J. Pharm. Pharmacol. 1999, 51: 1257–1265
Received March 23, 1999
Accepted May 25, 1999

Renal and Cardiovascular Role of the Neuropeptide Y Y_1 Receptor in Ischaemic Heart Failure Rats

XIAO-HE ZHAO, XIANG-YING SUN, ANDERS BERGDAHL*, LARS EDVINSSON* AND THOMAS HEDNER

*Department of Clinical Pharmacology, Sahlgrenska University Hospital, Göteborg, and *Department of Internal Medicine, University Hospital, Lund, Sweden*

Abstract

The cardiovascular role of the neuropeptide Y Y_1 receptors in-vivo and in-vitro in ischaemic heart failure was evaluated by using the novel neuropeptide Y Y_1 selective antagonist BIBP 3226 (*R*-N2-(diphenylacetyl)-N-[(4-hydroxyphenyl)methyl]-D-arginine-amide).

In pithed rats, incremental doses of BIBP 3226 inhibited the exogenous neuropeptide Y induced pressor response in a dose-related fashion and a bolus injection of BIBP 3226 ($0{\cdot}5\ mg\ kg^{-1}$) significantly shifted the pressor response curve of exogenous neuropeptide Y to the right. The potentiation effect to exogenous neuropeptide Y on the pressor response to preganglionic sympathetic nerve stimulation in ischaemic heart failure rats as well as on the contractile response to noradrenaline in renal arteries in sham-operated animals were also inhibited by the neuropeptide Y Y_1 antagonist. In conscious ischaemic heart failure rats, incremental doses of BIBP 3226 ($0{\cdot}125-1\ mg\ kg^{-1}$) significantly reduced basal blood pressure and heart rate.

Compared with sham-operated rats, neuropeptide Y by itself induced no contraction and no potentiation on noradrenaline elicited contraction in renal artery of the ischaemic heart failure rat. Furthermore, under in-vivo conditions, BIBP 3226 did not influence basal renal function or the response to exogenous neuropeptide Y on urinary volume, urinary sodium and urinary potassium.

Our results demonstrate that although there is a downregulation of the Y_1 receptors by ischaemic heart failure, Y_1 receptors are still mainly involved in cardiovascular actions of exogenous neuropeptide Y and play a role in maintaining basal blood pressure and heart rate in ischaemic heart failure. However, our data do not imply any significant role of Y_1 receptors on basal renal function in the ischaemic heart failure rat model.

During sympathetic activation, neuropeptide Y is released together with noradrenaline and adenosine triphosphate from the sympathetic nerve terminals (Lacroix et al 1989; Lundberg et al 1989). In concert these transmitters are considered to be involved in the physiological regulation of vascular smooth muscle tone and cardiac function (Lundberg et al 1983; Ekblad et al 1984; Lacroix et al 1989).

Postjunctional neuropeptide Y Y_1-receptor activation mediates vasoconstriction, an increase in blood pressure, and potentiation of pressor responses induced by several agonists (Edvinsson et al 1984; Dahlöf et al 1985; Linton-Dahlöf 1989). Furthermore, neuropeptide Y Y_1 receptors appear to be involved in the regulation of renal function (Leys et al 1987; Bischoff & Michael 1998).

In experimental and clinical ischaemic heart failure, the activity of the sympathetic nervous system is enhanced, resulting in an exaggerated release of noradrenaline and neuropeptide Y (Kasakov et al 1988; Torres et al 1992). The augmented neuropeptide Y activity alone or in concert with other neurohumoral alterations may be responsible for the reduction in organ and tissue perfusion as well as in changes of cardiac output which are characteristic of ischaemic heart failure. It has been suggested that an enhanced neuropeptide Y activity represents one of several neurohumoral alterations seen in heart failure as well as in other cardiovascular diseases (Grundemar & Håkanson 1994). Patients with ischaemic heart failure have elevated plasma concentrations of

Correspondence: X.-Y. Sun, Department of Clinical Pharmacology, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden.
E-Mail: sunxy@pharm.gu.se

neuropeptide Y (Edvinsson et al 1990; Valdemarsson et al 1994). However, firm evidence supporting a pathophysiological role of neuropeptide Y in experimental or clinical ischaemic heart failure is limited.

To investigate the potential role of neuropeptide Y and the neuropeptide Y Y_1 receptor, we have used the potent and selective antagonist BIBP 3226 (*R*-N2-(diphenylacetyl)-N-[(4-hydroxyphenyl) methyl]-D-arginine-amide) as a tool to elucidate the neuropeptide Y Y_1 receptor function in the ischaemic heart failure rat. We hypothesized that BIBP 3226 would antagonize vascular responses as well as the potentiation effects of several potent pressor agents to both endogenous and exogenous neuropeptide Y in ischaemic heart failure, providing an insight into the role of the sympathetic co-transmitters in cardiovascular regulation.

Materials and Methods

Experimental animals

Male Sprague–Dawley rats (ALAB, Sollentuna, Sweden), 160–200 g, were maintained on standard rat pellets and tap water was freely available. Groups of four rats were housed in cages at +26°C, with 60% humidity and a 0500–1900 h light regimen.

The Ethics Committee for Animal Experiments at the University of Gothenburg, Sweden, approved the study protocol.

Induction of ischaemic heart failure

During short-lasting methohexital sodium anaesthesia (75 mg kg^{-1}, i·p.), rats were catheterized and artificially ventilated with a respirator. A left thoracotomy was performed, exposing the left ventricular wall. The left coronary artery was ligated by positioning a suture between the pulmonary artery out-flow tract and the left atrium. Thereafter, the lungs were hyper-inflated using positive end-expiratory pressure, the thorax immediately closed and the rats were allowed to recover for at least five weeks before the experiments. Sham-operated rats (normal controls) underwent the same surgical procedure but without coronary artery ligation and were allowed to recover for four to five weeks.

