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Exogenous NPY modulation of cardiac autonomic reflexes and its pressor effect in the conscious rabbit

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1 Neuropeptide Y (NPY) may inhibit sympathetic and vagal transmission via presynaptic Y2 receptors and cause vasoconstriction via postsynaptic Y1 receptors. We examined the effects of NPY and related peptides on cardiovascular parameters and autonomic reflexes in the conscious rabbit. Further, the postjunctional effects of NPY and related peptides were assessed on acetylcholine (ACh) and isoprenaline agonist dose-chronotropic response curves.

2 In conscious rabbits the cardiac baroreceptor-heart rate reflex (baroreflex), Bezold-Jarisch like and nasopharyngeal reflexes were assessed in control, propranolol-treated or methscopolamine-treated (baroreflex only) groups, before and 30 min after i.v. administration of NPY ($10\ \mu g\ kg^{-1} + 5\ \mu g\ kg^{-1}\ min^{-1}$) or vehicle (saline, $10\ ml\ h^{-1}$). The effects of equivalent pressor doses of [Leu^{31}, Pro^{34}]NPY or methoxamine on the baroreflex were also examined. In separate animals, dose-heart rate (HR) response curves to isoprenaline or ACh were constructed before and 15 min after administration of NPY, [Leu^{31},Pro^{34}]NPY (ACh only) or [Leu^{31},Pro^{34}]NPY + sodium nitroprusside (ACh only).

3 Administration of NPY-receptor agonists caused sustained bradycardia (in the absence of methscopolamine) and rightward shifts of the barocurves in all 3 groups. The range of sympathetically-mediated tachycardia was significantly decreased by NPY or [Leu^{31},Pro^{34}]NPY in the methscopolamine-treated group. However, these changes in the baroreflex were no different from those elicted by equipressor doses of methoxamine. There was no vagal inhibition by any NPY-receptor agonist in all three autonomic reflexes examined. ACh or isoprenaline dose-HR response curves were not affected by NPY peptide administration.

4 We conclude that in the conscious rabbit, at a single dose that elicits a significant pressor response, exogenous NPY has no direct effect on modulation of cardiac and autonomic reflexes. Non-specific effects of exogenous NPY on the baroreflex may be fully explained by its pressor action. There was no effect of NPY on postjunctional ACh or isoprenaline agonist dose-response curves. Therefore, it is unlikely that endogenous NPY has a functional role in directly modulating cardiac autonomic neurotransmission in the rabbit.

Keywords: Baroreceptor-heart rate reflex; neuropeptide Y; cardiac neurotransmission; Bezold-Jarisch like reflex; nasopharyngeal reflex

Introduction

Neuropeptide Y (NPY), a 36 amino acid peptide originally isolated from the porcine brain (Tatemoto *et al*., 1982), has been shown to be abundant within the mammalian central and peripheral nervous systems. NPY is present in perivascular nerves throughout the mammalian vascular tree. In the heart, NPY-containing perivascular nerve fibres penetrate all regions and tissues (Warner & Levy, 1990; McDermott *et al*., 1993). However, the role of the endogenous NPY is not well understood. In particular, the presence of NPY in sinoatrial and atrioventricular nodal tissues of the heart (Warner & Levy, 1990; Steele & Choate, 1994) may suggest a neuromodulatory role in cardiac regulation. Previous studies in anaesthetized dogs (Potter, 1987; Hall *et al*., 1990; Yamasaki *et al*., 1991) and guinea-pig isolated atria (Potter, 1987) have shown that exogenous NPY may cause the 'vagal fade' phenomenon (inhibition of vagally-induced bradycardia) that is elicited by excessive sympathetic activation. This phenomenon is thought to be mediated by presynaptic Y2 receptors located on the vagus nerve. Presynaptic Y2 receptors are also present on sympathetic nerve fibres (Shine *et al*., 1994) and mediate presynaptic inhibition of neurotransmitter release. NPY may also act at postsynaptic Y1 receptors (Wahlestedt *et al*., 1990a). This receptor subtype mediates the direct pressor effect of NPY and may be involved in NPY-induced potentiation of responses to a variety of vasoconstrictor agents (Wahlestedt *et al*., 1990b).

The role of endogenous NPY in the peripheral regulation of cardiovascular reflexes such as the baroreceptor-heart rate reflex (baroreflex) has been little studied. The baroreflex is one of the most important cardiovascular control mechanisms as it is able to respond rapidly to abrupt transient changes in blood pressure. It may influence both peripheral vascular tone and the heart via efferent sympathetic and parasympathetic nerves. Bolus injection of exogenous NPY mimics the inhibitory effects of intense periods of sympathetic stimulation on cardiac vagal neurotransmission in the anaesthetized dog (Potter, 1985; 1987). Furthermore, endogenous NPY was recently put forward as a possible mediator of post-exercise depression of the vagal component of the baroreflex in man (Ulman *et al*., 1997). However, Minson *et al*. (1990) have shown that $10\ \mu g\ kg^{-1}$ i.v. bolus of NPY caused an increase in sensitivity of the baroreflex with no effect on the vagal component of the curve in the conscious rabbit. This result was in contrast to the inhibitory effect of exogenous NPY on vagally-mediated responses observed in other species (Lundberg *et al*., 1984; Potter, 1985).

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In light of the reported modulation of baroreflex gain by NPY and the apparent differences between the effects of exogenous NPY on the vagal component of the baroreflex in conscious rabbits (Minson *et al*., 1990), and the vagal inhibition observed in other species including man, we have explored the cardiovascular pharmacology of NPY at the cardiac neuroeffector junction in the rabbit. We used NPY and the putative NPY Y1-receptor-selective agonist [Leu^{31}, Pro^{34}]NPY (Potter & McCloskey, 1992) to define the NPY receptors involved in cardiac autonomic neurotransmission. Specifically, the baroreflex and two other vagally-mediated autonomic reflexes, the Bezold-Jarisch like and nasopharyngeal reflexes were studied. The Bezold-Jarisch like reflex, characterized by an acute vagally-mediated bradycardia and depressor response (Krayer, 1961), is activated by 5-hydroxytryptamine (5-HT)$_3$ receptors on vagal afferent C-fibres in the right ventricle of the heart (Fozard, 1984). Likewise, the nasopharyngeal reflex is characterized by apnoea in expiration, profound vagally-mediated bradycardia and generalized increase in sympathetic activity (White *et al*., 1974). This reflex is evoked by inhalation of cigarette smoke and other irritant vapours into the nasopharynx and is initiated by stimulation of trigeminal nerve afferents. Arterial baroreflexes have also been shown to contribute to the bradycardia observed (McRitchie & White, 1974). We examined the effect of NPY on the Bezold-Jarisch like and nasopharyngeal reflexes in conjunction with the baroreflex because previous studies have shown that the efferent pathways of these reflexes may be modulated differently (Wright & Angus, 1995). In order to clarify the prejunctional from the postjunctional actions of NPY, the effects of this peptide on chronotropic responses to muscarinic receptor (with acetycholine) and β-adrenoceptor stimulation (with isoprenaline) were also tested.

