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Intracerebroventricular responses to neuropeptide γ in the conscious rat: characterization of its receptor with selective antagonists

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1 The cardiovascular and behavioural effects elicited by the intracerebroventricular (i.c.v.) administration of neuropeptide γ (NPγ) in the conscious rat were assessed before and 5 min after i.c.v. pretreatment with antagonists selective for NK_1 (RP 67,580), NK_2 (SR 48,968) and NK_3 (R 820) receptors. In addition, the central effects of NPγ before and after desensitization of the NK_1 and NK_2 receptors with high doses of substance P (SP) and neurokinin A (NKA) were compared.

2 Intracerebroventricular injection of NPγ (10-780 pmol) evoked dose- and time-dependent increases in mean arterial blood pressure (MAP), heart rate (HR), face washing, head scratching, grooming and wet-dog shake behaviours. Similar injection of vehicle or 1 pmol NPγ had no significant effect on those parameters.

3 The cardiovascular and behavioural responses elicited by NPγ (25 pmol) were significantly and dose-dependently reduced by pretreatment with 650 pmol and 6.5 nmol of SR 48,968. No inhibition of NPγ responses was observed when 6.5 nmol of RP 67,580 was used in a similar study. Moreover, the prior co-administration of SR 48,968 (6.5 nmol) and RP 67,580 (6.5 nmol) with or without R 820 (6.5 nmol) did not reduce further the central effects of NPγ and significant residual responses (30-50%) remained.

4 No tachyphylaxis to NPγ-induced cardiovascular and behavioural changes was observed when two consecutive injections of 25 pmol NPγ were given 24 h apart.

5 Simultaneous NK_1 and NK_2 receptor desensitization reduced significantly central effects mediated by 25 pmol NPγ. However, significant residual responses persisted as seen after pretreatment with SR 48,968.

6 The results suggest that the central effects of NPγ are mediated partly by NK_2 receptors and by another putative tachykinin receptor subtype (NPγ receptor?) that appears to be different from NK_1 and NK_3 receptors.

Keywords: Tachykinin antagonists; neuropeptide γ; cardiovascular responses; behaviours; SR 48,968; RP 67,580; R 820

Introduction

Substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and N-terminally extended forms of NKA, neuropeptide γ (NPγ) and neuropeptide K (NPK) belong to the mammalian members of the tachykinin family of peptides. These biologically active peptides exert their actions through the activation of three receptors termed neurokinin-1 (NK_1), NK_2 and NK_3 (Guard & Watson, 1991; Regoli *et al.*, 1994). Although they are not highly selective agonists, SP, NKA and NKB are believed to be the endogenous ligands for the NK_1, NK_2 and NK_3 receptors, respectively (Regoli *et al.*, 1987). It is unclear whether the multiple NKA-related peptides, including NPK and NPγ interact with a single class of binding sites (NK_2) or whether they differentially interact with other undiscovered tachykinin receptor subtypes (Takeda & Krause, 1991).

NPγ, a 21 amino acid peptide isolated from rabbit intestine extracts by Kage *et al.* (1988) is encoded by only one of the four mRNAs generated from the primary transcript of the preprotachykinin (PPT) A gene, called γ-PPT. γ-PPT also codes for SP and NKA and comprises over 75% of all four alternate PPT-A splice variants (α-, β-, γ- and δ-PPT) expressed in all tissues of the rat (Carter & Krause, 1990). Moreover, β-PPT and γ-PPT together have been estimated to represent over 99% of all mRNA generated from PPT-A in rats and human subjects (Carter & Krause, 1990; Helke *et al.*, 1990; Marchand *et al.*, 1993). Even though physiological roles for NPγ are still poorly defined, this last discovered mammalian tachykinin member merits further investigation since it is relatively abundant in various rat tissues: brain (7.54 ± 0.50 pmol g^{-1} tissue), duodenum (9.81 ± 1.33 pmol g^{-1} tissue) and jejunum (7.48 ± 0.28 pmol g^{-1} tissue) (Takeda *et al.*, 1990). In all rat tissues, the endogenous levels of NKA-related peptides are NKA > NPγ = NPK > NKA(3-10) (Takeda *et al.*, 1990).