Determination of ischaemic heart failure

After the rat had been killed the heart was dissected out, cut into transverse slices and incubated in a solution of triphenyltetrazolium chloride in phosphate buffer (pH 7·4) for 5 min at 37°C. The incubated tissue faces (4–6 consecutive faces) were photographed in colour. Photographs of the gross left-ventricular slices were projected at 10 × magnification and the endocardial circumferences of fibrotic and normal areas were quantitated with a distance meter. Infarct size was expressed as a fraction of total cross-sectional endocardial circumference of the left ventricle.

Animals were included in the study protocol if the infarction size was over 30% (in all animals: the mean was 43·1%, range 32–51%). These rats had signs of heart failure, which included dilatation of the heart, pulmonary oedema, hepatic congestion, and pleural effusion. Previous work in our laboratory had shown that the plasma catecholamines (both plasma noradrenaline and adrenaline) were significantly increased in rats with such infarction sizes, compared with sham-operated controls. Furthermore, circulating plasma atrial natriuretic peptide concentrations were elevated (Feng 1993).

In-vivo study

Conscious and pithed ischaemic heart failure rats were used for the in-vivo study. In the conscious rat model, using methohexital sodium (70 mg kg^{-1}, i·p.) anaesthesia, the left femoral artery was cannulated with a polyethylene cannula (PE-50) for measurements of arterial blood pressure and heart rate via the arterial catheter by using a Statham P23 DC pressure transducer connected to a Grass Polygraph. The right jugular vein was cannulated (PE-50) for drug or saline administration. All catheters were tunnelled subcutaneously and exteriorized on the back of the neck. The urinary bladder was cannulated with a polyethylene catheter (PE-200) for collection of urine samples. The rats were allowed to recover overnight before they were placed in specially designed Plexiglas cylinders having openings at the bottom for the bladder catheter. The experiments were performed 24-h after cannulation. One hour after basal blood pressure and heart rate stabilization, BIBP 3226 or saline infusion was given for 30 min at a rate of 1·8 mL h^{-1}. Urine samples were collected in preweighed plastic tubes during the infusion of BIBP 3226 or saline. All urine volumes were measured gravimetrically. Urinary sodium ($U_{Na}V$) and potassium (U_KV) excretion were analysed by a flame spectrophotometer (Model FLM3, Radiometer, Copenhagen, Denmark).

In the pithed rat model, anaesthesia was induced with methohexital sodium (70 mg kg^{-1}, i·p.) and the animals were then tracheotomized. The left

carotid artery and both jugular veins were cannulated with polyethylene catheters (PE-50). The rats were then pithed by the procedure previously described (Sun et al 1991). The sympathetic nervous system was stimulated preganglionically (0·8 and 16 Hz, 1 ms, 65 V for 20 s) via a Grass model S4 stimulator. After the pithing procedure, 40 min was allowed before the experiments started.

In-vitro studies

The rats were put into a chamber containing frozen CO_2 to which water was added. During the CO_2 anaesthesia the chest was cut open and the heart was removed. The kidney was rapidly removed and put into a chilled buffer solution aerated with 5% CO_2. The buffer solution was composed of (in mM): NaCl 119; $NaHCO_2$ 15; KCl 4·6; $MgCl_2$ 1·2; NaH_2PO_4 1·2; $CaCl_2$ 1·5; glucose 5·5.

The renal arteries at the hilus of the kidney, normally divided in three separate branches before entering the hilus, were dissected free under a microscope and cut into cylindrical segments (1–2 mm long).

The vessel segments were mounted on two metal prongs (diam. 0·1 mm), one of which was connected to a force displacement-transducer (FT03C) and attached to a Macintosh IIvx computer, and the other to a displacement device. The position of the holder could be changed by means of a movable unit allowing fine adjustments of vascular tension by varying the distance between the metal prongs. The Macintosh software program Chart continuously recorded the experiments. The mounted specimens were immersed in temperature-controlled tissue baths containing the above buffer solution. The buffer solution was continuously gassed with 5% CO_2 in O_2, giving a pH of 7·4.

Study design and experimental protocols

To investigate the role of the neuropeptide Y Y_1 receptor, a selective Y_1 receptor antagonist BIBP 3226 was used as a tool in the following experiments.

In-vivo studies

Effect of BIBP 3226 on the pressor response in response to exogenous neuropeptide Y administration. Four different dose-levels (0·125, 0·25, 0·5 and 1·0 mg kg^{-1}, i.v.) were studied in pithed ischaemic heart failure and sham-operated rats. When blood pressure and heart rate recordings had been stabilized, saline or BIBP 3226 was pre-administered 2 min before neuropeptide Y (20 μg kg^{-1}, i.v.) administration. Each BIBP 3226 administration was followed by a 30-min recovery period.

Effect of BIBP 3226 on the cardiovascular actions of exogenous neuropeptide Y in pithed ischaemic heart failure and sham-operated rats. After a 40-min stable basal blood pressure recording period, BIBP 3226 (0·5 mg kg^{-1}) or saline was pre-administered as an intravenous bolus 5 min before incremental doses of neuropeptide Y (5, 10, 20, 20, 80, 160 μg kg^{-1}, i.v.) were given. One group of ischaemic heart failure rats received three doses of neuropeptide Y only.

In separate experiments, the action of BIBP 3226 on the potentiation effects of neuropeptide Y were studied in pithed ischaemic heart failure rats. The rats were divided in to two groups, which were randomly assigned to treatment with either BIBP 3226 or saline. The control preganglionic sympathetic nerve stimulation response (0·8 Hz) was obtained before neuropeptide Y administration. After 10 min, a neuropeptide Y infusion with a subthreshold dose (0·1 μg min^{-1} for 30 min) was given together with a bolus injection of BIBP 3226 (0·5 mg kg^{-1}, i.v.) or saline (0·3 mL). Two minutes after the onset of neuropeptide Y infusion, the preganglionic sympathetic nerve stimulation was repeated.

