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Pharmacology of H 394/84, a dihydropyridine neuropeptide Y Y_1 receptor antagonist, in vivo

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Abstract

The object of the present paper was to investigate the in vivo pharmacological profile of the dihydropyridine neuropeptide Y Y_1 receptor antagonist 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 (H 394/84). The renal vasoconstrictor response to neuropeptide Y in anaesthetized rats was dose-dependently antagonized by H 394/84 (ID_{50} value = 41 ± 4 nmol/kg/min), whereas the renal vascular responses to noradrenaline and angiotensin II were only slightly affected by H 394/84 (500 nmol/kg/min). In pigs pretreated with reserpine and transection of sympathetic nerves (depleted of noradrenaline), H 394/84 dose-dependently antagonized renal and femoral vasoconstrictor responses evoked by sympathetic nerve activation (neuronally released neuropeptide Y) and exogenous neuropeptide Y. Significant inhibition was seen already at 1.0 nmol/kg/min, when plasma levels of the antagonist reached 29 ± 4 nM. Around 70% of the antagonism remained 90 min after H 394/84 was given. The disposition of H 394/84 fits a biexponential model with initial and terminal half-lives of 2.6 and 48 min, respectively. H 394/84 (100 nmol/kg/min) did not inhibit vascular responses to neuropeptide Y Y_2 receptor-, α − adrenoceptor- or purinoceptor-activation in the pig in vivo. It is concluded that H 394/84 is a potent neuropeptide Y Y_1 receptor antagonist with rather long duration of action in vivo. The selectivity and specificity in vivo is more than 100-fold, and H 394/84 antagonizes vascular responses to exogenous and endogenous, neuronally released, neuropeptide Y with similar potency.

Keywords: Neuropeptide Y Y_1 receptor antagonist; Dihydropyridine; H 394/84; Sympathetic; Vasoconstriction

1. Introduction

Neuropeptide Y, a now well-known sympathetic co-transmitter, is co-stored and co-released with noradrenaline, especially upon stronger nerve activation (see Lundberg, 1996). In most vessels, neuropeptide Y acts on the neuropeptide Y Y_1 receptor to mediate vasoconstriction (see Malmström, 1997), but activation of neuropeptide Y Y_2 receptors is known to exert vasoconstrictor responses in some vascular beds, e.g. the pig spleen (Modin et al., 1991; Malmström, 1997). Studies in the pig in vivo have shown that while normally the neuronal release of neuropeptide Y seems restricted, presumably due to an effective prejunctional α-adrenergic inhibition (Lundberg, 1996), and noradrenaline seems to be the primary mediator of sympathetic vascular responses (Malmström and Lundberg, 1996), this situation may be reversed. Thus, in pigs pretreated with reserpine in combination with transection of sympathetic nerves, noradrenaline levels are depleted to the extent that neuropeptide Y is the primary mediator of sympathetic vasoconstrictor responses in several vascular beds (Lundberg and Modin, 1995; Malmström et al., 1996).

Although there exist several non-peptide antagonists for the neuropeptide Y Y_1 receptor (see Lundberg et al., 1996), the search for more potent tools is constantly in progress aiming to facilitate future studies on the involvement of neuropeptide Y in physiology and pathophysiology of sympathetic transmission. More recent developments include a series of non-peptidergic dihydropyridine neuropeptide Y receptor antagonists with high affinity for

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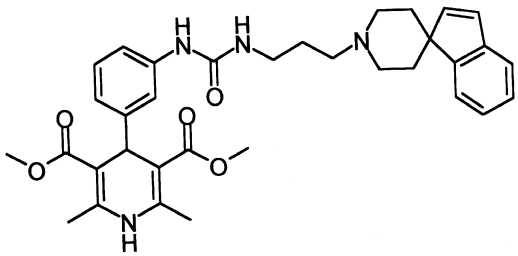

Fig. 1. Chemical structure of the dihydropyridine neuropeptide Y Y_1 receptor antagonist H 394/84.

the neuropeptide Y Y_1 receptor and purportedly much weaker affinities for α_1-adrenoceptors and Ca^{2+} channels (Poindexter et al., 1996). Hence, these compounds were considered selective neuropeptide Y Y_1 receptor antagonists.

In the present study, the antagonistic potency and specificity of one of these compounds, 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), was investigated in the anaesthetized rat. Furthermore, the antagonistic properties of H 394/84 were studied on vascular responses evoked by exogenous neuropeptide Y and endogenous, neuronally released, neuropeptide Y. For this purpose, the reserpine-treated pig in vivo model was used, where neurogenically released neuropeptide Y is the primary mediator of sympathetic vasoconstrictor responses in kidney and hind limb (Malmström et al., 1997). Furthermore, the short-lasting sympathetic vasoconstrictor response in kidney is mimicked by the renal vascular response to intravenous (i.v.) neuropeptide Y injections, whereas the long-lasting sympathetic vasoconstrictor response in hind limb is mimicked by the vascular response to local intra-arterial (i.a.) neuropeptide Y injection, giving a feasible comparison of the antagonistic effects (Malmström et al., 1997). The aim of the study was to compare the antagonistic effects of different doses of H 394/84 on these vascular responses, to correlate the actions to the circulating plasma levels of the compound and to present a pharmacokinetic profile of the antagonist in vivo.

2. Materials and methods

2.1. In vivo studies

These studies were approved by the local ethics committees for animal research.

2.2. Anaesthetized rats in vivo

Male Sprague–Dawley rats (350–425 g, Charles River Sweden) were anaesthetized with sodium pentobarbitone (70 mg/kg i.p.), and tracheotomized with a polyethylene catheter (PE240) to facilitate spontaneous breathing. A catheter (PE25) was inserted into the tail artery for recording of mean arterial pressure. Heart rate was measured by means of a ratemeter from the pulsating arterial pressure signal. The anaesthesia was maintained by a continuous infusion of pentobarbitone in the tail artery (10–12 mg/kg/h). Catheters (PE25) were inserted into the right and left jugular veins for infusion of drugs. The body temperature was measured by a rectal probe and maintained by means of a thermostatically controlled heating pad at 37.5°C. The left renal artery was dissected free and a small probe (Transonic 0,7 VB42) was placed around the artery for blood flow measurement.

