropeptide Y Y_1, Y_4 and Y_5 receptors, or at a variety of 60 other receptor types and enzyme systems (Doods et al., 1999; Berglund et al., 2000; Dumont et al., 2000b). The in vitro antagonistic properties of BIIE0246 have been investigated in various bioassays. BIIE0246 dose dependently antagonized neuropeptide Y Y_2 receptor-mediated responses in rat vas deferens (inhibition of twitch responses) and in dog saphenous vein (contraction) with pA_2 values of 8.1 and 8.6, respectively (Dumont et al., 2000b). In contrast, BIIE0246 did not affect neuropeptide Y responses at pre- and postjunctional neuropeptide Y Y_1 receptors in rabbit vas deferens and human cerebral arteries, or neuropeptide Y Y_4 receptor-mediated contraction of rat colon (Dumont et al., 2000b).

The pharmacological profile of BIIE0246 in vivo was investigated in the anaesthetized pig, where the antagonistic effects on neuropeptide Y Y_2 receptor-mediated splenic vasoconstriction were studied in detail. The neuropeptide Y Y_2 receptor agonist, *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–36), evoked a dose-dependent vasoconstriction in pig spleen that was potently and dose dependently antagonized by BIIE0246 (Fig. 4). Significant inhibition was already seen with 1 nmol/kg (equal to 1 μg/kg) of BIIE0246 and the ID_{50} value was 2.1 nmol/kg (Malmström, 2001b). It was consistent with this that BIIE0246 antagonized the neuropeptide Y Y_2 receptor-mediated part of the splenic vasoconstrictor response to peptide YY with similar potency (ID_{50} value 3.4 nmol/kg). After maximum inhibition by BIIE0246, there remains a part (about 50%) of the peptide YY-evoked splenic vasoconstriction that is neuropeptide Y Y_1 receptor-mediated (Fig. 4). The evidence for this was that addition (after BIIE0246 was given) of the neuropeptide Y Y_1 receptor antagonist, H 409/22, abolished this remaining part of the response (Fig. 4) (Malmström, 2001a). The ability of BIIE0246 to antagonize neuropeptide Y Y_2 receptor-mediated responses to endogenous neuropeptide Y has also been demonstrated in vivo. Thus, in the reserpine-treated pig in vivo, BIIE0246 antagonized the activation of prejunctional autoinhibitory neuropeptide Y Y_2 receptors by endogenous neuropeptide Y. This antagonistic effect markedly increased the sympathetic nerve-evoked release of neuropeptide Y from spleen and kidney (Malmström et al., 2002a).

The duration of action of BIIE0246 in vivo is rather short, approximately 60 min, based on data obtained with the anaesthetized pig and rat (Malmström, 2001b; Smith-White et al., 2001). Selectivity for the neuropeptide Y Y_2 receptor in vivo was demonstrated as BIIE0246 did not affect the splenic vasoconstriction evoked by the neuropeptide Y Y_1 receptor agonist, [Leu^{31},Pro^{34}]neuropeptide Y, in the pig (Fig. 4) (Malmström, 2001a,b). Moreover, BIIE0246 does not affect neuropeptide Y Y_1 receptor-mediated renal vasoconstrictor responses to peptide YY and [Leu^{31},Pro^{34}]neuropeptide Y in the pig (Malmström, 2001b). Specificity was shown, as BIIE0246 does not affect vascular responses to phenylephrine, α,β-methylene ATP and angiotensin II in the pig in vivo (Malmström, 2001b). BIIE0246, unlike many of the neuropeptide Y Y_1 receptor antagonists described above, does not seem to elicit non-specific hypotensive effects at high doses in vivo. Thus, BIIE0246 did not cause, e.g., splenic vasodilatation in pig under control conditions (when plasma levels of neuropeptide Y are in the low 20 pM range) (Malmström, 2001b; Malmström et al., 2002b). Interestingly, when plasma levels of neuropeptide Y rise to around 40 pM after treatment with reserpine or the α_2-adrenoceptor antagonist, yohimbine, BIIE0246 did evoke vasodilatation in pig spleen (Malmström et al., 2002a,b). From these results, it can be inferred that: (1) These enhanced plasma levels of neuropeptide Y were in a range where activation of neuropeptide Y Y_2 receptors significantly participates in the regulation of basal splenic vascular tone, and (2) The direct dilating effect of BIIE0246 seen under some conditions is probably explainable by the inhibition of splenic vasoconstriction exerted by circulating neuropeptide Y acting on the neuropeptide Y Y_2 receptor, rather than by a non-specific effect.