Effect of BIBP 3226 on endogenous neuropeptide Y responses. The effect of BIBP 3226 on basal blood pressure and heart rate was investigated in conscious ischaemic heart failure rats. After a 60-min stabilization period, BIBP 3226 was administered in increasing doses (0·125, 0·25, 0·5 and 1 mg kg^{-1}) as bolus intravenous injection. The control group received saline injection (0·3 mL) only. Each bolus injection was given with a 30-min interval.

In separate experiments, a subgroup receiving BIBP 3226 (0·5 mg kg^{-1}) in combination with prazosin (10 μg kg^{-1}) was compared with subgroups receiving either an intravenous bolus of BIBP 3226 (0·5 mg kg^{-1}) or prazosin (10 μg kg^{-1}).

Effects of BIBP 3226 (0·5 mg kg^{-1}) on the pressor response to an acute air jet was studied in conscious ischaemic heart failure rats. The air jet stimulation was given during 5 min at 5 bars pressure. The control group received saline (0·3 mL) only.

In pithed ischaemic heart failure rats, a control preganglionic sympathetic nerve stimulation (0·8 or 16 Hz, 65 V, 1 ms for 20 s) was applied. After an 8-min interval, a bolus injection of BIBP 3226 (0·5 mg kg^{-1}) or saline (0·3 mL) was given. Two

minutes later, the preganglionic sympathetic nerve stimulation (0·8 or 16 Hz) was repeated.

Renal effects of BIBP 3226 in conscious ischaemic heart failure rats. In the BIBP 3226-treated group, after a control urine collection during saline infusion, BIBP 3226 was administered at two different dose-levels, 3 and 6 mg kg^{-1} h^{-1}, given as 30-min intravenous infusions. Each urine collection (for 10 min) started 5 min after the onset of drug or saline infusion. Each infusion was followed by a 10-min recovery period. In the control group, the animals received saline infusion only during all steps.

In separate groups, the renal effects of exogenous neuropeptide Y alone or with BIBP 3226 were investigated. Saline infusion acted as the control. After a 30-min urine collection, saline (0·1 mL min^{-1}) or neuropeptide Y (0·4 and 0·8 μg min^{-1}) without and with BIBP 3226 (3 mg kg^{-1} h^{-1}) was administered as an infusion for 30 min. During the 30-min infusion as well as the two following 30-min periods, urine collections were performed.

In-vitro studies

Effect of neuropeptide Y on renal arteries with or without BIBP 3226. Segments of renal artery were given an initial tension of 3–4 mN and allowed to stabilize at this tension for 1 h. The contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution which had the same composition as the standard solution except that some of the NaCl was exchanged for an equimolar concentration of KCl. These contractions served as internal standards and were set as 100%. When two reproducible contractions had been achieved (variation less then 10%) the vessels were used for further studies. Noradrenaline was given in concentrations of 10^{-10} to 10^{-3} M. Vessels without treatment of BIBP 3226 and neuropeptide Y served as controls. In another group, BIBP 3226 (10^{-6} M) was added to the mounted vessels 10 min before neuropeptide Y was given. Neuropeptide Y (10^{-8} M) was thereafter added to BIBP 3226-incubated and non-BIBP 3226-incubated vessels in a parallel manner. After 2-min neuropeptide Y-pretreatment, noradrenaline was given in concentrations of 10^{-10} to 10^{-3} M.

Drugs

Methohexital sodium (Eli Lilly & Co., USA), pentobarbital sodium (Nord Vacc, Sweden), heparin (Lövens Läkemedel, Sweden), pancuronium bromide (Pancuron, The Netherlands), atropine sulphate (Sigma, St Louis, MO), noradrenaline (Sigma, St Louis, MO), neuropeptide Y (CRB, Cambridge, UK), prazosin (Sigma, St Louis, MO) and R-N2-(diphenylacetyl)-N-[(4-hydroxyphenyl) methyl]-D-arginine-amide (a neuropeptide Y Y_1 selective antagonist; BIBP 3226; Karl Thomae GmbH, Germany) were used. BIBP 3226 was dissolved in 0·9% NaCl and was prepared fresh before each experiment.

Statistics

In-vivo studies. All values are given as means ± s.e.m. Statistics were calculated using the Macintosh Statview program on a Macintosh computer. Group comparisons were performed using analysis of variance with either an unpaired or paired Student's *t*-test. $P < 0{\cdot}05$ was considered significant. In conscious model studies, analysis was made using two-way analysis of variance followed by unpaired Student's *t*-test between groups. One-way analysis of variance with repeated measurement followed by Scheffe's F test was used to compare within groups. $P \leq 0{\cdot}05$ was considered significant.

In-vitro studies. Results are given as the percentage of the potassium-induced contraction. Maximum effect of contraction = E_{max}%. The potency of the agonists is expressed as pEC50 values (negative logarithm of the molar concentration of agonist inducing half maximum response). n refers to the number of rats and N to the number of segments used in total. The data are expressed as mean values ± s.e.m. The Mann–Whitney U test was used when comparing sham and ischaemic heart failure rats, and the Wilcoxon signed rank test when comparing differences within each group. $P \leq 0{\cdot}05$ was considered significant.

Results

Effect of BIBP 3226 on the cardiovascular response to exogenous neuropeptide Y in pithed ischaemic heart failure rats

A bolus injection of neuropeptide Y (20 μg kg^{-1}, i.v.) induced a pressor response, with a blood pressure increase and a reduction of heart rate in control pithed ischaemic heart failure rats (Figure 1). Pretreatment with increasing doses of BIBP 3226 (0·125, 0·25, 0·5 and 1 mg kg^{-1}, n = 4) induced a significant and dose-dependent inhibition

of the pressor response to neuropeptide Y (Figure 1). There was no significant effect of BIBP 3226 on the heart rate response (Figure 1). In the pithed sham-operated rats, a dose of 0·5 mg kg^{-1} BIBP 3226 also significantly reduced the pressor response to 20 μg kg^{-1} neuropeptide Y from a control value of 67·3 ± 4·3 to 8·3 ± 1·5 mmHg (data not shown).