Methods

Animals and minor procedures

Male and female New Zealand white rabbits (mean 2.51 ± 0.03 kg, range 2.00–3.69 kg; $n = 108$) were used in the study. Rabbits were kept on a 12 h light/dark cycle and had free access to food and water. This study was approved by the University of Melbourne Animal Ethics and Experimentation Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia. On the day of each experiment, the central ear artery and marginal ear vein were cannulated under local anaesthesia (1% lignocaine, Xylocaine, Astra). The ear artery catheter was connected to a pressure transducer (CDX, Cobe) for the measurement of phasic and mean arterial blood pressure (MAP). The blood pressure signal triggered a rate meter for the measurement of heart rate (HR). The ear vein catheter was for administration of drugs. Following these minor operative procedures, rabbits rested quiety in polycarbonate restrainers for approximately 30 min before commencement of the experiment.

Protocols for autonomic reflexes: action of NPY and related peptides

The effects of NPY-related peptides on the baroreceptor-HR, Bezold-Jarisch like and nasopharyngeal reflexes were tested in separate experimental groups. The baroreceptor-HR reflex (baroreflex) study consisted of three treatment groups: (i) no pretreatment (Control); i.e. with both sympathetic and parasympathetic reflex components intact, (ii) β-adrenoceptors antagonized with propranolol (0.5 mg kg^{-1}, bolus, then 2.4 mg kg^{-1} h^{-1}, i.v. (Korner & Uther, 1969; West & Korner, 1975)); i.e. vagal reflex only intact; and (iii) muscarinic receptors antagonized via methscopolamine (50 μg kg^{-1}, bolus, then 50 μg kg^{-1} h^{-1}, i.v. (Wright & Angus, 1983)); i.e. sympathetic reflex only intact.

On each experimental day, the baseline baroreflex (with control, methscopolamine or propranolol pretreatment) was tested by the steady-state method elicited by alternate stepwise increases and decreases in MAP (Head & McCarty, 1987). The entire sigmoid MAP-HR relationship was assessed by eliciting a wide range of variations in MAP. Graded changes in MAP (± 5–40 mmHg from baseline) were achieved by ear vein bolus injections of 1–150 μl of phenylephrine (100 μg kg^{-1} ml^{-1}) and 5–250 μl of sodium nitroprusside (100 μg kg^{-1} ml^{-1}). Doses were chosen randomly, except that an increase in MAP was always followed by a decrease in MAP to prevent a shift in resting parameters. NPY, [Leu^{31},Pro^{34}]NPY, methoxamine or vehicle was then administered (see below) and when cardiovascular variables had stabilized (approx. 30 min), the baroreflex was retested.

Resting MAP, HR and the baroreflex (as above) were assessed before and 30 min after commencing administration of a starting bolus dose plus steady infusion of (a) NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v. (Minson *et al*., 1989b)); (b) the relatively selective Y1 receptor agonist [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v.); (c) the α_1-adrenoceptor-selective agonist methoxamine (25 μg kg^{-1} + 1.5 mg kg^{-1} h^{-1}, i.v.); or (d) vehicle (0.9% saline 10 ml h^{-1}). Infusions were maintained for the duration of the experiments. The bolus dose of NPY that was administered has been previously shown to cause an increase in the sensitivity of the baroreflex in conscious rabbits (Minson *et al*., 1990). Doses of [Leu^{31},Pro^{34}]NPY and methoxamine chosen were calculated in preliminary experiments to give equivalent pressor responses to NPY in the autonomically intact and ganglion blocked rabbit.

In a separate group of animals, the peak bradycardia responses elicited by either i.v. injection of 5-HT (1–30 μg kg^{-1}, 100 μl constant volume; Benzold-Jarisch like reflex (Krayer, 1961; Paintal, 1973)) or 30 ml of cigarette smoke drawn into a 50 ml syringe and directed in front of the rabbits' external nares (nasopharyngeal reflex (White *et al*., 1974)) were measured. The Bezold-Jarisch like and nasopharyngeal reflexes were assessed before and 30 min after administration of NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min, i.v.) or vehicle (0.9% saline 10 ml h^{-1}) in the absence or presence of β-adrenoceptor antagonism with propranolol (0.5 mg kg^{-1}, bolus, then 2.4 mg kg^{-1} h^{-1}, i.v.).

Effects of NPY on post-junctional cardiac receptors

In separate groups of rabbits, the effects of exogenous NPY and related peptides were examined on cardiac β-adrenoceptors and muscarinic receptors. Cardiovascular reflexes were obviated with the ganglion blocker mecamylamine, administered intravenously (4 mg kg^{-1}, bolus, then 2.5 mg kg^{-1} h^{-1}) for the duration of the experiment. This regimen has been previously shown to prevent reflex responses to cigarette smoke, intravenous 5-HT or noradrenaline (Ward & Angus, 1993). A 20 min stabilization period was allowed after the initial bolus before agonist curves were constructed. Following ganglion blockade, the β-adrenoceptor agonist isoprenaline (10–1000 ng kg^{-1}) was injected intravenously in a constant volume of 100 μl and peak changes in HR and MAP

measured. Once all cardiovascular parameters had returned to baseline, vehicle (0.9% saline; 10 ml h^{-1}) or NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}) were administered intravenously followed by a 15 min stabilization period. A second isoprenaline dose-response curve was then constructed in the presence of NPY or vehicle, as described above.

Acetylcholine dose-response curves

Cardiovascular reflexes were obviated as detailed above and rabbits allowed to stabilize for 20 min. Rabbits then received either (a) vehicle (0.9% saline, 10 ml h^{-1}, i.v.); (b) NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v.); (c) [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v.); or (d) [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v.) co-infused with sodium nitroprusside (SNP; 0.5 mg h^{-1}). A further 15 min stabilization period was allowed before construction of acetylcholine dose-response curves. Acetylcholine (ACh; 1–128 μg kg^{-1}) was administered intravenously in a constant volume of 100 μl and the peak bradycardia responses measured. With the higher doses of ACh, it was necessary to administer a bolus of methoxamine (100 μg ml^{-1}, i.v.) immediately following the peak bradycardia to prevent MAP falling below 20 mmHg. In early experiments without methoxamine, high doses of ACh caused the rabbits to die from hypotensive shock.

Drugs

Drugs were freshly prepared in 0.9% saline and included acetylcholine bromide (Sigma, St Louis, MO, U.S.A.), 5-hydroxytryptamine (5-HT, creatinine sulphate complex, Sigma), (−)-isoprenaline hydrochloride (Sigma), mecamylamine hydrochloride (Merck, Sharp & Dohme, Rahway, NJ, U.S.A.), methoxamine hydrochloride (Sigma), methscopolamine bromide (Upjohn, Rydalmere, NSW, Australia), neuropeptide Y and [Leu^{31},Pro^{34}]NPY (synthesized by Dr Roger Murphy, Department of Pharmacology, University of Melbourne, Victoria, Australia), (−)-phenylephrine hydrochloride (Sigma), propranolol hydrochloride (Sigma) and sodium nitroprusside (David Bull Laboratories, Clayton, Victoria, Australia). Immediately following dilution, acetylcholine was stored on ice and shielded from direct light.

Analysis and statistical methods

Parameter measurement and agonist dose-response curves Data are presented as mean ± 1 s.e.mean. Values for MAP and HR presented in the text and tables have been rounded to the nearest whole number. The peak change in resting cardiovascular parameters after administration of each peptide/agonist was compared within animals by Student's *t* test for paired data. Dose-HR response curves to 5-HT were compared within animals before and after NPY treatment by repeated measures ANOVA. The Greenhouse-Geisser estimate of epsilon was used as a correction for correlation (Ludbrook, 1994). Average s.e.mean within animals for the HR response to 5-HT was calculated from the ANOVA by use of the pooled estimate of error from the residual mean square as (error mean square/number of animals)$^{0.5}$ after subtraction of the sums of squares between animals and between doses from the total sums of squares for 5-HT (Wright *et al*., 1987). This error bar (±1 average s.e.mean) is located on the average 5-HT dose-response line (Figure 4).

