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Receptor subtypes Y_1 and Y_5 are involved in the renal effects of neuropeptide Y

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1 Systemic infusion of neuropeptide Y (NPY) reduces renal blood flow and can concomitantly increase diuresis, natriuresis and calciuresis in anaesthetized rats. The present study was designed to investigate whether the apparently contradictory NPY effects on renal blood flow and urine formation and composition are mediated by distinct NPY receptor subtypes.

2 NPY and its analogues, peptide YY (PYY), [Leu^{31}, Pro^{34}]NPY and NPY_{13-36}, were infused in incremental doses of 0.3, 1 and 3 μg kg^{-1} min^{-1} for 45 min each and the results compared to those obtained in vehicle-infused rats. Renal blood flow was monitored in 1–5 min intervals, while urine excretion and composition were determined in 15 min collection periods. Plasma renin activity and aldosterone concentrations were measured at the end of the final infusion period.

3 Relative to vehicle NPY, PYY and [Leu^{31}, Pro^{34}]NPY dose-dependently reduced renal blood flow and increased diuresis, natriuresis and calciuresis with roughly similar potency; NPY_{13-36} slightly but significantly increased renal blood flow but had no effect on diuresis, natriuresis and calciuresis. None of the peptides significantly affected endogenous creatinine clearance or kaliuresis.

4 Plasma renin activity was significantly reduced by PYY. Quantitatively similar reductions were observed with NPY and [Leu^{31}, Pro^{34}]NPY but failed to reach statistical significance with the given number of experiments. NPY_{13-36} did not reduce plasma renin activity. None of the peptides significantly affected plasma aldosterone concentrations.

5 In another series of experiments infusion of PYY_{3-36} (2 μg kg^{-1} min^{-1} for 120 min) did not reduce renal blood flow but significantly enhancd diuresis and natriuresis to a similar extent as the NPY 2 μg kg^{-1} min^{-1}.

6 In a final series of experiments the Y_1-selective antagonist, BIBP 3226 (1 or 10 μg kg^{-1} min^{-1}) dose-dependently antagonized reductions of renal blood flow elicited by bolus injections of NPY (0.1–30 μg kg^{-1}). BIBP 3226 (10 μg kg^{-1} min^{-1}) also inhibited the effects of a 120 min infusion of NPY (2 μg kg^{-1} min^{-1}) on renal blood flow but had only minor inhibitory effects on enhancements of diuresis and did not significantly affect enhancements of natriuresis.

7 We conclude that NPY reduces renal blood via a classical Y_1 subtype of NPY receptor. In contrast enhancements of diuresis, natriuresis and calciuresis occur via a distinct subtype which resembles the receptor that mediates NPY-induced enhancement of food intake.

Keywords: Neuropeptide Y; BIBP 3226; receptor subtypes; renal blood flow; diuresis; natriuresis

Introduction

Neuropeptide Y (NPY) is a cotransmitter of the sympathetic nervous system and affects cardiovascular function at multiple levels (for reviews see Dumont *et al*., 1991; Michel & Rascher, 1995). Thus, centrally or peripherally administered NPY alters systemic and local haemodynamics by effects on the brain, spinal cord, heart, vasculature and kidney. The kidney appears to play a prime role in cardiovascular regulation due to its infinite gain control mechanism of blood pressure (Guyton, 1991). NPY could potentially affect renal function via alterations of intrarenal haemodynamics, glomerular filtration, tubular transport processes, renin release and/or secondary to systemic effects; evidence for most of these possibilities has been presented. Thus, NPY-induced renal vasoconstriction has been demonstrated in a number of species by various investigators by *in vivo* as well as *in vitro* techniques (Allen *et al*., 1985; Hackenthal *et al*., 1987; Echtenkamp & Dandridge, 1989; Minson *et al*., 1989; Pernow & Lundberg, 1989; Persson *et al*., 1991; Rieß *et al*., 1995; Bischoff *et al*., 1996). Renal vasoconstriction by other hormones is typically accompanied by reduced diuresis and natriuresis, and intrarenal NPY administration in conscious dogs (Persson *et al*., 1991) or in primates can indeed reduce diuresis (Echtenkamp & Dandridge, 1989). On the other hand, it was found that NPY can enhance diuresis, natriuresis and calciuresis in anaesthetized rats (Smyth *et al*., 1988; Bischoff *et al*., 1996).

In order to solve the apparent contradiction between renal vasoconstriction and enhanced diuresis and natriuresis in the rat, we have recently compared NPY effects on renal blood flow (RBF) with those on diuresis and electrolyte excretion (Bischoff *et al*., 1996). Our data demonstrate that NPY-induced reductions of RBF and enhancements of urine and electrolyte excretions can occur concomitantly but can be differentiated regarding their dose-dependency and time course. While NPY maximally reduced RBF within the first 2–3 min of systemic infusion, enhancement of diuresis and electrolyte excretion required 45 min to develop fully. RBF reductions were detectable following as little as 0.1 μg kg^{-1} min^{-1} NPY, while enhancements of diuresis and natriuresis were not detectable with less than 1 μg kg^{-1} min^{-1}.

Similar to NPY big endothelin can reduce RBF and concomitantly enhance diuresis and natriuresis. However, reduction of RBF occurred via an ET_A subtype of endothelin receptor whereas enhancement of diuresis and natriuresis did not (Pollock & Opgenorth, 1994). NPY is also known to act via multiple receptor subtypes (Michel, 1991; Gehlert, 1994). At present, three subtypes of NPY receptor have been identified which are defined primarily based on the order of potency of selective agonists including NPY, its endogenous analogue peptide YY (PYY), [Pro34]-substituted NPY analogue such as

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[Leu^{31}, Pro^{34}]NPY, and C-terminal NPY or PYY fragments such as NPY_{13-36} or PYY_{3-36}. More recently, potent NPY antagonists have also been introduced. Among those BIBP 3226 has been shown to be selective for Y_1 over Y_2 receptors *in vitro* and *in vivo* (for review see Doods *et al*., 1996).

In analogy to the above data on big endothelin, we have hypothesized that NPY might cause its apparently contradictory renal effects by activation of multiple receptor subtypes. Therefore, we have compared the effects of NPY, PYY, [Leu^{31}, Pro^{34}]NPY, NPY_{13-36} and vehicle on systemic haemodynamics, RBF, endogenous creatinine clearance, diuresis, sodium, potassium and calcium excretion, plasma renin activity and plasma aldosterone. Due to the differential time course and dose-dependency of renovascular and tubular NPY effects (see above), we have chosen a study design in which three incremental doses of each of the above peptides (0.3–3 μg kg^{-1} min^{-1}) are infused for 45 min each. In additional experiments the agonistic effects of the Y_2-selective agonist, PYY_{3-36}, and the Y_1-selective antagonist, BIBP 3226, were investigated by use of 120 min continuous infusion and/or bolus injections.