Effect of BIBP 3226 on the cardiovascular actions of exogenous neuropeptide Y in ischaemic heart failure rats

In pithed ischaemic heart failure rats as well as sham-operated rats, BIBP 3226 inhibited the pressor responses to exogenous neuropeptide Y. A bolus injection of BIBP 3226 (0·5 mg kg^{-1}) was administered 2 min before incremental doses of neuropeptide Y (5, 10, 20, 40, 80 and 160 μg kg^{-1}, respectively, n = 5–10). BIBP 3226 significantly shifted the dose-dependent pressor response curve to neuropeptide Y rightward, while there was no significant effect on heart rate (Figure 2).

In separate experiments, neuropeptide Y administration at a non-pressor dose (0·1 μg min^{-1} infusion during 30 min) enhanced the pressor response to preganglionic sympathetic nerve stimulation (0·8 Hz) and this potentiation effect of neuropeptide Y was significantly inhibited by BIBP 3226 (n = 7 per subgroup, Table 1).

Effect of BIBP 3226 on endogenous neuropeptide Y actions

In conscious ischaemic heart failure rats, there was a significant reduction of baseline mean blood pressure and heart rate by BIBP 3226 (0·125–1 mg kg^{-1}, n = 9, Figure 3).

Basal blood pressure and heart rate were found to be significantly reduced after administration of BIBP 3226 (0·5 mg kg^{-1}, n = 8, $P = 0·0023$ in mean blood pressure and $P = 0·0122$ in heart rate)

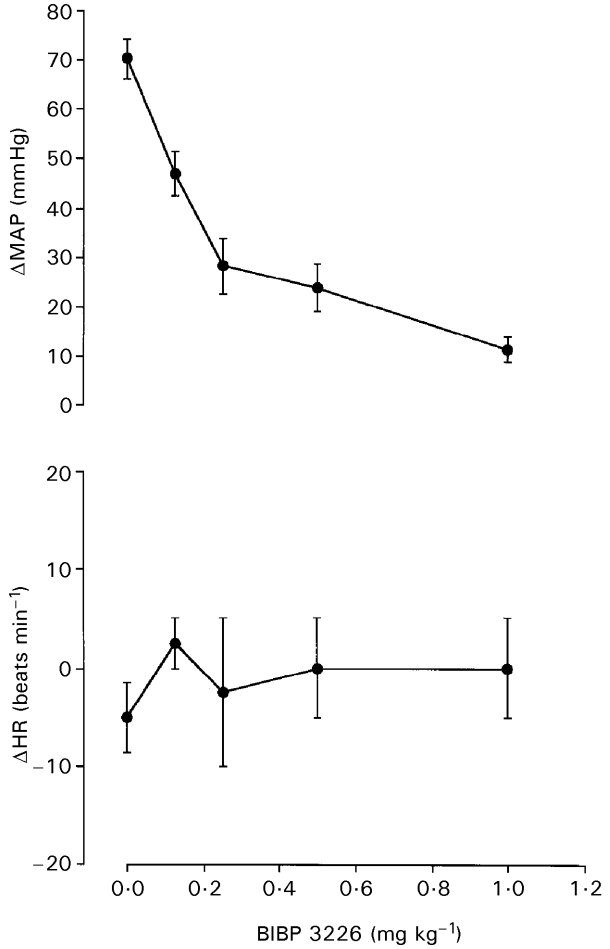

Figure 1. Dose dependent inhibitory effects of bolus BIBP 3226 on mean arterial pressure (MAP) response elicited by exogenous neuropeptide Y (20 μg kg^{-1}). HR, heart rate. Values are mean ± s.e.m., n = 4 rats.

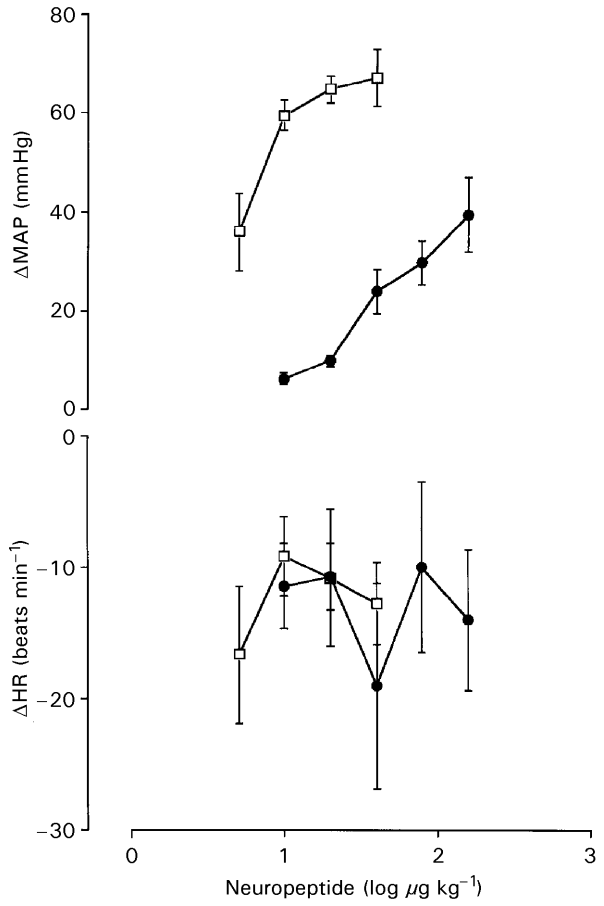

Figure 2. Effects of BIBP 3226 (●) (0·5 mg kg^{-1}) on the mean arterial pressure (MAP) response curve in relation to exogenous neuropeptide Y in pithed ischaemic heart failure rats. HR, heart rate. The control group (□) was pretreated with saline. Values are mean ± s.e.m., n = 5–10 rats.

