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SR 120107A antagonizes neuropeptide Y Y_1 receptor mediated sympathetic vasoconstriction in pigs in vivo

Rickard E. Malmström *, Agnes Modin, Jan M. Lundberg

Division of Pharmacology, Department of Physiology and Pharmacology, Karolinska Institute, S-17177 Stockholm, Sweden

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Abstract

The effects of the neuropeptide Y Y_1 receptor antagonist SR 120107A (1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*cis*-cyclohexylmethyl]amidino]phenyl]propionyl]pyrrolidine, (*S*,*R*) stereoisomer) on sympathetic non-adrenergic vasoconstriction in a variety of vascular beds were studied in reserpinized anesthetized pigs in vivo. The rapid vasoconstrictor response evoked by single impulse stimulation, in hind limb and nasal mucosa, was not affected by SR 120107A (1.5 mg kg^{-1} i.v.). In contrast, SR 120107A potently inhibited the long-lasting phase of vasoconstriction evoked by high frequency (60 impulses at 20 Hz) sympathetic nerve stimulation, in the main and deep femoral, the saphenous and the internal maxillary arteries, leaving merely the initial rapid peak of vasoconstriction in these vessels. Furthermore, the vasoconstrictor response was nearly abolished in the kidney and was attenuated in the spleen and main femoral artery, despite maintained neuropeptide Y overflow. The vasoconstrictor response evoked in the kidney by peptide YY, a neuropeptide Y Y_1 and Y_2 receptor agonist, was also nearly abolished in the presence of SR 120107A. This inhibitory effect on the response to exogenous agonist correlated well with the long-lasting inhibition of the response to nerve stimulation in the same tissue. The peptide YY-evoked vasoconstriction in the spleen was not altered by SR 120107A, in accordance with the view that the neuropeptide Y receptor population in this organ consists mainly of neuropeptide Y Y_2 receptors. SR 120107A did not influence the vasoconstrictor effects of α,β-methylene ATP (mATP) or phenylephrine in any of the tissues studied. We conclude that SR 120107A is a potent neuropeptide Y Y_1 receptor antagonist with long duration of action in vivo. Endogenous neuropeptide Y acting on the neuropeptide Y Y_1 receptor is likely to account for the long-lasting component of the reserpine-resistant sympathetic vasoconstriction upon high frequency stimulation in hind limb and nasal mucosa. Furthermore, the peak vasoconstriction in kidney, and to some extent in spleen, is also neuropeptide Y Y_1 receptor mediated.

Keywords: Neuropeptide Y Y_1 receptor antagonist; SR 120107A; Sympathetic vasoconstriction; (Pig)

1. Introduction

Neuropeptide Y is a 36-amino acid peptide, isolated from pig brain (Tatemoto, 1982). Neuropeptide Y has been demonstrated to be colocalized (Lundberg et al., 1982) and coreleased with noradrenaline from sympathetic perivascular nerve terminals (Lundberg et al., 1984), especially upon strong activation (Lundberg et al., 1985). Exogenous neuropeptide Y both exerts potent vasoconstriction (Lundberg and Tatemoto, 1982) and inhibits noradrenaline release (Lundberg and Stjärne, 1984). At least two types of neuropeptide Y receptors are involved in sympathetic vascular control: the neuropeptide Y Y_1 receptor situated mainly postjunctionally and mediating vasoconstriction, and the neuropeptide Y Y_2 receptor, which is located prejunctionally and inhibits noradrenaline release (Wahlestedt et al., 1986; Sheikh et al., 1989). However, the neuropeptide Y Y_1 receptor can also be prejunctional and inhibiting sympathetic transmitter release, as in rabbit vas deferens (Doods and Krause, 1991). The novel neuropeptide Y Y_1 receptor antagonists SR 120107A (1-[2-[2-(2-naphtylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*cis*-cyclohexylmethyl]amidino]phenyl]propionyl]pyrrolidine, (*S*,*R*) stereoisomer) (Serradeil-Le Gal et al., 1994) and BIBP 3226 (Rudolf et al., 1994) have recently been shown to strongly reduce non-adrenergic sympathetic vasoconstriction evoked by high frequency stimulation in guinea-pig vena cava in vitro (Malmström and Lundberg, 1995a,b), given final evidence for the involvement of endogenous neuropeptide Y in sympathetic vasoconstric-

* Corresponding author. Tel.: +46-8-7287958; fax: +46-8-332278.

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tion. However, both types of neuropeptide Y receptors can be postjunctional and mediate vasoconstriction (Lundberg et al., 1988; Modin et al., 1991). The relative contribution of neuropeptide Y Y_1 and Y_2 receptors mediating vasoconstriction differs in various vascular beds. Thus, neuropeptide Y Y_1 receptor activation causes an increase in blood pressure (Modin et al., 1991; Grundemar et al., 1992) due to general vasoconstriction, whereas neuropeptide Y Y_2 receptor agonists have especially prominent vasoconstrictory effects in pig spleen (Lundberg et al., 1988; Modin et al., 1991). Exogenous administration of neuropeptide Y evokes a slowly developing long-lasting vasoconstriction which is similar to that seen upon sympathetic nerve stimulation after blockade of adrenoceptors (Lundberg and Tatemoto, 1982). Considering the diversity of adrenoceptor subtypes, there is a risk that adrenoceptor blocking drugs do not completely block the receptors in vivo. Reserpine depletes nerve terminals of monoamines, including NA, via interference with the granular storage mechanism (Carlsson, 1965). However it also induces increased sympathetic nerve impulse activity (Pernow et al., 1988) resulting in a reduction of the tissue content also of neuropeptide Y due to prolonged and enhanced release in excess of peptide resupply (see Lundberg et al., 1990). This problem can be overcome if nerve impulse traffic is interrupted by transection of sympathetic nerves. The combination of reserpine administration and nerve transection creates a situation with markedly reduced tissue noradrenaline levels, but maintained levels of neuropeptide Y. In this experimental in vivo model, electrical stimulation of sympathetic nerves evokes a long-lasting vasoconstriction in several vascular beds in several species, which mimics the response evoked by exogenous neuropeptide Y administration (see Lundberg et al., 1989a, 1990). The aim of this study was to clarify the possible role of endogenous neuropeptide Y for this response by the use of the selective non-peptide neuropeptide Y Y_1 receptor antagonist, SR 120107A. SR 120107A has been described to possess high affinity for neuropeptide Y Y_1 receptors and virtually no affinity for neuropeptide Y Y_2 receptors in binding studies (Serradeil-Le Gal et al., 1994). Furthermore, SR 120107A has been postulated to have long duration of neuropeptide Y Y_1 receptor antagonizing actions in vivo (Serradeil-Le Gal et al., 1994). Thus, SR 120107A represents a very interesting tool for the study of supposed neuropeptide Y Y_1 receptor mediated phenomena in vivo. In this study we demonstrate the actions of SR 120107A on sympathetic vasoconstriction evoked by endogenously released neuropeptide Y in vivo. To rule out prejunctional effects of SR 120107A, we also measured nerve stimulation-evoked neuropeptide Y overflow from spleen and kidney. Finally, in order to investigate whether the non-adrenergic rapid vasoconstriction in response to single impulse stimulation is mediated by ATP, we used the P_{2x} purinoceptor antagonist suramin (Dunn and Blakeley, 1988), administered i.a. (nasal mucosa) to give local effects.