Cardiovascular parameters before and after cigarette smoke (nasopharyngeal reflex) were compared within animals by Student's *t* test for paired data. The effect of NPY-related peptides on the peak HR or MAP responses to smoke were also compared to the respective within animal controls by paired *t* test. MAP and HR responses to sodium nitroprusside and phenylephrine were measured as peak changes. Analysis of the baroreflex involved fitting the MAP and HR changes to a sigmoidal logistic equation characterised by four parameters: (a) HR range (beats min^{-1}) between upper and lower curve plateaux; (b) MAP at the midpoint of the HR range [MAP_{50} (mmHg)]; (c) average gain which corresponds to the linear portion of the sigmoid relationship between HR and MAP (beats min^{-1} $mmHg^{-1}$); and (d) lower HR plateau (beats min^{-1}). The effects of pressor agonists or vehicle on each barocurve parameter within animal were compared by Student's *t* test for paired data and between treatment groups by 1-way ANOVA, with a Bonferroni *post-hoc* test to correct for multiple comparisons (Ludbrook, 1994).

Sigmoid logistic dose-HR response curves for isoprenaline and ACh were constructed with absolute values (Nakashima *et al*., 1982). Values for the doses eliciting 50% of the maximum response (ED_{50}) were obtained from each fitted curve. Values for ED_{50} within animal were compared between treatments by Student's *t* test for paired data. ED_{50} values for ACh dose-response curves between treatment groups were compared by Student's *t* test for unpaired data or 1-way ANOVA when more than 2 groups were being compared. In all cases, statistical significance was accepted when $P<0.05$.

Results

Resting MAP and HR

NPY In the rabbits used to assess the baroreceptor-HR reflex, exogenous NPY caused significant increases in MAP in all three treatment groups and corresponding bradycardia (in the absence of methscopolamine) (Table 1). There was no significant difference in resting MAP and HR between treatment groups, except in methscopolamine-treated animals where resting HR was significantly higher than in the control and propranolol-treated groups ($P<0.0001$). In the group of rabbits used to assess the Bezold-Jarisch like and nasopharyngeal reflexes, administration of NPY caused MAP to increase from 71 ± 2 to 96 ± 3 mmHg ($n=6$; $P=0.0002$), with a corresponding decrease in HR from 220 ± 5 to 181 ± 5 beats min^{-1} ($P<0.0001$).

[Leu^{31},Pro^{34}]NPY and methoxamine Administration of [Leu^{31},Pro^{34}]NPY (LP-NPY) and methoxamine caused MAP to be increased in all groups and a corresponding bradycardia in the absence of methscopolamine (Tables 2 and 3, respectively). These cardiovascular changes were of similar magnitude as those after NPY administration in all three treatment groups.

Effects of agonists on the baroreceptor-HR reflex

NPY In the control group, administration of NPY caused the baroreflex curve to shift to the right and down (Figure 1a), corresponding to the changes in MAP and HR outlined above. There was a significant fall in the lower HR plateau ($n=6$; $P=0.03$), with no change in the HR range and MAP_{50}. Average gain showed a tendency to decrease after NPY administration. However, this failed to reach statistical significance (Table 1). In the propranolol-treated rabbits, effective antagonism of β-adrenoceptors was confirmed by the

lack of change in baseline MAP or HR in response to a single bolus of isoprenaline (1 μg kg^{-1}, i.v.; 100 μl volume). In the presence of this β-adrenoceptor antagonism, the HR range was decreased 53%. NPY had no effect on the upper HR plateau of the barocurve in the presence of propranolol (Figure 1b). However, NPY caused the lower HR plateau to be lowered significantly ($P=0.0004$). This downward shift of the lower HR plateau was almost twice the initial bradycardia (31±6 beats min^{-1}) causing a 64% increase in HR range ($P=0.0002$). MAP_{50} was also significantly increased after NPY infusion ($P=0.002$; Table 1). There was no change in average gain of the baroreflex after NPY treatment (Table 1).

In the methscopolamine-treated rabbits, HR range of the baroreflex was decreased to 27% of control (Figure 1c; $n=6$). NPY administration caused a further 11% decrease in the HR range ($P=0.02$) and a significant increase in MAP_{50} ($P=0.03$).

Table 1 Baroreflex curve parameters before and after NPY administration in conscious rabbits in the control ($n=6$), propranolol ($n=6$) and methscopolamine ($n=6$) treatment groups

Group		*MAP* (mmHg)	*HR* (beats min^{-1})	*HR range* (beats min^{-1})	*Average gain* (beats min^{-1} mmHg^{-1})	*MAP_{50}* (mmHg)	*Lower HR plateau* (beats min^{-1})
Control	Baseline	71±2	202±7	164±15	8.2±0.8	66±2	167±8
	NPY	86±4*	156±7*	177±16	6.4±2.0	75±5	111±9*
Propranolol	Baseline	77±1.5	211±7	77±6	4.1±0.5	74±1.5	187±8
	NPY	84±2.0*	181±6*	126±4*	5.2±0.5	82±2.0*	126±5*
Methscopolamine	Baseline	75±2	293±13	45±5	2.1±1.0	70±3	280±11
	NPY	100±6*	292±10	26±5*	0.7±0.3	99±9*	281±8

Values are mean±s.e.mean. NPY, neuropeptide Y. *n*, number of rabbits. HR, heart rate. MAP_{50}, MAP at half the HR range. Values shown are for baroreceptor-heart rate reflex curves for control, propranolol (0.5 mg kg^{-1}, bolus, then 2.4 mg kg^{-1} h^{-1}, i.v.) and methscopolamine (50 μg kg^{-1}, bolus, then 50 μg kg^{-1} h^{-1}, i.v.) groups before (baseline) and after treatment with NPY (10 μg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v.). *$P<0.05$, significant difference between corresponding values within group after NPY treatment; Student's *t* test for paired data.

Table 2 Baroreflex curve parameters before and after [Leu^{31},Pro^{34}]NPY administration in conscious rabbits in the control ($n=6$), propranolol ($n=6$) and methscopolamine ($n=6$) treatment groups

Group		*MAP* (mmHg)	*HR* (beats min^{-1})	*HR range* (beats min^{-1})	*Average gain* (beats min^{-1} mmHg^{-1})	*MAP_{50}* (mmHg)	*Lower HR plateau* (beats min^{-1})
Control	Baseline	77±3	231±16	136±12	8.7±1.1	73±3	197±14
	LP-NPY	83±2	183±14*	154±9	5.4±0.5*	74±2	151±14*
Propranolol	Baseline	71±2	224±4	76±5	4.3±0.7	69±2	192±7
	LP-NPY	77±2*	196±4*	104±10*	4.8±0.5	75±3*	153±10*
Methscopolamine	Baseline	75±3	269±8	60±5	2.8±0.3	67±3	258±7
	LP-NPY	91±2*	259±5	32±5*	2.1±0.5	70±3	257±5

Values are mean±s.e.mean. LP-NPY, [Leu^{31},Pro^{34}]NPY, neuropeptide Y analogue. *n*, number of rabbits. HR, heart rate. MAP_{50}, MAP at half the HR range. Values shown are for baroreceptor-heart rate reflex curves for control, propranolol (0.5 mg kg^{-1}, bolus, then 2.4 mg kg^{-1} h^{-1}, i.v.) and methscopolamine (50 μg kg^{-1}, bolus, then 50 μg kg^{-1} h^{-1}, i.v.) groups before (baseline) and after treatment with [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1}, bolus, then 5 μg kg^{-1} min^{-1}, i.v.). *$P<0.05$, significant difference between corresponding values within group after [Leu^{31},Pro^{34}]NPY treatment; Student's *t* test for paired data.

