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Cardiovascular responses to intrathecal neuropeptide γ in conscious rats: receptor characterization and mechanism of action

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1 In the conscious rat, cardiovascular responses to intrathecally (i.t.) administered neuropeptide γ (NPγ) were studied prior to and after the i.t. pretreatment with selective antagonists at NK_1 ((±)-CP 96345 and RP 67580), NK_2 (SR 48968) and NK_3 (R 486) receptors. Pretreatment with a mixture of peptidase inhibitors (phosphoramidon, captopril, bacitracin, phenanthroline) was also tested to ascertain whether or not the effect of NPγ was mediated by a metabolite. The involvement of peripheral catecholamines was examined with intravenous injection of α-adrenoceptor (phentolamine) and β-adrenoceptor (propranolol) antagonists.

2 NPγ (0.078–78 nmol) induced dose-dependent increases in heart rate (HR) and mean arterial blood pressure (MAP). The highest dose of 78 nmol did not induce an increase of MAP greater than that with 7.8 nmol but was preceded by a transient decrease of MAP (1–3 min). No desensitization was observed when three injections of 7.8 nmol NPγ were given at 90 min intervals.

3 Cardiovascular and behavioural (biting/scratching) effects evoked by 0.78 nmol NPγ were significantly reduced by the NK_1 antagonists, (±)-CP 96345 (65 nmol) or RP 67580 (7.8 and 78 nmol). However, cardiovascular responses to NPγ were not affected by (±)-CP 96345 (6.5 nmol), SR 48968 (7.8 and 78 nmol) or R 486 (25 nmol). Pretreatment with peptidase inhibitors significantly enhanced the cardiovascular and behavioural responses to NPγ.

4 The pressor response to 7.8 nmol NPγ was converted to a vasodepressor response by pretreatment with phentolamine (2 mg kg^{-1}, i.v.) while the chronotropic response was markedly reduced by propranolol (2 mg kg^{-1}, i.v.).

5 These results suggest that the cardiovascular responses to i.t. NPγ are mediated by NK_1 receptors in the spinal cord leading to the peripheral release of catecholamines from sympathetic fibres or the adrenal medulla. It is unlikely that the spinal action of NPγ results from its metabolic conversion into neurokinin A or another major metabolite.

Keywords: Neuropeptide gamma; spinal cord; cardiovascular responses; NK_1 receptors

Introduction

The tachykinins substance P (SP), neurokinin A (NKA), NKA (3-10), neurokinin B (NKB), neuropeptide K (NPK) and neuropeptide gamma (NPγ or γ-preprotachykinin-(72-92)) represent a family of structurally related peptides found primarily in neurones of the central and peripheral nervous system (Helke *et al.*, 1990). These peptides are encoded by two genes designated as preprotachykinin gene I (PPT-I) and PPT-II (the NKB gene). The PPT-I gene is alternatively spliced to yield four mRNA species, that encode four precursor proteins: α-, β-, γ and δ-PPT. Whereas SP is derived from all four precursors, β- and γ-PPT yield NKA in addition to NPK and NPγ, respectively (Helke *et al.*, 1990; Khan & Collins, 1994). NPγ, a 21-amino-acid peptide containing the sequence of NKA at its C-terminal end has been isolated from rabbit small intestine (Kage *et al.*, 1988). NPγ is present in several peripheral tissues as well as in the brain although at lower concentration than NKA (Takeda *et al.*, 1990). In many tissues, including the central nervous system, γ-PPT mRNA represents 75–80% of all mRNA expressed from the PPT-1 gene in the rat (Carter & Krause, 1990; Marchand *et al.*, 1993). Like SP, NPγ is a potent sialogogue in the rat (Takeda & Krause, 1989) and its intracerebroventricular (i.c.v.) injection leads to increases in mean arterial blood pressure (MAP) and heart rate (HR) in both the anaesthetized (Hagio *et al.*, 1991) and conscious rat (Picard & Couture, 1996), and to a raised plasma level of luteinizing hormone in male rats (Kalra *et al.*, 1992). In the conscious rat, the intrathecal (i.t.) injection of SP and NPK to the 9th thoracic spinal cord level (T9) produces dose-dependent increases in MAP and HR through the activation of the sympatho-adrenal system (Hassessian *et al.*, 1990; Pham *et al.*, 1993).

Three tachykinin receptors termed neurokinin$_1$, (NK_1), NK_2 and NK_3 have been pharmacologically characterized and cloned (for review see Regoli *et al.*, 1988; 1994). In various biological and radioligand binding assays, SP is the preferred agonist for the NK_1 receptor, while NKA, NPK and NPγ show higher affinities for the NK_2 receptor and NKB for the NK_3 receptor (Dam *et al.*, 1990; 1991; Guard & Watson, 1991; Regoli *et al.*, 1994). Several non-peptide antagonists selective for the NK_1 receptor have been described: (±)CP 96345 (Snider *et al.*, 1991), RP 67580 (Garret *et al.*, 1992) and SR 140333 (Jung *et al.*, 1994); RP 67580 exhibits higher affinity in rat than in guinea-pig or man (Garret *et al.*, 1992). SR 48968 is a selective non-peptide antagonist with high affinity for the NK_2 receptor (Emonds-Alt *et al.*, 1993) while R 486 is a peptide antagonist selective for the NK_3 receptor of the rat portal vein (pA_2 of 7.45) (Drapeau *et al.*, 1990). In a previous study, we have reported that intrathecally administered R 486 blocks, in a selective manner, the antinociceptive effect induced by the NK_3 receptor agonist, [$MePhe^7$]-NKB, in the rat tail-flick test (Couture *et al.*, 1993).

