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Autoinhibitory function of the sympathetic prejunctional neuropeptide Y Y_2 receptor evidenced by BIIE0246

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Abstract

The significance of neuropeptide Y Y_2 receptors in sympathetic nonadrenergic transmission was investigated using the novel selective antagonist 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). In anaesthetized pigs pretreated with reserpine, and after transection of sympathetic nerves (depleted of noradrenaline), electrical stimulation of renal and splanchnic sympathetic nerves evoked vasoconstriction in, and overflow of neuropeptide Y-like immunoreactivity from, kidney and spleen, respectively. In the presence of BIIE0246, the nerve-evoked overflows of neuropeptide Y-like immunoreactivity were markedly increased and the splenic vasoconstrictor response prolonged. In addition, BIIE0246 caused splenic vasodilatation per se in this model where basal levels of circulating neuropeptide Y exceed 40 pM. It is concluded that endogenous neurogenical neuropeptide Y regulates its own release via activation of sympathetic prejunctional inhibitory neuropeptide Y Y_2 receptors in both spleen and kidney in the reserpinized pig. Moreover, when circulating levels of neuropeptide Y are moderately increased, activation of neuropeptide Y Y_2 receptors seems to contribute to basal splenic vascular tone.

Keywords: BIIE0246; Neuropeptide Y Y_2 receptor; Prejunctional; Sympathetic transmission

1. Introduction

The 36 amino acid residue neuropeptide Y (Tatemoto et al., 1982) is abundantly present, co-localized with noradrenaline, within the sympathetic nervous system (see Lundberg, 1996). Neuropeptide Y is co-released with noradrenaline upon nerve activation and acts as a sympathetic transmitter predominantly on two receptor subtypes: the neuropeptide Y Y_1 and Y_2 receptors. These were initially classified as post- and prejunctional receptors based on the actions of a number of peptide neuropeptide Y analogues acting like agonists (Wahlestedt et al., 1986, 1990), but have since been further characterized by the use of nonpeptide antagonists. Thus, investigations using selective neuropeptide Y Y_1 receptor antagonists, e.g. BIBP3226 (Rudolf et al., 1994), demonstrated that the neuropeptide Y Y_1 receptor is the main receptor subtype involved in vascular responses to neuropeptide Y (see Malmström, 1997). However, in some tissues, there is involvement of the neuropeptide Y Y_2 receptor as well (Modin et al., 1991; Malmström, 2001a). Recently, the first nonpeptide 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) was presented (Doods et al., 1999). BIIE0246, possessing high affinity for the neuropeptide Y Y_2 receptor, is devoid of affinity for neuropeptide Y Y_1, Y_4 and Y_5 receptors (Doods et al., 1999). Furthermore, BIIE0246 also shows high potency and selectivity (vs. neuropeptide Y Y_1 and other receptors) for the neuropeptide Y Y_2 receptor in vivo (Malmström, 2001b). By using BIIE0246, evidence has now been presented that the neuropeptide Y Y_2 receptor exerts prejunctional inhibition of transmitter release (Malmström et al., 2002; Smith-White et al., 2001), although in some instances, this function can be mediated by the neuropeptide Y Y_1 receptor (Doods et al., 1995). Evidence for the involvement of other subtypes than neuropeptide Y Y_1 and Y_2 receptors in

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peripheral sympathetic transmission is lacking. A potential role for the neuropeptide Y Y_4 receptor in modulation of arterial neurogenic vasoconstriction in the rat has been suggested (Barrios et al., 1999), but needs to be further elucidated using a subtype-selective receptor antagonist.