Table 3 Baroreflex curve parameters before and after methoxamine administration in conscious rabbits in the control ($n=4$), propranolol ($n=4$) and methscopolamine ($n=4$) treatment groups

Group		*MAP* (mmHg)	*HR* (beats min^{-1})	*HR range* (beats min^{-1})	*Average gain* (beats min^{-1} mmHg^{-1})	*MAP_{50}* (mmHg)	*Lower HR plateau* (beats min^{-1})
Control	Baseline	73±2	222±6	145±10	6.8±0.8	67±2	189±6
	Meox	88±1*	153±12*	131±3	4.3±1.0*	80±4*	117±11*
Propranolol	Baseline	72±1	227±8	90±9	3.4±0.4	70±1	188±10
	Meox	99±3*	178±9*	132±9*	3.5±0.3	93±3*	138±11*
Methscopolamine	Baseline	74±3	307±21	54±5	3.1±0.6	65±3	301±21
	Meox	105±4*	305±25	21±2*	1.5±0.3†	80±5†	304±26

Values are mean±s.e.mean. Meox, Methoxamine; *n*, number of rabbits. HR, heart rate. MAP_{50}, MAP at half the HR range. Values shown are for baroreceptor-heart rate reflex curves for control, propranolol (0.5 mg kg^{-1}, bolus, then 2.4 mg kg^{-1} h^{-1}, i.v.) and methscopolamine (50 μg kg^{-1}, bolus, then 50 μg kg^{-1} h^{-1}, i.v.) groups before (baseline) and after treatment with methoxamine (25 μg^{-1} kg^{-1}, bolus, then 1.5 mg kg^{-1} h^{-1}, i.v.). *$P<0.05$, significant difference between corresponding values within group after methoxamine treatment; Student's *t* test for paired data. †$P=0.06$.

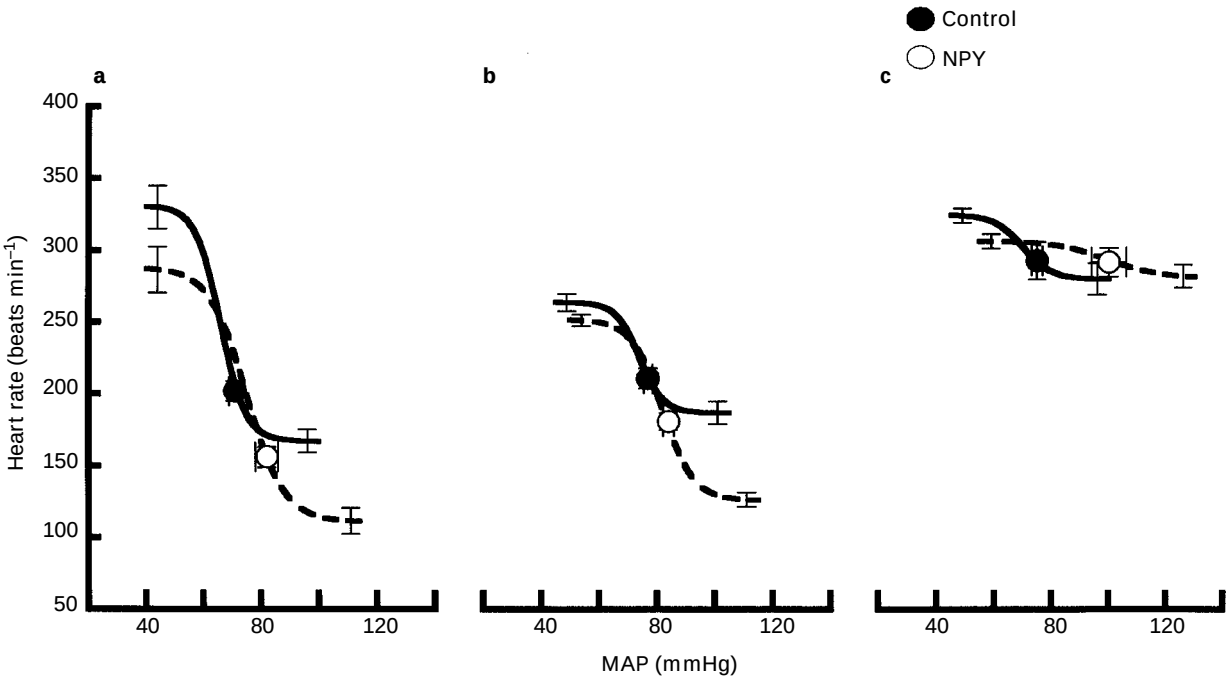

Figure 1 Effect of NPY on average baroreflex curves relating mean arterial pressure (MAP) to heart rate in conscious rabbits in the control (a, $n=6$), propranolol (0.5 mg kg^{-1} + 2.4 mg kg^{-1} h^{-1}, i.v., b, $n=6$) and methscopolamine (50 μg kg^{-1} + 50 μg kg^{-1} h^{-1}, i.v., c, $n=6$) treatment groups. Curves shown are before and 30 min after administration of NPY (10 μg kg^{-1}, bolus + 5 μg kg^{-1} min^{-1}, i.v.). The symbol on each curve represents the average resting value for MAP and heart rate. The values are mean and vertical lines show s.e.mean (those not shown are contained within the symbol). The error bars on the curves represent the s.e.mean of the lower heart rate plateau (right) and heart rate range (left).

The lower HR plateau and average gain did not change with NPY treatment, although there was a trend for average gain to be decreased similar to control (Table 1).

[Leu^{31},Pro^{34}]NPY After administration of the NPY Y1-receptor selective agonist [Leu^{31},Pro^{34}]NPY (LP-NPY) in the control group, the barocurve was shifted down and to the right ($n=6$; Figure 2a), corresponding to the changes in MAP and HR outlined above. In the presence of LP-NPY alone, there was no significant change in HR range or MAP_{50} of the barocurve compared to the within group baseline measurement (Figure 2; Table 2). The lower HR plateau was significantly decreased ($P=0.0005$), corresponding to the initial change in HR described above. Average gain was also decreased after infusion of LP-NPY ($P=0.02$). These changes were similar to those elicited by NPY on the baroreflex (Table 1). In the presence of β-adrenoceptor antagonism, the changes in the baroreflex elicited by LP-NPY were no different in comparison to the effect of NPY on the baroreflex in the propranolol-treated group (Figure 2b; Table 2). In the methscopolamine-treated group, LP-NPY again elicited changes in the baroreflex similar to those of NPY (Figure 2c). However, there was no change in the MAP_{50} after LP-NPY treatment (Table 2).