5. Pinpointing peripheral neuropeptide Y receptors with selective antagonists

5.1. Vascular neuropeptide Y receptors

Neuropeptide Y acts as a vasoconstrictor (Lundberg and Tatemoto, 1982) in a variety of vascular beds in vitro and in vivo (for review, see Malmström, 1997). In vessels not responsive to direct effects, neuropeptide Y may serve as a regulating factor enhancing the effects of other vasoactive substances, e.g., noradrenaline (Ekblad et al., 1984). Based on experiments with neuropeptide Y receptor agonists, it seemed that vascular responses were preferentially mediated via the neuropeptide Y Y_1 receptor subtype (Wahlestedt et al., 1986). However, in a few vascular beds, neuropeptide Y Y_2 receptors seemed to be involved (Modin et al., 1991; Pheng et al., 1997a).

The receptor subtypes involved in vascular effects of neuropeptide Y have been further characterized using selective antagonists. Evidence for the involvement of neuropeptide Y Y_1 receptors in (a) direct vasoconstrictor effects evoked by exogenous peptide, (b) potentiation of vascular responses to other vasoactive substances, and (c) vasoconstriction evoked by sympathetic nerve activation has been provided for several vascular beds and species both in vitro and in vivo. In addition, there is now also evidence for neuropeptide Y Y_2 receptor-mediated vasoconstriction. The most recent data, obtained using selective antagonists, in different species are summarized below.

The neuropeptide Y Y_1 receptor is the most abundant, and in some species perhaps the only, receptor subtype mediating vasoconstrictor responses to neuropeptide Y. This may be concluded based on the effects of selective neuropeptide Y Y_1 receptor antagonists on the elevation of mean arterial pressure caused by neuropeptide Y, or peptide YY (given i.v. to anaesthetized animals), roughly representing the sum of the vasoconstrictor responses evoked. Thus, the elevation of mean arterial pressure induced with neuropeptide Y/peptide YY in the rat is antagonized by BIBP-3226 (Doods et al., 1995; Modin et al., 1999), SR 120819A (Serradeil-Le Gal, 1997), and H 394/84 (Malmström et al., 2001a), in the guinea pig by SR120819A (Serradeil-Le Gal, 1997) and H 409/22 (Abrahamsson, 2000), in the cat by BIBP3226 and BIBO3304 (Capurro and Huidobro-Toro, 1999), in the dog by BIBP3226 and SR120107A (Malmström et al., 1998), and in the pig by BIBP3226 (Lundberg and Modin, 1995; Malmström et al., 1997), SR120107A (Malmström and Lundberg, 1996), H 409/22 (Malmström et al., 2000) and H 394/84 (Malmström et al., 2001a), but not by the neuropeptide Y Y_2 receptor antagonist BIIE0246 (Malmström, 2001b). The most abundant vascular neuropeptide Y receptor is thus of the Y_1 subtype. Separate species and vascular beds of interest are discussed further below.

In the rat, renal neuropeptide Y Y_1 receptor-mediated vasoconstriction was demonstrated in vivo (Bischoff et al., 1997a; Modin et al., 1999; Malmström et al., 2001a) and in isolated perfused kidney (Doods et al., 1995). Further, neuropeptide Y Y_1 receptor-mediated contractions were shown in rat coronary and cerebral arteries in vitro (Prieto et al., 1998; You et al., 2001). In addition, neuropeptide Y Y_1 receptor-mediated potentiation of adrenergic elevation of mean arterial pressure, renal and mesenteric vasoconstriction was demonstrated in rat in vivo (Bischoff et al., 1997b). This is also applicable for potentiation of adrenergic and sympathetic nerve-evoked vasoconstriction in the isolated perfused mesenteric artery (Doods et al., 1995; Han et al., 1998b), and in rat tail and caudal artery in vitro (Gicquiaux et al., 1996; Barrios et al., 1998). Finally, BIBP3226 inhibited part of the elevation of mean arterial pressure evoked by stimulation of the sympathetic trunk in the pithed rat, supporting the involvement of neuropeptide Y Y_1 receptors (Kennedy et al., 1997). This was supported by studies with BIBP3226 in isolated perfused mesenteric artery (Han et al., 1998b) and in mesenteric vessel preparations in vitro (Racchi et al., 1997).

In the guinea pig, neuropeptide Y Y_1 receptor-mediated contractions have been demonstrated in, e.g., vena cava, using BIBP3226 and SR120107A (Malmström and Lundberg, 1995a,b), and in basilar artery (Nilsson et al., 1996), using BIBP3226. Neuropeptide Y Y_1 receptor-mediated potentiation of contractions evoked by noradrenaline was shown in mesenteric artery (Nilsson et al., 1996). Activation of neuropeptide Y Y_1 receptors by endogenous neuropeptide Y accounts for part of the vasoconstrictor responses to sympathetic nerve stimulation in guinea pig vena cava in vitro, as demonstrated using BIBP3226 (Fig. 2) and SR120107A (Malmström and Lundberg, 1995a,b). In the rabbit, neuropeptide Y Y_1 receptor-mediated contractions were demonstrated in saphenous vein (Gicquiaux et al., 1996) and isolated perfused ear (Doods et al., 1995). In addition, neuropeptide Y potentiates sympathetic contractions of rabbit ear artery via the neuropeptide Y Y_1 receptor (Garcia-Villalon et al., 2000).