2. Material and methods

2.1. In vivo study

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

2.2. Surgical preparation

Pigs of either sex (19–23 kg, $n = 6$) were premedicated with ketamine (20 mg kg^{-1} i.m.) and atropine (0.02 mg kg^{-1} i.m.) and anesthetized with sodium pentobarbitone (20 mg kg^{-1} i.v.). Skeletal muscle relaxation was induced with pancuronium (0.5 mg kg^{-1} i.v.). The animals were intubated and ventilated by a respirator (Servo ventilator 900, Siemens-Elema, Sweden). The anesthesial 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, the postganglionic sympathetic nerves to the left kidney and the right sympathetic lumbar chain (level L3-L4) were exposed and sectioned. The left cervical sympathetic trunk was exposed in the neck, and thereafter cut. The incisions were closed, and before the anesthesia was terminated, reserpine (1 mg kg^{-1}) was administered i.v. The following day, the pigs were re-anesthetized as described above, with the exception that they were ventilated by the respirator via a tracheal tube. Into the left femoral vein, a catheter was inserted for infusion of drugs to maintain anesthesia (pentobarbitone 8 mg kg^{-1} h^{-1} i.v.), skeletal muscle relaxation (pancuronium 0.5 mg kg^{-1} h^{-1} i.v.), fluid balance (sodium chloride 154 mM and glucose 28 mM, 2 ml min^{-1}) and to prevent intravascular coagulation (heparin 250 IE kg^{-1} h^{-1}). For measurement of mean arterial blood pressure, a catheter, connected to a Statham P23 AC pressure transducer, was inserted into the left femoral artery. Heart rate was recorded by a tachograph unit triggered by the blood pressure. The blood flow of the splenic artery, the left renal artery, the right main and deep femoral artery, the right saphenous artery (supplying the skin beneath the knee) and the left internal maxillary artery (supplying mainly the nasal mucosa) was measured by ultrasonic flow probes (2RB) placed around the respective vessels. The flow probes were connected to Transonic flowmeters (T202, Transonic Instruments, Ithaca, NY, USA). The skin on the left side of the nose was shaved and a Laser Doppler probe (Periflux PF2, Perimed KB, Stockholm, Sweden) was placed perpendicular to the skin and fixed with tape, in order to monitor superficial blood flow. Bipolar platinum electrodes were placed around the distal branches of the cut postganglionic sympathetic nerves accompanying the splenic and renal arteries, the lumbar sympathetic chain and the cervical sympathetic trunk. One catheter was placed in the main splenic vein and another in the left main renal vein, via side branches, which allowed

local venous blood sampling. The left superficial temporal artery was cannulated with a catheter in a retrograde direction, for local i.a. infusion of drugs. Finally, a catheter was placed in the right brachial artery, for collection of systemic arterial blood. (For further details of the preparations see Lacroix et al., 1988; Pernow and Lundberg, 1989a,b; Modin et al., 1993a,c.) The abdomen was closed and the pigs were allowed to stabilize for 1 h before the experiments were undertaken.

2.3. Experimental procedures

The experiment was initiated by administration of atropine (0.5 mg kg^{-1} i.v.) to prevent any cholinergic vasodilatory response evoked by lumbar sympathetic stimulation in hind limb (Modin et al., 1993c). The same dose of atropine was readministered 4 h later, as the calculated duration of action of atropine is approximately that time. After 15 min, electrical stimulation of the spleen, the kidney, the lumbar sympathetic chain and the cervical sympathetic trunk was performed in series by a Grass stimulator. The stimulation was given as single impulse and as three high frequency bursts of 20 Hz for 1 s (5 ms, 25 V) with an interval of 10 s (a total of 60 impulses). Brief stimulations were chosen to avoid spontaneous decline of neuropeptide Y release (Modin et al., 1993b). During the 20 Hz stimulation of the spleen and kidney, blood samples were collected from the brachial artery and splenic or renal vein respectively, before, after the second burst, after the last burst, 30 s and 2 min after the stimulation for measurement of plasma levels of neuropeptide Y-like immunoreactivity. Nerve stimulations were followed by i.v. bolus injections of peptide YY (120 pmol kg^{-1}) to establish the degree of blockade of vascular neuropeptide Y receptors. Peptide YY was chosen as neuropeptide Y receptor agonist because it acts on both neuropeptide Y Y_1 and Y_2 receptors and because it does not interfere with the neuropeptide Y radioimmunoassay and therefore allows parallel release studies (see Pernow and Lundberg, 1989b). This was followed by i.v. bolus injections of α,β-methylene ATP (mATP) (20 nmol kg^{-1}) and phenylephrine (15 nmol kg^{-1}) to verify the selectivity of the neuropeptide Y receptor blockade. This series of nerve stimulations and exogenous agonists was repeated 10 min after i.v. administration of SR 120107A (1.5 mg kg^{-1}). Thereafter a recovery period of 3 h followed, after which the procedure was undertaken once again. After completion of the final set a 10 min i.a. infusion of suramin (15 mg min^{-1}), into the superficial temporal artery was commenced, during which the cervical trunk was stimulated with a single impulse, and mATP (same bolus as above) was given i.v. This was performed to give a local concentration of suramin high enough to have effects on the nasal vascular response to mATP and to investigate the possible involvement of ATP in the response to single impulse stimulation.