After completion of surgery, the rats were allowed to stabilize for 30 min whereafter the experiment started. Mean arterial pressure, heart rate, and renal blood flow were continuously recorded during the experiments. Neuropeptide Y (1 nmol/kg) was given twice as i.v. injections at 20-min intervals, the second of which served as control. H 394/84 was given as consecutive 20-min i.v. infusions at three increasing doses (5, 50 and 500 nmol/kg/min). In two rats, H 394/84 was given as 20-min infusions at 5, 16.5, 50 and 165 nmol/kg/min. H 394/84 was dissolved in a vehicle of polyethylene glycol, dimethylacetamide and saline (4–40%, 0.4–40%, 20–95.6% v/v), the composition of which depended on the H 394/84 dose. In a separate series, the vehicles alone were administered consecutively at the same infusion rate (50 μl/kg/min) and concentrations as during the infusions of H 394/84. Fifteen minutes after the start of infusion of each dose of H 394/84 or vehicle, neuropeptide Y was injected and the responses were recorded. In another series, either angiotensin II (48 pmol/kg) or noradrenaline (12 nmol/kg) was injected i.v. according to the same protocol as for the neuropeptide Y injections, and thus given before and 15 min into the consecutive 20-min infusions of either H 394/84 (5, 50 and 500 nmol/kg/min) or the corresponding vehicle.

2.3. Anaesthetized pigs in vivo

Male domestic pigs (17–19 kg) were premedicated with ketamine (20 mg/kg intramuscular, i.m.) and atropine (0.02 mg/kg i.m.) and thereafter anaesthetized with sodium pentobarbitone (20 mg/kg intravenously, i.v.), intubated and ventilated by a respirator (Servo ventilator 900, Siemens-Elema, Sweden), whereafter skeletal muscle relaxation was induced (pancuronium, 0.5 mg/kg i.v.). Before administration of pancuronium, the anaesthesial depth was checked by pinching the interdigital skin. The retroperitoneal space was reached via a flank incision below the left costal margin, where the postganglionic sympathetic nerves to the left kidney and the sympathetic lumbar chains of both sides (level L3–L4) were exposed

and sectioned. The incision was closed and reserpine (1 mg/kg i.v.) was administered before extubation.

The pigs, pretreated with reserpine, were re-anaesthetized (see above) 24 h later 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. Another catheter was placed into the left brachial artery, to allow collection of systemic bloodsamples. A catheter was also inserted into the right brachial vein for infusion of drugs to maintain anaesthesia (sodium pentobarbitone, 8 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 artery, the left renal artery and the femoral arteries of both sides, for continuous monitoring of local blood flows. Electrodes were placed on the distal ends of the cut left and right lumbar sympathetic chain (supplying hind limbs) and the left renal sympathetic nerve, to allow electrical stimulation. The saphenous arteries of both hind limbs were cannulated in a retrograde direction with catheters, for local intra-arterial (i.a.) injection of neuropeptide Y. The abdomen was then closed, whereafter the pigs were allowed to stabilize for 1 h before the experiments were commenced.

Atropine (0.5 mg/kg i.v.) was administered every fourth hour to prevent any cholinergic vasodilatory response in hind limb upon sympathetic nerve stimulation (Modin et al., 1993). Electrical sympathetic nerve stimulations (two 20-Hz bursts, 1 s each, at a 10-s interval (5 ms, 25 V)) were performed by a Grass stimulator. These were followed by an i.v. bolus injection of neuropeptide Y (230 pmol/kg) and i.a. bolus injections of neuropeptide Y (1.2 nmol) into the saphenous arteries bilaterally. The doses of neuropeptide Y were chosen to evoke roughly similar vascular responses as sympathetic nerve stimulation in kidney and hind limb, respectively. H 394/84 was given as consecutive 50-min i.v. infusions at increasing doses between 100 pmol/kg/min to 100 nmol/kg/min (equal to between 62 ng/kg/min and 62 μg/kg/min), each at 15-min intervals. Systemic arterial blood samples were collected before and at the 30th, 40th and 50th minute of each infusion, and furthermore 1, 2, 5, 10, 15, 30, 60, 90 and 120 min after cessation of the last infusion of H 394/84 (100 nmol/kg/min during 50 min), to determine the plasma levels of H 394/84 and to evaluate the pharmacokinetics of the compound. The set of nerve stimulations and neuropeptide Y injections was repeated between the 30th and 50th minute of each infusion, and once again 90 min after cessation of the last infusion of H 394/84. These vascular responses studied are reproducible and not susceptible to any spontaneous decline as has been demonstrated in a previous study (Malmström et al., 1997). In one series ($n = 4$), i.v. injections of α,β-methylene ATP (20 nmol/kg), the α_1-adrenoceptor agonist phenylephrine (15 nmol/kg) and the neuropeptide Y Y_2 receptor agonist *N*-acetyl [Leu^{28},Leu^{31}]neuropeptide Y-(24–36) (560 pmol/kg) were given before and during the third and fourth infusions of H 394/84 (10 and 100 nmol/kg/min) to investigate the specificity and selectivity of the putative neuropeptide Y Y_1 receptor blockade. The vehicle for H 394/84 was ethanol (0.01–10% v/v, depending on the dose given) and saline. In one series ($n = 3$), the set of nerve stimulations and neuropeptide Y injections was repeated during infusion of the high-dose H 394/84 vehicle (ethanol 10% v/v and saline) alone.