In the cat, there is evidence for neuropeptide Y Y_1 receptor-mediated vasoconstriction in skeletal muscle arteries in vivo (Ekelund and Erlinge, 1997). In the anaesthetized dog, neuropeptide Y Y_1 receptor-mediated vasoconstriction has been demonstrated in kidney and spleen (Malmström et al., 1998). This was supported by studies on canine splenic arteries in vitro, in which activation of neuropeptide Y Y_1 receptors accounts for part of the sympathetic nerve-evoked responses as well (Yang and Chiba, 2000). Moreover, neuropeptide Y Y_1 receptors seem to be involved in the canine coronary vasoconstriction elicited by sympathetic nerve activation in vivo (Tanaka et al., 1997). Finally, there is also evidence for neuropeptide Y Y_2 receptor-mediated vascular effects in the dog. Thus, BIIE0246 antagonized neuropeptide Y-evoked contractions of canine saphenous vein in vitro (Dumont et al., 2000b).

In the pig, the neuropeptide Y Y_1 receptor seems to be the sole receptor subtype mediating vasoconstrictor responses to neuropeptide Y in kidney (Fig. 5). Thus, renal vasoconstriction evoked by exogenous neuropeptide Y, as well as by endogenous neuropeptide Y released upon sympathetic nerve activation, is antagonized by BIBP3226 (Lundberg and Modin, 1995; Malmström et al., 1997), SR120107A (Malmström and Lundberg, 1996; Malmström et al., 1996), H 409/22 (Malmström et al., 2000), and H 394/84 (Fig. 5) (Malmström et al., 2001a). Similar results were seen with skeletal muscle of the hind limb, which thus represents a neuropeptide Y Y_1 receptor vascular bed (Fig. 3). In addition, mesenteric vasoconstriction is neuropeptide Y Y_1 receptor-mediated as demonstrated by the effects of H 409/22 (Malmström, 2000). In nasal mucosa, the neuropeptide Y Y_1 receptor mediates non-adrenergic sympathetic vasoconstriction as shown with BIBP3226 and SR120107A (Lundberg and Modin, 1995; Malmström et al., 1996).

The pig spleen, however, represents a vascular bed with a dual neuropeptide Y receptor population; neuropeptide Y Y_1 and Y_2 receptors are both involved in vasoconstrictor responses (Fig. 4). Splenic vasoconstriction elicited by endogenous neuropeptide Y (evoked by sympathetic nerve activation) seems predominantly mediated by the neuropeptide Y Y_1 receptor. Thus, BIBP3226 and SR120107A antagonized the splenic sympathetic vasoconstriction evoked in the reserpine-treated pig (Lundberg and Modin, 1995; Malmström et al., 1996). The involvement of the neuropeptide Y Y_2 receptor in this nerve-evoked vasoconstriction is difficult to establish, considering that this receptor subtype modulates the splenic sympathetic release of neuropeptide Y. Thus, BIIE0246 enhanced the splenic release of neuropeptide Y upon sympathetic nerve stimulation in the reserpine-treated pig, but did not inhibit the vascular response evoked (Malmström et al., 2002a). Hence, inhibition of any possible involvement of vascular neuropeptide Y Y_2 receptors in the nerve-evoked splenic response may have been counteracted by the increased release of neuropeptide Y. In contrast, both neuropeptide Y Y_1 and Y_2 receptors may clearly be involved in the splenic vasoconstriction evoked by exogenous/circulating neuropeptide Y. The vasoconstriction evoked in pig spleen by agonists to the neuropeptide Y Y_1, but not the Y_2, receptor is antagonized by BIBP3226 (Lundberg and Modin, 1995), SR120107A (Malmström et al., 1998), and H 409/22 (Fig. 4) (Malmström et al., 2000). In contrast, BIIE0246 antagonized the splenic vasoconstriction evoked by a neuropeptide Y Y_2, but not by a Y_1, receptor agonist (Fig. 4) (Malmström, 2001b). The splenic vasoconstriction evoked by neuropeptide Y and peptide YY is only partially (to about 50%) inhibited on blockade of only one of the two receptors at a time (Fig. 4) (Malmström and Lundberg, 1996; Malmström et al., 1997, 2000; Malmström, 2001b). Combined neuropeptide Y Y_1 and Y_2 receptor blockade (H 409/22 and BIIE0246) completely abolished the peptide YY-evoked vasoconstriction in pig spleen (Fig. 4) (Malmström, 2001a). Thus, evidence was presented for the existence of both vascular neuropeptide Y Y_1 and Y_2 receptors in pig spleen.