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The aim of the present study was threefold: First, to assess the effect of intrathecally administered NPγ on the cardiovascular system of the conscious freely moving rat; second, to characterize the receptor involved by use of antagonists selective for the NK_1, NK_2 and NK_3 receptors; and third to examine the peripheral mechanism underlying the cardiovascular effect of NPγ. A preliminary account of this work has been presented elsewhere (Poulat *et al.*, 1993).

Methods

Implantation of catheters and measurement of cardiovascular parameters

Male Wistar rats (Charles River, St Constant, Qué., Canada) weighing 270–350 g were anaesthetized with an intraperitoneal injection of 65 mg kg^{-1} sodium pentobarbitone (Somnotol, M.T.C. Pharmaceuticals, Cambridge, Ont., Canada) and two catheters were implanted. One was a stretched polyethylene catheter (PE-50;Intramedic, Clay Adams, NJ, U.S.A.) inserted into a femoral artery and pushed to the level of the abdominal aorta. This catheter was filled with physiological saline containing heparin (sodium salt, 50 iu ml^{-1}), tunnelled under the skin and exteriorized at the back of the neck. The catheter was placed inside a 20 cm long tether consisting of a steel spring sutured to the skin on the back of the rat. The second catheter for intrathecal injections consisted of a PE-10 tubing inserted via an incision in the *dura mater* at the atlanto-occipital junction, through the spinal subarachnoid space to the level of the ninth thoracic segment. After fixing this catheter on the skull with cyanoacrylate glue, its end was also brought through the tether. Following surgery, rats were housed individually with the tether drawn through the top of the grid cage and were given free access to food and tap water. Most animals (95%) showed apparently normal locomotor, drinking and eating behaviour. The few rats (less than 5%) displaying a flaccid paralysis of the hindlimbs were killed. The correct position of the intrathecal catheter tip was verified by post-mortem laminectomy.

Experiments were conducted in conscious rats at least 24 h after surgery. The rat was free to move in its cage which was partially covered with an opaque cloth to avoid visual stimuli. Blood pressure was monitored through the intra-arterial catheter with a Statham pressure transducer (P231D) while the heart rate derived from the blood pressure signal was measured with a cardiac tachometer (model 7P4) and both parameters were monitored on a Grass polygraph model 79D (Grass instruments Co., Quincy, MA, U.S.A.). The care of the animals and research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian council on animal care and approved by the Committee responsible for animal care at the Université de Montréal.

Experimental protocols

Once a stable tracing of blood pressure and heart rate had been obtained, the rats were given an i.t. injection of 30 μl artificial cerebro-spinal fluid (aCSF; composition in mM: NaCl 128.6, KCl 2.6, $MgCl_2$ 2.0 and $CaCl_2$ 1.4; pH adjusted to 7.2). Only rats without significant cardiovascular changes in response to i.t. aCSF were used. Peptides were dissolved in aCSF and injected at the T-9 spinal cord level in a volume of 10 μl. The catheter was then flushed with 20 μl aCSF, a volume corresponding to the volume of the catheter. Thus, the total volume of each injection represents 30 μl given within 30 s.

Dose-response effect of NPγ

The first series of experiments was designed to study the effects of four i.t. doses (0.078, 0.78, 7.8 and 78 nmol, $n=8-12$) of NPγ on MAP and HR. Each rat received increasing doses at intervals of 30 to 120 min to allow for the return to baseline values before the next dose of the peptide was given. In order to determine whether tachyphylaxis occurred, three consecutive doses of 7.8 nmol NPγ ($n=4$) were given 90 min apart. Five consecutive injections of 30 μl aCSF at 60 min intervals had no significant effect on MAP or HR (Hassessian *et al.*, 1993).

NK_1, NK_2, and NK_3 receptor antagonists versus NPγ

This series of experiments was designed to identify the receptor involved in the cardiovascular effect of NPγ. To this end, the rats received an i.t. injection of 0.78 nmol NPγ and 90 min later, a second injection of 0.78 nmol of NPγ was given 5 or 15 min after an i.t. pretreatment with an NK_1, NK_2 or NK_3 receptor antagonist. The rats received another dose of 0.78 nmol NPγ 24 h later, to test the reversibility or duration of the inhibition. Each antagonist was tested in separate groups of rats. (±)-CP 96345 (6.5 and 65 nmol, $n=6$) and RP 67580 (7.8 and 78 nmol, $n=6-8$) were used as NK_1 antagonists while SR 48968 (7.8 and 78 nmol, $n=5-8$) and R 486 (25 nmol, $n=7$; solubility in 30% dimethylsulphoxide could not be achieved at higher doses) were used as NK_2 and NK_3 antagonists, respectively. The doses of antagonists are based on previous studies showing selectivity of the inhibition toward the respective agonists (Pham & Couture, 1993; Couture *et al.*, 1995).