Methoxamine The changes in the baroreflex elicited by both NPY and [Leu^{31},Pro^{34}]NPY could also be mimicked by the α_1-adrenoceptor selective agonist methoxamine (Figure 3a). After methoxamine infusion, there was no change in the HR range of the barocurve. However, MAP_{50} was significantly increased ($P=0.03$; $n=4$). Lower HR plateau and average gain were also significantly decreased post-methoxamine ($P=0.0009$ and $P=0.05$, respectively). In both the propranolol-treated and methscopolamine-treated groups, the changes elicited by methoxamine were no different from those of NPY or [Leu^{31},Pro^{34}]NPY (Figure 3b and c, respectively; Table 3).

Vehicle Administration of vehicle (saline) had no effect on resting cardiovascular parameters, nor the baroreflex, in all three treatment groups (data not shown).

Effect of NPY on the Bezold-Jarisch like and nasopharyngeal reflexes

Intravenous administration of 5-HT elicited dose-dependent bradycardia (Figure 4). Exogenous NPY had no effect on the maximum fall in HR caused by the highest dose of 5-HT (30 μg kg^{-1}, i.v.) (80 ± 14 and 75 ± 5 beats min^{-1}, before and after NPY, respectively; $n=6$). At baseline, exposure to cigarette smoke caused a significant increase in MAP from 70 ± 3 to 83 ± 4 mmHg ($P=0.01$) and a profound bradycardia, HR falling from 214 ± 6 to 41 ± 5 beats min^{-1} (Figure 5). Administration of NPY had no effect on this reflex with the fall in HR elicited by cigarette smoke in the presence of NPY (42 ± 6 beats min^{-1}) being no different from the pre-NPY value. In the presence of NPY, MAP was also increased by a similar degree to control values (77 ± 6 to 88 ± 5 mmHg, baseline and peak MAP, respectively; $n=6$; $P=0.001$). Propranolol treatment had no effect on the HR response to 5-HT alone, or in the presence of NPY (Figure 4), nor did it affect the response to smoke (Figure 5).

Effects of NPY-related peptides on agonist dose-response curves

Effects of ganglion blockade on resting MAP and HR The effect of ganglion blockade on resting parameters was not different between groups, therefore results have been pooled.

Ganglion blockade caused MAP to fall markedly from 73 ± 1 to 63 ± 1 mmHg ($n=35$; $P<0.001$) with corresponding changes in HR of 199 ± 4 to 285 ± 7 beats min^{-1} ($n=35$; $P<0.001$).

Isoprenaline dose-response curves Isoprenaline caused dose-dependent tachycardia and falls in MAP in the presence of ganglion blockade (Figure 6). Following NPY treatment, MAP was significantly higher ($P<0.0001$) with no change in

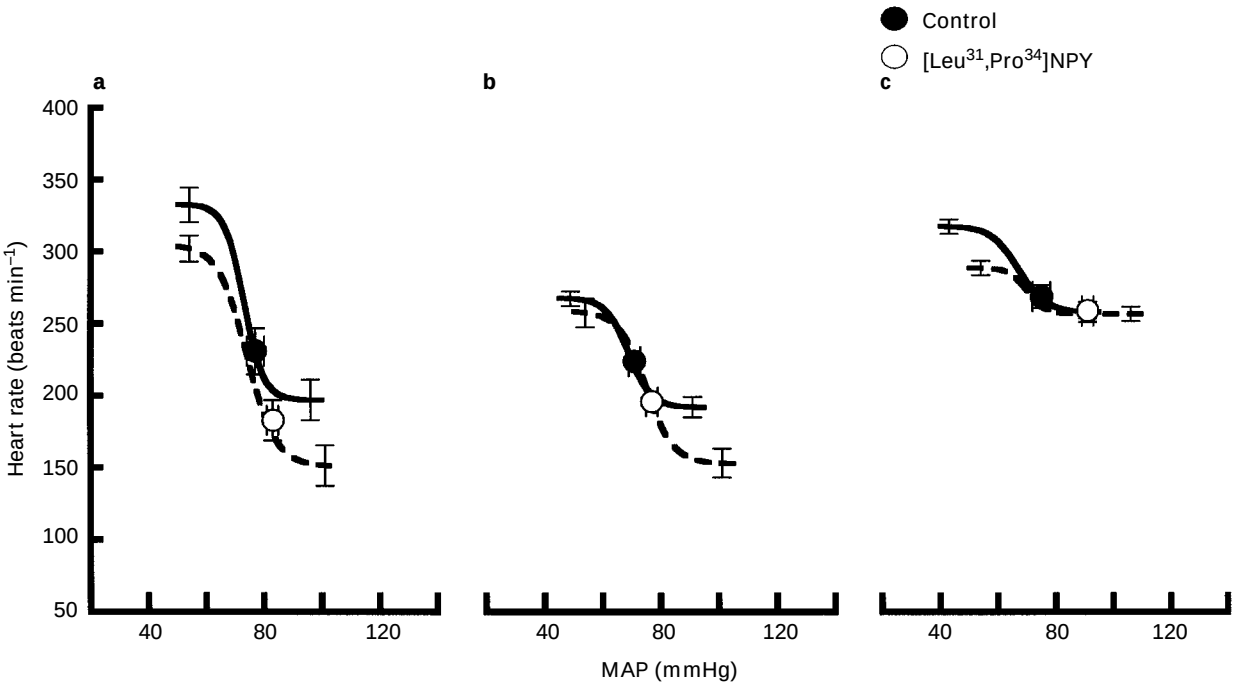

Figure 2 Effect of [Leu^{31},Pro^{34}]NPY on average baroreflex curves relating mean arterial pressure (MAP) to heart rate in conscious rabbits in the control (a, $n=6$), propranolol (0.5 mg kg^{-1} + 2.4 mg kg^{-1} h^{-1}, i.v., b, $n=6$) and methscopolamine (50 μg kg^{-1} + 50 μg kg^{-1} h^{-1}, i.v., c, $n=6$) treatment groups. Curves shown are before and 30 min after administration of [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1}, bolus + 5 μg kg^{-1} min^{-1}, i.v.). The symbol on each curve represents the average resting value for MAP and heart rate. The values are mean and vertical lines show s.e.mean (those not shown are contained within the symbol). The error bars on the curves represent the s.e.mean of the lower heart rate plateau (right) and heart rate range (left).