In man, splanchnic and renal neuropeptide Y Y_1 receptor-mediated vasoconstriction was demonstrated by the antagonism exerted by H 409/22 on responses to exogenous neuropeptide Y in vivo (Ahlborg et al., 1999). In vitro, neuropeptide Y Y_1 receptor-mediated contractions of human cerebral (Abounader et al., 1995) and subcutaneous (Nilsson et al., 1996) arteries and saphenous vein (Racchi et al., 1999) were shown, using BIBP3226. Further, the involvement of neuropeptide Y Y_1 receptors in both potentiation of contractions to noradrenaline in omental arteries (Bergdahl et al., 1996), and nerve-evoked contractions of saphenous vein (Racchi et al., 1999) and mesenteric vessels (Racchi et al., 1997), has been shown in vitro.

5.2. Prejunctional neuropeptide Y receptors

At the prejunctional level, neuropeptide Y may inhibit transmitter release, e.g., from sympathetic (Lundberg et al.,

1982b; Lundberg and Stjärne, 1984; Stjärne et al., 1986; Pernow and Lundberg, 1989a) and parasympathetic (Potter, 1985) nerves. The modulation of transmitter release was attributed to neuropeptide Y Y_2 receptor activation (Wahlestedt et al., 1986). However, the first prejunctional neuropeptide Y receptor to be pharmacologically classified using selective antagonists was of the Y_1 subtype. Thus, the inhibition of twitch responses evoked in rabbit vas deferens by neuropeptide Y was antagonized by BIBP3226 (Doods et al., 1995). This was further shown using SR120107A and SR120819A (Serradeil-Le Gal et al., 1994, 1995). BIBP3226, however, did not affect responses in the prototypical neuropeptide Y Y_2 receptor bioassay of rat vas deferens (Doods et al., 1995). In this preparation, and in guinea pig vas deferens also, inhibition of twitch responses by neuropeptide Y was in its turn established to be neuropeptide Y Y_2 receptor-mediated by using BIIE0246 (Doods et al., 1999; Dumont et al., 2000b; Smith-White et al., 2001).

The release of noradrenaline and neuropeptide Y from sympathetic nerves in the pig kidney is modulated by neuropeptide Y (Pernow and Lundberg, 1989a), but not affected by neuropeptide Y Y_1 receptor antagonists (Lundberg and Modin, 1995; Malmström and Lundberg, 1996; Malmström et al., 1996). The presence of a prejunctional neuropeptide Y Y_2 receptor on renal sympathetic nerves that, upon its activation by exogenous stimulation, inhibits transmitter release was established using BIIE0246 (Malmström et al., 2002b). It was demonstrated that, in the absence of noradrenaline (after reserpine treatment), endogenous neuropeptide Y inhibits its own release via activation of prejunctional neuropeptide Y Y_2 receptors in both pig kidney and spleen. Thus, the release of neuropeptide Y was greatly enhanced upon sympathetic nerve activation after BIIE0246 treatment (Malmström et al., 2002a). Moreover, the neuropeptide Y-evoked inhibition of cholinergic transmission in rat heart is mediated via activation of neuropeptide Y Y_2 receptors as shown with BIIE0246 (Smith-White et al., 2001).

6. Neuropeptide Y and sympathetic transmission

Since the discovery of neuropeptide Y, the presence of which was demonstrated in sympathetic nerves (Lundberg et al., 1982b), indirect evidence supporting its role as a sympathetic co-transmitter had accumulated. It was initially shown that neuropeptide Y mimicked the prolonged vasoconstriction evoked by high-frequency sympathetic nerve stimulation in the presence of adrenoceptor blockade (Lundberg and Tatemoto, 1982) or after reserpine treatment, when the tissue content of noradrenaline is reduced by >90%, (Lundberg et al., 1986b; Lundblad et al., 1987; Lacroix et al., 1988; Pernow and Lundberg, 1989b). That neuropeptide Y may mediate non-adrenergic sympathetic vasoconstriction was supported by several additional observations: (1) inhibition of non-adrenergic sympathetic contractions by antiserum to neuropeptide Y (Laher et al., 1994), (2) tachyphylaxis to neuropeptide Y or neuropeptide Y Y_1 receptor agonists reduces non-adrenergic sympathetic nerve responses (Öhlén et al., 1990; Morris, 1991), (3) neuropeptide Y overflow is highly correlated with non-adrenergic vasoconstrictor responses (Lundberg et al., 1989), and (4) the development of supersensitivity to neuropeptide Y-evoked vasoconstriction after sympathetic denervation (Neild, 1987; Lacroix and Lundberg, 1989). Experiments using selective neuropeptide Y Y_1 receptor antagonists have now finally established the role of neuropeptide Y as a sympathetic co-transmitter and mediator of non-adrenergic vasoconstriction.