Methods

Animal surgery and experimental protocol

Male Wistar rats (strain: Hsd/Cpb:WU) were obtained from Harlan CPB (Zeist, Netherlands) and instrumented as previously described (Smits *et al*., 1983; Bischoff *et al*., 1996). Briefly, rats were unilaterally nephrectomized (left kidney) 7–10 days before the experiment. On the day of the experiment the animals were anaesthetized with an initial i.p. injection of sodium pentobarbitone (60 mg kg^{-1}) and additional doses of 3 mg i.v. were administered every 30–45 min. The animals were placed on a heating pad to maintain the body temperature at 37°C. Following tracheotomy to facilitate ventilation, the left femoral artery and vein were cannulated for monitoring mean arterial pressure (MAP) and heart rate (HR) and for infusion of vehicle and peptide solutions, respectively. Following an abdominal midline incision, the ureter was cannulated for urine sampling. Connective tissue was carefully dissected from the right renal artery, and an electromagnetic blood flow sensor was placed on the renal artery. Before the start of the experiment animals were allowed 3 h of recovery, during which time 60–80 μl min^{-1} of 0.9% saline were infused via the femoral vein. MAP, HR, RBF and urine formation had stabilized at the end of this equilibration period.

Following recovery from surgery, vehicle (0.9% saline), peptide or antagonist solutions were infused via the femoral vein at a rate of 60–80 μl min^{-1}. In study 1 each rat was infused for three consecutive experimental periods of 45 min each with incremental peptide doses of 0.3, 1 and 3 μg kg^{-1} min^{-1}. Forty-five minute infusion periods were chosen because our previous study had shown that this time is necessary to observe maximal effects for NPY-induced alterations of renal function (Bischoff *et al*., 1996). In study 2 each rat obtained a continuous infusion of the antagonist, BIBP 3226 (1 or 10 μg kg^{-1} min^{-1}), or vehicle starting 90 min after completion of surgery. Starting 30 min thereafter the rats received bolus injections (100 μl 100 g^{-1} body weight) of the indicated NPY doses or vehicle in 30 min intervals. Animals for study 2 did not undergo ureter catherization. In study 3 each rat received a continuous infusion of BIBP 3226 (10 μg kg^{-1} min^{-1}) or vehicle starting 90 min after completion of surgery. An infusion of NPY or PYY_{3-36} (2 μg kg^{-1} min^{-1}) or vehicle was started 30 min thereafter and continued for 120 min. In all three studies MAP, HR and RBF were measured every 5 min for the whole experiment; during the first 10 min of each experimental period or after each bolus injection RBF was quantitated every minute. Urine was collected in preweighed tubes in 15 min intervals. At the end of the experiment a blood sample was taken from the abdominal aorta, and subsequently the rat was killed with an overdose of pentobarbitone.

Biochemical analysis

Urine formation was quantitated gravimetrically assuming a specific gravity of 1.0 kg l^{-1}, and samples were stored at 4°C until analysis. Serum and plasma were prepared from the aortic blood sample by centrifugation (10 min at 5000 *g*) and stored at −20°C until analysis. Urinary sodium, potassium and calcium concentrations were determined with an Eppendorf flame photometer. Urinary and serum creatinine was determined with an automated analysis system for clinical chemistry (Hitachi 707). Plasma renin activity was measured as previously described (Hackenthal, 1985). Plasma aldosterone concentrations were determined with a commercially available radioimmunoassay (Sorin Biomedica, Saluggia, Italy).

Chemicals

Rat/human NPY, PYY, [Leu^{31}, Pro^{34}]NPY, NPY_{13-36} and PYY_{3-36} were obtained from Saxon Biochemicals GmbH (Hannover, Germany) and sodium pentobarbitone (Nembutal) from Sanovi (Hannover, Germany). BIBP 3226 ((R)-N^2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl) methyl]argininamide) (Doods *et al*., 1996) was a kind gift of Dr Karl Thomae GmbH (Biberach an der Riss, Germany).

Data analysis

The average MAP, HR, and RBF during the last 30 min and urine formation during the last 45 min before the start of the experiment in each animal was taken as baseline; baseline data for study 1 are shown in Table 1, baseline data for studies 2 and 3 were similar (data not shown). All other experimental data are expressed as alteration relative to the baseline obtained in the individual animal. Initially rats were subdivided on a random basis into five groups receiving vehicle, NPY, PYY, [Leu^{31}, Pro^{34}]NPY or NPY_{13-36}. Since baseline RBF, diuresis and electrolyte excretion in the PYY group differed considerably from those in the other groups, a sixth group also receiving PYY was later added to the study. Baseline values in the sixth group were similar to those in the other groups (Table 1). Since PYY-induced alterations were not significantly different between the two PYY groups (data not shown), pooled data for both groups are presented throughout the manuscript except for Table 1.

Data are presented as mean ± s.e.mean of *n* experiments. Statistical significance of differences between experimental groups were determined by one-way analysis of variance followed by unpaired two-tailed *t* tests with Bonferroni corrections for multiple comparisons or two-way analysis of variance as indicated. A $P<0.05$ was considered significant. Statistical calculations were performed with the InStat and Prism progammes (GraphPAD Software, San Diego, CA).

Results

Study 1

Intravenous infusion of NPY, PYY and [Leu^{31}, Pro^{34}]NPY dose-dependently increased MAP relative to vehicle-infused rats with maximal increases of ≈30 mm Hg achieved by infusion of 3 μg kg^{-1} min^{-1}NPY (Figure 1). The rank order of potency of MAP elevations was NPY ⩾ PYY ⩾ [Leu^{31}, Pro^{34}]NPY with NPY_{13-36} being without effect. Peptide-induced MAP increases were accompanied by reductions of HR (data not shown) indicating baroreflex activation.

Infusion of NPY, PYY and [Leu^{31}, Pro^{34}]NPY dose-dependently reduced RBF (Figure 2). Peak reductions of RBF always occurred within 2–3 min, and were followed by a partial tachyphylaxis. Maximal RBF reductions of

Table 1 Baseline values of systemic haemodynamics, urinary parameters and body weight

	Vehicle	*NPY*	*PYY1*	*PYY2*	*[Leu^{31},Pro^{34}] NPY*	*NPY$_{13-36}$*
Mean arterial pressure	118±5	118±4	119±5	119±5	117±6	123±5
Renal blood flow	8.7±0.7	8.2±0.9	6.8±0.4	8.9±0.6	9.4±0.7	7.8±0.6
Urine volume	152±25	195±20	355±94	151±23	133±13	138±21
Sodium excretion	22.6±5.4	30.4±4.5	59.3±17.1	15.3±3.3	16.5±3.3	12.5±4.0
Potassium excretion	25.7±3.5	30.6±2.0	24.3±1.3	47.1±20.3	31.3±4.2	51.6±25.3
Calcium excretion	0.354±0.049	0.450±0.085	1.033±0.194	0.612±0.116	0.317±0.062	0.279±0.076
Creatinine clearance	1.4±0.2	1.4±0.2	1.1±0.1	1.5±0.1	1.4±0.1	1.2±0.1
Body weight	311±8	302±5	281±8	348±10	325±7	324±11

Data are mean±s.e.mean of 6–9 animals. Mean arterial pressure is given in mmHg, renal blood flow in ml min^{-1}, urine volume in μl 15 min^{-1}, sodium, potassium and calcium excretion in μmol 15 min^{-1}, creatinine clearance in ml min^{-1} and body weight in g. Mean arterial pressure and renal blood flow data are the average of data monitored in the last 30 min and urinary values were the average of the last three collecting periods prior to start of peptide or vehicle infusion; body weights were determined on the morning of the experiment. Note that the group PYY2 was studied after completion of all other groups because the group PYY1 had considerably different baselines values from the other groups.