In addition to receptor classification, the use of neuropeptide Y Y_1 receptor antagonists has presented evidence for the involvement of endogenous, neuronally released, neuropeptide Y in sympathetic vasoconstriction (see Malmström, 1997). However, normally, the release of neuropeptide Y seems restricted, presumably due to an effective prejunctional α-adrenergic inhibition (see Lundberg, 1996), and the effects of endogenous neuropeptide Y seem not clear cut even after adrenoceptor blockade (Malmström et al., 2002; see Malmström, 1997). Considering the diversity of adrenoceptor subtypes, there is a risk that adrenoceptor blocking agents do not completely block the receptors in vivo. However, treatment with reserpine in combination with transection of sympathetic nerves creates a situation where noradrenaline levels are depleted to the extent that neuropeptide Y is the primary mediator of sympathetic vasoconstrictor responses in several vascular beds in the pig (Lundberg and Modin, 1995; Malmström et al., 1996). In parallel, studies with BIIE0246 have demonstrated the existence of prejunctional neuropeptide Y Y_2 receptors that may, when activated by exogenous stimulation, inhibit transmitter release (Malmström et al., 2002; Smith-White et al., 2001). However, in these studies, BIIE0246 did not affect the transmitter release per se (Malmström et al., 2002; Smith-White et al., 2001), even after adrenoceptor blockade (Malmström et al., 2002), indicating that the effects of endogenous neuropeptide Y could not be established. Therefore, in this study, we have used the reserpine-treated pig model to study the possible significance of neuropeptide Y Y_2 receptors in sympathetic nonadrenergic transmission using the neuropeptide Y Y_2 receptor antagonist BIIE0246. Two vascular beds were studied in detail: (1) the kidney where only postjunctional neuropeptide Y Y_1 receptors to date have been identified (see Malmström, 1997) and (2) the spleen where both neuropeptide Y Y_1 and Y_2 receptors may participate in vasoconstrictor responses (Modin et al., 1991; Malmström, 2001a). In kidney, a prejunctional neuropeptide Y Y_2 receptor has been localized that inhibits transmitter release upon exogenous receptor activation (Malmström et al., 2002). In neither of these vascular beds, neuropeptide Y Y_1 receptors seem to affect transmitter release (Lundberg and Modin, 1995; Malmström and Lundberg, 1996; Malmström et al., 1996).

2. Materials and methods

2.1. In vivo studies

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

2.2. Surgical preparations

Domestic pigs (15–18 kg) of either sex, premedicated with ketamine (20 mg/kg intramuscular, i.m.) and atropine (0.02 mg/kg i.m.), were anaesthetized with sodium pentobarbitone (20 mg/kg intravenously, i.v.), intubated and ventilated by a respirator (Servo ventilator 900, Siemens-Elema, Sweden); skeletal muscle relaxation (pancuronium, 0.5 mg/kg i.v.) was induced after. The anaesthesial depth was checked by pinching the interdigital skin before administration of pancuronium. The retroperitoneal space was reached via a flank incision below the left costal margin, where the left major splanchnic nerve and the postganglionic sympathetic nerves to the left kidney were exposed and sectioned. The incision was closed and reserpine (1 mg/kg i.v.) was administered before extubation.

The following day, the pigs were re-anaesthetized (see above) and ventilated by the respirator via a tracheal tube. A catheter, connected to a Statham P23 AC pressure transducer, was inserted into the right brachial artery for measurement of mean arterial pressure. Heart rate was recorded by a tachograph unit triggered by the blood pressure. Catheters were placed in the left brachial artery, and the main splenic and left renal veins via side branches, to allow systemic and local venous blood sampling. A catheter was also inserted into the right brachial vein for infusion of drugs to maintain anaesthesia (fentanyl, 10 μg/kg/h, and midazolam, 0.2 mg/kg/h), skeletal muscle relaxation (pancuronium, 0.5 mg/kg/h), fluid balance (sodium chloride 154 mM and glucose 28 mM, 2 ml/min) and to prevent intravascular coagulation (heparin 250 IU/kg/h). Ultrasonic flow probes (2RB, 4RB), connected to Transonic flowmeters (T202, T206, Transonic Instruments, Ithaca, NY, USA), were placed around the splenic and left renal arteries, for continuous monitoring of local blood flows. Electrodes were placed on the distal ends of the cut splanchnic and left renal sympathetic nerves to allow electrical stimulation. The abdomen was then closed, and the pigs were then allowed to stabilize for 1 h before the experiments were commenced.