6.1. Neuropeptide Y mediates sympathetic vasoconstriction in vitro

Evidence for the involvement of neuropeptide Y in sympathetic vasoconstriction was first presented under in vitro conditions (Fig. 2) (Malmström and Lundberg, 1995a,b). Transmural electrical field stimulation of perivascular sympathetic nerves in the guinea pig vena cava evokes a biphasic contractile response with an initial rapid peak followed by a prolonged contraction (Fig. 2). Pretreatment with neuropeptide Y Y_1 receptor antagonists, BIBP3226 or SR120107A, largely abolished the long-lasting phase of contraction (Fig. 2), leaving only the initial rapid peak (Malmström and Lundberg, 1995a,b). BIBP3435, the inactive enantiomer of BIBP3226, did not affect the contractions—establishing the selectivity of the antagonistic effect (Malmström and Lundberg, 1995b). Thus, endogenous neuropeptide Y, released from perivascular sympathetic nerves, mediates long-lasting neurogenic vasoconstriction in the guinea pig vena cava. The rapid phase of sympathetic contraction, resistant to neuropeptide Y Y_1 receptor blockade, was virtually abolished after addition of the α-adrenoceptor antagonist, phentolamine (Fig. 2) (Malmström and Lundberg, 1995b). Together, these findings support the concept of sympathetic co-transmission with noradrenaline and neuropeptide Y. Additional in vitro data corroborating the findings in guinea pig caval vein were obtained later using human, rat, and canine vessels. Both contraction of rat mesenteric artery (Racchi et al., 1997), and elevation of perfusion pressure in the isolated perfused rat mesenteric artery (Han et al., 1998b), evoked by sympathetic nerve stimulation, were partially inhibited by BIBP3226. Further, BIBP3226 partially inhibits sympathetic nerve-evoked contractions of human mesenteric vessels and saphenous vein (Racchi et al., 1997, 1999). In the latter preparation, and in canine splenic artery, a combination of purinoceptor–, adrenoceptor– and neuropeptide Y Y_1 receptor blockade strongly reduced the nerve-evoked response (Racchi et al., 1999; Yang and Chiba, 2000), which supports co-transmission with ATP, noradrenaline and neuropeptide Y.

6.2. Neuropeptide Y mediates sympathetic vasoconstriction in vivo

Evidence for neuropeptide Y as a mediator of sympathetic non-adrenergic vasoconstriction was also presented under in vivo conditions (Lundberg and Modin, 1995; Malmström et al., 1996). In pigs pre-treated with reserpine and with transection of sympathetic nerves (noradrenaline reduced by >90%), the vasoconstrictor responses evoked by high-frequency sympathetic nerve stimulation were studied in kidney, spleen, nasal mucosa, and skeletal muscle of the hind limb. Kidney (Fig. 5) and spleen respond to sympathetic nerve activation with rapid and rather short-lasting vasoconstriction whereas, in hind limb (Fig. 3) and nasal mucosa, the rapid phase is followed by a sustained long-lasting response. BIBP3226 and SR120107A strongly antagonized renal and splenic nerve-evoked responses (Lundberg and Modin, 1995; Malmström et al., 1996). Moreover, both antagonists abolished the long-lasting sympathetic vasoconstrictor responses evoked in hind limb (Fig. 3) and nasal mucosa, leaving merely the initial rapid phase of vasoconstriction (Lundberg and Modin, 1995; Malmström et al., 1996). Thus, evidence was presented that endogenous neuropeptide Y, released from sympathetic nerves, mediates non-adrenergic vasoconstriction, acting on the neuropeptide Y Y_1 receptor, in several vascular beds in the reserpine-treated pig. These results gained support from the fact that the neuropeptide Y Y_1 receptor antagonists, BIBP3226, H 409/22, and H 394/84 (Fig. 5), all exert dose-dependent antagonism on the sympathetic nerve-evoked vasoconstrictor responses in kidney and hind limb of the reserpine-treated pig in vivo (Malmström et al., 1997, 2000, 2001a). Moreover, BIBP3435 and H 510/45, the inactive enantiomers of BIBP3226 and H 409/22, respectively, did not affect any of these sympathetic vascular responses, demonstrating the selectivity of the antagonistic effects described above (Lundberg and Modin, 1995; Malmström et al., 2000). A small portion of the splenic and renal (Fig. 5) non-adrenergic sympathetic vasoconstrictor responses, as well as all of the initial rapid phase of the non-adrenergic sympathetic vasoconstrictor response in nasal mucosa and hind limb (Fig. 3), remain after neuropeptide Y Y_1 receptor blockade (Lundberg and Modin, 1995; Malmström et al., 1996, 1997, 2000, 2001a). These rapid sympathetic vasoconstrictor responses, resistant to treatment with reserpine and neuropeptide Y Y_1 receptor antagonists, may very well be mediated by purinergic mechanisms. It is consistent with this that α,β-methylene ATP evokes rapid vasoconstrictor effects in all these vascular beds (Malmström et al., 1996). Potent purinoceptor antagonists for in vivo use will be needed to solve this issue.