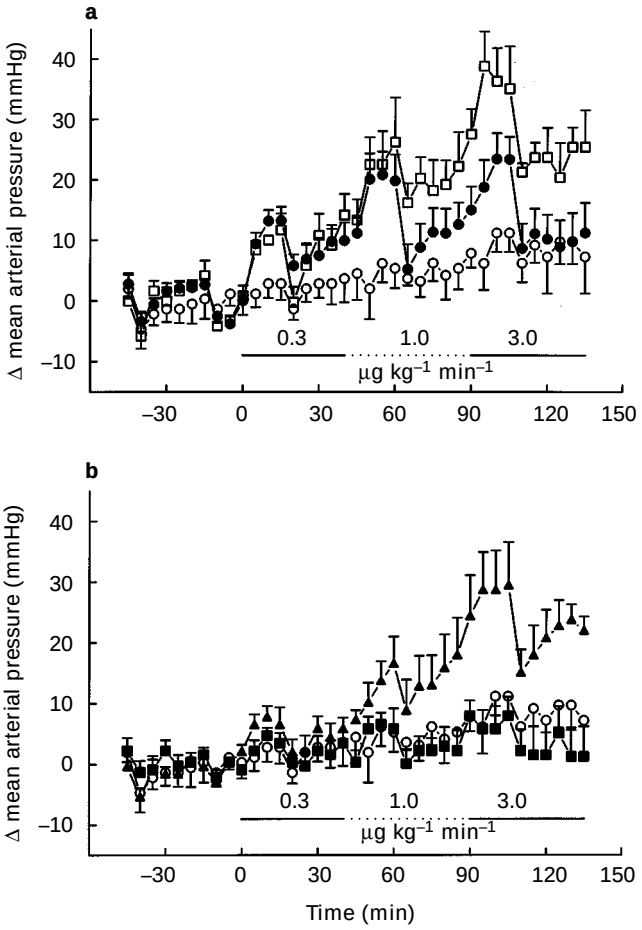

Figure 1 Effects of (a) NPY (□, $n=6$), PYY (●, $n=15$); (b) [Leu^{31}, Pro^{34}]NPY (▲, $n=8$), NPY$_{13-36}$ (■, $n=8$) and (a and b) vehicle (○, $n=6$) on mean arterial pressure in anaesthetized rats. Data are mean arterial pressure elevations relative to baseline values shown in Table 1; vertical lines show s.e.mean. Note that transient pressure reductions every 30–45 min are related to additional i.v. injections of pentobarbitone. Mean arterial pressure elevations by NPY, PYY and [Leu^{31}, Pro^{34}]NPY were significant ($P<0.05$) vs values in vehicle-infused rats by a two-way analysis of variance while those of NPY$_{13-36}$ were not. Infused peptide doses are shown in the bottom of each panel.

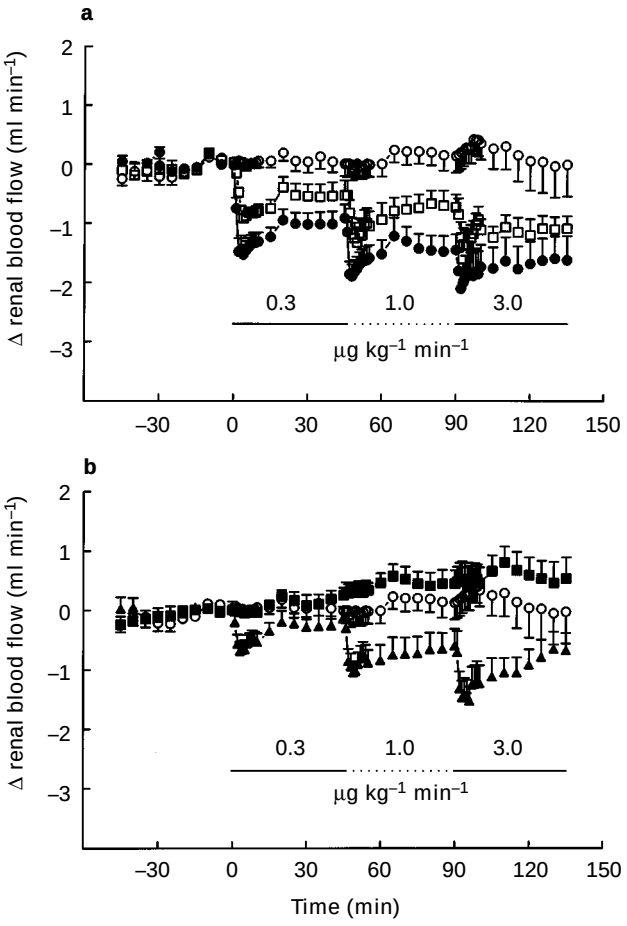

Figure 2 Effects of (a) NPY (□, $n=8$), PYY (●, $n=17$); (b) [Leu^{31}, Pro^{34}]NPY (▲, $n=9$), NPY$_{13-36}$ (■, $n=8$) and (a and b) vehicle (○, $n=8$) on renal blood flow in anaesthetized rats. Data are mean renal blood flow alterations relative to baseline values shown in Table 1; vertical lines show s.e.mean. Alterations of renal blood flow by all four peptides were statistically significant ($P<0.05$) vs vehicle-infused rats by a two-way analysis of variance with NPY, PYY and [Leu^{31}, Pro^{34}]NPY causing decreases and NPY$_{13-36}$ causing an increase. Infused peptide doses are shown in the bottom of each panel.

$\approx$2 ml min^{-1} were achieved by infusion of 3 μg kg^{-1} min^{-1} PYY. The short C-terminal fragment, NPY$_{13-36}$, did not reduce RBF slightly but significantly increased it (Figure 2). Neither of the four peptides significantly affected endogenous creatinine clearance (data not shown).

Infusion of NPY, PYY and [Leu^{31}, Pro^{34}]NPY dose-dependently increased urine formation (Figure 3), sodium (Figure 4) and calcium excretion (Figure 5) relative to vehicle-infused rats. The rank order of potency of diuresis, natriuresis and calciuresis enhancements was PYY$\geqslant$NPY$\geqslant$[Leu^{31}, Pro^{34}]

NPY while NPY_{13-36} did not significantly affect any of these parameters. Neither of the four peptides significantly affected potassium excretion (Figure 6).