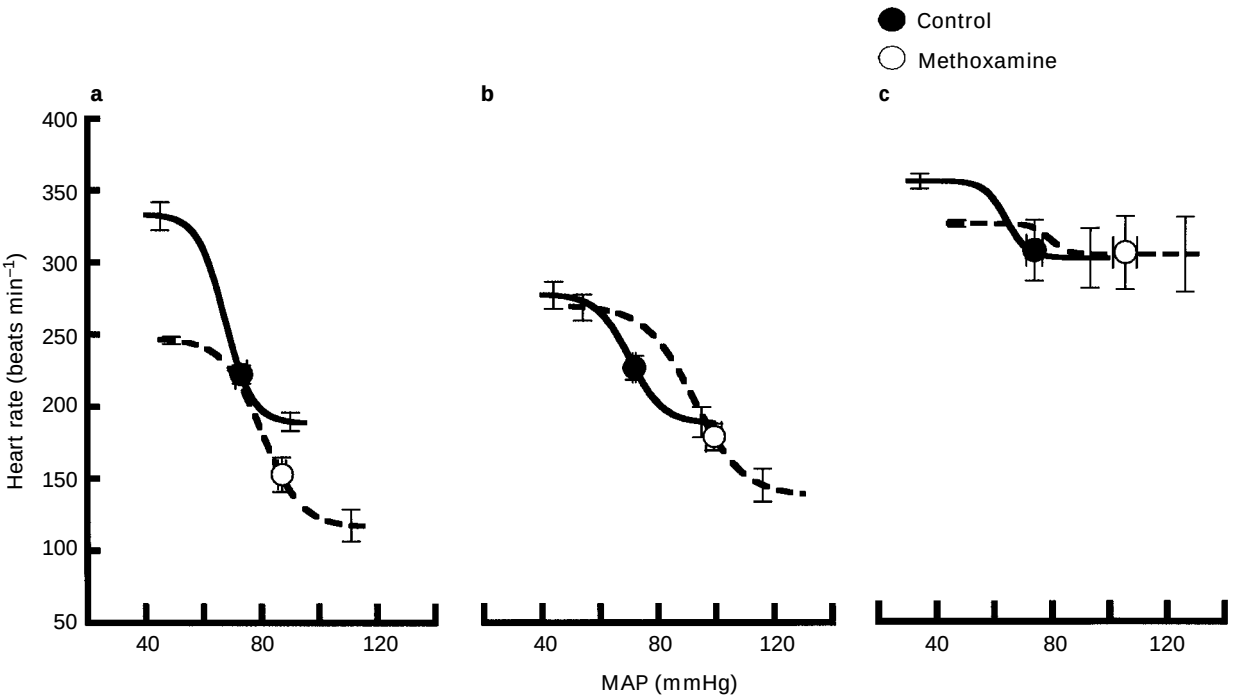

Figure 3 Effect of methoxamine on average baroreflex curves relating mean arterial pressure (MAP) to heart rate in conscious rabbits in the control (a, $n=6$), propranolol (0.5 mg kg^{-1} + 2.4 mg kg^{-1} h^{-1}, i.v., b, $n=6$) and methoscopolamine (50 μg kg^{-1} + 50 μg kg^{-1} h^{-1}, i.v., c, $n=6$) treatment groups. Curves shown are before and 30 min after administration of the α_1-adrenoceptor agonist methoxamine (25 μg kg^{-1}, bolus + 1.5 mg kg^{-1} h^{-1}, i.v.). The symbol on each curve represents the average resting value for MAP and heart rate. The values are mean and vertical lines show s.e.mean (those not shown are contained within the symbol). The error bars on the curves represent the s.e.mean of the lower heart rate plateau (right) and heart rate range (left).

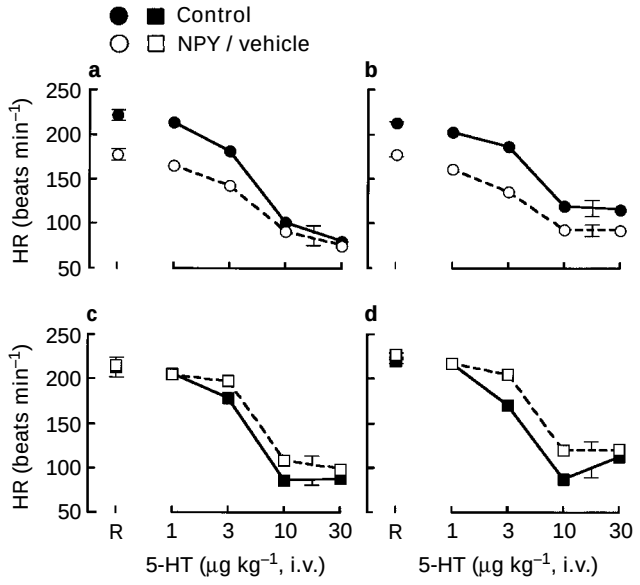

Figure 4 Effect of NPY on average dose-heart rate (HR) response curves to 5-hydroxytryptamine (5-HT; 1–30 μg kg^{-1} i.v., bolus, constant volume 100 μl) in conscious rabbits in control (a, c; $n=6$ each) and propranolol (0.5 mg kg^{-1} + 2.4 mg kg^{-1} h^{-1}, i.v., b, d; $n=6$ each) treatment groups. (a and b) Before and 30 min after NPY (10 μg kg^{-1} bolus + 5 μg kg^{-1} min^{-1}, i.v.). (c and d) Data from the vehicle time controls ($n=6$, control and propranolol-treated animals, respectively). R is the average resting HR. Error bars on R are s.e.mean and on lines are average s.e.mean (see Methods).

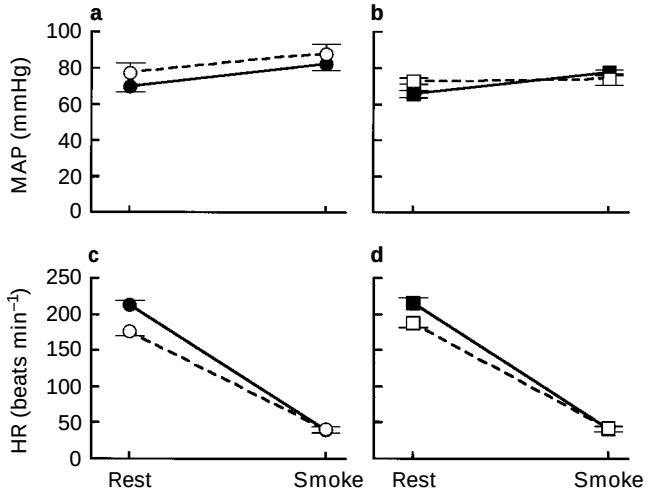

Figure 5 Nasopharyngeal reflex elicited by cigarette smoke in conscious rabbits in control (a, c, $n=6$) and propranolol (0.5 mg kg^{-1} + 2.4 mg kg^{-1} h^{-1}, i.v., b, d, $n=6$) treatment groups before (solid symbols) and 30 min after (open symbols) administration of NPY (10 μg kg^{-1} bolus + 5 μg kg^{-1} min^{-1}, i.v.). (a and b) Mean arterial pressure (MAP) and (c and d) heart rate (HR). Error bars are s.e.mean.

HR. NPY had no effect on isoprenaline-elicited tachycardia, response range and sensitivity to β-adrenoceptor stimulation in the presence of NPY being no different from the pretreatment control curve. There was no change in response range, nor sensitivity of the dose-HR response curves to isoprenaline in vehicle-treated animals. In the presence of NPY there was also no change in sensitivity to vascular β-adrenoceptor stimulation (log ED_{50} 2.1$\pm$0.10 ng kg^{-1}; $n=7$) compared to the corresponding vehicle-treatment curves (log ED_{50} 1.9$\pm$0.10 ng kg^{-1}; $n=4$).