Table 1. Inhibition by BIBP 3226 (0·5 mg kg^{-1}) of the potentiation effect of neuropeptide Y on the pressor response to preganglionic sympathetic nerve stimulation in ischaemic heart-failure pithed rats.

	Change in mean arterial pressure (mmHg)	Change in heart rate (beats min^{-1})
Control	38 ± 4	54 ± 4
Neuropeptide Y	56 ± 5[a]	76 ± 9
Neuropeptide Y + BIBP 3226	44 ± 7[b]	71 ± 19

Mean ± s.e.m. [a]$P < 0·05$ compared with control, [b]$P < 0·05$ compared with experiments without BIBP 3226.

or prazosin (10 μg kg^{-1}, n = 6, $P = 0·0003$ in mean blood pressure) compared with the controls in the same subgroup (Figure 4). However, the co-administration of prazosin with BIBP 3226 (n = 6) resulted in only a minor decrease in basal mean blood pressure in conscious ischaemic heart failure rats (Figure 4). Thus, there was no significant synergistic effect observed when BIBP 3226 was administered together with the α-adrenoceptor antagonist. Interestingly, prazosin only changed the mean blood pressure while BIBP 3226 altered both basal mean blood pressure and heart rate (Figure 4).

In conscious ischaemic heart failure rats, and acute air jet was given with 5 bars pressure for 5 min. The basal mean blood pressure was significantly increased during air jet stimulation in the saline pretreated group (n = 5, $P = 0·0001$). How-

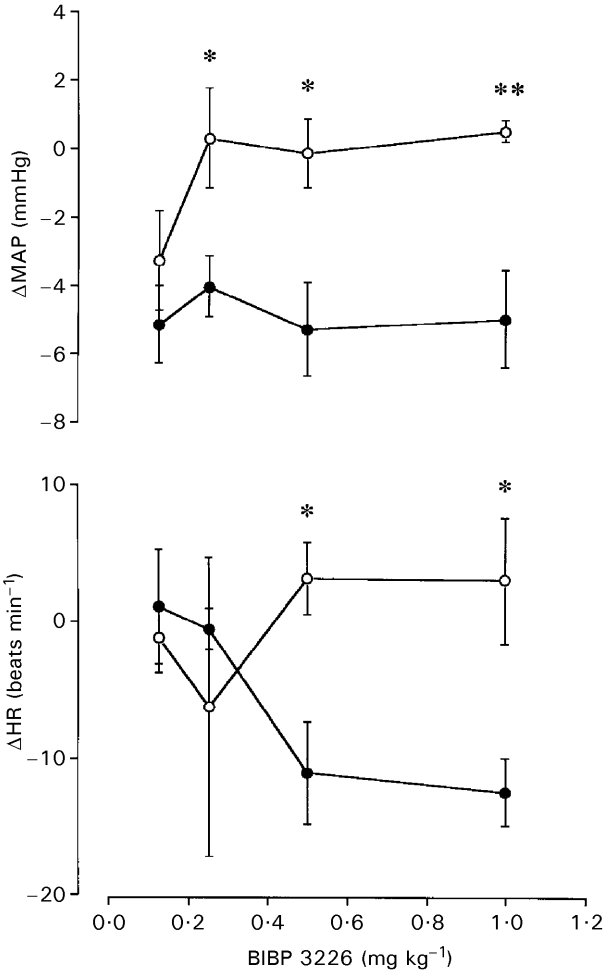

Figure 3. The effects of BIBP 3226 on basal mean arterial pressure (MAP) and heart rate (HR) in conscious ischaemic heart failure rats. The zero value for each group was taken as the control. Values are mean ± s.e.m., n = 9 rats. Statistics by two way analysis of variance followed by Student's *t*-test. *$P < 0·05$, **$P < 0·01$ compared with saline group (n = 8). When comparing saline (○) and BIBP 3226 (●) there was a significant difference in BIBP 3226 doses of 0·25 to 1·0 mg kg^{-1} on both mean arterial pressure and heart rate.

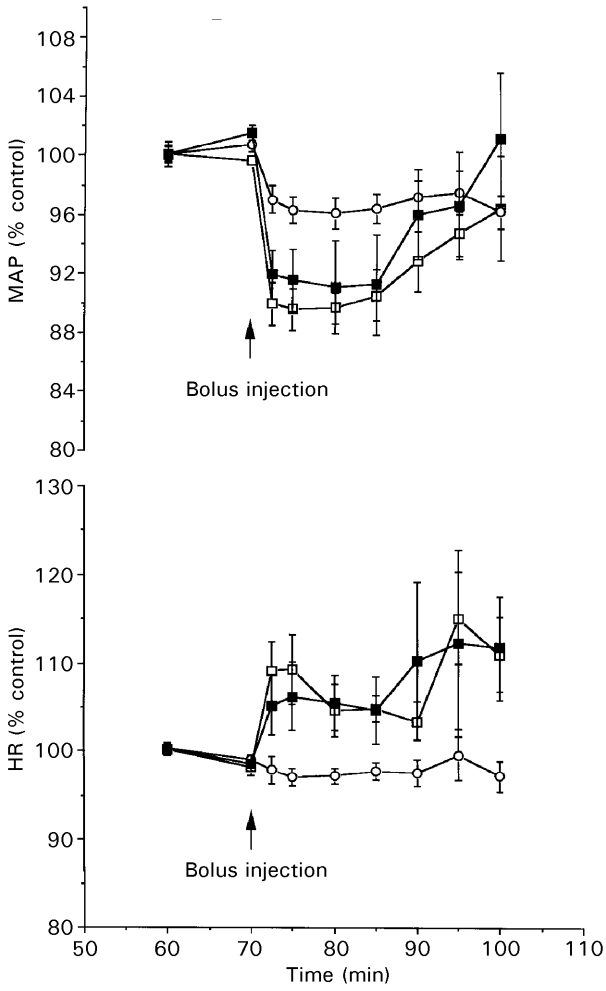

Figure 4. The influence of prazosin (□) (10 μg kg^{-1}, n = 6 rats), BIBP 3226 (○, 0·5 mg kg^{-1}, n = 6–10 rats) or the combination (■) on the basal mean arterial pressure (MAP) and heart rate (HR) in conscious ischaemic heart failure rats. Values are mean ± s.e.m. There were no significant differences between prazosin alone and prazosin + BIBP 3226.