At the end of the 3 μg kg^{-1} min^{-1} infusion period blood samples were obtained in which plasma renin activity and plasma aldosterone were determined. Plasma renin activity was significantly reduced by $\approx$70% by PYY (Figure 7). NPY and [Leu^{31}, Pro^{34}]NPY caused quantitatively very similar reductions of plasma renin activity but those failed to reach statistical significance with the considerably smaller number of samples tested for those peptides. NPY_{13-36} did not reduce plasma renin activity and if anything enhanced it. In contrast neither of the four peptides significantly affected plasma aldosterone concentration (Figure 7).

Study 2

These experiments were designed to establish the dose-dependency of BIBP 3226 inhibition of renovascular NPY effects. Before the first bolus injection RBF was 7.4$\pm$0.3 (n=12), 7.6$\pm$0.5 (n=6) and 8.2$\pm$0.7 ml min^{-1} (n=12) in the groups receiving vehicle, low and high dose of BIBP 3226 (1 and 10 μg kg^{-1} min^{-1}), respectively (not significantly different). Bolus injections of vehicle did not significantly affect RBF (data not shown). In contrast bolus injections of NPY (0.1–30 μg kg^{-1}) dose-dependently lowered RBF with maximal reductions of $\approx$4 ml min^{-1} (Figure 8). Coinfusion of 1 μg kg^{-1} min^{-1} BIBP 3226 caused a slight shift of the NPY dose-response curve to the right towards high doses (Figure 8). Coinfusion with 10 μg kg^{-1} min^{-1} BIBP 3226 abolished the RBF-lowering effects of NPY (Figure 8).

Study 3

These experiments were designed to expand further the subtype-characterization performed in study 1. For this purpose an infusion of NPY (2 μg kg^{-1} min^{-1}) or vehicle in the absence and presence of the high dose of BIBP 3226 or vehicle

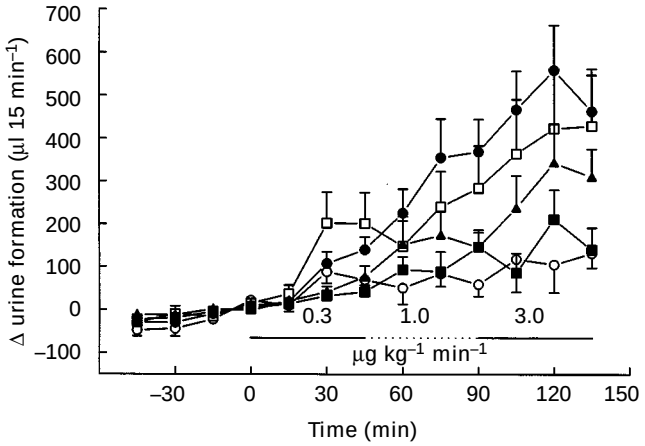

Figure 3 Effects of NPY (□, n=8), PYY (●, n=18), [Leu^{31}, Pro^{34}]-NPY (▲, n=9), NPY_{13-36} (■, n=8) and vehicle (○, n=6) on urine formation in anaesthetized rats. Data are mean urine volume alterations relative to baseline values shown in Table 1; vertical lines show s.e.mean. Urine formation increases by NPY, PYY and [Leu^{31}, Pro^{34}]NPY were significant (P<0.05) vs values in vehicle-infused rats by a two-way analysis of variance while those of NPY_{13-36} were not. Infused peptide doses are shown at the bottom.

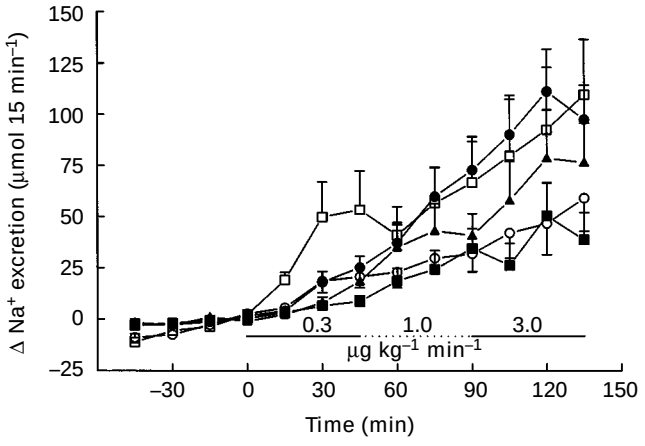

Figure 4 Effects of NPY (□, n=8), PYY (●, n=18), [Leu^{31}, Pro^{34}]-NPY (▲, n=8), NPY_{13-36} (■, n=8) and vehicle (○, n=8) on Na^+ excretion in anaesthetized rats. Data are mean Na^+ excretion alterations relative to baseline values shown in Table 1; vertical lines show s.e.mean. Na^+ excretion increases by NPY, PYY and [Leu^{31}, Pro^{34}]NPY were significant (P<0.05) vs values in vehicle-infused rats by a two-way analysis of variance while those of NPY_{13-36} were not. Infused peptide doses are shown at the bottom.

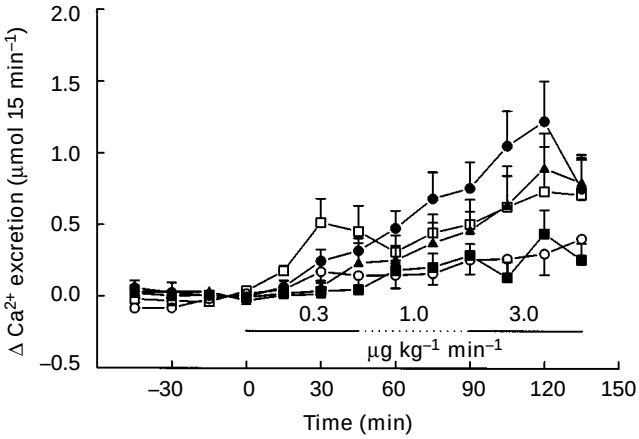

Figure 5 Effects of NPY (□, n=8), PYY (●, n=18), [Leu^{31}, Pro^{34}]-NPY (▲, n=8), NPY_{13-36} (■ n=8) and vehicle (○, n=8) on Ca^{2+} excretion in anaesthetized rats. Data are mean Ca^{2+} excretion alterations relative to baseline values shown in Table 1; vertical lines show s.e.mean. Ca^{2+} excretion increases by NPY, PYY and [Leu^{31}, Pro^{34}]NPY were significant (P<0.05) vs values in vehicle-infused rats by a two-way analysis of variance while those of NPY_{13-36} were not. Infused peptide doses are shown at the bottom.