NPγ is a preferential NK_2 receptor agonist but like the other endogenous tachykinins, it is poorly selective and can also stimulate the NK_1 receptor (Helke *et al.*, 1990). In the hamster urinary bladder, the pharmacological profile of NPγ was similar to that of NKA and NPK on NK_2 receptor binding stimulation of phosphatidylinositol hydrolysis and smooth muscle contraction (van Giersbergen *et al.*, 1992). Moreover, NPγ was one of the most potent tachykinins to contract the human isolated bronchus (Burcher *et al.*, 1991) and to increase guinea-pig total lung resistance (Yuan *et al.*, 1994). These effects on lung functions and human bronchus were inhibited by SR 48,968, suggesting that NPγ exerts its effects via NK_2 receptors (Qian *et al.*, 1994; Yuan *et al.*, 1994). On the other hand, when administered intrathecally (T-9 level) in the conscious rat, NPγ (78 pmol-78 nmol) induced dose-dependent increases in heart rate and mean arterial blood pressure; these

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effects were blocked by RP 67,580 (selective NK_1 receptor antagonist), but not by SR 48,968 or R 486 (selective NK_3 antagonist) (Poulat *et al.*, 1996).

In the urethane-anaesthetized rat, NPγ (i.c.v.; 0.5 nmol) induced increases in blood pressure and heart rate, effects blocked by pentolinium but not by a vasopressin antagonist (Hagio *et al.*, 1991). Phentolamine also blocked the pressor response induced by NPγ. These results indicate that haemodynamic responses occurring following central administration of NPγ are secondary to the activation of the sympathetic nervous system. However, it is not known whether the central actions of NPγ are mediated by NK_2 or NK_1 receptors or both, or via a novel yet undiscovered tachykinin receptor subtype showing high affinity for NPγ. With the use of selective NK_1 (RP 67,580), NK_2 (SR 48,968) and NK_3 (R 820) receptor antagonists and a protocol of desensitization for NK_1 and NK_2 receptors, the present study addresses this issue. These antagonists have been selected for their ability to block in a selective manner the intracerebral effects evoked by exogenous tachykinin agonists (SP, NKA, senktide) as previously reported (Cellier *et al.*, 1995; Couture *et al.*, 1995). The optimal doses required to achieve maximal inhibition with the antagonist were derived from those extensive studies. These experiments were performed in the conscious freely moving rat to avoid the potential for confounding interaction of NPγ with general anaesthesia and to assess simultaneously both the cardiovascular and behavioural changes elicited by central injection of NPγ.

Methods

Animal preparation

The animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care and the CDEA of the Université de Montréal. Male Wistar rats weighing 300–350 g were purchased from Charles River (St. Constant, Québec, Canada). The animals were kept in a room at 20–25°C in individual plastic cages (40 cm × 23 cm × 20 cm) and were submitted to a 12 h light/dark cycle (lights on 06 h 00 min–18 h 00 min) with free access to commercial food and tap water.

Rats were temporarily anaesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbitone, 65 mg kg^{-1} (Somnotol; M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada) to allow implantation of a polyethylene i.c.v. cannula (PE-20; Intramedic, Clay Adams, NJ, U.S.A.). The i.c.v. catheter was inserted with a guide cannula into the right lateral brain ventricle by use of a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.) as previously described (Picard *et al.*, 1994).

The animals were returned to their resident plastic cages for a recovery period of 72 h. After this period, rats were re-anaesthetized with sodium pentobarbitone 65 mg kg^{-1} and a second cannula (PE-50) implanted into the left femoral artery all the way to the abdominal aorta for the measurement of blood pressure and heart rate (HR). The intraarterial catheter was pre-siliconized to avoid long term blood clotting, filled with physiological saline containing heparin sodium salt (100 iu ml^{-1}) and exteriorised subcutaneously at the back of the neck about 1 cm caudal to the i.c.v. cannula. The rats intracerebroventricularly implanted require a minimum of 5 days to recover from surgery and anaesthesia as indicated by the re-establishment of their normal body weight and renal functions (Yuan, 1995). Prolonged femoral artery catheterization increased considerably the number of rats having blood clotting complications. Thus, to avoid extensive post-operative care, the intra-arterial surgery, which is less stressful than the i.c.v. implantation, was performed 2 days prior to experiments which were conducted in conscious freely moving rats kept in their resident plastic cages with the top grid removed. Heparin (100 iu ml^{-1}) was re-injected through the intrafemoral catheter at the beginning of each experimental day.