2.4. Determination of H 394/84 in plasma

Blood samples were collected in prechilled tubes containing EDTA (final concentration of 10 mM), centrifuged for 10 min (+4°C), whereafter the plasma was pipetted off and stored at −20°C until being analysed for H 394/84 by high performance liquid chromatography. Two hundred-microliter plasma was extracted with 4 ml of a mixture of dichloromethane (25%), diethylether (74%) and 2-butanol (1%). The organic phase was transferred to a new tube and evaporated to dryness under nitrogen. The residue was redissolved in mobile phase and injected onto the liquid chromatography-system. H 394/84 was detected by a fluorescence detector monitored at excitation wavelength 378 nm and emission wavelength 435 nm, respectively. Limit of quantitation was 25 nM. With use of an adequate internal standard, the s.d. of repeatability (the intra-day coefficient) was 0.9–2.4% during the analyses.

2.5. Calculations

Vascular responses are calculated as minimum remaining vascular conductance, i.e. blood flow divided by mean arterial pressure (see Stark, 1968), in percentage of basal vascular conductance (prior to vascular response), and expressed in percentage of the control vascular response (seen before H 394/84 was given). 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 or the Student's *t*-test (paired samples) where applicable. The pharmacokinetic variables for H 394/84 were estimated by non-linear regression analysis, using WinNonlin™ Pro version 1.5 (Scientific Consulting, Apex, NC, USA). The calculations were based on concentrations at the two highest dose levels, since other concentrations were just above or below the limit of quantitation. The time course of H 394/84 concentration was best described by the biexponential function $C = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t}$ where the coefficients C_1 and C_2 and the exponents λ_1 and λ_2 describe the mono-

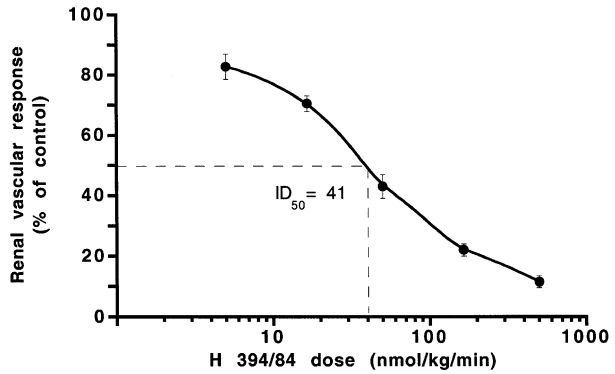

Fig. 2. Inhibitory effects of increasing doses of H 394/84 on the renal vasoconstrictor response evoked in anaesthetized rats by neuropeptide Y (1 nmol/kg, i.v.). Data are presented as means ± S.E.M., $n = 5$ ($n = 2$ for H 394/84 16.5 and 165 nmol/kg/min). The dashed line indicates the ID_{50} value of the antagonism exerted by H 394/84.

exponential decline of the plasma concentration (C) of H 394/84, with time (t). Weighting was performed according to $1/C_{pred^2}$, where C_{pred} is the predicted concentration. All pigs were individually fitted. All pharmacokinetic parameters were obtained from these computer fits.

2.6. Drugs

Ketamine (Parke-Davis, CA, USA), sodium pentobarbitone (NordVacc, Sweden), atropine (premedication) and sodium heparin (KabiVitrum, Sweden), pancuronium bromide (Organon, The Netherlands), reserpine, atropine chloride, phenylephrine hydrochloride, angiotensin II, noradrenaline and α,β-methylene ATP (Sigma, MO, USA), neuropeptide Y, *N*-acetyl [Leu^{28},Leu^{31}]neuropeptide Y-(24–36) (Auspep, Australia). 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) (AstraZeneca R&D Mölndal, Sweden).

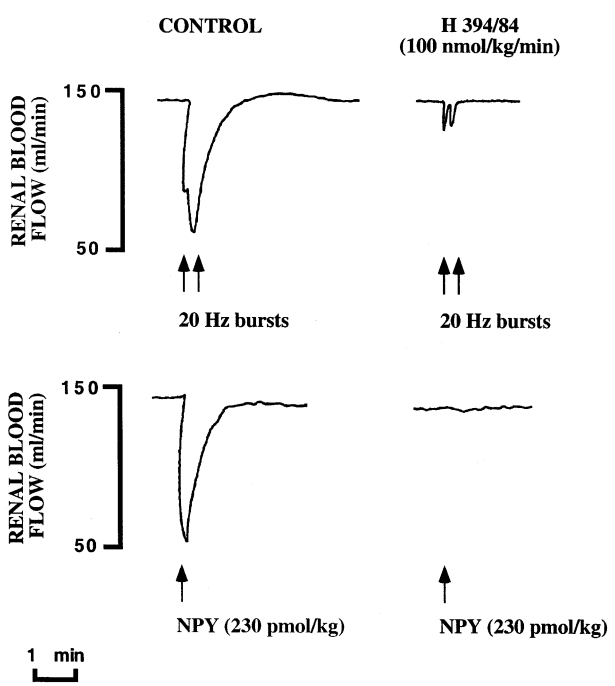

Fig. 3. Original recording of renal arterial blood flow in the reserpine-treated pig in vivo. Vasoconstrictor responses to high frequency stimulation of the renal sympathetic nerves (two 1-s bursts at 20 Hz at 10-s intervals) and exogenous neuropeptide Y (230 pmol/kg, i.v.) are shown before (control) and during infusion of H 394/84 (100 nmol/kg/min).

3. Results

3.1. Effects of H 394/84 in the anaesthetized rat

Basal heart rate, mean arterial pressure and renal blood flow in the rat were not affected by the lower doses of H 394/84 (5 and 50 nmol/kg/min), whereas at the highest dose (500 nmol/kg/min) mean arterial pressure decreased