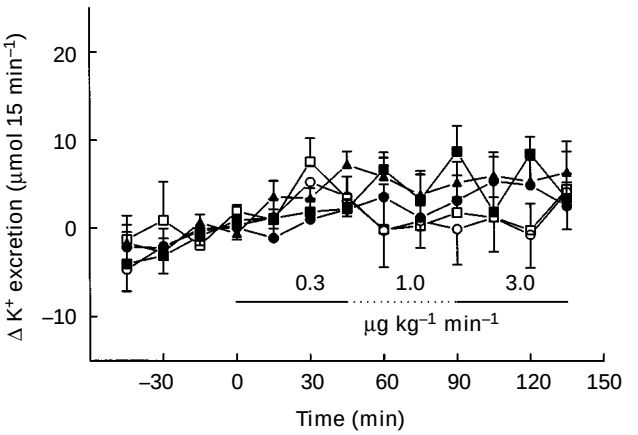

Figure 6 Effects of NPY (□, n=8), PYY (●, n=18), [Leu^{31}, Pro^{34}]NPY (▲, n=9), NPY_{13-36} (■, n=8) and vehicle (○, n=8) on K^+ excretion in anaesthetized rats. Data are mean K^+ excretion alterations relative to baseline values shown in Table 1 vertical lines show s.e.mean. None of the peptides significantly altered K^+ excretion relative to baseline values as assessed by a two-way analysis of variance. Infused peptide doses are shown at the bottom.

was performed for 120 min; in parallel other rats received a 120 min infusion of the long C-terminal fragment, PYY_{3-36}. Infusion of BIBP 3226 alone did not significantly alter MAP (Figure 9). In contrast infusion of 2 μg kg^{-1} min^{-1} NPY elevated MAP by $\approx$20 mmHg relative to vehicle-infused rats, and this increase was markedly inhibited by coinfusion of BIBP 3226 (Figure 9). Infusion of PYY_{3-36} did not significantly alter MAP relative to vehicle-infused rats (Figure 9).

Similar to study 2, infusion of BIBP 3226 did not significantly alter basal RBF values (8.0$\pm$0.3 vs 8.5$\pm$0.5 ml min^{-1}, n=20 and 15, respectively). However, a comparison of RBF values in the absence and presence of BIBP 3226 throughout the whole 120 min infusion period in a two-way analysis of variance revealed a significant enhancement of RBF ($P<0.031$; Figure 10). NPY rapidly reduced RBF with peak reductions of $\approx$1.8 ml min^{-1} in the first 3 min and a partial tachyphylaxis thereafter (Figure 10). The RBF-lowering effects of NPY were markedly inhibited upon coinfusion with BIBP 3226 (Figure 10). In contrast the long C-terminal fragment, PYY_{3-36}, did not reduce RBF but rather significantly enhanced it relative to vehicle-infused rats ($P<0.0001$; Figure 10).

Diuresis (119 μl 15 min^{-1}) remained stable upon vehicle infusion and was not markedly altered upon infusion of BIBP 3226 (Figure 11). In contrast infusion of NPY increased diuresis, and this enhancement was only partially blocked by coinfusion of BIBP 3226 (Figure 11). Infusion of PYY_{3-36} enhanced diuresis to a similar extent and with a similar time course as did NPY (Figure 11). Natriuresis (6.0 μmol 15 min^{-1}) also remained stable upon vehicle infusion and was not affected by BIBP 3226 infusion (Figure 12). Natriuresis

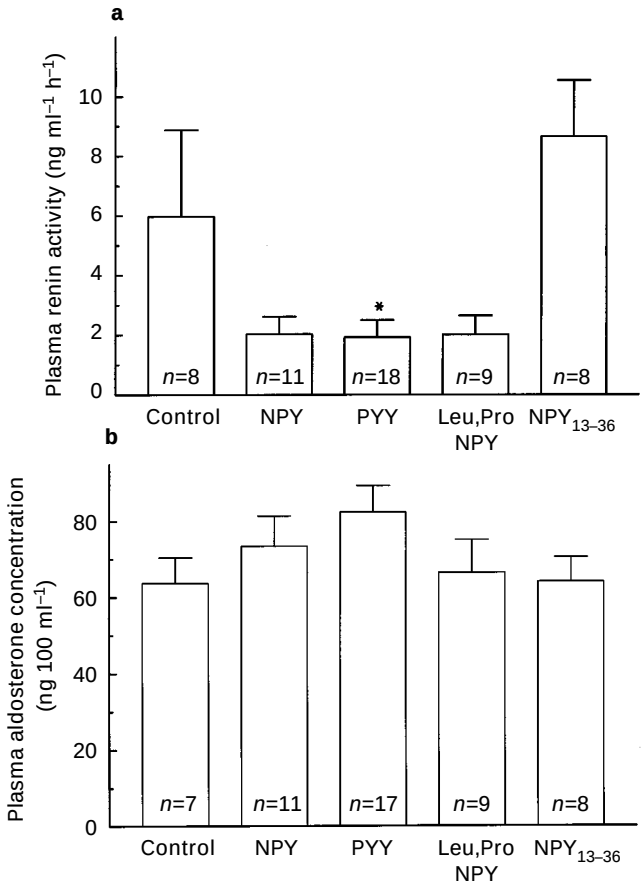

Figure 7 Effects of NPY, PYY, [Leu^{31,} Pro^{34}]NPY ('Leu,Pro NPY'), NPY_{13-36} and vehicle (control) on (a) plasma renin activity and (b) plasma aldosterone concentrations in anaesthetized rats. Data are mean$\pm$s.e.mean of the number of indicated experiments. Values were determined in plasma taken from an aortic blood sample at the end of the 3 μg kg^{-1} min^{-1} infusion period. *$P<0.05$ vs control by a one-way analysis of variance followed by Dunnett's multiple comparison test.

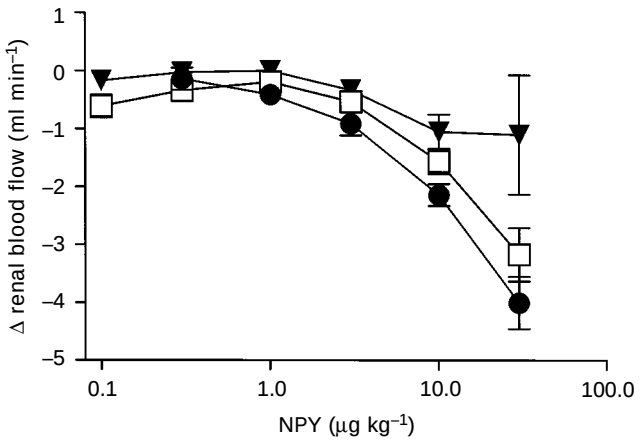

Figure 8 Effects of bolus injections of NPY on renal blood flow in anaesthetized rats infused with vehicle (●, n=12), 1 μg kg^{-1} min^{-1} (□, n=6) or 10 μg kg^{-1} min^{-1} BIBP 3226 (▲, n=12). Data are mean renal blood flow alterations relative to baseline values as determined before each bolus injection; vertical lines show s.e.mean.