ever, there was no significant difference in the increase of mean blood pressure between the BIBP 3226-treated and non-treated groups. The heart rate was only slightly altered by BIBP 3226 during the stress stimulus (data not shown).

In pithed ischaemic heart failure rats, the effects of BIBP 3236 on the pressor responses to preganglionic sympathetic nerve stimulation at two frequencies (0·8 and 16 Hz, n = 7) were studied. No significant inhibitory effect was observed (data not shown).

The urinary volume (U_V), urinary sodium ($U_{Na}V$) and urinary potassium (U_KV) after BIBP 3226 (n = 7) pretreatment were compared with saline-pretreated ischaemic heart failure rats (n = 9). After incremental doses of BIBP 3226 ($3-6\,mg\,kg^{-1}\,h^{-1}$, during a 30-min infusion) there was no significant difference between the two groups (Table 2).

In separate experiments, ischaemic heart failure rats were co-infused with exogenous neuropeptide Y and saline or BIBP 3226 (n = 7). The results show that U_V and $U_{Na}V$ but not U_KV were slightly, but not significantly, higher in the group given neuropeptide Y and BIBP 3226 administration than the non-BIBP 3226-treated group (data not shown).

Effect of neuropeptide Y on renal arteries

Noradrenaline induced a reproducible concentration-dependent contraction of renal arteries in both sham-operated and ischaemic heart failure rats (Figure 5). However, there were no significant contractile effects of neuropeptide Y on renal vessels (data not shown).

Table 2. Effects of increasing doses of BIBP 3226 on urinary volume, urinary sodium excretion and urinary potassium excretion in rats.

	Volume ($\mu L\,min^{-1}$)	Sodium ($\mu mol\,min^{-1}$)	Potassium ($\mu mol\,min^{-1}$)
Predose			
Saline control	35 ± 6	1·9 ± 0·9	1·8 ± 0·3
Experimental	47 ± 15	5·0 ± 2·4	2·0 ± 0·2
First infusion ($3\,mg\,kg^{-1}h^{-1}$)			
Saline control	52 ± 17	6·3 ± 2·8	3·8 ± 0·4
Experimental	59 ± 12	10·5 ± 2·1	3·7 ± 0·5
Second infusion ($6\,mg\,kg^{-1}h^{-1}$)			
Saline control	47 ± 11	6·9 ± 1·9	3·0 ± 0·6
Experimental	61 ± 13	11·1 ± 1·9	2·7 ± 0·4
Recovery			
Saline control	31 ± 5	4·5 ± 0·9	2·2 ± 0·3
Experimental	40 ± 11	8·4 ± 2·1	1·4 ± 0·2

Means ± s.e.m.

Effect of BIBP 3226 on the neuropeptide Y potentiation of noradrenaline in renal arteries

In sham-operated animals, neuropeptide Y significantly shifted the noradrenaline-induced concentration–response curve of renal arteries to the left without changes in maximal contraction