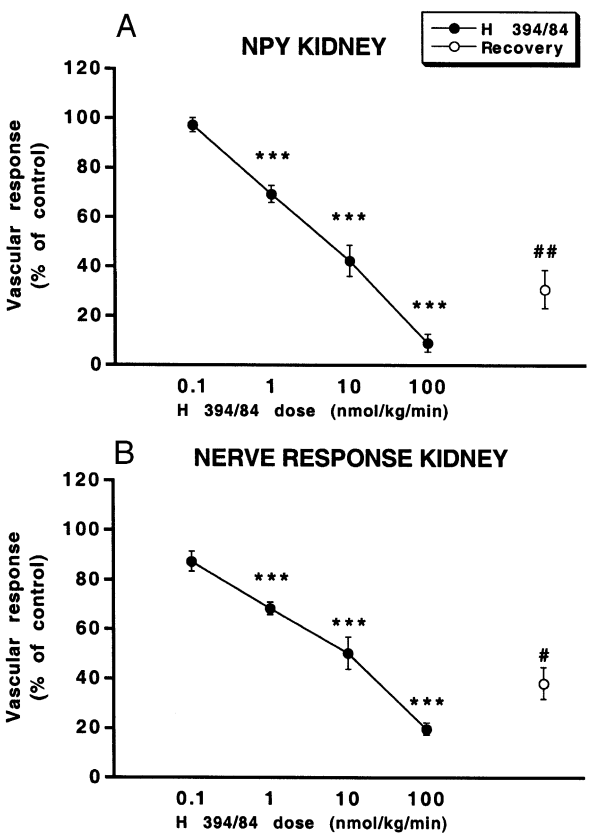

Fig. 4. Inhibitory effects of increasing doses of H 394/84 on the renal vasoconstrictor responses to i.v. neuropeptide Y administration (230 pmol/kg) (A) and sympathetic nerve stimulation (B) in the reserpine-treated pig in vivo. The vascular responses (expressed as % of the control response) are shown during consecutive 50-min infusions of H 394/84 (0.1–100 nmol/kg/min) and after a recovery period of 90 min. Data are given as means ± S.E.M., $n = 6$. Significant differences compared to control are indicated $^{***}P < 0.001$. Significant differences between H 394/84 (100 nmol/kg/min) and recovery are indicated $^{\#}P < 0.05$, $^{\#\#}P < 0.01$.

from 99 ± 4 to 90 ± 3 mm Hg, without any significant effects on heart rate and renal blood flow. This effect was not different from vehicle (polyethylene glycol/dimethylacetamide/saline (% v/v) 40/40/20) alone. Neuropeptide Y (1 nmol/kg, i.v.) evoked elevation of mean arterial pressure (by 23 ± 2 mm Hg) and vasoconstriction in the kidney (renal blood flow reduced by 52 ± 2%). H 394/84 exerted dose-dependent antagonism on the renal vasoconstrictor effect to neuropeptide Y (Fig. 2). Significant inhibition was seen during the lowest dose of H 394/84 when 83 ± 4% ($P < 0.05$) of the renal vasoconstrictor effect remained. The ID_{50} value was 41 ± 4 nmol/kg/min. Angiotensin II (48 pmol/kg, i.v.) and noradrenaline (12 nmol/kg, i.v.) elevated mean arterial pressure (by 28 ± 2 and 42 ± 2 mm Hg, respectively) and reduced renal blood flow by 60 ± 3% and 33 ± 4%, respectively. These renal vasoconstrictor effects were not affected by the lower doses of H 394/84, whereas during the highest dose of the antagonist (500 nmol/kg/min), 67 ± 8% ($P < 0.01$, $n = 4$) and 67 ± 3% ($P < 0.01$, $n = 4$) remained of these vascular responses, respectively. The vehicles exerted no inhibitory effects per se, although a non-significant 10% reduction of all vascular responses was seen upon the high-dose vehicle (polyethylene glycol/dimethylacetamide/saline (% v/v) 40/40/20).

3.2. Effects of H 394/84 per se in the pig

The two lower doses of H 394/84 (0.1 and 1.0 nmol/kg/min) did not evoke any cardiovascular effects per se. The third infusion of H 394/84 (10 nmol/kg/min) was accompanied by a slight decrease in splenic vascular conductance (to 84 ± 3% of basal) without any effect on mean arterial pressure. The last infusion of H 394/84 (100 nmol/kg/min) lowered splenic vascular conductance to 53 ± 5% of basal and elevated mean arterial pressure to 107 ± 2% of basal. These effects were not different from vehicle (ethanol 10% v/v and saline) alone. The blood flows of kidney and hind limb were not, or only marginally, affected by the infusions of vehicle or H 394/84.

3.3. Vascular responses to exogenous agonists in the pig

Neuropeptide Y (230 pmol/kg, i.v.) evoked vasoconstrictor responses in kidney (Fig. 3) and spleen. Mean arterial pressure was elevated by 33 ± 3 mm Hg and vascular conductance in spleen and kidney was reduced to 29 ± 1% of basal and 34 ± 8% of basal, respectively. The neuropeptide Y-evoked effects on mean arterial pressure and renal vascular conductance were gradually inhibited upon increasing doses of H 394/84 (Fig. 4). Significant antagonistic effects on the neuropeptide Y-evoked renal vasoconstriction was seen during the second (1.0 nmol/kg/min), and on the elevation of mean arterial pressure during the third (10 nmol/kg/min) infusion of H 394/84 (Fig. 4). The greatest inhibitory effects were seen during the last (100 nmol/kg/min) infusion of H 394/84, when the elevation of mean arterial pressure was limited to 20 ± 4%, and the renal vasoconstrictor response was only 9 ± 4%, of the control responses elicited by neuropeptide