Measurement of cardiovascular and behavioural responses

The arterial blood pressure was monitored through the intra-arterial catheter with a Statham pressure transducer (P231D) while the HR derived from the blood pressure signal was measured with a cardiac tachometer (model 7P4) and both variables were registered on a Grass polygraph model 79D (Grass Instruments Co., Quincy, MA, U.S.A.). Experiments were performed during the day and started when the animal reached a resting state and stable basal mean arterial blood pressure (MAP) and HR.

The behavioural activity of the rat was assessed for over a 30 min period starting immediately after i.c.v. injection. The frequency of individual behaviours: face washing (FW), head scratching (HS) and grooming (G) was determined according to the 15 s sampling procedure of Gispen *et al.* (1975). During every consecutive period of 15 s, a score of 1 or 0 was given depending on whether the animal showed the specific type of behaviour or not, whatever its frequency, intensity or span during that interval. Summation of scores for 30 min following the i.c.v. injection gave the behavioural score for face washing, head scratching and grooming in each experiment. The maximal theoretical score for these three behaviours was 120 (15 s intervals × 30 min). The wet-dog shake behaviour was measured according to the number of episodes (less than 1 s each) during the 30 min period, whatever the intensity. Rats were injected i.c.v. with 25 pmol angiotensin II to verify the patency of the i.c.v. cannula. Only those animals that responded with an immediate sharp rise in blood pressure, associated with an intense dipsogenic activity as reported earlier (Kirby *et al.*, 1992), were included in the study. The correct position of the i.c.v. cannula was also verified histologically by *post-mortem* dissection. The rate of successful implantation was close to 95%.

Experimental protocols

In the first series of experiments, the animals ($n=7$ to 9 per group) received i.c.v. injection of increasing doses of NPγ (1 pmol, 10 pmol, 25 pmol, 78 pmol, 780 pmol) diluted in a volume of 1 μl and flushed with 4 μl of artificial cerebrospinal 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). The injection period lasted about 30 s and was immediately followed by cardiovascular and behavioural measurements for 30 min. Each dose was administered at intervals of 48 h to avoid tachyphylaxis (Itoi *et al.*, 1992; Picard *et al.*, 1994). An additional group ($n=22$) of control rats received 5 μl aCSF only. This dose-response curve to NPγ was performed to estimate adequately its potency and to select the dose in the protocol using the selective tachykinin antagonists.

A second series of experiments was designed to estimate possible tachyphylaxis after two consecutive injections of NPγ given 24 h apart. Therefore, at the same time, on day 1 and day 2, a group of rats ($n=8$) were injected with 25 pmol NPγ and the effects were measured both on the cardiovascular system and on behavioural activity. This control experiment was required to confirm the adequacy of this schedule in the protocol using the antagonists against that dose of NPγ.

In the third part of this study, one or a combination of three antagonists (RP 67,580, SR 48,968 or R 820) was given i.c.v. in a volume of 1 μl as a pretreatment 5 min prior to the injection of 25 pmol NPγ. On the first day of the experiment, the vehicle aCSF containing dimethylsulphoxide (DMSO), used to dissolve the tested antagonist, was injected i.c.v. 5 min prior to NPγ (25 pmol). On the second day, one of the following pretreatments was given, prior to NPγ, to five groups of rats: group 1 ($n=8$), 650 pmol SR 48,968; group 2 ($n=8$), 6.5 nmol

SR 48,968; group 3 ($n=8$), 6.5 nmol RP 67,580; group 4 ($n=8$), 6.5 nmol SR 48,968 + 6.5 nmol RP 67,580; group 5 ($n=8$), 6.5 nmol SR 48,968 + 6.5 nmol RP 67,580 + 6.5 nmol R 820. Doses of antagonists used in these experiments are based on a previous study (Picard *et al.*, 1994). Each animal received only one pretreatment. On the third day, NPγ was injected alone to evaluate the reversibility of any inhibition observed on day 2. The inherent activity of the antagonists was tested in separate experiments ($n=8$). Baseline MAP and HR values were calculated 1 min before the injection of NPγ.