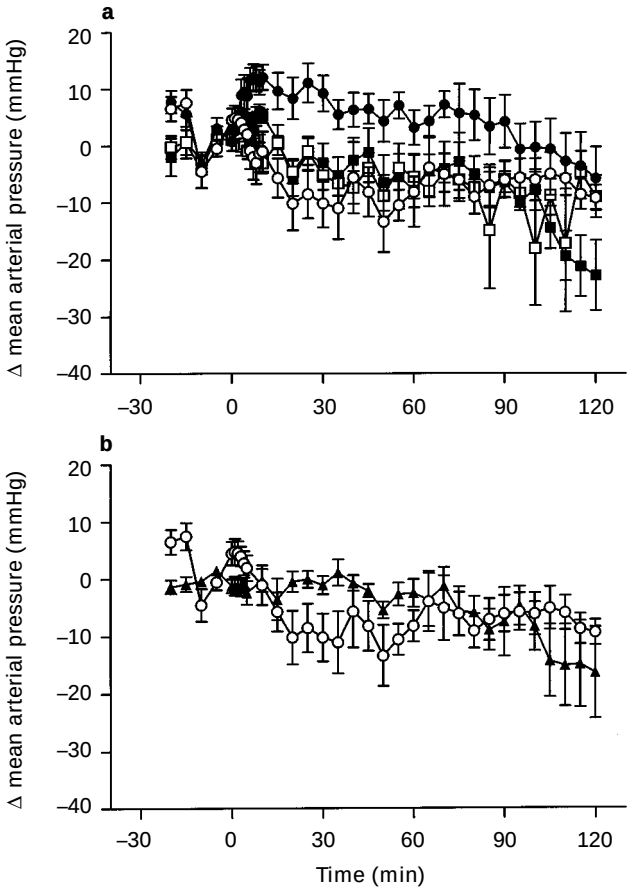

Figure 9 Effects of vehicle (a and b, ○, n=6) (a) 10 μg kg^{-1} min^{-1} BIBP 3226 (□ n=8), 2 μg kg^{-1} min^{-1} NPY alone (●, n=7), NPY + BIBP 3226 (■, n=7), and (b) 2 μg kg^{-1} min^{-1} PYY_{3-36} (▲, n=7) on mean arterial pressure in anaesthetized rats. Data are mean arterial pressure elevations relative to baseline values determined in each animal; vertical lines show s.e.mean. Note that transient pressure reductions every 30–45 min are related to additional i.v. injections of pentobarbitone. Mean arterial pressure elevations by NPY were significant ($P<0.05$) vs values in vehicle-infused rats, by two-way analysis of variance, while those of NPY in the presence of BIBP 3226 or of PYY_{3-36} were not.

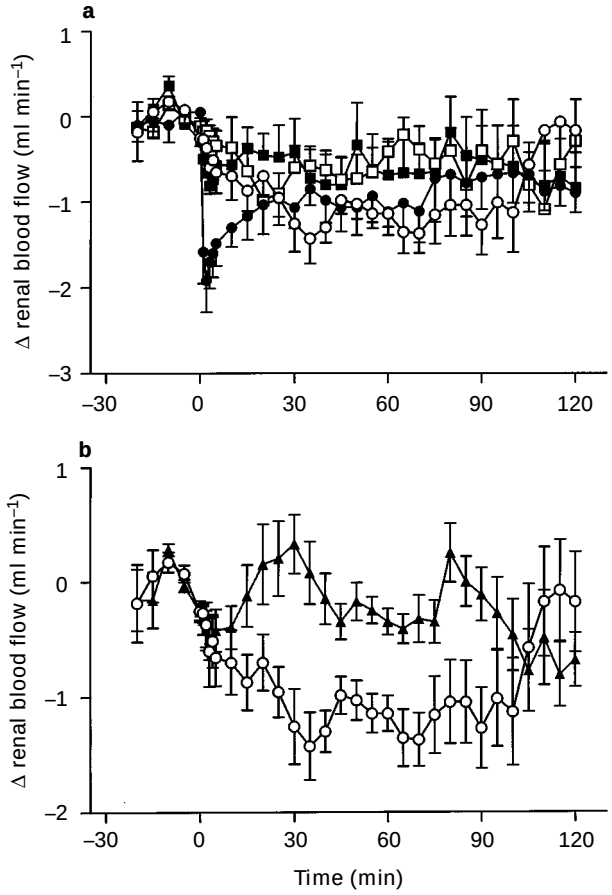

Figure 10 Effects of vehicle (a and b, ○, $n=6$), (a) 10 μg kg^{-1} min^{-1} BIBP 3226 (□, $n=8$), 2 μg kg^{-1} min^{-1} NPY alone (●, $n=7$), NPY + BIBP 3226 (■, $n=7$), and (b) 2 μg kg^{-1} min^{-1} PYY_{3-36} (▲, $n=7$) on renal blood flow in anaesthetized rats. Data are mean renal blood flow alterations relative to baseline values determined in each animal; vertical lines show s.e.mean. Renal blood flow reductions by NYP alone and increases by PYY_{3-36} were significant ($P<0.05$) vs values in vehicle-infused rats by a two-way analysis of variance while the effects of NPY in the presence of BIBP 3226 were not.