2.4. Determination of neuropeptide Y-like immunoreactivity in plasma

The blood samples were collected in ice-cold tubes containing EDTA (final concentration of 10 mM), centrifuged 10 min (+4°C) whereafter the plasma was pipetted off and stored at −20°C. Neuropeptide Y-like immunoreactivity was determined with radioimmunoassay (using antibody N1) after ethanol extraction. This method is described and evaluated by Theodorsson-Norheim et al. (1985). For characterization of neuropeptide Y-like immunoreactivity in plasma from pig splenic venous effluent see Lundberg et al. (1989b).

2.5. Calculations

Vascular responses to electrical nerve stimulation and exogenous agonist administration are expressed as minimum remaining vascular conductance (calculated as blood flow divided by mean arterial blood pressure, see Stark, 1968), in percentage of basal vascular conductance (prior to vascular response). Vascular responses in the skin, measured as Laser Doppler flow, are expressed in arbitrary units and calculated as percent of basal values. As an aspect of the duration of the vasoconstriction, we use the time it takes for the vascular bed in question to regain 75% of its basal blood flow (prior to stimulation). As indication of transmitter release from the spleen and kidney, the total overflow of neuropeptide Y-like immunoreactivity was calculated as the integrated area of the splenic or renal veno-arterial plasma difference, multiplied by the local arterial plasma flow. The hematocrit was determined after centrifugation of the blood samples. Data in the 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.

2.6. Drugs

Ketamine (Parke-Davis, CA, USA), sodium pentobarbitone (NordVacc, Sweden), atropine (premedication) and sodium heparin (KabiVitrum, Sweden), pancuronium bromide (Organon, Netherlands), reserpine, atropine chloride, α,β-methylene adenosine 5′-triphosphate and phenylephrine hydrochloride (Sigma, St Louis, MO, USA), peptide YY-(1–36) (Peninsula Lab. Europe, Merseyside, UK), suramin (Bayer, Leverkusen, Germany). SR 120107A was synthesized by Albany Molecular Research, Albany, USA. All drugs were dissolved in saline.

3. Results

3.1. Sympathetic nerve stimulation

Single impulse stimulation of the cervical trunk and the lumbar chain (but not of kidney or spleen) evoked rapid

vasoconstrictor responses in corresponding vascular beds. Vascular conductance was reduced to between 81% (saphenous artery) and 89% (deep femoral artery) of basal.

SR 120107A did not have any effect on these vascular responses. Basal vascular conductance was not influenced by SR 120107A except in the spleen, where it increased by 30%. This was accompanied by a slight fall in blood pressure (by an average of 5 mm Hg).

Nerve stimulation at high frequency (three 1 s bursts at 20 Hz) of the splenic, renal, cervical and lumbar sympathetic nerves evoked rapidly developing vasoconstriction in the corresponding vascular bed (Fig. 1). Especially in the main and deep femoral artery, the saphenous artery, the nasal mucosa and skin, this rapid phase was followed by a long-lasting, slowly declining vasoconstriction (Fig. 1). The maximal reductions in vascular conductance ranged from 30% (kidney) to 60% (saphenous artery). The time to recovery of 75% of basal vascular flow varied from 260 s (nasal mucosa) to 370 s (saphenous artery). SR 120107A nearly abolished the vasoconstriction evoked by high frequency stimulation in the kidney (Figs. 1 and 2). The maximal vasoconstriction in spleen and main femoral artery was attenuated whereas the peak responses were unchanged in all other vascular beds, in the presence of SR 120107A (Fig. 2). Three hours after administration of SR 120107A the vascular response in the kidney to high frequency stimulation had partially recovered: only one third of the attenuation remained. The same trend was also observed in the spleen and main femoral artery (although this was not statistically significant) (Fig. 2).

SR 120107A potently inhibited the slowly declining long phase of vasoconstriction in all vascular beds where this was seen after the control stimulation (Fig. 1). The time to recovery of 75% of basal blood flow was much shorter varying from 39 s (deep femoral artery) to 78 s (nasal mucosa), in the presence of SR 120107A. Three hours after administration of SR 120107A the vasoconstriction was partially normalized in the main femoral artery, but the time to recovery of 75% of basal vascular flow was nonetheless shorter than under basal conditions. The same trend toward a recovery of basal vasoconstrictor responses was seen in the other vascular beds as well (although not statistically significant) (Fig. 3).

3.2. Neuropeptide Y-like immunoreactivity overflow

Basal arterial levels of plasma neuropeptide Y-like immunoreactivity were 54 ± 4 pM before and 52 ± 3 pM after SR 120107A just prior to nerve stimulations.