Another group of rats ($n=6$) was used in a fourth series of experiments designed to rule out the participation of NK_1 receptors in the central effects of NPγ and to gain more information concerning the substantial cardiovascular and behavioural component that is resistant to NK_2 receptor blockade after NPγ injection. Therefore, on the first day, NPγ (25 pmol) was injected i.c.v. and the cardiovascular and behavioural responses were measured over a period of 30 min. On the second day, SP and NKA were co-administered 3–5 times at 30 min intervals with high doses (6.5 nmol) to cause rapid desensitization of NK_1 and NK_2 receptors. The lack of effects evoked by SP and NKA after this protocol indicated that a complete desensitization to SP and NKA occurred. At this point, NPγ (25 pmol) was injected i.c.v. and its central effects were compared to those observed 24 h earlier. One hour after NPγ injection, SP and NKA were co-administered again to ascertain the level of desensitization.

Peptides and non-peptides

The non-peptide NK_1 antagonist, RP 67,580 (racemic form of 7,7-diphenyl-2[1-imino-2 (2-methoxy-phenyl)-ethyl]perhydro-isoindol-4-one (3aR, 7aR); mol. wt.: 475.0 for the hydrochloride salt) was a gift from Dr C Garret, Rhône-Poulenc Rorer, Paris, France. The NK_2 antagonist, SR 48,968 ((S)-N-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2- (3,4-dichlorophenyl)-butyl]benzamide; mol. wt.: 570.0) was a gift of Dr J.-C. Brelière, Sanofi, Montpellier, France. The compound R 820 (3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl), synthesized in the laboratory of Dr D. Regoli (Sherbrooke University, Sherbrooke, Canada), is a selective antagonist of the rat NK_3 receptor showing higher affinity and metabolic stability than R 486 and R 487 (two peptide NK_3 antagonists) (Regoli *et al.*, 1994). Moreover, R 486 maintained a residual agonistic effect on NK_1 and NK_2 receptors when injected i.c.v. in the conscious rat (Picard *et al.*, 1994). R 820 was selected in this study because of its lack of direct effect upon i.c.v. administration (Cellier *et al.*, 1995). NPγ, SP, NKA and angiotensin II were purchased from Hükabel Scientific Ltd, Montréal, Canada. Heparin sodium salt Grade II from porcine intestinal mucosa was purchased from Sigma chemicals (St. Louis, MO, U.S.A.). The antagonists were dissolved in DMSO (Fisher) and aCSF was added to obtain the desired solution (the final solution contained a maximum of 20% of DMSO). NPγ, SP, NKA and angiotensin II were dissolved directly in aCSF. The stock solutions (1–10 mg ml^{-1}) of peptides and non-peptides were divided into 100 μl aliquots and stored at −20°C until used. Daily dilutions were made in aCSF before each experiment.

Statistical analysis of data

The results are expressed as mean ± s.e.mean. Statistical differences were evaluated with Student's *t* test for paired samples or Wilcoxon-Mann-Whitney (U) test for unpaired samples on non parametric values (behaviour frequency). When more than one comparison was made, the significance of differences among groups was evaluated with a one-way or two-way analysis of variance (ANOVA) in conjunction with Bonferroni confidence intervals. Only probability values (*P*) less than 0.05 were considered to be statistically significant.

Results

Central cardiovascular and behavioural effects induced by NPγ

The time course of MAP and HR variations elicited by the i.c.v. injection of NPγ are illustrated in Figure 1. While 1 and 10 pmol NPγ failed to modify MAP when compared to aCSF, higher doses (25, 78 and 780 pmol) of NPγ elicited dose- and time-dependent increases in MAP. The pressor response reached a maximum at 3–5 min (25 and 78 pmol) or 7–9 min (780 pmol) before returning gradually to pre-injection values. In contrast to the MAP response, HR was significantly ($P<0.05$) increased with 10 pmol of NPγ. Higher doses of NPγ (25–780 pmol) raised HR in a dose- and time-dependent fashion. The tachycardia reached a maximum within 3 min and returned to basal values in parallel with the pressor response.