Mechanism underlying the cardiovascular effect of NPγ

The role of the sympathetic nervous system was investigated with an antagonist of α-adrenoceptors (phentolamine-HCl, $n=8$) and β-adrenoceptors (propranolol, $n=6$). First, the rats received an i.t. injection of 7.8 nmol NPγ and 90 min later, 2 mg kg^{-1} of either antagonist was injected i.v. 15 min prior to the second injection of NPγ.

Effect of peptidase inhibitors on the cardiovascular effects of NPγ

In this experiment, the effect of peptidase inhibitors was examined on the response to NPγ to test the possibility that NPγ-induced cardiovascular effects are due to one of its metabolites. First, the rats received an i.t. injection of 0.78 nmol NPγ ($n=7$) and 30 min later, 10 μl of a mixture of four peptidase inhibitors (150 nmol phosphoramidon, 80 nmol captopril, 80 nmol 1–10 phenanthroline, 40 μg bacitracin) was injected i.t., 30 min prior to the second injection of NPγ.

Peptides and other compounds

NPγ was purchased from Hükabel Scientific Ltd (Montréal, Québec, Canada) and phentolamine-HCl, heparin sodium salt (grade II), propranolol, phosphoramidon, captopril, bacitracin and 1–10 phenantroline were all purchased from Sigma Chemicals (St Louis, MO, U.S.A.). The non-peptide NK_1 antagonist, RP 67580 (racemic form of 7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl] perhydro-isoindol-4-one (3aR, 7aR); mol. wt: 475 for the hydrochloride salt) was donated by Dr C. Garret, Rhône-Poulenc Rorer, Paris, France. (±)-CP 96345 (the racemic mixture of (±)-*cis*-3-(2-methoxybenzylamino)-2-benzhydryl-quinuclidine) was provided by Drs B.D. Gitter and J.J. Howbert at Lilly Research Lab., Indianapolis, U.S.A. The NK_2 antagonist, SR 48968 ((S)-N-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide; mol. wt: 570) was given by Dr J-C Brelière, Sanofi, Montpellier, France. R 486 (H-Asp-Ser-Phe-Trp-β-Ala-Leu-Met-NH_2; mol. wt: 868) was a gift from Dr D. Regoli at Sherbrooke University, Sherbrooke, Canada. The antagonists were dissolved in dimethylsulphoxide (DMSO, Fisher) and aCSF was added to obtain the desired solution (The final solution contains a maximum of 30% DMSO). Higher concentrations of DMSO reduced the responses to NPγ and therefore limited the final concentration of

antagonist tested (particularly R 486). NPγ and peptidase inhibitors were dissolved in aCSF. Phentolamine and propranolol were dissolved in saline containing 1% of tetramethylenesulphone (Sigma).

Statistical analysis of data

Results were expressed as means ± s.e.mean. The values for MAP and HR represent the difference between values at the designed time and the baseline values 30 s before the injection. The time-course effects were analyzed, for up to 30 min after injection, with a two-way analysis of variance (ANOVA) for repeated measures, in conjunction with Bonferroni confidence intervals. Statistical significance of differences between maximal values were analysed with a one-way ANOVA followed by a Tukey's test for multiple comparisons between groups. Only probability values (P) smaller than 0.05 were considered to be statistically significant.

Results

Cardiovascular responses to NPγ

The time courses of MAP and HR changes elicited by the i.t. injection of four doses (0.078 – 78 nmol) of NPγ are depicted in Figure 1. While aCSF had no appreciable effects, 78 and 780 pmol NPγ evoked dose-dependent and time-related increases in MAP that peaked at 5 – 7 min post-injection. However, 7.8 and 78 nmol NPγ induced a transient decrease in MAP that was significant at 78 nmol between 1 – 3 min post-injection. The vasodepressor response peaked at 1 min and was followed by a pressor response that peaked at 15 – 20 min. The dose of 78 nmol NPγ failed to induce a greater pressor response than 7.8 nmol. The pressor responses to all 4 doses of NPγ were statistically significant when compared to aCSF values (Figure 1). The chronotropic response to NPγ was dose-dependent and time-related. At 78 nmol NPγ, the cardiovascular response returned to pre-injection values only after 4 h. At all doses, i.t. injection of NPγ induced biting and scratching behaviour and a strong vasodilatation of the ears as previously observed with SP (Hassessian & Couture, 1989). No motor impairment was observed at any doses.

The NPγ-induced cardiovascular effect was resistant to desensitization because three injections of 7.8 nmol NPγ at 90 min intervals produced equivalent cardiovascular responses. Whereas the initial vasodepressor response was slightly enhanced at the third injection, the secondary pressor response was not affected by repeated injections of NPγ (data not shown).