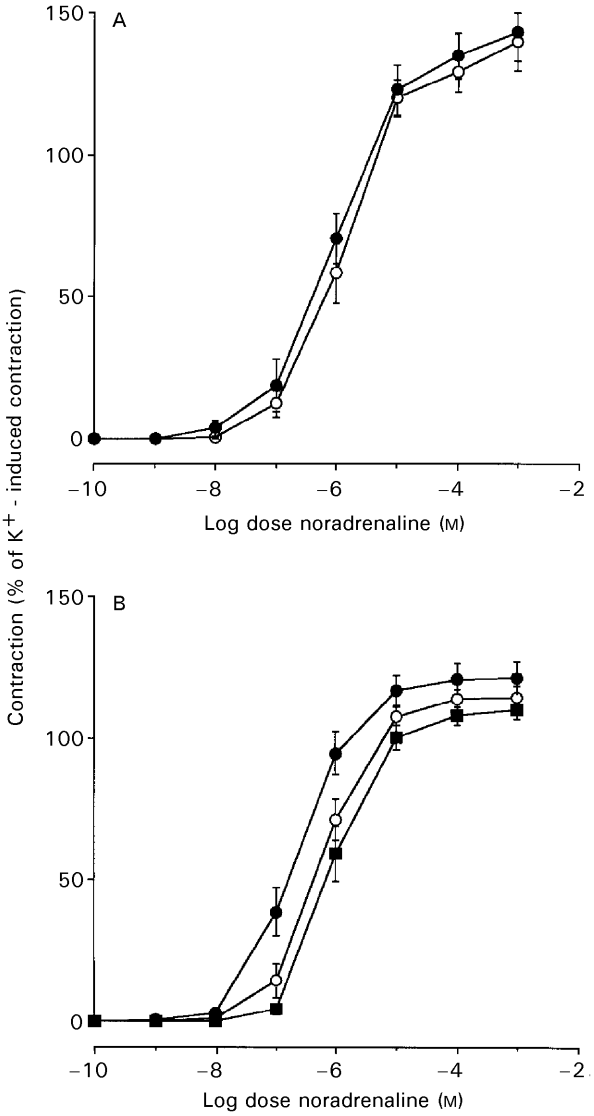

Figure 5. A. Dose–response curves showing the vascular contraction in renal arteries from ischaemic heart failure rats in response to noradrenaline without (○) or with neuropeptide Y (●, 10^{-8} M, n × N = 6 × 9, n refers to number of rats used and N to number of vascular segments used in each group). No potentiation is seen in the presence of neuropeptide Y. B. Dose–response curve showing the vasoconstrictor responses in renal arteries from sham operated rats in response to noradrenaline only (○) (n × N = 9 × 13), noradrenaline in the presence of neuropeptide Y (●) (10^{-8} M, n × N = 9 × 13) and noradrenaline with neuropeptide Y and the selective neuropeptide Y Y_1-receptor antagonist BIBP 3226 (■) (10^{-6} M, n × N = 5 × 10). A significant potentiation of the noradrenaline contractile effect was obtained in the presence of neuropeptide Y. This effect was completely abolished in the presence of BIBP 3226.

(pEC50 values: 6·2 ± 0·2 and 6·6 ± 0·1, Table 3). The selective neuropeptide Y Y_1-antagonist BIBP 3226 completely abolished the potentiation of neuropeptide Y in renal arteries while the maximal contraction to noradrenaline was unaffected (Figure 5B and Table 3).

Effect of neuropeptide Y on the noradrenaline contraction in renal arteries of ischaemic heart failure rats

In renal arteries from ischaemic heart failure rats, neuropeptide Y did not induce any potentiation of noradrenaline-induced vasoconstriction. Furthermore, there were no differences in pEC50 values or maximal contraction compared with control segments (Figure 5A).

In the presence of neuropeptide Y there was a significant difference in pEC50 values between ischaemic heart failure and sham renal arteries (Table 3) further indicating the lack of response to neuropeptide Y in renal arteries from ischaemic heart failure rats.

Discussion

In using BIBP 3226 as a tool to elucidate a potential role of neuropeptide Y Y_1 receptors in experimental ischaemic heart failure, we found that there was a slight but significant adjustment in the haemodynamics after the administration of BIBP 3226 to ischaemic heart failure rats. Administration of BIBP 3226 inhibited the cardiovascular actions of exogenous neuropeptide Y in pithed animals with ischaemic heart failure and reduced the basal blood pressure in conscious ischaemic heart failure rats. This may indicate that the neuropeptide Y Y_1 receptors are mainly involved in the exogenous neuropeptide Y cardiovascular action and play a role in maintaining the basal blood pressure during conditions of ischaemic heart failure. Why BIBP 3226 had no effect on heart rate induced by exogenous neuropeptide Y but significantly inhibited the basal heart rate is not clear. It might be because BIBP 3226 inhibited the potentiation of endogenous neuropeptide Y on the cardiovascular regulation of noradrenaline. Although high concentrations of neuropeptide Y and noradrenaline are present in circulating blood in ischaemic heart failure, our results show that combined blockade gave no further evidence for a reciprocal interaction between neuropeptide Y Y_1 and α_1-adrenergic receptors in the peripheral vascular system.

Table 3. Vasoconstrictor responses to noradrenaline alone and in combination with neuropeptide Y in renal arteries from sham-operated and ischaemic heart failure rats.

	N (n)	E_{max} (%)	pEC50
Sham + noradrenaline	9 (13)	114·3 ± 3·8	6·2 ± 0·1
Sham + noradrenaline + neuropeptide Y	9 (13)	121·2 ± 5·7	6·6 ± 0·1[a]
Sham + noradrenaline + neuropeptide Y + BIBP 3226	5 (10)	110·1 ± 3·3	6·0 ± 0·1[b]
Ischaemic heart failure + noradrenaline	6 (9)	136·7 ± 9·1	5·9 ± 0·1
Ischaemic heart failure + noradrenaline + neuropeptide Y	6 (9)	138·6 ± 9·1	6·1 ± 0·2[c]

N = number of rats used, n = number of vascular segments used in each group. [a]$P < 0·01$ compared with corresponding experiment without neuropeptide Y, [b]$P < 0·05$ compared with corresponding experiment without BIBP 3226, [c]$P < 0·05$ corresponding sham-operated experiment.

BIBP 3226 had no effect on renal urinary volume and sodium in the ischaemic heart failure rat model. This indicates that the neuropeptide Y Y_1 receptor is not a major factor involved in regulation of renal urinary volume and sodium excretion. This is in contrast to the functional neuropeptide Y inhibitor α-trinositol which exhibits diuretic and natriuretic properties (Sun et al 1995). Since α-trinositol may involve blockade of specific inositol-related mechanisms, the renal effects of this agent may not necessarily be attributed to a specific neuropeptide Y receptor inhibition. It might be due to endogenous neuropeptide Y tonically activating the renovascular but not tubular neuropeptide Y receptors (Bischoff & Michael 1998). However, our in-vitro data clearly showed that neuropeptide Y did not induce any contractile effect of its own on renal arteries from ischaemic heart failure or sham-operated control rats. Furthermore, it might be because other types of neuropeptide Y receptors are involved in the renal actions of neuropeptide Y (Bischoff et al 1997).

This study illustrates that there is a modulation of neuropeptide Y receptors in ischaemic heart failure rats: the potentiating effects of neuropeptide Y were lost in renal arteries while significant potentiation was seen in sham-operated controls which could be blocked by BIBP 3226. This is hypothesized to be due to a desensitization or reduction in the expression of the neuropeptide Y Y_1 receptors. Our previous work demonstrated that the functional non-competitive neuropeptide Y inhibitor, α-trinositol, not only significantly inhibited the pressor response to exogenous neuropeptide Y but also antagonized the renal effects of neuropeptide Y (Sun et al 1995). This lends some support to the

idea that neuropeptide Y could contribute to the haemodynamic abnormalities observed in ischaemic heart failure. Interestingly, data from rat as well as man show that neuropeptide Y has a mitogenic effect in vascular smooth muscle cells (Erlinge et al 1993) and an angiogenic effect on vascular endothelium (Zukowska-Grojec et al 1998). Thus, it might be that the high circulating levels of neuropeptide Y (and noradrenaline/adenosine triphosphate) seen in ischaemic heart failure could contribute to hypertrophy in the vascular bed, an effect that is not completely abolished by drug therapy.