The cardiovascular responses to i.c.v. NPγ were accompanied by a marked increase in behavioural activity. As shown in Table 1, face washing, head scratching, grooming and wet-dog shake increased dose-dependently following injection of NPγ (10–780 pmol). While grooming was significantly enhanced by 10 pmol NPγ, the threshold dose for face washing

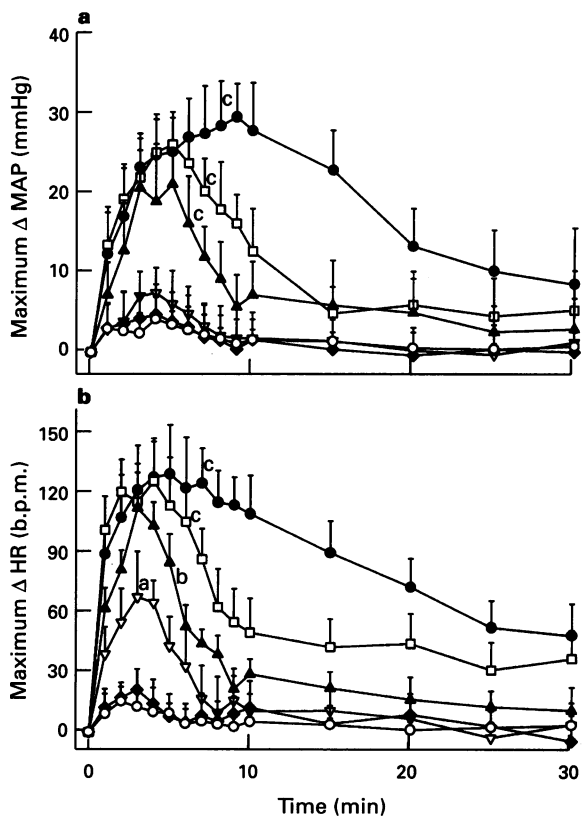

Figure 1 Time course of changes in (a) mean arterial blood pressure (MAP) and (b) heart rate (HR) evoked by i.c.v. injections of NPγ at the dose of 1 pmol (◆), 10 pmol (▽), 25 pmol (▲), 78 pmol (□) and 780 pmol (●) in conscious freely moving rats. Cardiovascular effects mediated by aCSF (○) are also shown. Each point represents the mean ± s.e.mean of 7–9 rats. Baseline MAP and HR values are 105.3 ± 9.3 mmHg and 342.5 ± 16.0 b.p.m. for the aCSF group; 103.2 ± 10.3 mmHg and 361.5 ± 17.1 b.p.m. for the 1 pmol group; 97.5 ± 9.9 mmHg and 337.8 ± 21.9 b.p.m. for the 10 pmol group; 105.4 ± 13.1 mmHg and 362.0 ± 27.2 b.p.m. for the 25 pmol group; 101.0 ± 13.5 mmHg and 353.4 ± 25.3 b.p.m. for the 78 pmol group and 106.3 ± 8.7 mmHg and 351.8 ± 18.0 b.p.m. for the 780 group. Statistical comparison to the vehicle for the period of 1–10 min following i.c.v. injection is indicated by $^{a}P<0.05$; $^{b}P<0.01$; $^{c}P<0.001$.

and head scratching was 25 pmol and that for the wet-dog shake was 78 pmol. In addition, the occurrence of face washing, head scratching and grooming seemed to be in close parallel with the cardiovascular responses and showed a similar time course.

Reproduction of the central effects of NPγ

This series of experiments was undertaken to evaluate the possible desensitization to NPγ-induced cardiovascular and behavioural effects when two i.c.v. injections of 25 pmol are given one day apart. Using this protocol, the changes in MAP, HR, face washing, head scratching, grooming and wet-dog shake produced by the first and second injection of NPγ were not significantly different from each other (Figure 2).

Effects of selective tachykinin antagonists versus the central effects of NPγ

No statistical difference was observed between baseline MAP and HR values on day 1 (without antagonist), day 2 (5 min after antagonist injection) and day 3 (24 h after antagonist injection) in each of the five groups of rats tested with antagonist (data not shown). The NK_1 receptor antagonist, RP 67,580 (6.5 nmol) was inactive against the central cardiovascular and behavioural effects induced by 25 pmol NPγ (Figure 3 and Table 2). In contrast, the pressor as well as the face washing, head scratching and grooming behaviours induced by 25 pmol NPγ were significantly and dose-dependently reduced when rats were pretreated with the NK_2 selective antagonist SR 48,968 (650–6500 pmol) (Figure 3 and Table 2). Whereas the positive chronotropic response to NPγ was significantly reduced by the highest dose of SR 48,968 (6.5 nmol), the wet-dog shake behaviour remained resistant to this treatment. The inhibitory effect of the antagonist on the cardiovascular system was no longer observed when the agonist was readministered 24 h later (Figure 3).