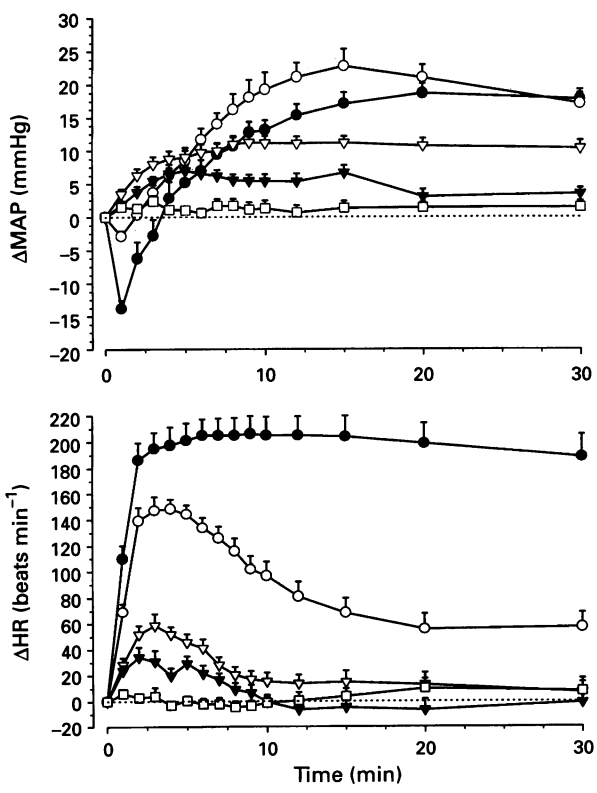

Figure 1 Time course of mean arterial blood pressure (MAP) and heart rate (HR) changes evoked by the i.t. injection of aCSF (□) or NPγ at increasing doses of 0.078 (▼), 0.78 (▽), 7.8 (○) and 78 (●) nmol in conscious rats. Each point represents the mean ± s.e.mean of 8 – 12 rats. Statistical comparisons to aCSF values were for (a) the pressor response: 0.078 nmol (1 – 30 min, $F(1,19) = 16$; $P < 0.01$), 0.78 nmol (1 – 30 min, $F(1,18) = 48$; $P < 0.001$), 7.8 nmol (1 – 30 min, $F(1,18) = 56$; $P < 0.001$) and 78 nmol (4 – 30 min, $F(1,15) = 20$; $P < 0.01$ and 1 – 4 min, $F(1,15) = 11$; $P < 0.05$); and (b) the tachycardia: 0.078 nmol (1 – 10 min, $F(1,19) = 9$; $P < 0.05$), 0.78 nmol (1 – 20 min, $F(1,20) = 13$; $P < 0.01$), 7.8 nmol (1 – 30 min, $F(1,19) = 131$; $P < 0.001$) and 78 nmol (1 – 30 min, $F(1,15) = 187$; $P < 0.001$).

Effects of NK_1 antagonists on the cardiovascular response to NPγ

Figure 2 shows the effects of two NK_1 antagonists on the maximal pressor (4 – 8 min) and chronotropic (2 – 3 min) ef-

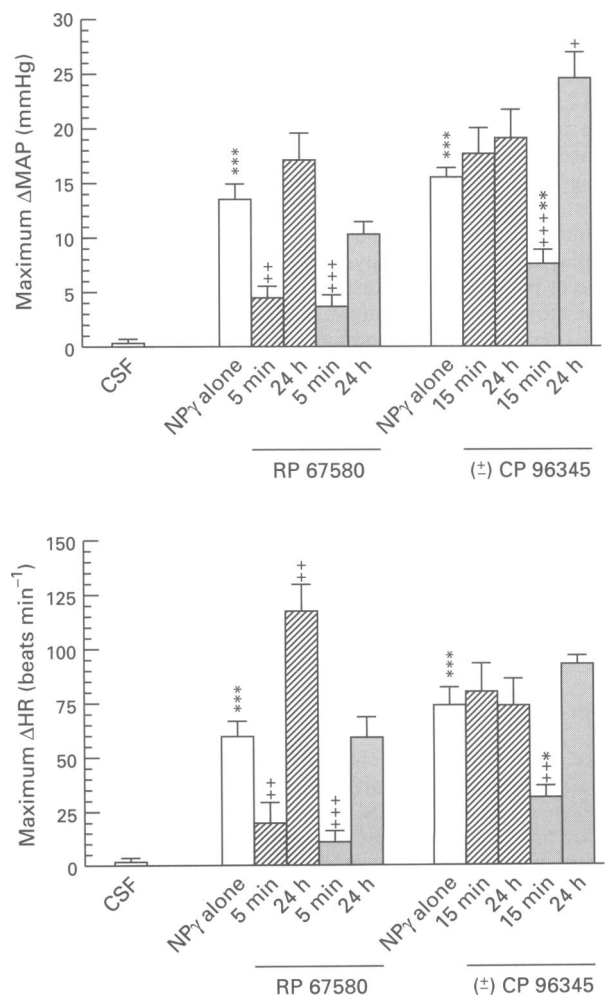

Figure 2 Effects of the prior i.t. injection of two NK_1 antagonists RP 67580 (hatched column, 7.8 nmol or stippled column 78 nmol) and (±)-CP 96345 (hatched column, 6.5 nmol or stippled column 65 nmol), on maximal cardiovascular changes induced by 0.78 nmol NPγ. Each column represents the mean ± s.e.mean of 6 – 8 rats. NPγ vs aCSF (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$); antagonist vs NPγ ($^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$).