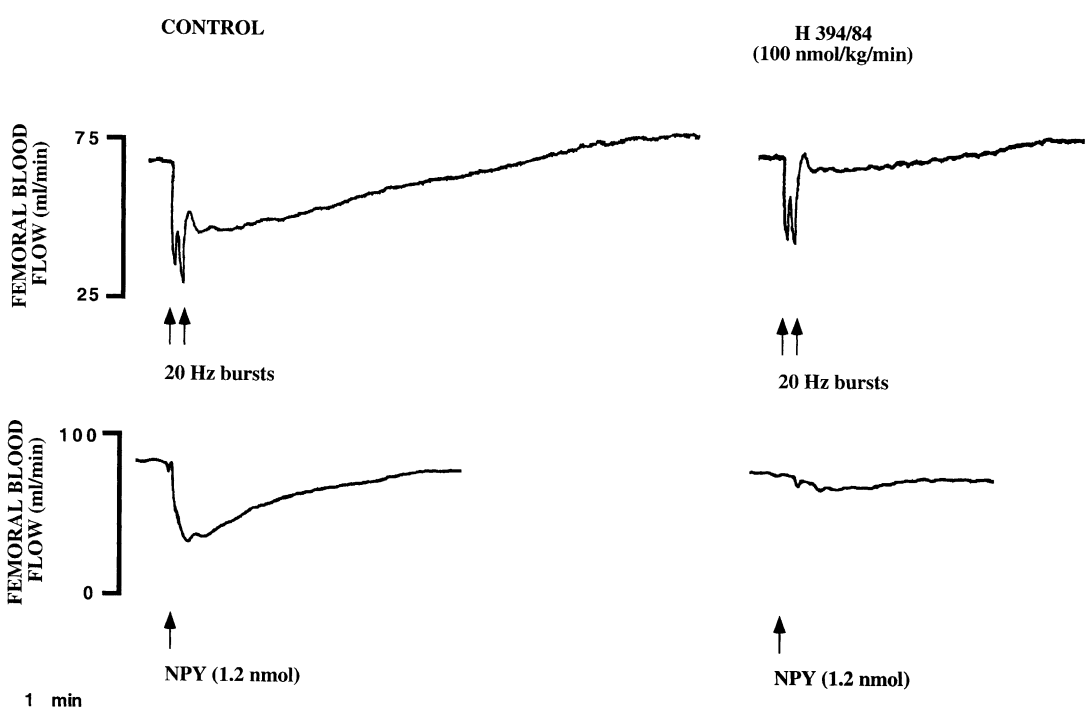

Fig. 5. Original recording of arterial blood flow of hind limb in the reserpine-treated pig in vivo. Vascular responses to high frequency stimulation of lumbar sympathetic nerves (two 1-s bursts at 20 Hz at 10-s intervals) and exogenous neuropeptide Y (1.2 nmol, i.a.) are shown before (control) and during infusion of H 394/84 (100 nmol/kg/min).

Y (Fig. 4). After the recovery period (90 min), the neuropeptide Y-evoked renal vascular response and elevation of mean arterial pressure had returned to 31 ± 8% and 47 ± 7% of the control responses, respectively (Fig. 4). The splenic vascular response to neuropeptide Y was not affected by any of the doses of H 394/84.

Local injection of neuropeptide Y (1.2 nmol, i.a.) into the saphenous artery evoked vasoconstriction in the hind limb (Fig. 5), reducing the vascular conductance to 47 ± 3% of basal. This neuropeptide Y-evoked response was gradually attenuated in the presence of increasing doses of H 394/84, the antagonistic effect of which reaching significance at 10 nmol/kg/min, and the greatest inhibition was seen during infusion of the highest dose (100 nmol/kg/min) when 32 ± 4% of this vascular response remained (Fig. 6). Following a recovery period (90 min), the vasoconstrictor response evoked in hind limb by neuropeptide Y had returned to 70 ± 4% of the control response (Fig. 6). The vehicle (ethanol 10% v/v and saline) alone did not affect any of the neuropeptide Y-evoked vascular responses studied.

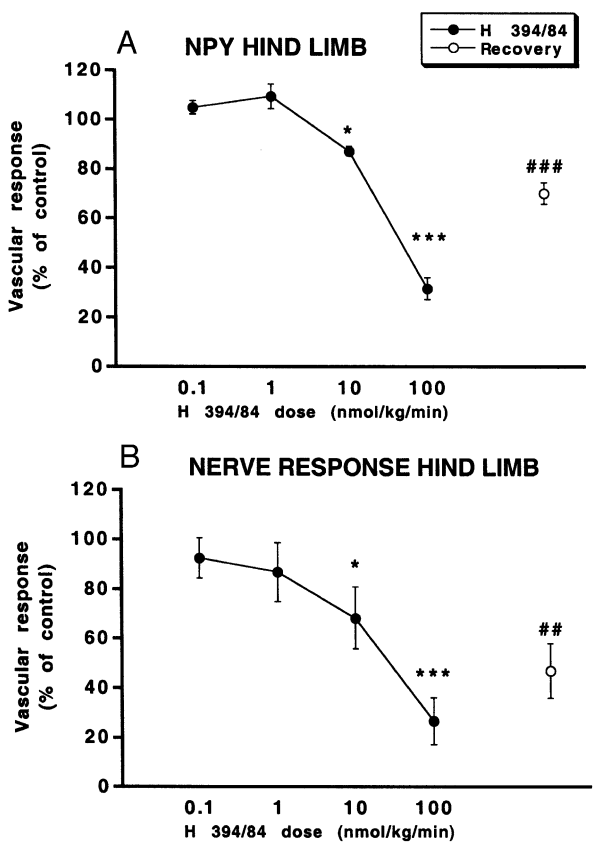

Fig. 6. Inhibitory effects of increasing doses of H 394/84 on the vasoconstrictor responses evoked in hind limb by neuropeptide Y (1.2 nmol, i.a.) (A) and sympathetic nerve stimulation (B) in the reserpine-treated pig in vivo. The vascular responses (expressed as % of the control response) are shown during consecutive 50-min infusions of H 394/84 (0.1–100 nmol/kg/min) and after a recovery period of 90 min. Data are given as means ± S.E.M., $n = 9–10$. Significant differences compared to control are indicated $^{*}P < 0.05$, $^{***}P < 0.001$. Significant differences between H 394/84 (100 nmol/kg/min) and recovery are indicated $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$.