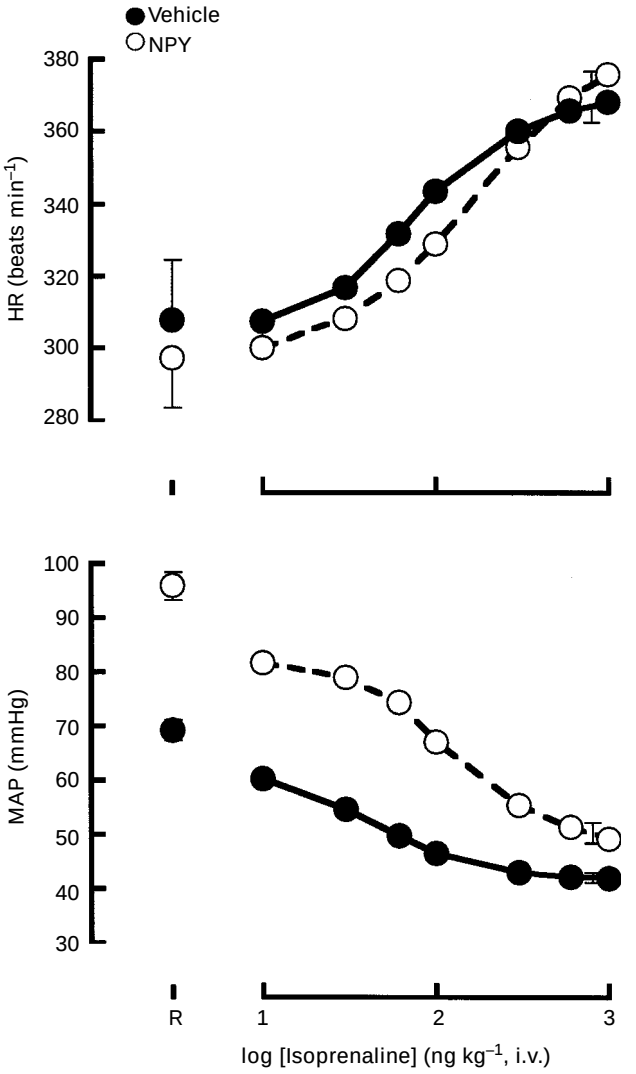

Figure 6 (a) Heart rate (HR) and (b) mean arterial pressure (MAP) following isoprenaline (10–1000 ng kg^{-1}, i.v.) in the vehicle (saline, $n=4$) and NPY (10 μg kg^{-1} + 5 μg kg^{-1} min^{-1}, i.v., $n=6$) treatment groups. R is the average resting HR (a) or MAP (b). Error bars on R are s.e.mean and on lines are average s.e.mean (see Methods).

Acetylcholine dose-response curves Acetylcholine caused a dose-dependent bradycardia (log ED_{50} 4.51$\pm$0.08 ng kg^{-1}; $n=6$; Figure 7a) followed by a sustained fall in MAP in the absence of autonomic reflexes. NPY administration caused MAP to increase by 44 mmHg from 64$\pm$2 to 108$\pm$3 mmHg ($n=6$). However, there was no change in HR. The dose of [Leu^{31},Pro^{34}]NPY was chosen to give an approximately equal pressor response as that observed with NPY. Following administration of [Leu^{31},Pro^{34}]NPY, MAP increased by 41 mmHg from 59$\pm$2 to 99$\pm$6 mmHg ($n=6$) with no change in HR. In the presence of NPY or [Leu^{31},Pro^{34}]NPY, the ACh dose-HR response curves were significantly left-shifted by approximately 3 fold (log ED_{50} 4.02$\pm$0.08 and 4.15$\pm$0.06 ng kg^{-1}, NPY ($n=6$; $P<0.001$) and [Leu^{31},-Pro^{34}]NPY ($n=6$; $P<0.01$) treated groups, respectively). However, when SNP was co-infused with [Leu^{31},Pro^{34}]NPY to return MAP to the pre-drug baseline (baseline 60$\pm$3 mmHg and 65$\pm$1 mmHg post-SNP infusion), ACh-induced bradycardia was no different from control curves in the absence of NPY (Figure 7b; log ED_{50} 4.45$\pm$0.04 ng kg^{-1}; $n=6$).

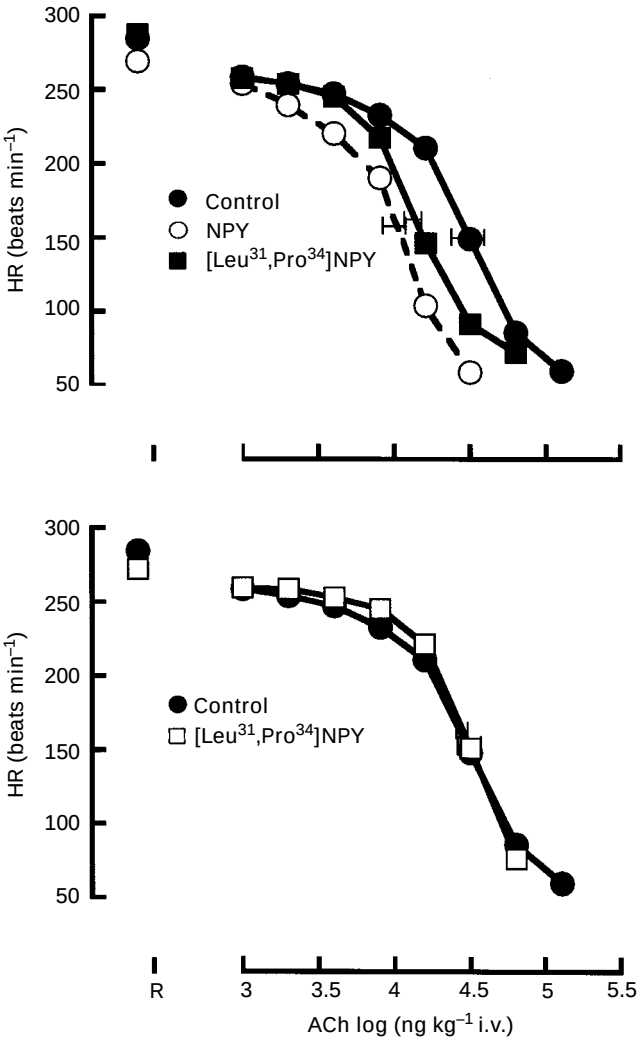

Figure 7 (a) Heart rate (HR) response to acetylcholine (ACh 1–128 μg kg^{-1}, i.v.) in control ($n=6$), NPY (10 μg kg^{-1} + 5 μg kg^{-1} min^{-1}, i.v., $n=6$) and [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1} + 5 μg kg^{-1} min^{-1}, i.v., $n=6$) treated groups. (b) HR response to acetylcholine (ACh, 1–128 μg kg^{-1}, i.v.) in control ($n=6$) and [Leu^{31},Pro^{34}]NPY (10 μg kg^{-1} + 5 μg kg^{-1} min^{-1}, i.v.) + SNP (0.5 mg h^{-1}, i.v., $n=6$) treated groups. R is average resting HR. Error bars shown are ± 1 s.e.mean on R and ED_{50}.

Discussion

This study demonstrated that NPY elicits changes to the baroreceptor-HR reflex that are non-specific and may be mimicked by other pressor agonists that elicit comparable increases in MAP in the conscious rabbit. Therefore, at least in the rabbit, our work does not support a direct neuromodulatory effect of NPY on cardiac sympathetic and vagal neurotransmission at the level of the heart. Exogenous NPY caused a significant increase in MAP of similar magnitude in the absence or presence of β-adrenoceptor antagonism. Corresponding with this pressor effect, in the absence of methscopolamine, there was a significant bradycardia, shifting the baroreflex down and to the right. This bradycardia would appear to be baroreflex-independent as it lasted for the duration of the NPY infusion in both control and propranolol-treated groups, and (in the control group) the curve was shifted downward with no change in HR range.