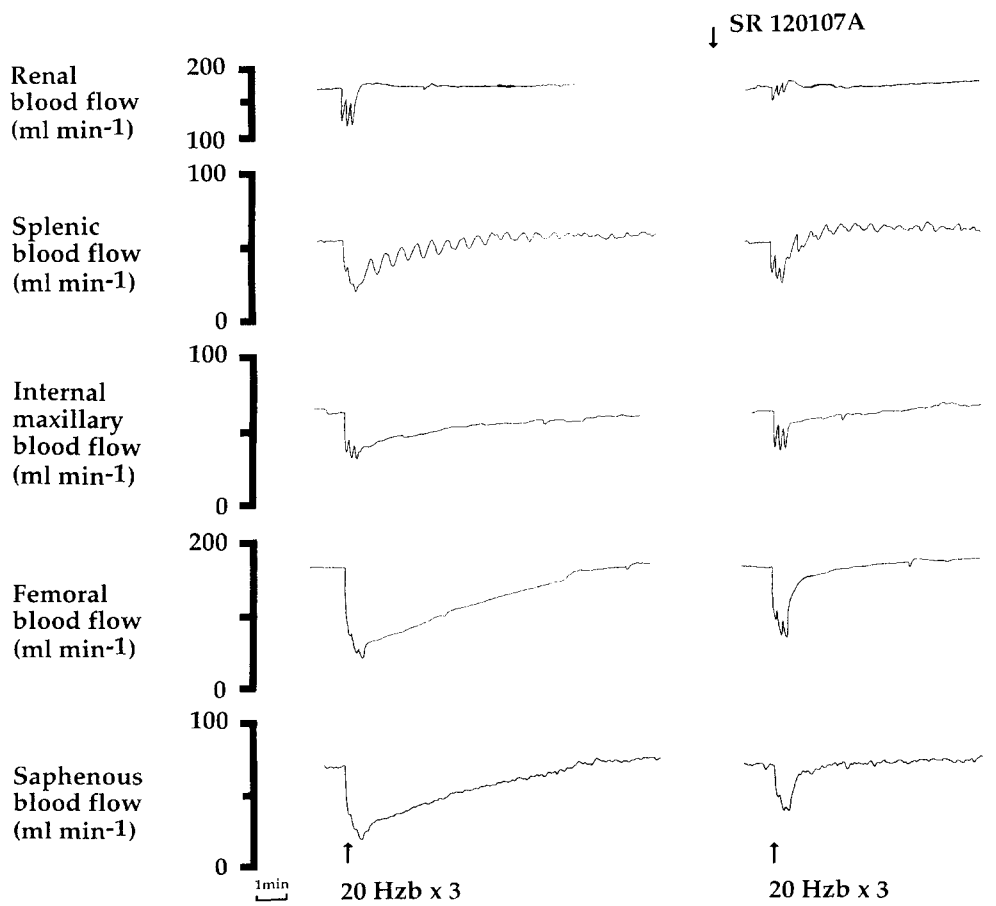

Fig. 1. Original recording of the arterial blood flow of the kidney, spleen, nasal mucosa and hind limb in reserpinized pigs at high frequency sympathetic nerve stimulation with three 1 s bursts of 20 Hz at 10 s intervals before and after SR 120107A (1.5 mg kg^{-1} i.v.).

The overflow of neuropeptide Y-like immunoreactivity from the spleen upon the three 20 Hz burst stimulation was 35 ± 13 pmol. In the presence of SR 120107A the overflow was 31 ± 10 pmol. On the third stimulation (after a 3 h recovery period) the overflow was 19 ± 3 pmol.