fects elicited by 0.78 nmol NPγ. (±)-CP 96345 (6.5 nmol) injected i.t. 15 min and 24 h before NPγ had no significant effect on the maximal cardiovascular response to NPγ. However, the maximal cardiovascular response induced by NPγ was significantly reduced by about 50% when 65 nmol (±)-CP 96345 was administered i.t., 15 min before 0.78 nmol NPγ. Although the maximal HR response to NPγ was restored 24 h later to pretreatment level, the pressor response was significantly enhanced at that time. The i.t. injection of 7.8 or 78 nmol RP 67580 abolished the maximal cardiovascular responses to 0.78 nmol NPγ when injected 5 min earlier as the residual responses were not significantly different from those observed with aCSF. The inhibition was greater than that observed with 65 nmol (±)-CP 96345. The inhibitory effect of 78 nmol RP 67580 was no longer observed when NPγ was re-administered alone 24 h later while the chronotropic response was significantly potentiated at that time after 7.8 nmol RP 67580. All behavioural manifestations (scratching and biting) accompanying the cardiovascular response to NPγ were simultaneously and reversibly blocked by both NK_1 receptor antagonists (data not shown).

Effects of NK_2 and NK_3 antagonists on the cardiovascular response to NPγ

SR 48968 (7.8 or 78 nmol), the NK_2 receptor antagonist, injected i.t. 15 min or 24 h before NPγ had no significant effect on the maximal cardiovascular response to 0.78 nmol NPγ (Figure 3). Similarly, R 486 (25 nmol), the NK_3 receptor antagonist, injected i.t. 15 min or 24 h before NPγ had no significant effect on the maximal cardiovascular response to 0.78 nmol NPγ (Figure 3). None of the NPγ-induced behavioural changes was significantly affected by SR 48968 or R 486 (data not shown).

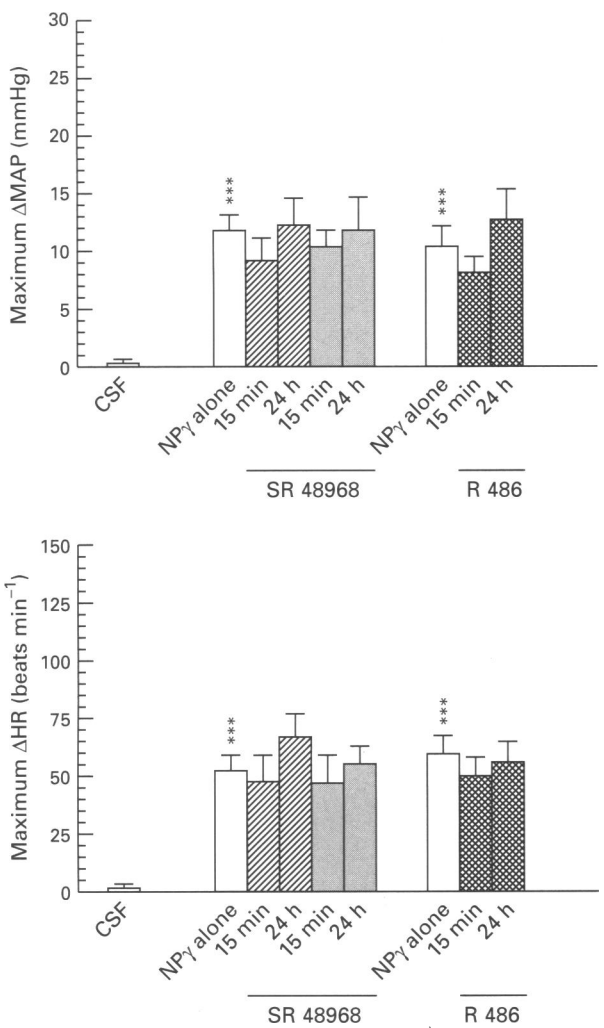

Figure 3 Effects of the prior i.t. injection of the NK_2 antagonist SR 48968 (hatched column, 7.8 nmol and stippled column 78 nmol) or the NK_3 antagonist R 486 (cross-hatched column, 25 nmol) on maximal cardiovascular changes induced by 0.78 nmol NPγ. Each point represents the mean ± s.e.mean of 6–8 rats. NPγ vs aCSF (***$P < 0.001$).

Effects of adrenoceptor inhibitors on the cardiovascular response to NPγ

Intravenous pretreatment with phentolamine (2 mg kg^{-1}) 15 min earlier, blocked significantly the pressor and chronotropic effects induced by 7.8 nmol NPγ (Figure 4). The blockade of the chronotropic response by phentolamine could be artefactual since baseline HR was enhanced to 505 beats min^{-1} in the presence of the α-adrenoceptor inhibitor (Table 1). Moreover, phentolamine enhanced the initial vasodepressor response induced by NPγ. On the other hand, i.v. injection of propranolol (2 mg kg^{-1}) reduced markedly the chronotropic effect of 7.8 nmol NPγ without affecting significantly the pressor response (Figure 4). Note that the small and transient initial vasodepressor component induced by NPγ is not altered by propranolol.