Evidence for the involvement of neuropeptide Y in sympathetic reserpine-resistant vasoconstriction in the pig is clear-cut. The importance of neuropeptide Y in sympathetic vascular responses evoked in the control pig (with intact noradrenaline levels) is less obvious, however. SR120107A inhibits part of the renal sympathetic vasoconstrictor responses evoked by nerve stimulation at different frequencies in the control pig (Malmström and Lundberg, 1996). Thus, neuropeptide Y seems involved in pig renal sympathetic vascular control under normal conditions. Subsequent addition of adrenoceptor antagonists largely abolished the sympathetic nerve-evoked vasoconstrictor responses evoked in kidney (Malmström and Lundberg, 1996). These results strongly support the concept of sympathetic co-transmission with noradrenaline and neuropeptide Y in vivo. Apart from this, other sympathetic vasoconstrictor responses evoked in the control pig were not affected by SR120107A but were strongly inhibited by adrenoceptor antagonists. Thus, noradrenaline seems to be the primary mediator of sympathetic vascular responses in the pig under normal conditions. Noradrenaline also exerts prejunctional α_2-adrenoceptor-mediated effects (Pernow and Lundberg, 1989a), restricting the sympathetic release of neuropeptide Y. As a consequence, the postjunctional effects of neuropeptide Y become less significant in the control than in the reserpine-treated pig, in which neuropeptide Y release is markedly (5- to 10-fold) increased (Malmström and Lundberg, 1996; Malmström et al., 1996). In the anaesthetized dog, BIBP3226 seems to inhibit sympathetic nerve-evoked coronary vasoconstriction in the presence, but not in the absence, of adrenoceptor blockade (Tanaka et al., 1997). Again, this shows the difficulty of demonstrating the involvement of endogenous neuropeptide Y in sympathetic vascular control under normal conditions. Furthermore, the inhibition by BIBP3226 of the pressor response evoked by sympathetic trunk stimulation in the pithed rat is more pronounced after complete adrenoceptor blockade (Kennedy et al., 1997). However, in this latter preparation, BIBP3226 itself exerted clear-cut inhibition of the response to high-frequency nerve stimulation, indicating that the release of neuropeptide Y was enough for significant vascular effects. In the anaesthetized cat, BIBP3226 and BIBO3304 partially inhibit the pressor response to baroreflex activation of sympathetic nerves (Capurro and Huidobro-Toro, 1999). This inhibition is also more pronounced in combination with adrenoceptor blockade, supporting co-transmission with noradrenaline and neuropeptide Y.

6.3. Neuropeptide Y and basal haemodynamics

Studies with neuropeptide Y Y_1 receptor antagonists did not demonstrate any involvement of this receptor subtype in regulating basal haemodynamics. Thus, neither basal mean arterial pressure nor regional blood flows was affected on administration of BIBP3226, SR120107A, H 409/22, and H 394/84 in the rat and pig (Doods et al., 1995; Lundberg and Modin, 1995; Malmström and Lundberg, 1996; Malmström et al., 2000, 2001a), at least not via selective effects that could be attributed to blockade of neuropeptide Y Y_1 receptors (see above).

Experiments with BIIE0246 revealed that endogenous circulating neuropeptide Y might be involved in regulation of basal vascular tone under certain conditions (Malmström et al., 2002a,b). Thus, in the anaesthetized pig, BIIE0246 per se evokes clear-cut vasodilation in spleen in two situations: (1) after reserpine treatment and (2) after treatment with the α_2-adrenoceptor antagonist, yohimbine. Under these conditions, circulating neuropeptide Y levels are increased to 40–50 pM (Malmström et al., 2002a,b). In contrast, BIIE0246 did not exert any splenic vascular effects per se under normal conditions, when circulating neuropeptide Y levels are in the low 20 pM range (Malmström, 2001b; Malmström et al., 2002b). These findings suggest that, in situations where circulating levels of neuropeptide Y are moderately increased, activation of vascular neuropeptide Y Y_2 receptors in pig spleen participates significantly in the regulation of basal vascular tone. In the pig, the vascular bed most sensitive to i.v. administered neuropeptide Y is the splenic circulation (Rudehill et al., 1987). As discussed above, both vascular neuropeptide Y Y_1 and Y_2 receptors mediate splenic vasoconstriction (Malmström, 2001a), the latter subtype being the most sensitive to circulating neuropeptide Y. The neuropeptide Y Y_1 receptor is activated by nerve-released neuropeptide Y, and by high doses of i.v. administered neuropeptide Y (high circulating levels of neuropeptide Y) predominantly, as shown by the effects of neuropeptide Y Y_1 receptor antagonists. The neuropeptide Y Y_2 receptor is readily activated at low i.v. doses of neuropeptide Y (low circulating levels of neuropeptide Y) as shown with BIIE0246. These facts may explain why experiments with neuropeptide Y Y_1 receptor antagonists did not show any significant participation of neuropeptide Y Y_1 receptors in basal vascular tone (in, e.g., kidney and spleen) under conditions similar to those above. However, the situation may obviously be different under conditions with more than moderate increases of plasma neuropeptide Y levels.