The neuropeptide Y-like immunoreactivity overflow

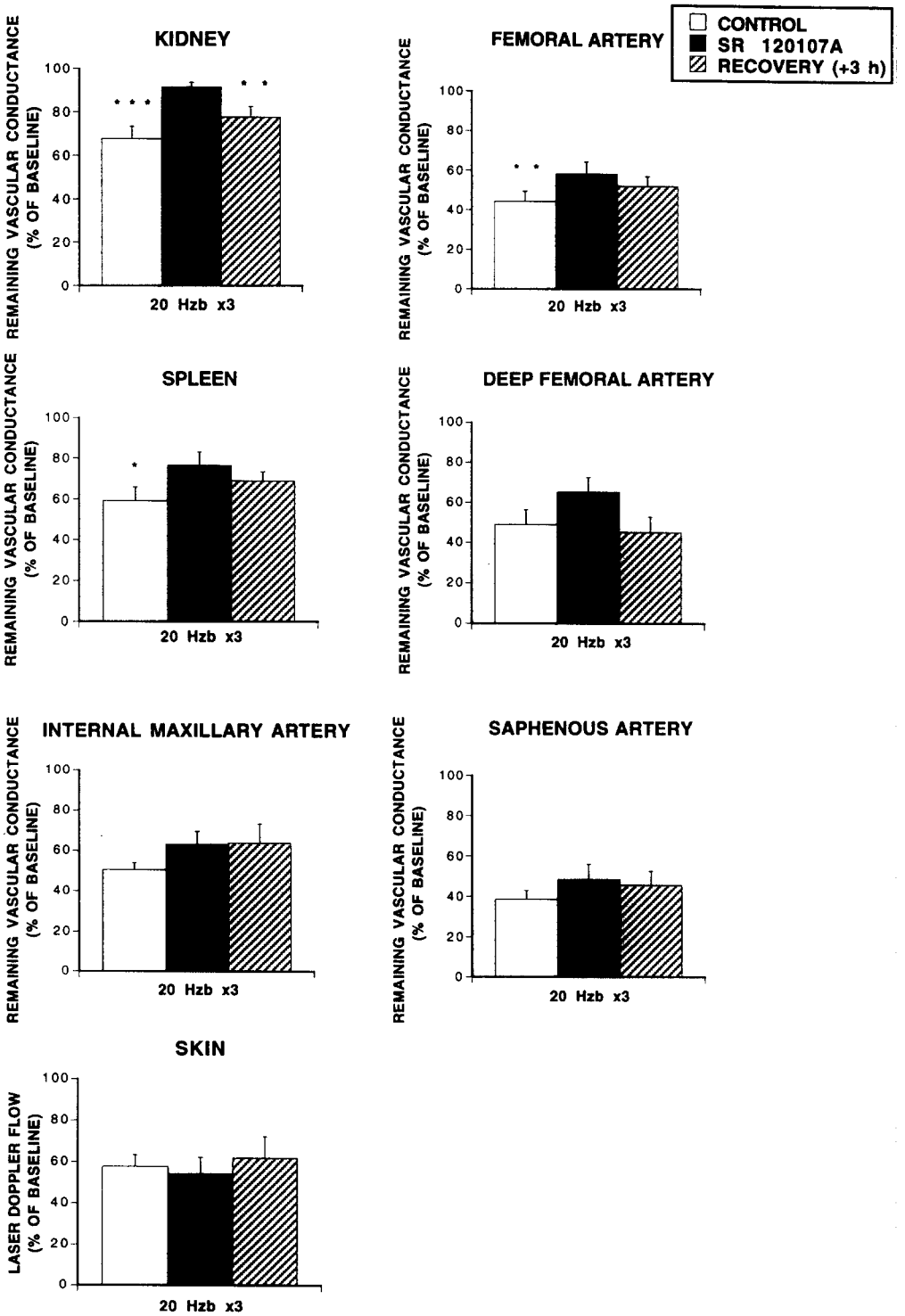

Fig. 2. Changes in vascular conductance or Laser Doppler flow (skin) upon electrical stimulation of sympathetic nerves in the spleen, kidney, cervical trunk and lumbar chain with three 1 s bursts of 20 Hz at 10 s intervals in reserpinized pigs. The vascular response is shown before and after SR 120107A (1.5 mg kg^{-1} i.v.) and 3 h later (recovery). Data are given as means ± S.E.M., $n = 6$. Significant differences compared with the response after SR 120107A are indicated * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

from the kidney upon nerve stimulation was 5.8 ± 0.8 pmol in the control, 6.8 ± 2.6 pmol in the presence of SR 120107A and 4.7 ± 1.3 pmol after the 3 h recovery period (Fig. 4).

3.3. Effects of exogenous agonists

Intravenous administration of peptide YY (120 pmol kg^{-1}) evoked an elevation of mean arterial blood pressure (by almost 30 mm Hg) and vasoconstriction in kidney and spleen (Fig. 5) resulting in a reduction of vascular conductance to 45% and 22% of that prior to drug administration, in the kidney and spleen, respectively. Only marginal effects were seen in the other vascular beds (Table 1). In the presence of SR 120107A, the effect of peptide YY was nearly abolished on arterial blood pressure and in the kidney, whereas in the spleen the response was largely unaltered (Fig. 5). After a 3 h recovery, peptide YY again exerted some effects in the kidney: vascular conductance decreased to 75% of that prior to peptide YY administration (Table 1). There was a strong correlation between the vascular responses evoked by sympathetic nerve stimulation and peptide YY in the kidney: the effects of both types of stimuli were similarly inhibited by SR 120107A, acutely as well as after the 3 h recovery (Fig. 6).

Phenylephrine (15 nmol kg^{-1}) and mATP (20 nmol kg^{-1}) evoked elevated arterial blood pressure (by 40 mmHg and 35 mmHg, respectively) and clearcut vasoconstriction in all vascular beds. SR 120107A had no effect upon these responses (Fig. 5, Table 1).