RP 67,580 (6.5 nmol) and SR 48,968 (6.5 nmol) were co-injected and tested against the NPγ-mediated effects. This pretreatment was as effective as SR 48,968 alone (6.5 nmol) in reducing NPγ-induced responses (Figure 3 and Table 2). The residual effects of NPγ were still significantly different from vehicle ($P < 0.05$) except for face washing which was reduced to the levels in vehicle treated rats (Figure 3 and Table 2).

Finally, a combination of SR 48,968 (6.5 nmol), RP 67,580 (6.5 nmol) and R 820 (6.5 nmol) was administered as a pretreatment to NPγ (25 pmol) to block NK_1, NK_2 and NK_3 receptors. This cocktail of antagonists failed to suppress further the NPγ-induced cardiovascular and behavioural effects when compared to the reduction by SR 48,968 (6.5 nmol) alone. The only exception is the wet-dog shake effect which was reduced to the levels in vehicle-treated rats (Figure 3 and Table 2).

None of the treatments with antagonists or NPγ showed any apparent toxic effects. Moreover, we have reported in an earlier study that 6.5 nmol of RP 67,580 or SR 48,968 had no significant effect on MAP, HR or on the individual behaviours (Picard *et al.*, 1994). R 820 ($n = 8$) was also found to be devoid of inherent activity; cardiovascular and behavioural responses to 6.5 nmol R 820 were not significantly different from vehicle values (maximum Δ HR: 13.9±8.2 b.p.m. at 3 min post-injection; maximum Δ MAP: 5.7±3.1 mmHg at 3 min post injection; face washing: 2.1±2.3 score 30 min^{-1}; head scratching: 1.8±1.3 score 30 min^{-1}; grooming 4.4±2.0 score 30 min^{-1}; wet-dog shake: 8.1±3.2 episodes 30 min^{-1}).

Effects of NK_1 and NK_2 receptor desensitization on central effects of NPγ

A rapid and complete desensitization to SP and NKA was observed after 3–5 co-injections of 6.5 nmol SP and NKA at

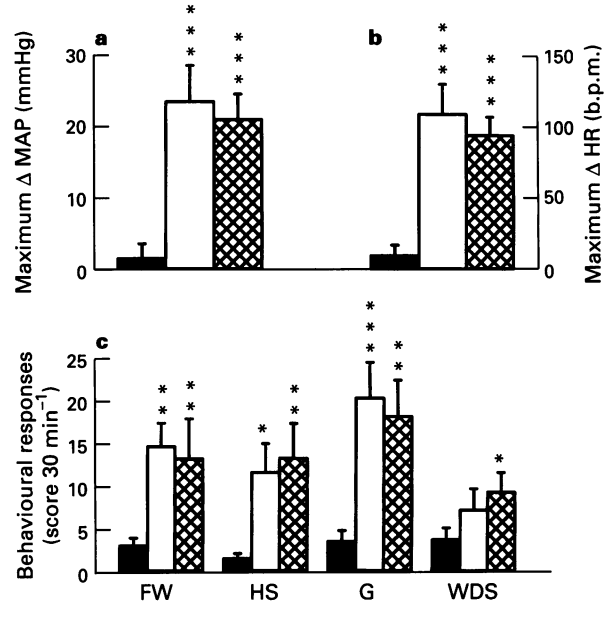

Figure 2 Cardiovascular and behavioural effects of i.c.v. injection of aCSF (solid columns, $n = 22$) or 25 pmol neuropeptide γ (NPγ) on day 1 (open columns, $n = 8$) and day 2 (cross-hatched columns, $n = 8$) in the same rats. Shown are maximal changes in (a) mean arterial blood pressure (MAP), (b) heart rate (HR) and (c) behavioural activity for a 30 min period. FW = face washing, HS = head scratching, G = grooming and WDS = wet dog shake. Each column represents the mean±s.e.mean of (n) rats. Baseline MAP and HR values are 100.8±11.5 mmHg and 329.0±23.7 b.p.m. for the day 1 group; 107.8±9.4 mmHg and 377.5±25.3 b.p.m. for the day 2 group and 103.4±12.5 mmHg and 358.0±19.4 b.p.m. for the day 3 group. Differences in MAP, HR and behaviours between the two injections of NPγ were non-significant. Statistical comparison to aCSF are indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Table 1 Behavioural responses elicited by i.c.v. injection of neuropeptide γ (NPγ) in conscious rats