2.3. Experimental procedures

Electrical stimulation of the splanchnic and left renal sympathetic nerves (three 20-Hz bursts, 1 s each, at 10-s intervals (5 ms, 25 V)) were performed by a Grass stimulator (model S11). To determine overflow as an estimate of release of neuropeptide Y-like immunoreactivity upon sympathetic nerve activation, arterial and splenic or renal venous blood samples were collected before, upon the second and third burst, and at 30 s, 1, 2 and 5 min after the electrical nerve stimulation. The overflows of neuropeptide Y-like immunoreactivity were studied upon nerve stimulation under control conditions, 30 min into an infusion of the neuropeptide Y Y_2 receptor antagonist BIIE0246, and again 2 h after the infusion of BIIE0246 was completed. BIIE0246 was administered as an i.v. injection (100

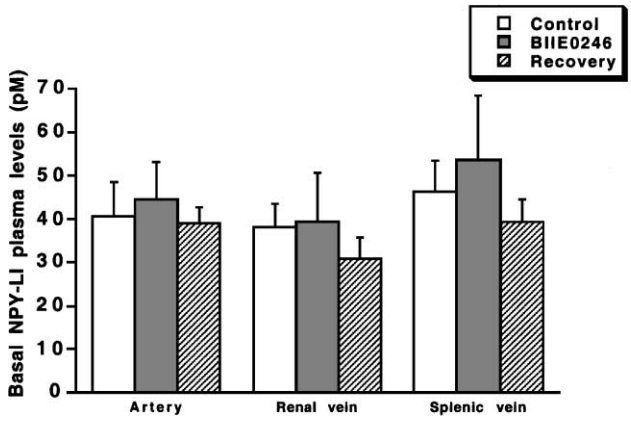

Fig. 1. Arterial, renal and splenic venous plasma levels of neuropeptide Y-like immunoreactivity are shown in the reserpine-treated pig under control conditions (empty bars), in the presence of the neuropeptide Y Y_2 receptor antagonist BIIE0246 (grey bars), and after a 2-h recovery period (hatched bars). Data are given as means ± S.E.M., $n = 6–10$. There were no significant differences compared to control.

nmol/kg equal to 100 μg/kg) followed by an i.v. infusion (5 nmol/kg/min) of the compound. The 2 h recovery period was chosen according to the duration of action of BIIE0246 in vivo (Malmström, 2001b). The dose of BIIE0246 was chosen according to a previous study (Malmström, 2001b). A bolus dose (200 pmol/kg, i.v.) of the neuropeptide Y Y_2 receptor agonist *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–36) was given 15 min before each set of nerve stimulations. Inhibition of the splenic vasoconstrictor response to *N*-acetyl [Leu^{28},Leu^{31}]neuropeptide Y-(24–36) was used as indication of an established neuropeptide Y Y_2 receptor blockade. The overflows of neuropeptide Y-like immunoreactivity are reproducible and not susceptible to any spontaneous decline as has been demonstrated in earlier studies (see Malmström, 1997).

2.4. Determination of neuropeptide Y-like immunoreactivity in plasma

The blood samples were collected in prechilled tubes containing EDTA (final concentration of 10 mM), centrifuged 10 min (+4 °C) and the plasma was pipetted off and stored at −20 °C. Neuropeptide Y-like immunoreactivity was determined with radioimmunoassay (using antibody N1) after ethanol extraction. The determination limit was 7.8 pM. The N1 antiserum shows no (< 0.1%) cross-reactivity to structurally related peptides such as peptide YY and *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–36). For further details, see Theodorsson-Norheim et al. (1985).

2.5. Calculations

To give a measure of neuropeptide Y release from the kidney and spleen, the total overflows of neuropeptide Y-like immunoreactivity were calculated as the integrated area of the renal and splenic veno-arterial plasma differences, multiplied by the local arterial plasma flow at each sample point. The hematocrit was determined after centrifugation of the blood samples. The vascular responses are expressed as changes in vascular conductance, calculated as blood flow divided by mean arterial pressure (Stark, 1968). Data in the