Phenylephrine (15 nmol/kg, i.v.) and α,β-methylene ATP (20 nmol/kg, i.v.) evoked vasoconstrictor effects in all vascular beds studied and elevation of mean arterial pressure (by 62 ± 3 and 56 ± 6 mm Hg, respectively). These vascular responses were not affected by the third dose of H 394/84 (10 nmol/kg/min). During the fourth dose of H 394/84 (100 nmol/kg/min), the phenylephrine-evoked response in kidney was unaffected (100 ± 7% of control), while the responses evoked in spleen (92 ± 4% of control) and hind limb (88 ± 13% of control) were slightly, but non-significantly, attenuated. The splenic vascular response evoked by α,β-methylene ATP was unaltered (99 ± 1% of control), while those evoked in kidney (83 ± 11% of control) and hind limb (85 ± 14% of control) were slightly, but non-significantly, lesser during the fourth dose of H 394/84. *N*-acetyl [Leu^{28},Leu^{31}]neuropeptide Y-(24–36) (560 pmol/kg, i.v.) evoked splenic vasoconstriction (vascular conductance reduced to 44 ± 9% of basal), with only marginal effects in other vascular beds, and a modest elevation of mean arterial pressure (by 15 ± 2 mm Hg). This vascular response was not affected (106 ± 7% of control) by H 394/84 (100 nmol/kg/min).

3.4. Vascular responses to sympathetic nerve stimulation in the pig

A rapid vasoconstrictor response was evoked in kidney upon high frequency stimulation (two 1-s bursts at 20 Hz) of the renal sympathetic nerves (Fig. 3). Renal vascular conductance was reduced to 68 ± 7% of basal upon the control stimulation. In hind limb, lumbar sympathetic nerve stimulation elicited an initial rapid vasoconstrictor effect (vascular conductance reduced to 50 ± 4% of basal), which was followed by a slowly declining long-lasting vasoconstriction (Fig. 5), peaking about a minute after the initial rapid phase, when vascular conductance was decreased to 73 ± 3% of basal. The vascular response in kidney to sympathetic nerve stimulation was gradually attenuated in the presence of increasing doses of H 394/84 (Fig. 4). Significant antagonistic effects were seen at the second H 394/84 infusion (1.0 nmol/kg/min), while the greatest inhibition was seen during the last infusion (100 nmol/kg/min), when 19 ± 2% of the control nerve-response remained (Figs. 3 and 4). The initial rapid phase of the sympathetic nerve-evoked vascular response in hind limb was not affected, whereas the long-lasting phase of this vasoconstriction was gradually attenuated in the presence of increasing doses of H 394/84 (Fig. 6). Significant antagonism of the long-lasting phase of sympathetic vasoconstriction in hind limb was seen at 10 nmol/kg/min, and greatest inhibition was seen at the highest dose of H 394/84, when 27 ± 9% remained of the control response (Figs. 5 and 6).

There was a partial recovery of the vascular responses to sympathetic nerve stimulation 90 min after the last infusion of H 394/84 (100 nmol/kg/min). Hence, the renal response had returned to 38 ± 6% of control (Fig. 4) and the peak of the long-lasting phase of sympathetic vasoconstriction in the hind limb was back to 47 ± 11% of control (Fig. 6).

The vehicle alone did not affect any of the vascular responses to sympathetic nerve stimulation studied.

3.5. Plasma levels and pharmacokinetics of H 394/84

No plasma levels of H 394/84 could be measured during the lowest dose infusion, as they presumably fell below the 25 nM detection limit of the present assay. During the second and third infusion of H 394/84 (1.0 and 10 nmol/kg/min), when significant effects were observed on the neuropeptide Y-evoked vascular responses in kidney and hind limb, respectively, plasma levels reached 29 ± 4 and 200 ± 20 nM, respectively, and remained stable from the 30th to the 50th minute of each infusion (Fig. 7). During the last infusion of H 394/84 (100 nmol/kg/min), plasma levels were steady during the last 20 min of the infusion at 1760 ± 130 nM (Fig. 7). The pharmacokinetics of H 394/84 was characterized by a high plasma clearance (CL_p) of 54 ± 7 ml/min/kg. The initial volume of distribution (V_1) was 0.3 ± 0.1 l/kg, and the volume of distribution at steady state (V_{ss}) was 1.3 l/kg, both relatively low. The elimination of H 394/84 from plasma was biphasic (Fig. 7), with a very rapid distribution half-life ($t_{1/2\lambda1}$) of 2.6 ± 0.6 min and a terminal half-life ($t_{1/2\lambda2}$) of 48 ± 7 min. The values of the coefficients C_1 and C_2 (for the biexponential function) were 36 ± 10 and 1.0 ± 0.4 nmol/l, respectively.

4. Discussion

In recent years, a handful of antagonists for the neuropeptide Y Y_1 receptor, e.g. ((*R*)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-argininamide) (BIBP 3226) (Rudolf et al., 1994) and (1-[2-[2-(2-naphthylsulfamoyl)-3-phenylpropionamido]-3-[4-[*N*-[4-(dimethylaminomethyl)-*trans*-cyclohexylmethyl]amidino] phenyl]propionyl]pyrrolidine, (*R*,*R*) stereoisomer) (SR 120107A) (Serradeil-Le Gal et al., 1994), has been introduced and pharmacologically characterized (see Malmström, 1997). Although non-peptide, these compounds share some of their structural characteristics with the C-terminal part of neuropeptide Y itself (see Lundberg et al., 1996). Presumably due to this fact, these antagonists also shared with neuropeptide Y the tendency (upon high doses) to elicit hypotensive effects (Doods et al., 1995; Lundberg and Modin, 1995; Malmström et al., 1996), a phenomenon that has been attributed to the basic moieties within the C-terminal part of neuropeptide Y causing release of histamine from mast-cells (Grundemar and Håkanson, 1991; Mousli et al., 1994). However, in cardiovascular research, so far neuropeptide Y Y_1 receptor antagonists have been invaluable in establishing the receptor (Y_1) responsible for most vascular responses to neuropeptide Y (see Malmström, 1997), and also in presenting evidence for the involvement of endogenous neuropeptide Y in sympathetic vasocon-