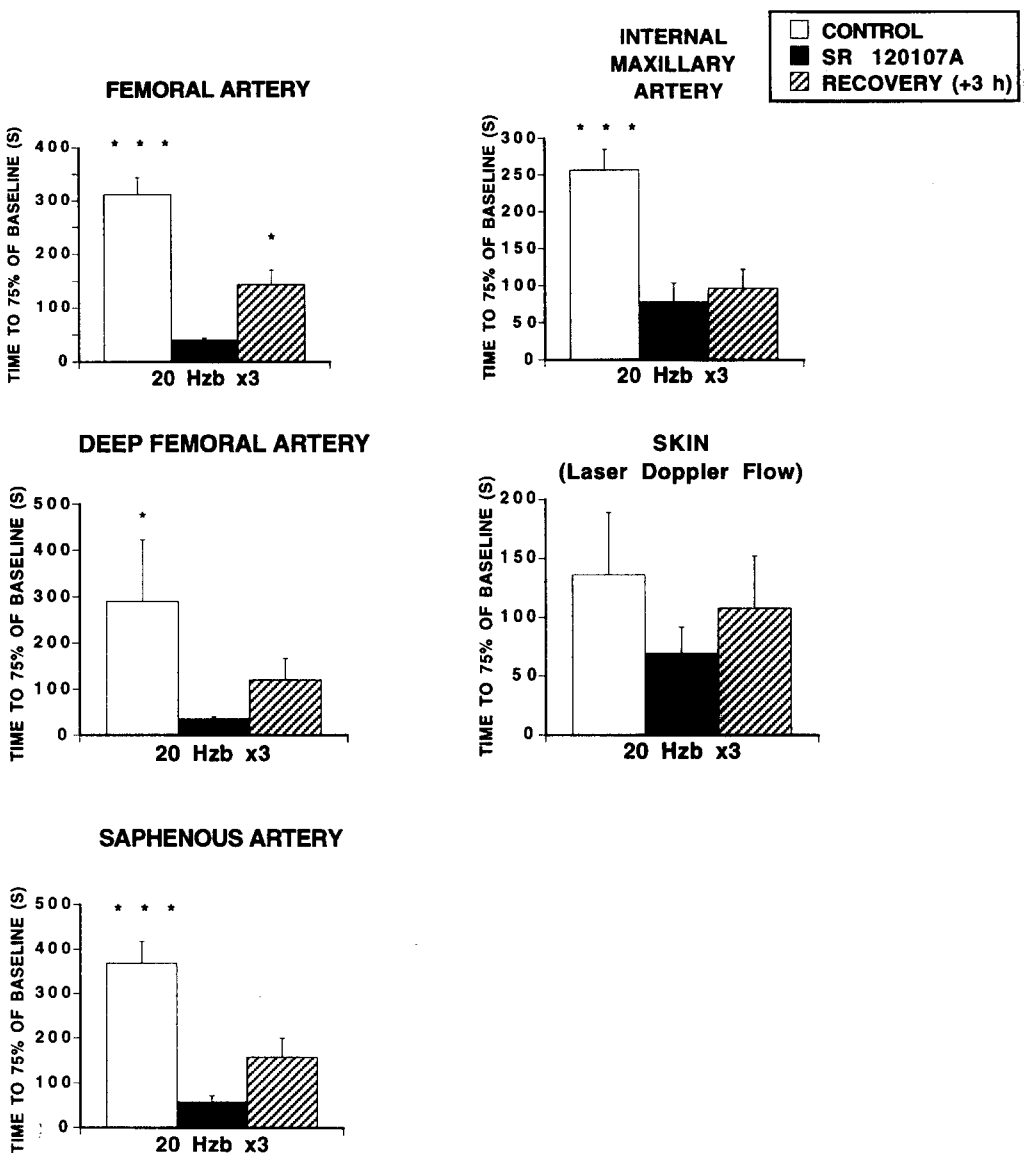

Fig. 3. Time to recovery of 75% of basal blood flow or Laser Doppler flow (skin) after electrical stimulation of sympathetic nerves in the cervical trunk and lumbar chain with three 1 s bursts of 20 Hz at 10 s intervals in reserpinized pigs. The vascular blood flow recovery time is shown before and after SR 120107A (1.5 mg kg^{-1}) and 3 h later (recovery). Data are given as means ± S.E.M., $n = 6$. Significant differences compared with the 75% blood flow recovery time after SR 120107A are indicated * $P < 0.05$, *** $P < 0.001$.

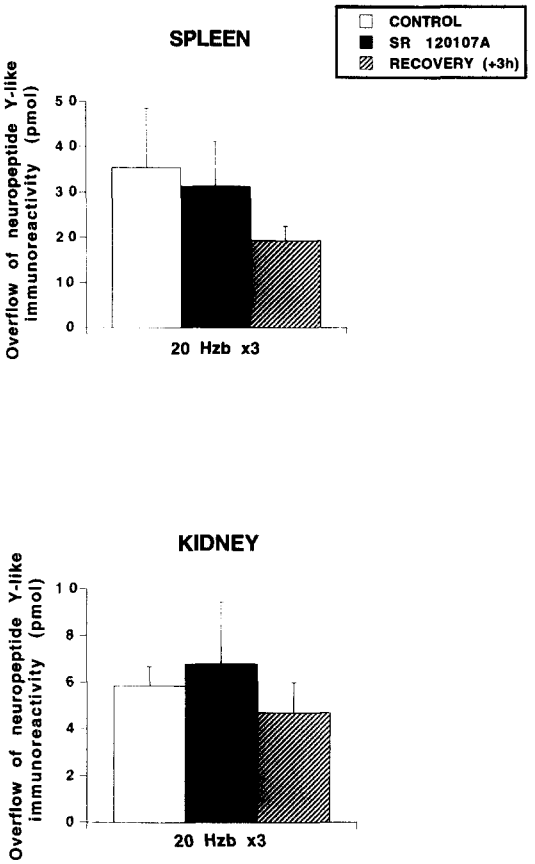

Fig. 4. Integrated overflow of neuropeptide Y-like immunoreactivity from the spleen and kidney evoked by electrical sympathetic nerve stimulation, with three 1 s bursts of 20 Hz at 10 s intervals. The neuropeptide Y-like immunoreactivity overflow is shown before and after SR 120107A (1.5 mg kg^{-1}) and 3 h later (recovery). Data are given as means ± S.E.M., $n = 4$. No significant differences in the neuropeptide Y-like immunoreactivity overflow were observed.

Local i.a. infusion of the P_{2x} purinoceptor antagonist suramin into the superficial temporal artery (15 mg min^{-1}, for 10 min) inhibited the vasoconstrictor response in the nasal mucosa to i.v. mATP. Thus, the vascular conductance remaining after injection of mATP was higher in the presence than in the absence of suramin, 86% vs. 56% of baseline ($P < 0.05$). However, suramin did not inhibit the vasoconstrictor response evoked by single impulse stimulation of the cervical trunk.

4. Discussion

The present study shows that the selective neuropeptide Y Y_1 receptor antagonist SR 120107A nearly abolished the reserpine resistant long-lasting phase of the vasoconstriction evoked in pig nasal mucosa and hind limb by high-frequency sympathetic stimulation. The maximal vasoconstrictor response evoked by sympathetic nerve stimulation was attenuated by SR 120107A, most strongly in the kidney, but also to some extent in the spleen and the main femoral artery. The selectivity of action of SR 120107A on neuropeptide Y Y_1 receptors is proven by its lack of effect on vascular responses evoked by mATP, phenylephrine (present data) and angiotensin II (unpublished data). That endogenous neuropeptide Y acting on the neuropeptide Y Y_1 receptor mediates this long-lasting nerve mediated vasoconstriction is supported by earlier findings like the ability of exogenous neuropeptide Y to mimic these responses (Lundberg et al., 1986) and that neuropeptide Y is preferentially released at high frequency stimulation (Lundberg et al., 1989b) or strong reflex sympathetic activation (Lundberg et al., 1985). The inhibitory effects of SR 120107A seem to be exclusively postjunctional at vascular neuropeptide Y Y_1 receptors, since the neuropeptide Y-like immunoreactivity overflow at stimulation of kidney and spleen was unaltered in its presence. Therefore, prejunctional effects – at least in these vascular beds – can be ruled out. In contrast to the non-peptide endothelin receptor antagonist bosentan, which elevates basal plasma endothelin levels in the pig (Weitzberg et al., 1994), SR 120107A did not influence basal neuropeptide Y levels. This suggests that circulating neuropeptide Y is not depen-

Table 1
Remaining vascular conductance in percentage of basal (prior to agonist action)