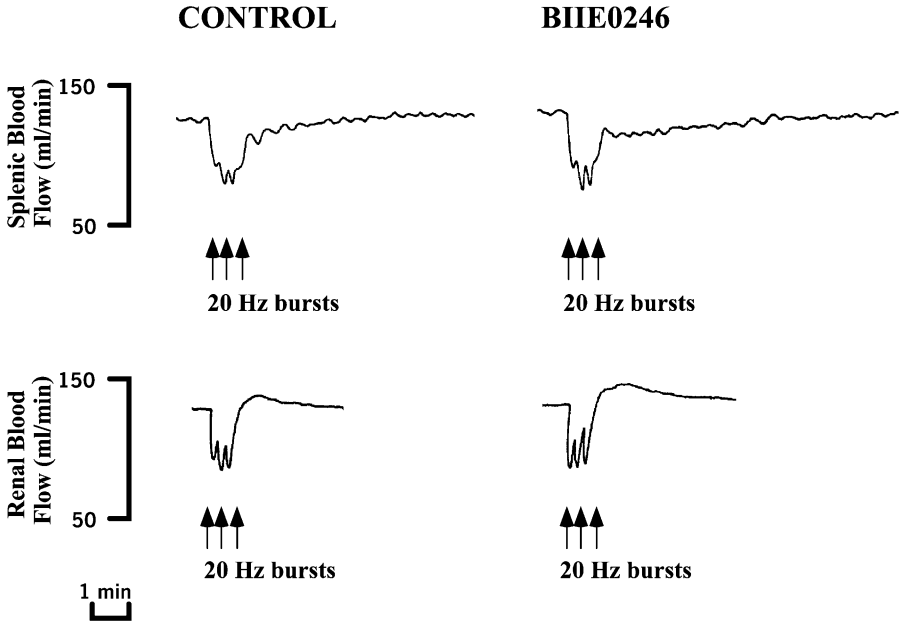

Fig. 2. Original recording of vascular responses evoked in spleen and kidney by sympathetic nerve stimulation in the reserpine-treated pig in vivo. Vascular responses are shown under control conditions and in the presence of the neuropeptide Y Y_2 receptor antagonist BIIE0246. Note the prolongation of the slowly declining phase of splenic vasoconstriction in the presence of BIIE0246.

text are given as means ± S.E.M., and statistical significance was calculated with the multiple analysis of variance (ANOVA) followed by the post test of Tukey, or with the Student's *t*-test (paired samples) where applicable.

2.6. Drugs

Ketamine (Parke-Davis, CA, USA), sodium pentobarbitone (NordVacc, Sweden), atropine and sodium heparin (KabiVitrum, Sweden), pancuronium bromide (Organon, The Netherlands), fentanyl (Pharmalink, Sweden), midazolam (Roche, Sweden), reserpine (Sigma, MO, USA), *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–36) (Auspep, Australia). 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, (Boehringer Ingelheim Pharma, Biberach, Germany). All drugs were dissolved in either saline or glucose (5%, w/v) solution.

3. Results

Basal splenic and renal blood flows, mean arterial pressure and heart rate were 91 ± 13 and 199 ± 36 ml/min, 85 ± 2 mm Hg, and 83 ± 9 beats/min, respectively. Splenic vasodilatation (blood flow increased to 98 ± 13 ml/min, $P < 0.01$ vs. basal, $n = 10$) was observed upon administration of BIIE0246. Renal blood flow (199 ± 37 ml/min), mean arterial pressure (83 ± 2 mm Hg) and heart rate (85 ± 9 beats/min) were not affected by BIIE0246. Basal arterial, splenic and renal venous plasma levels of neuropeptide Y-like immunoreactivity were 41 ± 8, 46 ± 7 and 38 ± 6 pM, respectively (Fig. 1). These plasma levels were not significantly enhanced by BIIE0246 (Fig. 1).

A rapid vasoconstrictor response was evoked in kidney upon electrical stimulation (three 1-s bursts at 20 Hz) of the renal sympathetic nerves. Renal vascular conductance was reduced by 37 ± 4% upon the control stimulation (Fig. 2). In spleen, splanchnic sympathetic nerve stimulation elicited an initial rapid vasoconstrictor effect (vascular conductance reduced by 39 ± 4%), which was followed by a rather slowly declining phase of vasoconstriction (basal blood flow was reached after 204 ± 24 s) (Fig. 2). The vascular responses evoked by electrical nerve stimulation in spleen and kidney were accompanied by neuropeptide Y-like immunoreactivity overflows of 4.5 ± 0.8 pmol (Fig. 3A) and 2.9 ± 1.1 pmol (Fig. 3B), respectively. In the presence of BIIE0246, the overflows of neuropeptide Y-like immunoreactivity upon nerve stimulation in spleen and kidney were increased to 7.2 ± 1.2 pmol (Fig. 3A) and 5.1 ± 1.8 pmol (Fig. 3B), respectively. The vascular response evoked by nerve stimulation in kidney was not affected in the presence of BIIE0246 (vascular conductance reduced by 33 ± 4%), and neither was the initial rapid nerve-evoked