6.4. Autoinhibitory neuropeptide Y Y_2 receptors

Finally, there is now also evidence for the involvement of prejunctional neuropeptide Y Y_2 receptors in sympathetic transmission (Malmström et al., 2002a). In the reserpine-treated pig, blockade of neuropeptide Y Y_2 receptors with BIIE0246 greatly enhanced the release of neuropeptide Y upon renal and splanchnic sympathetic nerve activation (Malmström et al., 2002a). As a consequence of the increased neuropeptide Y release, the sympathetic non-adrenergic (neuropeptide Y Y_1 receptor-mediated, see above) vasoconstriction evoked in spleen was augmented (Malmström et al., 2002a). Thus, in the reserpine-treated pig, sympathetic transmission with neuropeptide Y is auto-inhibitory-modulated via the prejunctional neuropeptide Y Y_2 receptor. The activation of prejunctional neuropeptide Y Y_2 receptors by endogenous neuropeptide Y in modulation of transmitter release, as for postjunctional neuropeptide Y Y_1 receptors in sympathetic vasoconstriction, is less obvious in the control pig with normal noradrenaline levels (Malmström et al., 2002b). Thus, in the control pig, BIIE0246 did not itself affect the renal sympathetic release of noradrenaline or neuropeptide Y per se indicating that, under the conditions of these experiments, endogenous neuropeptide Y does not seem to play a role in modulating transmitter release upon nerve activation (Malmström et al., 2002b). Instead, stimulation of the prejunctional neuropeptide Y Y_2 receptor by exogenous peptide YY was required to elicit inhibition of transmitter release, which in turn could be antagonized by BIIE0246 (Malmström et al., 2002b). However, the presence of a sympathetic prejunctional neuropeptide Y Y_2 receptor that, whenever activated, inhibits transmitter release was thus established. Consistent with this, BIIE0246 inhibited modulation of cholinergic or purinergic transmission in anaesthetized rats and guinea pig vas deferens upon exogenous neuropeptide Y Y_2 receptor stimulation, but not on its own (Smith-White et al., 2001).

7. Neuropeptide Y and cardiovascular pathophysiology

Altered expression, tissue content or release of, and ultimately of the sensitivity to, neurotransmitters, parallels several cardiovascular disorders. Consistent with this, stress increases neuropeptide Y mRNA (Nankova et al., 1996). At a prehypertensive stage, spontaneously hypertensive rats have a denser perivascular innervation with neuropeptide Y-containing fibres compared to that in control rats (Dhital et al., 1988; Lee et al., 1988; Kawamura et al., 1989). Moreover, spontaneously hypertensive rats have greater pressor responsiveness to neuropeptide Y, paralleling the development of hypertension (Miller and Tessel, 1991). BIBP3226 did not affect basal mean arterial pressure in spontaneously hypertensive rats, indicating that neuropeptide Y does not modulate basal blood pressure in this model (Doods et al., 1995). However, BIBP3226 inhibited the increase in mean arterial pressure upon cold stress provocation to a greater extent in borderline hypertensive rats than in normotensive rats (Zukowska-Grojec et al., 1996), suggesting an enhanced role of neuropeptide Y in stress-induced hypertension (Han et al., 1998a). This extends earlier in vitro data in support of an increased role of neuropeptide Y in sympathetic vasoconstriction in spontaneously hypertensive rats (Daly et al., 1988). Age and hyperlipidaemia may also enhance vasoconstrictor responses to neuropeptide Y (Corr et al., 1993). Age, hypertension, and hyperlipidaemia are paralleled by a loss of endothelium-dependent release of nitric oxide (Mombouli and Vanhoutte, 1999). Possibly, this is one explanation for the augmented neuropeptide Y responses under these conditions. Thus, nitric oxide may function as a basal inhibitory modulator of neuropeptide Y-evoked vasoconstriction, and the effects of neuropeptide Y are enhanced after inhibition of nitric oxide synthase (Malmström et al., 2001b). In addition,

the function of the neuropeptide Y Y_1 receptor is also inhibited at the mRNA level by nitric oxide donors in vitro (Dötsch et al., 1997).

An enhanced role of neuropeptide Y has also been suggested in congestive heart failure. Thus, in vivo venous responsiveness to neuropeptide Y is increased in patients with chronic heart failure (Feng et al., 2000). Furthermore, the inhibitory effect of the neuropeptide Y Y_1 receptor antagonist, H 409/22, on renal sympathetic nerve-evoked vasoconstriction is increased in rats with heart failure compared to controls (DiBona and Sawin, 2001). In contrast to in normal rats, neuropeptide Y also seems involved in maintaining basal blood pressure in rats with heart failure, as shown by the hypotensive effects of BIBP3226 (Zhao et al., 1999). Circulating neuropeptide Y levels are elevated, compared with those in normal subjects, in patients with cardiovascular disease such as acute myocardial infarction, angina pectoris (Ullman et al., 1990), heart failure (Hulting et al., 1990), and hypertension (Chalmers et al., 1989; Solt et al., 1990; Lettgen et al., 1994), where sympathetic nerve activity is increased (Anderson et al., 1989). Hypertension in phaeochromocytoma is associated with highly elevated neuropeptide Y levels (Corder et al., 1985; Lundberg et al., 1986a), especially on surgical manipulation of the tumour (Lundberg et al., 1986a), when the elevation of neuropeptide Y plasma levels correlates with the systemic vascular resistance (Eurin et al., 2000). It can thus be speculated that neuropeptide Y may contribute to the haemodynamic disturbances observed in these states of sympathetic hyperactivity. In addition to vasoconstrictor effects, long-standing increases in neuropeptide Y release may also play a role in the development of cardiovascular hypertrophy since chronic neuropeptide Y receptor stimulation may enhance DNA synthesis in vascular smooth muscle cells (Shigeri and Fujimoto, 1993; Zukowska-Grojec et al., 1998) and cardiomyocytes (Millar et al., 1994). These mitogenic effects of neuropeptide Y are partially inhibited by BIBP3226 (Zukowska-Grojec et al., 1998), but need further characterization with reference to the receptor subtypes involved.