Effects of peptidase inhibitors on the cardiovascular response to NPγ

The i.t. injection of a mixture of peptidase inhibitors altered the NPγ-mediated responses (Figure 5). The pressor response induced by 0.78 nmol NPγ was significantly increased between 8–30 min post-injection in the presence of peptidase in-

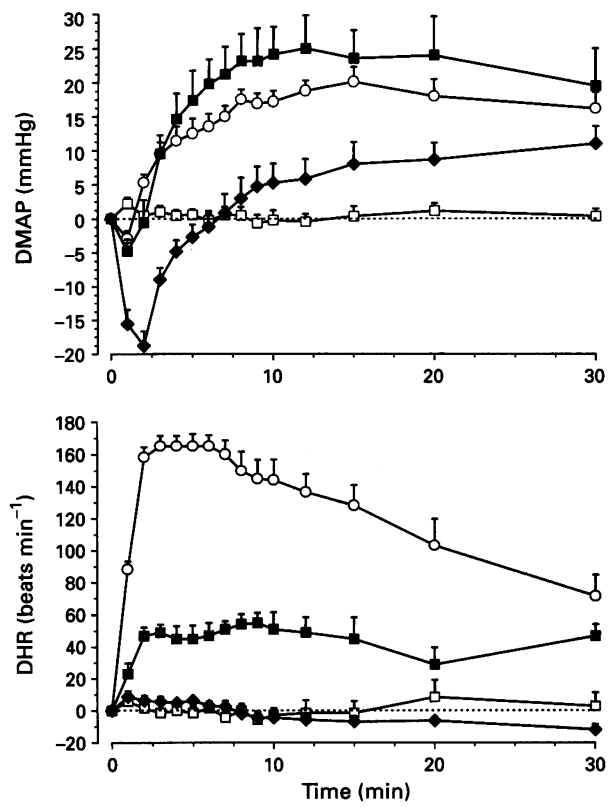

Figure 4 Time course of MAP and HR changes elicited by the i.t. injection of aCSF (□), or 7.8 nmol NPγ before (○), and 15 min after an i.v. injection of 2 mg kg^{-1} phentolamine (◆, $n=8$) or propranolol (■, $n=6$) in conscious rats. Each point represents the mean ± s.e.mean of n rats. Statistical comparisons to control NPγ values were significant for the pressor (1–30 min, $F(1,14)=27.9$, $P<0.001$) and cardiac response (1–30 min, $F(1,14)=42.37$, $P<0.001$) in phentolamine-treated rats, and for the cardiac response (1–30 min, $F(1,10)=76.9$, $P<0.001$) in propranolol-treated rats.

Table 1 Effects of various treatments on baseline MAP and HR in conscious rats

Treatment	n	MAP (mmHg) before	MAP (mmHg) after	HR (beats min^{-1}) before	HR (beats min^{-1}) after
(±)-CP 96345					
6.5 nmol i.t.	7	96.6±3.6	93.8±3.7	368±8	342±8
65 nmol i.t.	6	93.0±3.5	95.1±3.7	348±6	392±16
RP 67580					
7.8 nmol i.t.	8	101.7±2.0	108.6±2.6	346±6	395±17*
78 nmol i.t.	7	91.4±3.6	95.6±4.6	354±10	342±14
SR 48968					
7.8 nmol i.t.	8	101.9±3.8	109.4±4.7	370±13	393±13
78 nmol i.t.	6	110.1±2.8	110.4±3.3	368±11	386±12
R 486					
25 nmol i.t.	7	102.7±3.1	103.3±2.9	342±6	336±11
Phentolamine i.v.	8	98.2±3.5	66.6±4.4***	350±13	505±7***
Propranolol i.v.	6	91.6±6.2	97.4±6.3	353±16	302±16
Mixture of inhibitors i.t.	7	106.7±4.5	116.4±4.1	313±11	328±9

The values are expressed as means ± s.e.mean of *n* rats. See Methods for treatments. Statistical significance of differences between values before and after treatments were calculated with Student's t test for paired samples and are indicated by: *$P<0.05$; ***$P<0.001$.

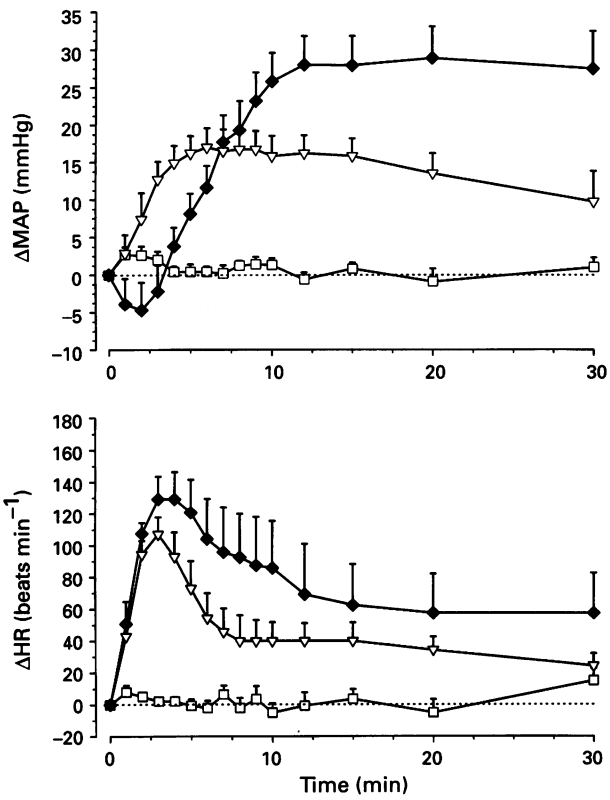

Figure 5 Time course of MAP and HR changes elicited by the i.t. injection of aCSF (□), or 0.78 nmol NPγ before (▽), or 30 min after an i.t. injection of peptidase inhibitors (◆) in conscious rats. Each point represents the mean ± s.e.mean of 7 rats. Statistical comparisons to control NPγ values were statistically significant only for MAP: first component (1–6 min), $F(1,12)=5.49$; $P<0.05$, and second component (8–30 min), $F(1,12)=5.06$; $P<0.05$.