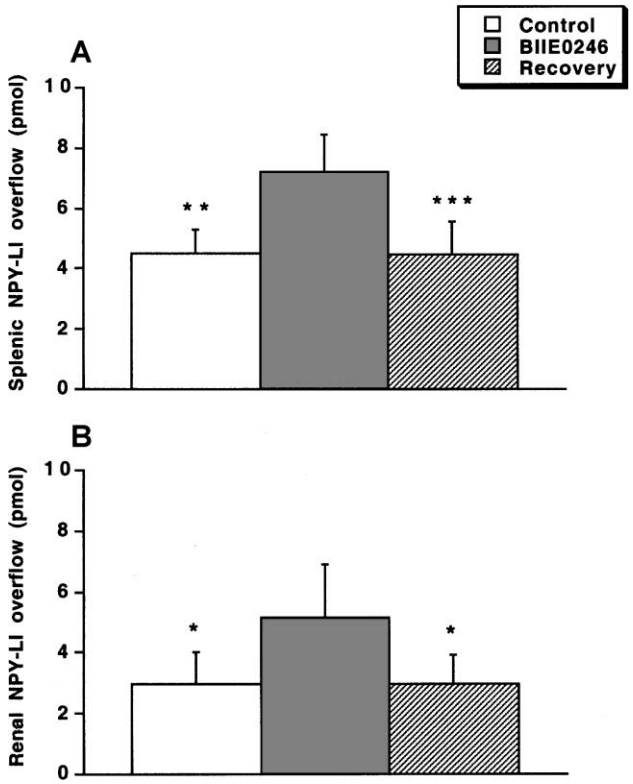

Fig. 3. Overflows of neuropeptide Y-like immunoreactivity from the (A) spleen and (B) kidney upon sympathetic nerve stimulation (three 1-s bursts at 20 Hz) in the reserpine-treated pig in vivo. Overflows of neuropeptide Y-like immunoreactivity are shown under control conditions (empty bars), in the presence of the neuropeptide Y Y_2 receptor antagonist BIIE0246 (grey bars), and after a 2-h recovery period (hatched bars). Data are given as means ± S.E.M., $n = 6–10$. Significant differences compared to overflows in the presence of BIIE0246 are indicated * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

vasoconstriction in spleen (vascular conductance reduced by 41 ± 3%) (Fig. 2). However, the slowly declining phase of vasoconstriction evoked in spleen by nerve stimulation was prolonged (basal blood flow was reached after 341 ± 29 s, $P < 0.001$ vs. the control response, $n = 10$) in the presence of BIIE0246 (Fig. 2). Two hours after termination of the BIIE0246 infusion, these enhancing effects had subsided. Hence, the neuropeptide Y-like immunoreactivity overflows upon nerve stimulation in spleen and kidney had returned to 4.4 ± 1.1 pmol (Fig. 3A) and 3.0 ± 1.0 pmol (Fig. 3B), respectively. The rapid vasoconstrictor responses evoked by nerve stimulation in kidney (vascular conductance reduced by 40 ± 2%) and spleen (vascular conductance reduced by 38 ± 2%) were still not different compared to control. The slowly declining phase of vasoconstriction evoked in spleen by nerve stimulation was reduced to 150 ± 13 s ($P < 0.001$ vs. the response in the presence of BIIE0246, $n = 10$).

N-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–36) (200 pmol/kg, i.v.) evoked splenic vasoconstriction but no, or only marginal, effects in kidney and on mean arterial pressure. Splenic vascular conductance was reduced by 22 ± 5% upon *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–

36) and this vasoconstrictor response was completely abolished in the presence of BIIE0246. Two hours after termination of the BIIE0246 infusion, the vasoconstrictor response evoked in spleen by *N*-acetyl[Leu^{28},Leu^{31}]neuropeptide Y-(24–36) was normalised as splenic vascular conductance was reduced by 16 ± 4%.

4. Discussion

In the present study, it was demonstrated that neuronally released endogenous neuropeptide Y exerts autoinhibition via sympathetic prejunctional neuropeptide Y Y_2 receptors. Evidence for this was presented using the novel selective nonpeptide neuropeptide Y Y_2 receptor antagonist BIIE0246 in the reserpine-treated pig in vivo. Moreover, circulating endogenous neuropeptide Y participates, via activation of postjunctional vascular neuropeptide Y Y_2 receptors, in the basal regulation of splenic vascular tone in the reserpine-treated pig (in which plasma levels of neuropeptide Y exceed 40 pM).