In conclusion, although there is modulation/downregulation of neuropeptide Y_1 receptors in ischaemic heart failure, neuropeptide Y Y_1 receptors are still mainly involved in cardiovascular actions of exogenous neuropeptide Y and play a role in maintaining the basal blood pressure and heart rate in ischaemic heart failure. However, Y_1 receptors had no significant effect in this study on basal renal function or on the cardiovascular response to stress (air jet stimulation) in the ischaemic heart-failure rat model.

Acknowledgements

This study was supported by the Swedish Medical Research Council (projects no 8642, 5958), the Medical Faculty (LUA), University of Göteburg, Sweden.

References

Bischoff, A., Michael, M. C. (1998) Renal effects of neuropeptide Y. Pflugers Arch. 435: 443–453

Bischoff, A., Avramidis, P., Erdbrügger, W., Münter, K., Michel, M. C. (1997) Receptor subtypes Y_1 and Y_5 are involved in the renal effects of neuropeptide Y. Br. J. Pharmacol. 120: 1335–1343

Dahlöf, C., Dahlöf, P., Lundberg, J. M. (1985) Neuropeptide Y (NPY): enhancement of blood pressure increases upon alpha-adreno receptor activation and direct pressor effects in pithed rats. Eur. J. Pharmacol. 109: 289–292

Edvinsson, L., Ekblad, E., Håkanson, R., Wahlestedt, C. (1984) Neuropeptide Y potentiates the effect of various vasoconstrictor agents on rabbit blood vessels. Br. J. Pharmacol. 83: 519–525

Edvinsson, L., Ekman, R., Hedner, P., Valdemarsson, S. (1990) Congestive heart failure: involvement of perivascular peptides reflecting activity in sympathetic, parasympathetic and afferent fibers. Eur. J. Clin. Invest. 20: 85–89

Ekblad, E., Edvinsson, L., Wahlestedt, C., Uddman, R., Håkanson, R. (1984) Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular fibers. Regul. Pept. 8: 225–235

Erlinge, D., Yoo, H., Edvinsson, L., Reis, D. J., Wahlestedt, C. (1993) Mitogenic effects of ATP on vascular smooth muscle cells vs. other growth factors and sympathetic cotransmitters. Am. J. Physiol. 265: H1089–H1097

Feng, Q. P. (1993) Sympathetic Regulation of Cardiovascular and Renal Function in Rats with Experimental Congestive Heart Failure. Thesis, Göteborg University, Sweden, pp 1–86

Grundemar, L., Håkanson, R. (1994) Neuropeptide Y effector systems: perspectives for drug development. Trends Pharmacol. Sci. 15: 153–159

Kasakov, L., Ellis, J., Kirkpatick, K., Milner, P., Burnstock, G. (1988) Direct evidence for concomitant release of noradrenaline, adenosine 5′-triphosphate and neuropeptide Y from sympathetic nerves supplying the guinea-pig vas deferens. J. Auton. Nerv. Syst. 22: 75–78

Lacroix, J. S., Stjärne, P., Änggård, A., Lundberg, J. M. (1989) Sympathetic vascular control of the pig nasal mucous (III): co-release of noradrenaline and neuropeptide Y. Acta. Physiol. Scand. 135: 17–28

Leys, K., Schachter, M., Sever, P. (1987) Auto radiographic localisation of neuropeptide Y receptors in rabbit kidney: comparison with rat, guinea-pig and human. Eur. J. Pharmacol. 134: 233–237

Linton-Dahlöf, P. (1989) Modulatory interactions of neuropeptide Y (NPY) on sympathetic neurotransmission. Acta. Physiol. Scand. 137 (Suppl. 586): 1–85

Lundberg, J. M., Terenius, L., Hökfelt, L., Hökfelt, T., Goldstein, M. (1983) High levels of neuropeptide Y (NPY) in peripheral noradrenergic neurones in various mammals including man. Neurosci. Lett. 42: 167–172

Lundberg, J. M., Rudehill, A., Sollevi, A., Hamberger, B. (1989) Evidence for co-transmitter role of neuropeptide Y in the pig spleen. Br. J. Pharmacol. 96: 675–687

Sun, X. Y., Dahlöf, C., Edvinsson, L., Hedner, T. (1991) D-myo-inositol-triphosphate is a selective antagonist of neuropeptide Y-induced pressor response in the pithed rat. Eur. J. Pharmacol. 204: 281–286

Sun, X. Y., Feng, Q. P., Zhao, X. H., Edvinsson, L., Hedner, T. (1995) Cardiovascular and renal effects of alpha-trinositol in ischaemic heart failure rats. Life Sci. 57: 1197–1211

Torres, G., Bitran, M., Huidobro-Toro, J. P. (1992) Co-release of neuropeptide Y (NPY) and noradrenaline from sympathetic nerve terminals supplying the rat vas deferens; influence of calcium and the stimulation intensity. Neurosci. Lett. 148: 39–42

Valdemarsson, S., Bergdahl, A., Edvinsson, L. (1994) Relationships between plasma levels of catecholamines and neuropeptides and the survival time in patients with congestive heart failure. J. Intern. Med. 235: 595–601

Zukowska-Grojec, Z., Karwatowska-Prokopczuk, E., Rose, W., Rone, J., Movafagh, S., Ji, H., Yeh, Y., Chen, W. T., Kleinman, H. K., Grouzmann, E., Grant, D. S. (1998) Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. Circ. Res. 83: 187–195