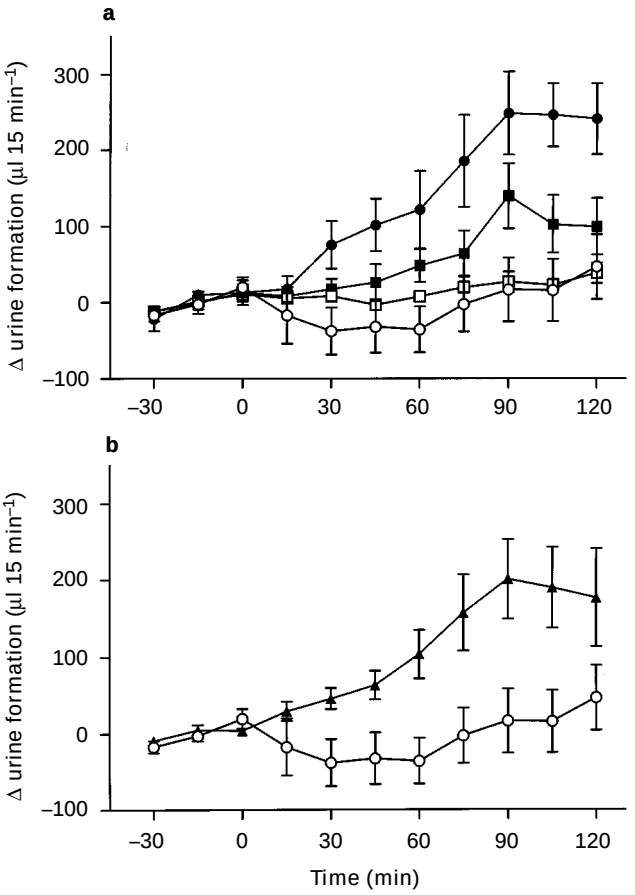

Figure 11 Effects of vehicle (a and b, ○, $n=6$), (a) 10 μg kg^{-1} min^{-1} BIBP 3226 (□, $n=8$), 2 μg kg^{-1} min^{-1} NPY alone (●, $n=7$), NPY + BIBP 3226 (■, $n=7$), and (b) 2 μg kg^{-1} min^{-1} PYY_{3-36} (▲, $n=7$) on diuresis in anaesthetized rats. Data are mean urine formation relative to baseline values determined in each animal; vertical lines show s.e.mean. Diuresis enhancements by NPY alone, NPY + BIBP 3226 and PYY_{3-36} were significant ($P<0.05$) vs values in vehicle-infused rats by a two-way analysis of variance; the inhibitory effect of BIBP 3226 vs NPY alone was also statistically significant ($P<0.05$).

was considerably increased by NPY infusion, and this was not significantly inhibited by coinfusion of BIBP 3226 (Figure 12). Infusion of PYY_{3-36} increased natriuresis to a similar extent and with a similar time course as did infusion of NPY (Figure 12).

Discussion

Our data confirm that systemic infusion of NPY increases MAP, decreases RBF and concomitantly increases diuresis, natriuresis and calciuresis in the anaesthetized rat; kaliuresis and endogenous creatinine clearance, an indicator of glomerular filtration rate, are not significantly affected. Our previous (Bischoff *et al*., 1996) and present data indicate that NPY-induced RBF reductions rapidly develop tachyphylaxis. Therefore, the present design of incremental infusion doses tests for NPY-induced RBF alterations in a preparation which may already partially be desensitized towards NPY. Our previous (Bischoff *et al*., 1996) and present data also indicate that NPY-induced enhancements of diuresis, natriuresis and calciuresis are slow in onset, i.e. requiring at least 45 min to reach maximum values; similarly, reversal of NPY-induced diuresis, natriuresis and calciuresis is also slow requiring at least 30 min to be complete. Thus, the present design of study 1 cannot exclude the possibility that the effects of the second and third dose of NPY at least partially also reflect effects of the preceding dose which have not yet fully waned. Thus, at each dose the order of potency of the four peptides can be compared, but unequivocal dose-response curves for an individual peptide cannot be derived from these data. However, this appears to be the most adequate study design for the comparison of the receptor subtypes involved in renovascular and tubular NPY effects due their the differential time courses and dose-dependencies.

It is now widely accepted that at least three subtypes of NPY receptor exist (Michel, 1991; Gehlert, 1994). The definition of subtypes of NPY receptors still mainly rests on the order of potency of agonistic NPY analogues. Thus, NPY, its endogenous analogue PYY, its [Pro^{34}]-substituted analogues (e.g.[Leu^{31}, Pro^{34}]NPY) and its C-terminal fragments (e.g. NPY_{13-36} or PYY_{3-36}) are used to describe NPY receptor subtypes. In this scheme a Y_1 receptor is defined by the rank order of potency PYY $\geqslant$ NPY $\geqslant$ [Pro^{34}]-substituted analogue $>>$ C-terminal fragment. A Y_2 receptor is defined by the rank order of potency PYY $\geqslant$ NPY $\geqslant$ C-terminal fragment $>>$ [Pro^{34}]-substituted analogue. At Y_3 receptors PYY is considerably less potent than NPY. In addition subtype-selective antagonists have recently become available. Among these BIBP 3226 has been shown to be selective for Y_1 over Y_2 receptors *in vitro* and *in vivo* (for review see Doods *et al*., 1996). Therefore, the present study has compared the potency of

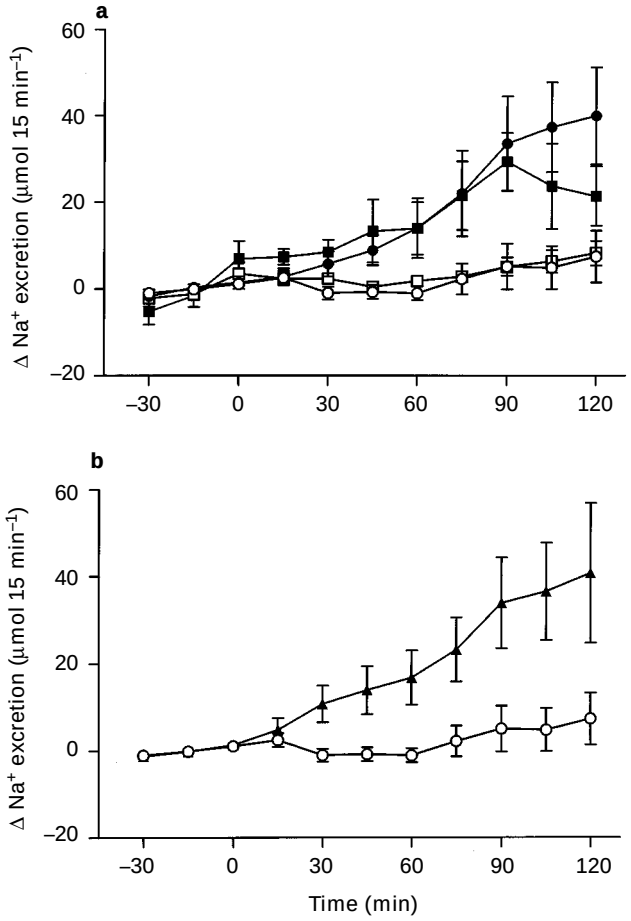

Figure 12 Effects of vehicle (a and b, ○, $n=6$), (a) 10 μg kg^{-1} min^{-1} BIBP 3226 (□, $n=8$), 2 μg kg^{-1} min^{-1} NPY alone (●, $n=7$), NPY + BIBP 3226 (■, $n=7$), and (b) 2 μg kg^{-1} min^{-1} PYY_{3-36} (▲, $n=7$) on natriuresis in anaesthetized rats. Data are mean sodium excretion relative to baseline values determined in each animal; vertical lines show s.e.mean. Enhancements of sodium excretion by NPY alone, NPY + BIBP 3226 and PYY_{3-36} were significant ($P<0.05$) vs values in vehicle-infused rats by two-way analysis of variance. BIBP 3226 did not significantly affect NPY-induced enhancements in this analysis.

NPY, PYY, [Leu^{31}, Pro^{34}]NPY, NPY_{13-36} and PYY_{3-36} to alter MAP, RBF and urine formation and composition. The ability of BIBP 3226 to inhibit NPY-induced alterations of renal function was also investigated.