Agonist	Tissue	Control	SR 120107A	Recovery (+3 h)
Peptide YY	Kidney	44.8 ± 3.9	93.8 ± 2.4 [a]	74.8 ± 2.9 [a]
(120 pmol kg^{-1})	Spleen	22.2 ± 5.1	21.7 ± 4.0	33.5 ± 3.5
	Main femoral artery	98.5 ± 5.4	107.0 ± 4.1	99.3 ± 4.8
	Internal maxillary artery	90.6 ± 5.2	101.4 ± 4.7	98.6 ± 4.5
mATP	Kidney	25.7 ± 3.1	22.0 ± 4.4	29.5 ± 5.0
(20 nmol kg^{-1})	Spleen	14.7 ± 3.3	12.2 ± 2.0	16.7 ± 1.9
	Main femoral artery	48.8 ± 7.0	62.2 ± 12.4	49.2 ± 5.6
	Internal maxillary artery	55.5 ± 5.4	56.3 ± 4.6	62.7 ± 8.2
Phenylephrine	Kidney	56.2 ± 2.5	61.5 ± 5.7	63.2 ± 5.7
(15 nmol kg^{-1})	Spleen	23.2 ± 4.1	31.5 ± 7.7	36.2 ± 7.6
	Main femoral artery	37.5 ± 4.9	46.7 ± 9.4	41.5 ± 7.7
	Internal maxillary artery	48.7 ± 3.7	52.3 ± 4.9	54.7 ± 4.9

Values are means ± S.E.M., $n = 6$. Significant differences compared with control are indicated. [a] $P < 0.001$.

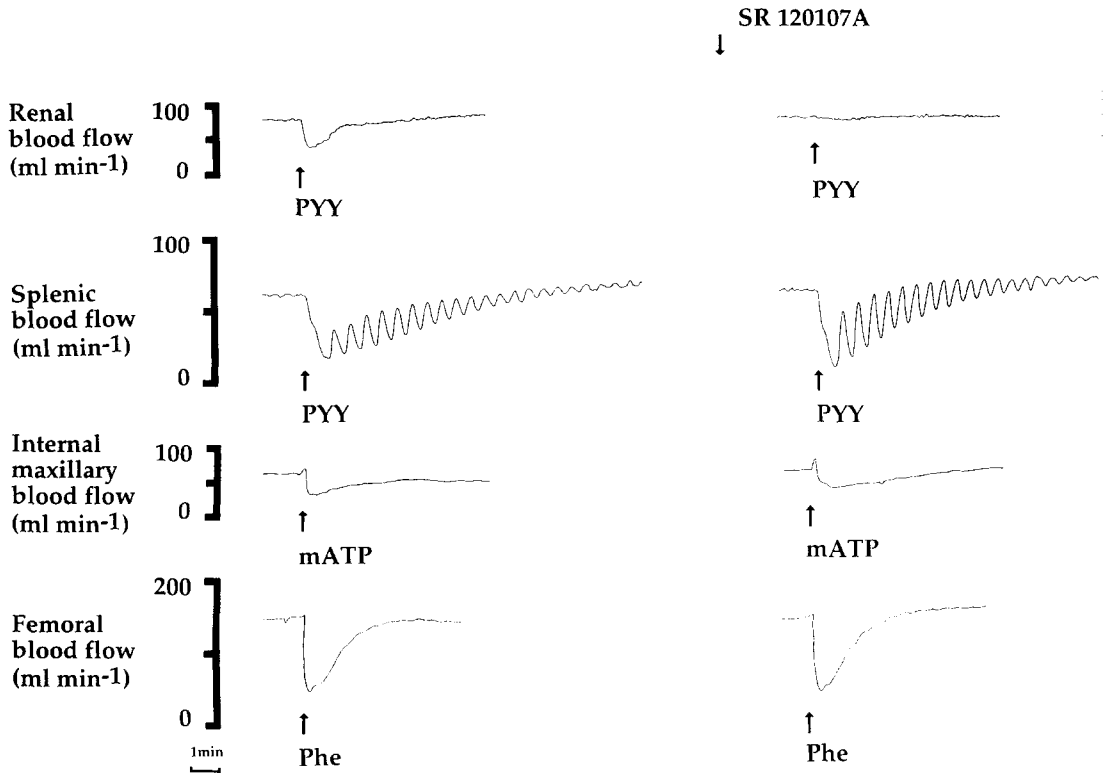

Fig. 5. Original recording of arterial blood flow in the kidney, spleen, nasal mucosa and hind limb in a reserpinized pig given i.v. peptide YY (120 pmol kg^{-1}), mATP (20 nmol kg^{-1}) and phenylephrine (15 nmol kg^{-1}), where indicated, before and after SR 120107A (1.5 mg kg^{-1}).

dent on binding to tissue receptors displaceable by SR 120107A. Unchanged neuropeptide Y-like immunoreactivity overflow upon high frequency nerve stimulation in the presence of SR 120107A implies that the total peptide overflow reaches levels where any extra venous overflow of neuropeptide Y, due to transmitter not binding to receptors occupied by SR 120107A, can not possibly be distinguished.

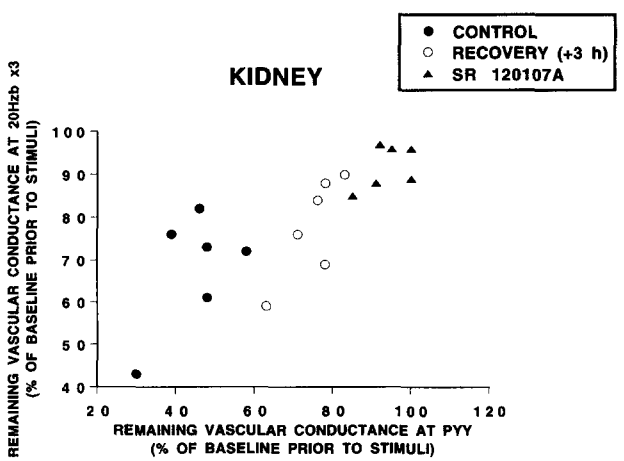

Fig. 6. Correlation between changes in vascular conductance in the kidney evoked by i.v. administered peptide YY (120 pmol kg^{-1}) and sympathetic nerve stimulation (three 1 s bursts of 20 Hz at 10 s intervals) before and after SR 120107 A and 3 h later (recovery).