hibitors. However, the onset of the pressor response was significantly delayed between 1–6 min by the same treatment. Therefore, the time-course of the pressor response was somewhat similar to that observed with 78 nmol NPγ (Figure 1). The HR response to NPγ was not significantly increased in intensity, but the effect was more sustained following the administration of peptidase inhibitors. Moreover the biting and scratching responses to NPγ were markedly enhanced in intensity by this treatment.

Baseline MAP and HR values

I.t. injection of (±)-CP 96345 and SR 48968 did not affect baseline MAP and HR values while 7.8 nmol RP 67580 significantly increased baseline HR (Table 1). R 486 induced a short-lasting cardiovascular effect as reported earlier (Picard *et al.*, 1994), but this effect was over before the injection of NPγ as shown by the presence of normal baseline MAP and HR values (Table 1). Systemic injection of phentolamine reduced MAP and increased HR while propranolol had no significant effect on baseline cardiovascular values (Table 1). Finally, the mixture of peptidase inhibitors had no appreciable effect on baseline MAP and HR (Table 1).

Discussion

Receptor subtype mediating the spinal action of NPγ

In unrestrained conscious rats, intrathecal injections of NPγ induced increases in MAP and HR. It can be postulated that these effects are mediated by NK_1 receptors in the spinal cord on the basis of the following considerations: (i) (±)-CP 96345 and RP 67580, two antagonists at NK_1 receptors, markedly reduced the cardiovascular responses induced by NPγ; the fact that RP 67580 was more potent than (±)-CP 96345 in blocking the cardiovascular effects induced by NPγ is in agreement with the greater affinity of RP 67580 at the NK_1 receptor in the rat (Garret *et al.*, 1992; Regoli *et al.*, 1994); (ii) SR 48968 and R 486, antagonists at NK_2 and NK_3 receptors, respectively, did not significantly alter the cardiovascular responses to NPγ. In this model, it was previously observed that SP and NPK also act via an NK_1 receptor whereas NKA, NKB as well as NK_2 and NK_3 receptor-selective agonists have little or no effects (Hassessian *et al.*, 1988; Pham & Couture, 1993; Couture *et al.*, 1995). The distribution of the various tachykinin receptor subtypes is in agreement with the cardiovascular effect mediated by those peptides. Indeed, NK_1 receptors (or SP preferring-receptors) are present in the intermediolateral cell column (IML) (Charlton & Helke, 1985; Buck *et al.*, 1986; Mantyh *et al.*, 1989; Yashpal *et al.*, 1990; Vigna *et al.*, 1994), particularly on sympathetic preganglionic neurones (Helke *et al.*, 1986), although they are also found in dorsal and ventral horns (Charlton & Helke, 1985; Buck *et al.*, 1986; Mantyh *et al.*, 1989; Yashpal *et al.*, 1990; Vigna *et al.*, 1994). On the other hand, the NK_2 and NK_3 receptors are mostly located in the dorsal horn (Buck *et al.*, 1986; Mantyh *et al.*, 1989; Yashpal *et al.*, 1990). However, NK_2 receptor

mRNA has been reported to be either undetectable (Tsuchida *et al.*, 1990) or present in a very low amount (Takeda & Krause, 1991) in the rat spinal cord whereas NK_1 receptor mRNA was detected by the mRNA blot hybridization technique (Tsuchida *et al.*, 1990).

Since its discovery, NPγ has been considered to be a selective NK_2 agonist in binding assays (Dam *et al.*, 1990; 1991; Badgery-Parker *et al.*, 1993; Zeng *et al.*, 1994) and this peptide has been used *in vivo* and *in vitro* to substantiate the presence of NK_2 receptors (Kalra *et al.*, 1992; Van Giersbergen *et al.*, 1992; Qian *et al.*, 1994; Zeng *et al.*, 1994). To our knowledge, our study is the first to show that NPγ behaves as a potent NK_1 agonist in functional studies in the CNS. Nevertheless, NPγ was also found to be a potent sialogogue in the rat (Takeda & Krause, 1989), an effect that is also believed to be mediated by the direct activation of NK_1 receptors on salivary glands (Snider *et al.*, 1991; Jung *et al.*, 1994). It is worth noting that the order of potency of tachykinins in inducing salivation NPK > NPγ > SP > NKA > NKB (Takeda & Krause, 1989) is very similar to that obtained in our paradigm (Couture *et al.*, 1995).