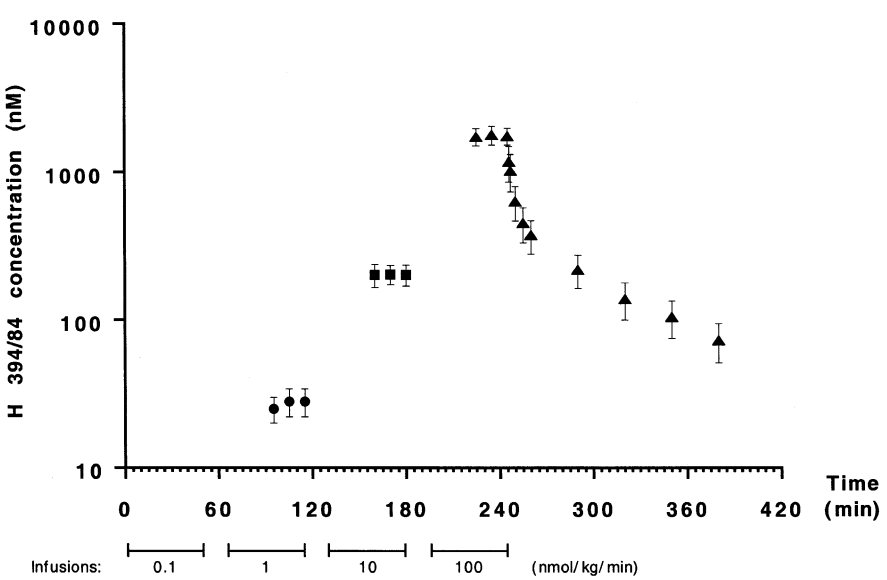

Fig. 7. Plasma levels of H 394/84 in the pig in vivo, plotted against time. H 394/84 was given as four consecutive 50-min i.v. infusions at increasing doses (0.1–100 nmol/kg/min), each 15 min apart. The plasma concentrations during the last 20 min of each of the last three infusions (1–100 nmol/kg/min), and after cessation of the last infusion (100 nmol/kg/min) are shown. No plasma concentrations could be detected during the first infusion (0.1 nmol/kg/min) as they presumably fell below the limit of quantitation (25 nM). Data are given as means ± S.E.M., $n = 6$.

strictor responses (see Malmström, 1997). The development of new antagonists is constantly in progress, aiming for compounds with higher potency and less side effects, to facilitate future studies on neuropeptide Y mechanisms. More recent developments include a series of non-peptidergic dihydropyridines that possessed high affinity for the neuropeptide Y Y_1 receptor but much weaker affinities for the α_1-adrenoceptor and Ca^{2+} channels (Poindexter et al., 1996). The pharmacological profile in vivo of H 394/84, a compound from this series, was investigated in the present study.

In order to investigate the selectivity of H 394/84 in functional experiments, the effects of the antagonist were tested on neuropeptide Y-evoked vasoconstrictor responses in two separate vascular beds in the pig. Neuropeptide Y mediates vascular responses predominantly via the neuropeptide Y Y_1 receptor in most vascular beds (see Malmström, 1997), e.g. the pig kidney (Malmström and Lundberg, 1996; Malmström et al., 1998). In contrast, the neuropeptide Y Y_2 receptor is the preferred subtype for such vascular events in the pig spleen (Modin et al., 1991; Malmström et al., 1998). In the current study, neuropeptide Y elicited vasoconstrictor responses both in pig spleen and kidney, only the latter of which was antagonized by H 394/84. Thus, while H 394/84 exerted dose-dependent inhibition of the neuropeptide Y Y_1 receptor mediated vasoconstriction in kidney, the neuropeptide Y Y_2 receptor mediated dito in spleen was not affected. Furthermore, the selective neuropeptide Y Y_2 receptor agonist *N*-acetyl [Leu^{28},Leu^{31}]neuropeptide Y-(24–36) elicited vasoconstriction in spleen only, and this response was not affected by H 394/84. Thus, H 394/84 seems selective for the neuropeptide Y Y_1 vs. the neuropeptide Y Y_2 receptor in vivo. These results are strengthened by receptor binding studies in vitro showing high affinity of H 394/84 for the neuropeptide Y Y_1 receptor in rat cortex (IC_{50} 30 nM), while in contrast, H 394/84 possesses low affinity for neuropeptide Y Y_2, Y_4 and Y_5 receptors as demonstrated by IC_{50} values at >10, 5 and 10 μM in pig splenic membranes (Y_2) and human recombinant neuropeptide Y Y_4 and Y_5 receptor binding assays, respectively (K. Gedda, personal communication).

It was also investigated whether H 394/84 shows specificity for neuropeptide Y receptors in two in vivo models. In the anaesthetized rat, it was demonstrated that, 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 needed for significant inhibition of the neuropeptide Y-evoked vascular response, slight inhibitory effects were seen upon the responses to angiotensin II and noradrenaline. Furthermore, a moderate dose of H 394/84 did not attenuate the vasoconstrictor responses evoked by α,β-methylene ATP and the α_1-adrenoceptor agonist phenylephrine in the pig in vivo. In parallel with the observations above, at a dose 100 times higher than was sufficient for significant neuropeptide Y Y_1 receptor antagonism in the pig in vivo, there was a tendency of H 394/84 to slightly (but non-significantly) attenuate some of the vascular responses to α,β-methylene ATP and phenylephrine. Thus, 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), and significant inhibition of non-neuropeptide Y receptor mediated responses was seen at 500 nmol/kg/min (rat). Judging from these data, the specificity in vivo of H 394/84 for the neuropeptide Y Y_1 receptor is at least 100-fold vs. other vascular receptors studied. H 394/84 belongs to a class of dihydropyridines known to have significant affinity for Ca^{2+} channels. Although described to possess much weaker affinity for the Ca^{2+} channel (Poindexter et al., 1996), it cannot be excluded that, upon doses as high as those giving inhibition of non-neuropeptide Y receptor mediated events in the rat, H 394/84 may display some Ca^{2+} antagonistic properties. From the results of the present in vivo studies, it was not possible to extract any hypotensive effects that might have been anticipated if H 394/84 possesses significant Ca^{2+} antagonistic properties, because at higher doses, the vehicles per se were not without effects. Thus, the infusion of 10% ethanol caused slight hypertensive effects in the pig in vivo, secondary to splenic vasoconstriction. Furthermore, the vehicle used in the rat (polyethylene glycol and dimethylacetamide) caused, at a high dose, slight hypotensive effects. However, because these effects were similar in the presence or absence of H 394/84, it seems unlikely that the antagonist would exert very substantial, Ca^{2+} antagonist-related, hypotensive effects per se.