The reserpine-resistant vasoconstriction in the kidney and spleen is of relatively short duration, which may be due to the presence of effective autoregulation of blood flow, especially in the kidney. The endothelium in these tissues is much more permeable than that in nasal mucosa and hind limb, and this may also contribute, since the more permeable the endothelium, the more easily will neuropeptide Y diffuse away from receptor sites in the organ. Although the maximal vasoconstriction in kidney in response to sympathetic nerve stimulation is likely to be mediated to a major extent by neuropeptide Y acting on neuropeptide Y Y_1 receptors, this is not the case in the other vascular beds. Nor do the rapid vasoconstrictor responses in hind limb and nasal mucosa to single impulse stimulation in reserpinized pigs seem to be mediated by neuropeptide Y. The lack of effect of SR 120107A on these small vasoconstrictor responses represents further evidence of selectivity and is in accordance with the view that peptides in autonomic motor nerves are mainly released upon high frequency stimulation (Lundberg et al., 1989a). These findings suggest that some sympathetic transmitter other than noradrenaline and neuropeptide Y, or some receptor other than the neuropeptide Y Y_1 receptor mediates these actions, although it cannot be completely excluded that the neuropeptide Y Y_1 receptor blockade is incomplete during the initial phase of the nerve response when neuropeptide Y release and local tissue concentration ought to be at its peak. Since the reserpine dosage used in

this study is known to cause a marked (about 90%) depletion of tissue noradrenaline in the pig (Lundberg et al., 1990) the mediator of rapid vascular responses to sympathetic stimulation in reserpinized pigs with neuropeptide Y Y_1 receptor blockade is unlikely to be noradrenaline. One possible alternative mediator is ATP. The P_{2x} purinoceptor antagonist suramin given locally in the internal maxillary artery potently inhibited the nasal vascular response evoked by exogenous administration of mATP. In contrast, no inhibitory effect was seen on the rapid vasoconstriction evoked by single impulse stimulation. This apparent discrepancy might be due to the fact that endogenous ATP released by nerve stimulation probably reaches higher concentrations locally in the neuromuscular junction than does mATP given intravenously. Furthermore, the antagonist suramin is a large molecule that may not easily diffuse into the tissue where it exerts its effects. Thus, in our view there is nothing in the present data that excludes the possibility that ATP is the mediator of the rapid vascular response to sympathetic stimulation that remains in reserpinized pigs with neuropeptide Y Y_1 receptor blockade. However, earlier experiments using in vivo tachyphylaxis with mATP have not favored ATP as the mediator of the vascular response evoked by single impulse stimulation in pig nasal mucosa (Lacroix et al., 1989). Further experiments and more potent P_{2x} purinoceptor antagonists will be needed to resolve this issue. The release of ATP upon high-frequency stimulation, in contrast to single impulse stimulation, is likely to be enhanced after reserpine treatment since the prejunctional inhibitory action on ATP release via noradrenaline acting on α_2-adrenoceptors (Ramme et al., 1987) is eliminated in this experimental model. In addition, suramin has been reported to inhibit not only vascular P_{2x} receptors, but also prejunctional P_{2y} receptors, which may further alter transmitter release (Von Kügelgen et al., 1993).

SR 120107A abolished the vasoconstrictor response evoked by i.v. administered peptide YY in the kidney, but did not affect the response in the spleen. This result further supports the selectivity of this compound and is in accordance with the suggestion that different neuropeptide Y receptor subtypes mediate the peptide YY effect in kidney (Y_1) and spleen (Y_2) (see Lundberg et al., 1988). Since the maximal vasoconstriction in response to sympathetic nerve stimulation in the spleen, but not the response to the exogenous neuropeptide Y Y_1 and Y_2 receptor agonist peptide YY given i.v. was inhibited by SR 120107A, neuronally released neuropeptide Y seems to preferentially activate neuropeptide Y Y_1 receptors also in this tissue. In the kidney, there was a strong correlation between the inhibitory actions of SR 120107A on the vasoconstrictor responses evoked by sympathetic nerve stimulation and those evoked by exogenous peptide YY. Not only does this suggest that the vasoconstriction which is attenuated is mediated by neuropeptide Y Y_1 receptors, it also indicates that a significant proportion of the inhibitory actions of SR 120107A remains 3 h after administration. In a parallel study on this reserpinized pig model, where instead the neuropeptide Y Y_1 receptor antagonist BIBP 3226 was used, largely similar conclusions were drawn (Lundberg and Modin, 1995). The most notable difference between BIBP 3226 and SR 120107A is that BIBP 3226 clearly possesses a shorter duration of action as antagonist at neuropeptide Y Y_1 receptors. Apart from this, both these neuropeptide Y Y_1 receptor antagonists attenuate the maximal response to high frequency stimulation in kidney and spleen, while it is mainly the duration of the response that is influenced in other vascular beds. A slight fall in mean arterial blood pressure and an increase in splenic blood flow are observed after both antagonists. These effects may not be related to neuropeptide Y Y_1 receptor blockade, however. Thus, also the enantiomer to BIBP 3226, BIBP 3435, which lacks effect on the neuropeptide Y Y_1 receptor, has this effect on splenic blood flow and mean arterial blood pressure (see Lundberg and Modin, 1995). Therefore neuropeptide Y Y_1 receptors are less likely to be involved in basal blood pressure regulation in this reserpinized pig model.

We conclude that SR 120107A is a potent neuropeptide Y Y_1 receptor antagonist with long duration of action in vivo. Furthermore, we have given further strong support for the suggestion that endogenous neuropeptide Y acting on the neuropeptide Y Y_1 receptor mediates the long-lasting reserpine resistant vasoconstriction in response to high frequency stimulation in several vascular beds, as well as the maximal vasoconstrictor response, especially in the kidney, in pigs in vivo.

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