In the present study NPY, PYY and [Leu^{31}, Pro^{34}]NPY rapidly increased MAP while NPY_{13-36} and PYY_{3-36} were ineffective. Moreover, BIBP 3226 antagonized the MAP-elevating effect of NPY. This and numerous studies by other investigators (for review see Michel & Rascher, 1995) and ourselves (Michel *et al*., 1992) demonstrate that NPY increases MAP via a Y_1 receptor in rats. MAP alterations can affect renal function by a mechanism termed 'pressure natriuresis' (Firth *et al*., 1990). While pressure natriuresis may contribute somewhat to the renal NPY effects, we have previously found that NPY-induced diuresis and natriuresis are maintained when renal perfusion pressure is kept constant and thus occur at least partly independent of pressure natriuresis (Bischoff *et al*., 1996).

The rank order of potency for RBF reductions was PYY $\geqslant$ NPY $\geqslant$ [Leu^{31}, Pro^{34}]NPY; NPY_{13-36} and PYY_{3-36} did not reduce RBF but rather caused small but statistically significant increases of RBF relative to vehicle-infused rats. The RBF-lowering effects of NPY were antagonized by the Y_1-selective antagonist, BIBP 3226. Our experiments with bolus injections demonstrate that antagonism was dose-dependent with an almost complete blockade at 10 μg kg^{-1} min^{-1} BIBP 3226. Y_1 receptor-mediated renal vasoconstriction has also been described based on the effect of agonists in a rat isolated perfused kidney preparation (Oellerich & Malik, 1993), *in vivo* in pigs (Modin *et al*., 1991), and in the rabbit isolated renal artery (Rieß *et al*., 1995). Renal vasoconstriction via a Y_1 receptor has also been demonstrated based on the effects of antagonists in the rat isolated kidney with BIBP 3226 (Doods *et al*., 1995) or the Y_1-selective antagonist 1229U91 (Hedge *et al*., 1995). Thus, all available data suggest that renal vasoconstriction by NPY and related peptides occurs via a classical Y_1 receptor. The small but consistent enhancements of RBF by BIBP 3226 infusion indicate that at least in the anaesthetized rat a tonic endogenous stimulation of renovascular Y_1 NPY receptors may exist.

In contrast to NPY the Y_2-agonists, NPY_{13-36} and PYY_{3-36}, caused small but significant increases of RBF relative to vehicle infused rats. NPY inhibits noradrenaline release in most model systems (Michel & Rascher, 1995) including human and rabbit kidney (Rieß *et al*., 1995) via a Y_2 receptor. Noradrenaline is a potent renal vasoconstrictor (Wolff *et al*., 1989). Therefore, we propose that the small but significant increases in RBF induced by the Y_2-selective agonists may occur secondary to activation of presynaptic NPY receptors on sympathetic nerve terminals resulting in reduced noradrenaline release and thus less α-adrenoceptor-mediated renal vasoconstriction. A definitive proof of this hypothesis in rats was beyond the scope of the present study, and other possibilities, e.g. vasodilatation by mast cell-derived histamine should be considered.

To extend our previous studies we have now also investigated the effects of NPY and its analogues on plasma renin activity and aldosterone concentrations. By use of a variety of *in vivo* and *in vitro* models many investigators have shown that NPY can reduce plasma renin activity or inhibit renin release in rats, most probably by a direct inhibitory effect on renin release from the juxtaglomerular cells (Hackenthal *et al*., 1987; Aubert *et al*., 1988; 1992). The inhibition of renin release can already be observed at NPY doses which lack effects on systemic haemodynamics in rats (Aubert *et al*., 1992) and cats (Corder *et al*., 1989). In the present study a significant reduction of plasma renin levels was detected upon PYY infusion; NPY and [Leu^{31}, Pro^{34}]NPY caused quantitatively similar inhibition but their effects did not reach statistical significance with the given number of rats, while NPY_{13-36} had no inhibitory effects on plasma renin activity. Similarly, Aubert *et al*. (1992) found that NPY and [Pro^{34}]NPY inhibited β-adrenoceptor-stimulated renin release in rats while NPY_{13-36} had no effect. Thus, inhibition of renin release in anaesthetized rats appears to occur via a Y_1 receptor. While some studies on isolated adrenals have suggested that NPY may also modulate aldosterone release (Neri *et al*., 1990; Bernet *et al*., 1994), the present study did not find altered plasma aldosterone levels in rats treated with NPY or its analogues, indicating that the regulation of plasma aldosterone *in vivo* may be complex.

Our attempts to define the receptor subtype mediating tubular NPY effects yielded surprising and, at first inspection, contradictory results: thus, the order of potency for enhancement of diuresis, natriuresis and calciuresis observed in study 1 was PYY $\geqslant$ NPY $\geqslant$ [Pro^{34}]NPY $>>$ NPY_{13-36}. This is consistent with mediation via a Y_1 subtype (Michel, 1991; Gehlert, 1994). On the other hand, PYY_{3-36} also enhanced diuresis and natriuresis with a time course and an efficacy identical to that of NPY, despite the fact that PYY_{3-36} is at least as selective for Y_2 over Y_1 receptors as short C-terminal fragments such as PYY_{13-36} or NPY_{13-36} (Wieland *et al*., 1995). Moreover, BIBP 3226, in a dose where it markedly inhibited renovascular and MAP effects of NPY, only partially inhibited NPY-induced diuresis and lacked statistically significant inhibition of NPY-induced natriuresis. These findings with PYY_{3-36} and BIBP 3226 are obviously not consistent with the pharmacological profile of a classical Y_1 NPY receptor.

NPY is a very potent stimulator of food intake in the central nervous system (Stanley & Leibowitz, 1984; Levine & Morley, 1984). Stimulation of food intake by NPY occurs by a receptor which is similar to but clearly distinct from the Y_1 subtype: thus, NPY, PYY and [^{34}Pro]-substituted analogues are potent agonists while short C-terminal fragments, e.g. NPY_{13-36} are not (Stanley *et al*., 1985; 1992). On the other hand, long C-terminal fragments such as PYY_{3-36} (Conze *et al*., 1995) or NPY_{3-36} (Doods, personal communication) are also agonists for this response. Moreover, the Y_1 antagonist, BIBP 3226, does not block the response to NPY (Doods *et al*., 1996). This situation is very similar to that observed for enhancements of diuresis and natriuresis in the present study. To the best of our knowledge no other responses have been described in peripheral tissues which are mediated by a receptor subtype similar to that mediating food intake. Very recently the cloning of an additional NPY receptor subtype has been obtained which has the above characteristics and has been designated Y_5 (Gerald *et al*., 1996).

In conclusion the present study demonstrates that NPY reduced RBF via a classical Y_1 receptor. In contrast enhancements of diuresis, natriuresis and calciuresis may occur by a similar but distinct receptor which is activated by PYY_{3-36} and not inhibited by BIBP 3226. This receptor resembles the one mediating enhancements of food intake in the CNS and may possibly represent a novel subtype.

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