In healthy volunteers, sympathetic activation by exercise causes cardiac release of noradrenaline and neuropeptide Y (Morris et al., 1997). However, upon exercise under hypoxic conditions, the cardiac overflow of neuropeptide Y, relative to that of noradrenaline, is enhanced (Kaijser et al., 1994). Further, the role of endogenous neuropeptide Y in sympathetic vasoconstriction is enhanced after short-term renal ischaemia, as demonstrated by the inhibition exerted by BIBP3226 (Malmström and Lundberg, 1997b). This observation indicates that metabolic degradation of neuropeptide Y is reduced in ischaemic regions, although an increased release of neuropeptide Y, relative to that of noradrenaline, may contribute. Importantly, there is evidence that neuropeptide Y Y_1 receptor-mediated vasoconstriction is greatly prolonged after short-term ischaemia (Fig. 6), in further support of inhibited local neuropeptide Y degradation

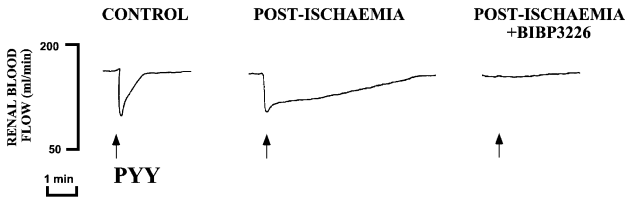

Fig. 6. Effects of short-term (15-min) ischaemia (renal arterial occlusion) on the vasoconstrictor response to peptide YY (230 pmol/kg) evoked in pig kidney in vivo. The renal vasoconstrictor response to peptide YY was markedly prolonged 5 min into reperfusion after short-term ischaemia, which could indicate reduced metabolic degradation of peptide YY (and neuropeptide Y) in ischaemic regions. BIBP3226 (2 μmol/kg) largely abolished the post-ischaemic renovascular response to peptide YY, demonstrating that this was a neuropeptide Y Y_1 receptor-mediated effect.

(Malmström and Lundberg, 1997b). This may suggest that the effects of neuropeptide Y become more important under ischaemic conditions, such as in patients with coronary artery disease. Indeed, intracoronary infusion of neuropeptide Y in patients with angina pectoris induces myocardial ischaemia with typical chest pain and ECG changes (Clarke et al., 1987). The involvement of endogenous neuropeptide Y in sympathetic coronary vasoconstriction resistant to adrenoceptor blockade was demonstrated in the dog (Tanaka et al., 1997). Furthermore, there is a correlation between the plasma levels of neuropeptide Y, but not those of noradrenaline, and the degree and duration of ST-segment depression after exercise in patients with coronary artery disease, which suggests that neuropeptide Y may contribute to myocardial ischaemia in these patients (Gullestad et al., 2000). It would thus be of interest to study the effects of neuropeptide Y Y_1 receptor antagonists on stress-evoked myocardial ischaemia. Furthermore, because of neuropeptide Y Y_2 receptor-mediated inhibition of vagal activity, a neuropeptide Y Y_2 receptor antagonist may be of relevance in the treatment of stress-evoked cardiac arrhythmias. Obviously, one potential advantage to be gained with neuropeptide Y receptor antagonists would be that these are less likely to influence basal vascular tone or mild reflex adjustments, relative to drugs influencing other transmitters involved in sympathetic transmission.

8. Concluding remarks

The status of neuropeptide Y as a sympathetic transmitter has been established. So have also fundamental cardiovascular effects of its receptor subtypes Y_1 and Y_2. Although probably not a modulator of basal haemodynamics, neuropeptide Y seems to be of increased importance in various cardiovascular disorders, e.g., hypertension, heart failure and ischaemia. There now exist several non-peptide antagonists with high selectivity for either neuropeptide Y Y_1 or Y_2 receptors, the usefulness of which in vivo has also been proven. With these tools, the questions surrounding the

extent to which neuropeptide Y contributes to cardiovascular pathophysiology have begun to be unravelled.

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