The existence of prejunctional neuropeptide Y receptors inhibiting transmitter release was suggested by Lundberg et al. (1982). Later, the neuropeptide Y-evoked modulation of noradrenergic (Lundberg and Stjärne, 1984) and purinergic (Stjärne et al., 1986) transmission was extended also to be valid for the release of neuropeptide Y itself (Pernow and Lundberg, 1989). Although prejunctional effects of neuropeptide Y were suggested to be neuropeptide Y Y_2 receptor mediated (Wahlestedt et al., 1986), the first prejunctional neuropeptide Y receptor to be pharmacologically classified using a selective antagonist was of the Y_1 subtype in rabbit vas deferens (Doods et al., 1995). Since the introduction of the first selective neuropeptide Y Y_2 receptor antagonist BIIE0246 (Doods et al., 1999), evidence has been presented for prejunctional neuropeptide Y Y_2 receptors regulating transmitter release in several tissues including rat vas deferens (Smith-White et al., 2001) and pig kidney (Malmström et al., 2002). However, in these studies (Malmström et al., 2002; Smith-White et al., 2001), stimulation of the receptor by exogenous agonists was required to elicit inhibition of transmitter release, which in turn could be antagonized by BIIE0246. In contrast, the antagonist BIIE0246 did not alter transmission per se. This indicates that, under the conditions of these experiments, endogenous neuropeptide Y was not significantly involved in the modulation of transmitter release. In apparent parallel, the involvement of endogenous neuropeptide Y in sympathetic vasoconstriction is not clear cut under control conditions (Malmström, 1997). Thus, while neuropeptide Y Y_1 receptor antagonists readily antagonized vasoconstrictor responses exerted by exogenous agonists, this was not entirely true for sympathetic nerve-evoked responses (Malmström and Lundberg, 1996; Malmström, 1997). Hence, in the normal situation, neuropeptide Y seems to exert in comparison to noradrenaline rather marginal effects, presumably due to an effective prejunctional α-adrenergic inhibition restricting the release of neuropeptide Y (see Lundberg, 1996). Depletion of noradrenaline (reserpine treatment in combination with transection of sympathetic nerves) was required to present evidence that endogenous, neuronally released, neuropeptide Y mediates sympathetic vasoconstriction (Lundberg and Modin, 1995; Malmström et al., 1996). In the present study, this approach was used to investigate the role of neuropeptide Y Y_2 receptors in nonadrenergic sympathetic transmission. It is here shown that, in the presence of the neuropeptide Y Y_2 receptor antagonist BIIE0246, the sympathetic nerve-evoked release of neuropeptide Y is markedly increased both in kidney and spleen. Most importantly, this demonstrates that endogenous, neuronally released, neuropeptide Y activates prejunctional neuropeptide Y Y_2 receptors to exert autoinhibition of its own release. Thus, the result from an earlier study (Malmström et al., 2002) showing the existence of a renal prejunctional neuropeptide Y Y_2 receptor activated upon exogenous receptor activation was here extended to be applicable also to endogenous activation. In addition, evidence was also presented for the presence of a prejunctional neuropeptide Y Y_2 receptor in pig spleen. The results in the present study are strengthened by the fact that a complete recovery of the responses was observed. Thus, the sympathetic nerve-evoked release of neuropeptide Y (as well as the splenic vasoconstrictor response exerted by the neuropeptide Y Y_2 receptor agonist) had returned to basal levels 2 h after the infusion of BIIE0246 was completed. This recovery reflects the duration of the antagonistic effects exerted by BIIE0246 in vivo, and correlates well to what was observed when investigating the effects of the compound on neuropeptide Y Y_2 receptor-mediated vasoconstriction in vivo (Malmström, 2001b).