Agonist	n	*Face washing* (score 30 min^{-1})	*Head scratching* (score 30 min^{-1})	*Grooming* (score 30 min^{-1})	*Wet-dog shakes* (episodes 30 min^{-1})
aCSF	22	2.9±0.9	1.4±0.6	3.4±1.3	3.6±1.4
NPγ 10 pmol	8	4.9±2.7	3.9±2.1	8.9±3.2*	6.7±3.7
NPγ 25 pmol	9	10.8±4.2*	11.4±3.2*	20.0±5.6**	7.8±4.0
NPγ 78 pmol	7	22.0±7.3***	18.4±7.9***	47.3±9.5***	12.7±5.4*
NPγ 780 pmol	7	36.9±12.9***	26.9±13.8***	55.9±8.2***	16.8±8.9*

Values represent the mean±s.e.mean of (n) rats for a period of 30 min. Statistical comparison to aCSF was evaluated with a one-way ANOVA and a *post-hoc* Wilcoxon-Mann-Whitney (U) test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

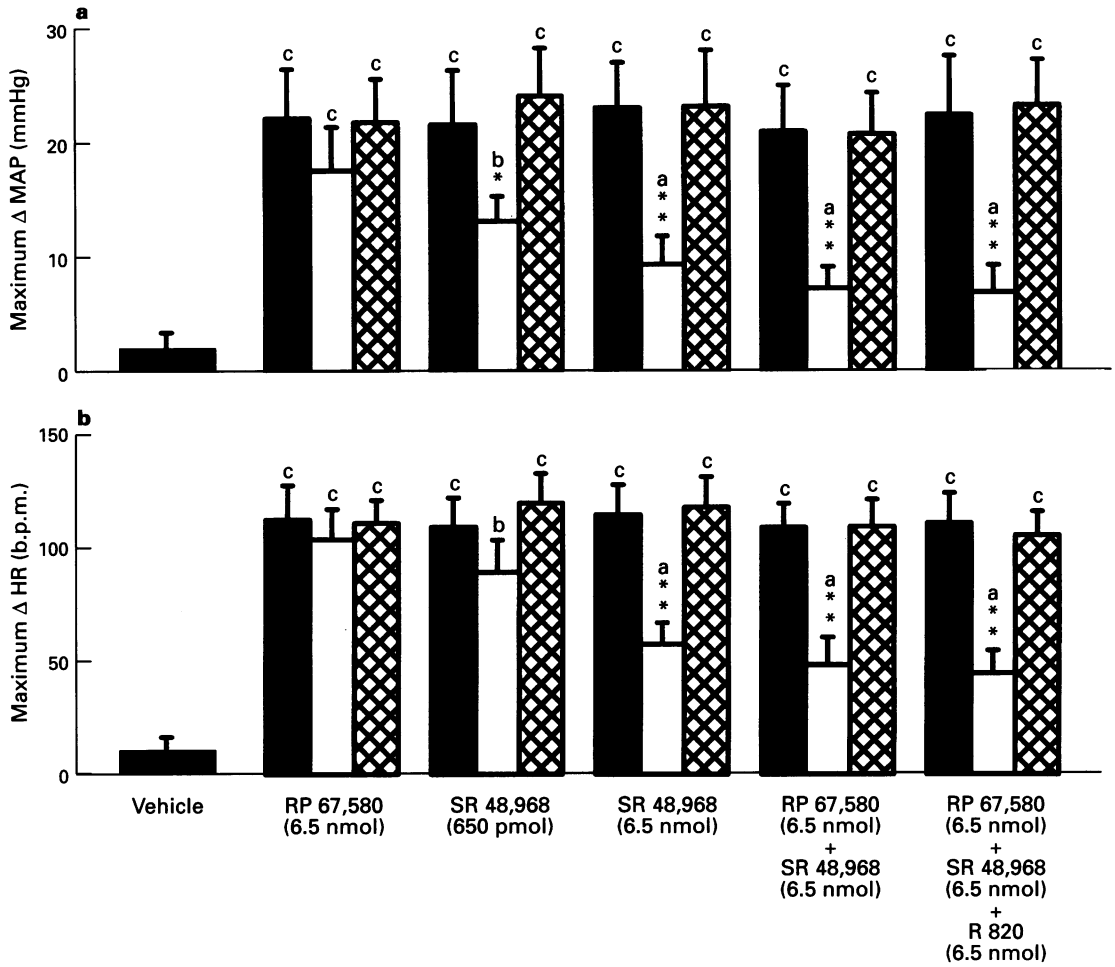

Figure 3 Effects of selective tachykinin receptor antagonists on maximal changes in (a) mean arterial blood pressure (MAP) and (b) heart rate (HR) induced by the i.c.v. injection of vehicle (grey-shaded columns) or 25 pmol NPγ in conscious rats. NPγ was injected alone on day 1 (solid columns), 5 min after the antagonist on day 2 (open columns) or alone on day 3 (cross-hatched columns). Values represent the mean ± s.e.mean of 7–8 rats. Baseline MAP and HR values are 103.2 ± 8.0 mmHg and 351.2 ± 17.9 b.p.m. for the RP 67,580 group, 108.6 ± 7.7 mmHg and 371.4 ± 21.6 b.p.m. for the SR 48,968 (650 pmol) group; 97.6 ± 9.1 mmHg and 342.5 ± 20.7 b.p.m. for the SR 48,968 (6.5 nmol) group: 115.3 ± 8.3 mmHg and 380.7 ± 23.0 b.p.m. for the RP 67,580 + SR 48,968 group and 104.3 ± 8.0 mmHg and 376.2 ± 23.8 b.p.m. for the RP 67,580 + SR 48,968 + R 820 group. Statistical comparison to the vehicle (a,b,c) or to the agonist alone on day 1 (*; solid column) is indicated by $*,^{a}P < 0.05$; $**,^{b}P < 0.01$; $^{c}P < 0.001$.

30 min intervals. At the end of this protocol, no central responses could be elicited by co-injection of both peptides. Moreover, the cardiovascular effects as well as the face washing, head scratching and grooming behaviours induced by 25 pmol NPγ were significantly decreased when compared to the effects with NPγ measured on the preceding day (Figure 4). However, NPγ maintained significant residual effects when compared with aCSF values while the wet-dog shake behaviour remained unaffected, suggesting a partial and incomplete cross-desensitization between SP/NKA and NPγ-induced central effects in rats.