In the presence of muscarinic receptor antagonism the pressor effect of NPY was more pronounced, presumably due to the absence of reflex bradycardia compensating for the increase in MAP. However, in the presence of methscopolamine NPY caused a significant attenuation of the sympathetically-mediated tachycardia as indicated by a decrease in the HR range of the curve. There was no evidence that NPY inhibits cardiac vagal action as neither the vagal plateau of the baroreflex, nor the two other vagally-mediated reflexes studied (Bezold-Jarisch like reflex and the nasopharyngeal reflex), were attenuated by exogenous NPY. Therefore our data support the findings of Minson *et al.* (1990) where, at a dose that elicits a significant pressor response, exogenous NPY has no effect on the vagal component of the baroreflex. The aforementioned changes elicited by NPY on the baroreflex (i.e. decreased range of sympathetic tachycardia) were also mimicked by the relatively selective Y1 receptor agonist [Leu^{31},Pro^{34}]NPY. This initially suggested to us that there may be prejunctional Y1 receptors mediating the inhibition of the sympathetic component of the baroreflex. However, infusion of the α_1-adrenoceptor agonist methoxamine was also able to imitate the actions of NPY on the baroreflex. If the decrease in HR range that was observed in the methscopolamine-treated groups was a result of presynaptic inhibition by either of the three agonists, then the sympathetic component of the HR range in control groups should have been decreased by a similar magnitude to methscopolamine-treated animals. However, there was no change in range of the barocurves after agonist administration in the absence of methscopolamine, suggesting a mechanism other than presynaptic inhibition may be causing the changes observed. The obvious difference in the response to agonist administration between the three experimental groups was the magnitude of the pressor response to either peptide or methoxamine. Thus the changes observed in the baroreflex in the presence of methscopolamine may be a consequence of the increase in MAP rather than a direct effect of NPY or [Leu^{31},Pro^{34}]NPY on this reflex.

Previously, Minson *et al.* (1990) demonstrated that baroreflex measurements made 5 min following a single bolus dose of 10 μg kg^{-1} exogenous NPY showed an increase in the gain of the MAP-heart period relationship which was suggested to be secondary to a decrease in β-adrenoceptor-mediated tone. However, measurements at 25 min, after cardiovasulcar parameters had returned to baseline, were no different from control. In our experiments we saw no significant change in sensitivity, nor range of the baroreflex, in control animals following NPY when the same dose was administered with steady infusion. However, there was a tendency for gain to be decreased, seen with infusion of both [Leu^{31},Pro^{34}]NPY and methoxamine.

In the present study, we chose a priming dose plus steady-state i.v. infusion of NPY to ensure that the reflex was being tested under stable haemodynamic conditions. The profound bradycardia that occurred following NPY administration has also been observed by Minson *et al.* (1990). This bradycardia appears to be independent of baroreflexes as no resetting of the HR was evident for the duration of the experiment. Baroreceptor resetting (operation of the baroreceptor-HR reflex around a new basal MAP) has been shown to occur within 10 min of sustained changes to basal MAP (Eckberg & Sleight, 1992). In our study the baroreflex was tested 30 min after administration of NPY, therefore any resetting of the reflex should have occurred by this time. By assessing the baroreflex after a transient pressor response to NPY, baroreflex-mediated changes in HR may have influenced the observations made by Minson *et al.* (1990). Indeed, they concluded that the increase in gain following NPY administration was similar to changes seen after administration of

propranolol and therefore may reflect a decrease in β-adrenoceptor-mediated tone. However, if the bradycardia they observed when assessing their initial 5 min barocurve was partly baroreflex-mediated, then the instability of the cardiac autonomic reflex, or non-steady state conditions under which their measurements were made, may preclude any firm conclusions regarding changes in gain of the reflex. By testing the effects of agonists on the baroreflex at 30 min, our data should not have been confounded by the superimposition of baroreflex-mediated changes in both cardiac vagal and sympathetic tone in response to the pressor effect of NPY.

An earlier study by Minson *et al.* (1989a) suggested that the NPY-elicited bradycardia was, in-part, independent of autonomic neural mechanisms. This may have been due to a decreased rate of sinoatrial discharge subsequent to decreased perfusion (Minson *et al.*, 1987). Indeed, in the rabbit isolated Langendorff heart preparation NPY has been shown to elicit significant vasoconstriction of coronary resistance vessels resulting in decreased myocardial contractility secondary to myocardial ischaemia (Allen *et al.*, 1983). However, in the present study antagonism of the vagus by methscopolamine prevented any HR changes in response to NPY administration. This would suggest that the bradycardia was reflex in origin as any decrease in heart rate caused by sino-atria node ischaemia should have been evident in the absence of vagally-mediated bradycardia. Furthermore, this would suggest that in our study, adequate perfusion of the sinoatrial node must have been maintained during the infusion of all three agonists studied. The sustained pressor response to the NPY infusion may have been sufficient to activate cardiac receptors located in the left ventricular wall. In a study by Ludbrook (1984), in which the baroreceptor-HR reflex and the cardiac receptor-HR reflex were compared in the conscious rabbit, the cardiac receptor-HR reflex was shown to be activated by non-physiological increases in MAP and left ventricular pressure, HR abruptly decreasing when left ventricular end diastolic pressure (LVEDP) reached 25 mmHg. Minson *et al.* (1987, 1989a) have demonstrated that a 10 μg kg^{-1}, i.v., bolus of NPY causes a significant increase in LVEDP. However, LVEDP was only increased to 3.7 mmHg which is below the threshold for activation of the left-sided cardiac receptors according to Ludbrook (1984). An infusion of NPY, such as the one administered in our study may serve to increase LVEDP further as total peripheral resistance is increased and maintained, possibly allowing the threshold of the reflex to be attained. A characteristic of non-arterial baroreceptors which would support the hypothesis that cardiac receptors are being activated by the agonist-induced increases in MAP is that they undergo resetting to a much lesser extent than arterial baroreceptors (Head, 1995). The lack of any HR resetting following administration of all three agonists further supports the possibility of cardiac receptor-HR reflex involvement in the bradycardic response.

To ensure that our interpretation of the action of NPY was not obscured by postjunctional receptor interactions, we performed a series of experiments with the β-adrenoceptor agonist isoprenaline and the muscarinic receptor agonist acetylcholine in autonomically blocked rabbits. These results suggest that the dose of exogenous NPY used in this study had no effect on cardiac β-adrenoceptor-mediated tachycardia elicited by isoprenaline or vascular β-adrenoceptors that mediated the depressor response to isoprenaline. Furthermore, administration of [Leu^{31},Pro^{34}]NPY had no effect on cardiac muscarinic receptors, as shown by no change in the sensitivity to acetylcholine after [Leu^{31},Pro^{34}]NPY administration (once the changes to baseline MAP had been corrected by SNP). These results allow us to discount the possibility that any neuroinhibition by NPY was countered by a potentiation of postsynaptic responses to endogenous noradrenaline or acetylcholine.

In conclusion, exogenous NPY caused a decrease in the range of sympathetically-mediated tachycardia without affecting either the vagal component of the baroreceptor-HR reflex, or the Bezold-Jarisch like and nasopharyngeal reflexes in the conscious rabbit. This action of NPY on the baroreflex was probably a non-specific consequence of the increase in MAP, as both [Leu^{31},Pro^{34}]NPY and methoxamine were able to mimic this effect. Furthermore, it would be unlikely for NPY to play a role in the modulation of cardiac vagal neurotransmission elicited by excessive activation of cardiac sympathetic tone, as demonstrated in other species.

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