In contrast, the increase in MAP and HR by the i.c.v. injection of NPγ (Hagio *et al.*, 1991; Picard & Couture, 1995) is partly via activation of the NK_2 receptor (Picard & Couture, 1995) while the cardiovascular responses to i.c.v. SP and NKA are mediated by both NK_1 and NK_2 receptors (Picard *et al.*, 1994). While NPγ is active in the low pmol range by the i.c.v. route (Picard & Couture, 1995), higher doses (high pmol to nmol range) are required by the i.t. route to evoke cardiovascular changes. The preferential selectivity of NPγ for NK_1 or NK_2 receptors *in vivo* may therefore rely on the sensitivity of the system. Although a supraspinal site of action for intrathecally administered NPγ cannot be completely ruled out, this is unlikely since the spinal and supraspinal cardiovascular effects of this peptide are mediated by the NK_1 and NK_2 receptors, respectively. It is not the first time that a putative natural NK_2 agonist has been found to have an affinity for NK_1 receptors. Previous binding and biological assays showed that NPK was an NK_2 agonist (Beaujouan *et al.*, 1988; Van Giersbergen *et al.*, 1992), yet the cardiovascular responses induced by i.t. and i.c.v. injections of NPK were reported to be entirely mediated by an NK_1 receptor (Pham & Couture, 1993; Prat *et al.*, 1994).

Sources and metabolism of NPγ

The anatomical distribution of NPγ in the spinal cord has not yet been studied but it cannot be ruled out that some of the NKA detected by immunocytochemistry or radioimmunoassay could be attributed to NPγ since the NKA sequence is included in that of NPγ. Indeed, studies have shown that NKA antibodies cross-react totally with NPγ (Wang *et al.*, 1993). Therefore, NPγ could be present: (1) in bulbospinal 5-hydroxytryptaminergic neurones projecting to the IML, where NKA-like immunoreactivity and γ-PPT mRNA have been shown, along with SP-like immunoreactivity (Harlan *et al.*, 1989; Marchand *et al.*, 1993; Nevin *et al.*, 1994). NKA-like immunoreactivity has also been detected by radioimmunoassay in the rat IML (Takano *et al.*, 1986). (2) In primary sensory C-fibres where NKA-like immunoreactivity has been detected in coexistence with SP-like immunoreactivity (Dalsgaard *et al.*, 1985; Helke & Niederer, 1990) in addition to the γ-PPT mRNA (Marchand *et al.*, 1993). However, to date, it is not known whether NPγ could be present alone or in combination with NKA.

It has been shown that the degradation of tachykinins can be inhibited by phosphoramidon (Warner *et al.*, 1990; Qian *et al.*, 1994), captopril and bacitracin (Couture & Regoli, 1981; Mauborgne *et al.*, 1991). For this reason, a mixture of peptidase inhibitors was administered to block the possible degradation of NPγ. The efficacy of these inhibitors was tested on the cardiovascular response induced by the i.t. injection of 6.5 nmol SP. The cardiovascular effects induced by SP were markedly and significantly increased in the presence of these peptidase inhibitors (data not shown). In the presence of peptidase inhibitors, we observed a delayed increase in the MAP response to 0.78 NPγ, so that the response resembled that obtained with 78 nmol NPγ in untreated rats indicating that the NPγ concentration had been increased in the aCSF by the peptidase inhibitors. Moreover, the HR response to NPγ in the presence of peptidase inhibitors was prolonged. Hence, it seems unlikely that the cardiovascular responses to i.t. NPγ are due to the generation of a major metabolite.

Mechanism underlying the effects of NPγ on MAP and HR

As the pressor and chronotropic responses induced by NPγ were reduced by phentolamine and propranolol, respectively, it is likely that the effects of NPγ are mediated by the peripheral release of catecholamines from sympathetic fibres and/or the adrenal medulla. The cardiovascular effects of i.t. SP and NPK were also found to be mediated by the peripheral release of catecholamines (Hassessian *et al.*, 1990; Pham *et al.*, 1993). In addition, it was observed that i.t. NPK can induce the release of neuropeptide Y from sympathetic fibres and/or the adrenal medulla (Pham *et al.*, 1993). However, the short-lasting vasodepressor effect observed with 7.8 nmol NPγ was not blocked by propranolol. Therefore, it is unlikely that β-adrenoceptors are involved in this vasodepressor response. A similar vasodepressor response was observed after i.t. injection of SP in pentobarbitone-anaesthetized rat (Couture *et al.*, 1988) and in conscious rats injected with high doses of SP (16.25–32.5 nmol) (Hassessian & Couture, 1989; Hassessian *et al.*, 1990). In the pentobarbitone-anaesthetized rat, this vasodepressor component was found to be resistant to inhibitors of α- or β-adrenoceptors, muscarinic, histamine, opioid and 5-hydroxytryptamine ($5\text{-}HT_2$) receptors and could not be prevented by bilateral adrenalectomy (Couture *et al.*, 1988). Thus, the exact mechanism of this vasodilatation elicited by SP and NPγ remains to be elucidated in future studies.

Conclusion

Intrathecal injections of NPγ produce dose-dependent increases in MAP and HR that are probably due to the activation of NK_1 receptors in the rat spinal cord and consequently to the peripheral release of catecholamines. It can be concluded that NPγ can also serve as an NK_1 agonist in certain *in vivo* systems and particularly in the spinal cord. Since NPγ has been reported to activate selectively NK_2 receptors, this bivalent property of NPγ should be considered in future studies using this tachykinin.

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