When administered i.v. to the rat, neuropeptide Y produced an increase in blood pressure and renal vasoconstriction, the latter of which was studied in detail, and is known to be mediated via neuropeptide Y Y_1 receptor activation (Bischoff et al., 1997). In the present study, H 394/84 dose-dependently inhibited the renal vasoconstrictor effect evoked by neuropeptide Y. Significant inhibitory effects were seen already upon the lowest dose (5 nmol/kg/min) given, whereas at the highest dose (500 nmol/kg/min), the neuropeptide Y-evoked renal vasoconstriction was almost abolished. The calculated ID_{50} value was 41 ± 4 nmol/kg/min, indicating potent neuropeptide Y Y_1 receptor antagonism in the rat in vivo.

Neuropeptide Y-evoked vasoconstriction in the kidney and hind limb of the pig are considered to be exclusively neuropeptide Y Y_1 receptor mediated events (see Malmström, 1997). Furthermore, when, after pretreatment with reserpine and preganglionic transection of sympathetic nerves, the neuronal noradrenaline levels are strongly reduced, neuropeptide Y, acting on the neuropeptide Y Y_1 receptor, is the primary mediator of sympathetic vasoconstrictor responses in these two vascular beds (see Malmström, 1997). In the current study, H 394/84 was found to be very potent in antagonizing the vascular

responses evoked in kidney by neuropeptide Y and sympathetic nerve activation. Importantly, H 394/84 antagonized vascular responses to exogenous and endogenous, neuronally released, neuropeptide Y with equal potency. Thus, significant inhibition of both the renal vasoconstrictor effect to i.v. neuropeptide Y and to sympathetic nerve stimulation was seen upon a low dose of H 394/84 (1 nmol/kg/min), giving very modest plasma levels (29 ± 4 nM) of the antagonist. Interestingly, H 394/84 was a 10-fold less potent antagonist in the hind limb, again equally potent on the vascular responses to endogenous and exogenous neuropeptide Y, as significant inhibitory effects on the vasoconstrictor effect to i.a. neuropeptide Y and sympathetic nerve stimulation were seen upon a higher dose (10 nmol/kg/min), giving plasma levels at 200 ± 20 nM. This phenomenon has not been reported for previously studied neuropeptide Y Y_1 receptor antagonists, e.g. BIBP 3226 and (2*R*)-5-([amino(imino)methyl]amino)-2-[(2,2-diphenylacetyl)amino]-*N*-[(1*R*)-1-(4-hydroxyphenyl)-ethyl]-pentanamide (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 the endothelial permeability. Hence, the vessels of skeletal muscle do not, in contrast to the kidney, possess a fenestrated endothelium (Bennett et al., 1959), and could because of this be less permeable to larger molecules. In line with this, H 394/84 is a larger molecule than BIBP 3226 and H 409/22, and since there exists other differences in their structural characteristics as well, there obviously may be other reasons for their different ability to reach the target receptor within the tissue. In parallel, differences in endothelial permeability may also explain why a large molecule like neuropeptide Y more potently evokes vasoconstriction in, e.g. kidney than in hind limb. Thus, moderate systemic doses of neuropeptide Y readily produces e.g. renal and splenic vasoconstrictor responses but do not evoke such responses in hind limb (Malmström et al., 1997, present study). Higher i.v. doses, or local i.a. administration, are needed for neuropeptide Y to exert vascular responses in skeletal muscle (Malmström et al., 1997, present study). Furthermore, neuropeptide Y produces characteristically long-lasting vasoconstrictor responses (see Malmström, 1997) in hind limb (in contrast to e.g. kidney), which, in turn, could depend on a less effective (due to the endothelium) "wash-out" of neuropeptide Y; a transmitter that otherwise is cleared by enzymatic degradation (see Medeiros and Turner, 1996) and possesses a rather long half-life in plasma (Pernow et al., 1987; Rudehill et al., 1987). Fully in line with previous studies, using other neuropeptide Y Y_1 receptor antagonists (Malmström et al., 1997, 2000), it was shown that H 394/84, at doses that strongly inhibited the neuropeptide Y-mediated, long-lasting phase, did not affect the initial rapid phase of vasoconstriction evoked by sympathetic nerve stimulation in hind limb. Based on these findings, it seems more likely that another (purinergic?) mechanism than the neuropeptide Y Y_1 receptor is mediating this rapid vascular response.

H 394/84 was found to possess a decidedly longer plasma half-life in vivo than previously reported neuropeptide Y Y_1 receptor antagonists (Malmström et al., 1997, 2000). In addition, the antagonistic effects of H 394/84 were also long-lasting. For example, 70 ± 8% of the neuropeptide Y-evoked renal vasoconstrictor response was still inhibited 90 min after the last infusion of H 394/84. In comparison, BIBP 3226 possesses rather short-lasting antagonistic effects in vivo (Malmström et al., 1997). Thus, 90 min after the last infusion of H 394/84, most of the antagonistic actions remained; the inhibition exerted was still stronger than what was observed during the third infusion of the antagonist.

It is concluded that the dihydropyridine compound H 394/84 is a highly potent neuropeptide Y Y_1 receptor antagonist with long duration of action in vivo. However, the antagonistic potency of H 394/84 seems to differ between vascular beds, being highly potent in the kidney. H 394/84 exerts selective neuropeptide Y Y_1 receptor antagonism at low and intermediate doses, but what is possibly Ca^{2+} antagonistic properties can be yielded at high doses, the specificity in vivo being at least 100-fold. Importantly, H 394/84 antagonizes vascular responses to exogenous and endogenous, neuronally released, neuropeptide Y with equal potency, facilitating future studies on the involvement of neuropeptide Y Y_1 receptor mediated effects in sympathetic transmission.

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