Rather surprising was that even though the nerve-evoked release was increased, the peak vascular responses evoked by endogenous neuropeptide Y were not augmented by BIIE0246. The nerve response in spleen was, however, prolonged, indicating enhanced effects of the neuronally released neuropeptide Y. The lack of effects on the peak nerve responses in kidney and spleen may have different explanations though. Importantly, there is a difference between these two vascular beds concerning the postjunctional neuropeptide Y receptor population: (1) pig kidney presumably possesses only the neuropeptide Y Y_1 receptor subtype (Malmström, 1997), whereas (2) pig spleen contains both neuropeptide Y Y_1 and Y_2 receptors (Modin et al., 1991; Malmström, 2001a). The splenic neuropeptide Y Y_1 receptor is activated by endogenous neuropeptide Y and upon higher doses of circulating neuropeptide Y predominantly (Malmström and Lundberg, 1996; Malmström et al., 1996). Low circulating levels of neuropeptide Y readily activate the splenic neuropeptide Y Y_2 receptors (Malmström, 2001b). The involvement of neuropeptide Y Y_2 receptors in nerve-evoked vasoconstrictor effects has not been demonstrated yet, and may not be that easy considering that the release of

neuropeptide Y most likely also will be affected upon blockade of this receptor subtype. Hence, the lack of effect of BIIE0246 on the peak nerve response in spleen may be explained by (1) the fact that postjunctional neuropeptide Y Y_2 receptors may actually be involved in this response and blockade of these would mask the effects of an enhanced release. Alternatively, (2) the release of neuropeptide Y may, in the control situation, already have been in excess and an increased release would in that case not render an augmented response. Interestingly though, the nerve-evoked splenic response was prolonged upon the increased release of neuropeptide Y, indicating enhanced effects of endogenous neuropeptide Y thus acting on the neuropeptide Y Y_1 receptor subtype during this phase of the nerve response. Considering the unaltered peak response in kidney, the second explanation seems the most applicable. Thus, there is no evidence for postjunctional (vascular) renal neuropeptide Y Y_2 receptors since agonists selective for this subtype do not evoke clear-cut vasoconstrictor effects in kidney (Malmström, 1997). Instead, an infusion of the neuropeptide Y Y_2 receptor agonist *N*-acetyl[Leu^{28},Leu^{31}]-neuropeptide Y-(24–36) merely inhibited the peak non-adrenergic renal sympathetic vasoconstriction evoked by nerve stimulation in the reserpine-treated pig (unpublished data) in further support of the prejunctional actions of this receptor subtype.

Upon administration of the neuropeptide Y Y_2 receptor antagonist BIIE0246 in the reserpine-treated pig in vivo, moderate but clear-cut splenic vasodilatation was observed. As discussed above, the splenic neuropeptide Y Y_2 receptors seem to be readily activated upon low to moderate circulating levels of neuropeptide Y to mediate vasoconstriction, whereas the participation of neuropeptide Y Y_1 receptors in such a response becomes significant at somewhat higher levels. The cause of this phenomenon is unknown but may be related to the distribution of receptors within the tissue. In this study, circulating levels of neuropeptide Y were in the low 40 pM range. The results with BIIE0246 suggest that these levels were high enough to significantly exert vasoconstrictor effects via activation of neuropeptide Y Y_2 receptors in the spleen. In accord with this, BIIE0246 caused splenic vasodilatation also after treatment with an α_2-adrenoceptor antagonist (Malmström et al., 2002), which increased circulating levels of neuropeptide Y to a similar extent as was seen in the present study. In contrast, BIIE0246 does not exert any vascular effects in spleen during control conditions (Malmström, 2001b; Malmström et al., 2002), when circulating neuropeptide Y levels are in the low 20 pM range.

In summary, it was demonstrated that the novel neuropeptide Y Y_2 receptor antagonist BIIE0246 markedly increased sympathetic nerve-evoked release of neuropeptide Y from spleen and kidney of the reserpine-treated pig. In spleen, this was paralleled with a prolonged vasoconstrictor response in support of enhanced effects of neuronal neuropeptide Y. Thus, evidence was here presented that the release of neuropeptide Y from sympathetic nerves is subjected to autoinhibitory modulation by endogenous neuropeptide Y acting on a prejunctional neuropeptide Y Y_2 receptor. Furthermore, BIIE0246 evoked splenic vasodilatation per se, suggesting that the circulating levels of neuropeptide Y observed were in a range ($\approx$ 40 pM) where activation of postjunctional neuropeptide Y Y_2 receptors significantly contributes to regulation of basal splenic vascular tone.

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