Discussion

The results of the present study reveal that the i.c.v. administration of NPγ in the awake unrestrained rat induces dose- and time-dependent increases in heart rate, blood pressure and behavioural activity (face washing, head scratching and grooming) through the activation of NK_2 receptors and possibly another yet unidentified tachykinin receptor that does not belong to the NK_1 or the NK_3 receptor subtype. Contrary to the above behavioural responses induced by NPγ, the wet-dog shake is not observed in all groups of rats receiving 25 pmol NPγ and is only slightly enhanced at higher doses (78 and 780 pmol). Therefore, the wet-dog shake response which is believed to be mediated by central NK_3 receptors and a 5-hydroxytryptamine mechanism (Stoessl *et al.*, 1988) is unlikely to be under the control of endogenous NPγ.

The cardiovascular and behavioural effects of i.c.v. NPγ comply with a general trend that is common to all endogenous PPT-A derived tachykinins. Indeed, dose-dependent increases in MAP and HR were elicited by i.c.v. injection of SP and NKA (Itoi *et al.*, 1992; Picard *et al.*, 1994) and NPK (Prat *et al.*, 1994). In the case of SP, NKA and NPγ, these effects were ascribed to sympathetic nervous system stimulation (Unger *et al.*, 1981; 1985; Takano *et al.*, 1990; Hagio *et al.*, 1991). A behavioural arousal reaction including increased locomotion and intense scratching and grooming behaviour accompanies these haemodynamic effects (Itoi *et al.*, 1992; Tschöpe *et al.*, 1992; Picard *et al.*, 1994; Prat *et al.*, 1994). Surprisingly, the order of potency for these natural tachykinins on the cardiovascular system after central administration is NPK > NPγ > SP > NKA > NKB (Couture *et al.*, 1995). Therefore, the most potent tachykinins are the N-extended

Table 2 Effects of selective tachykinin antagonists on behavioural responses elicited by the i.c.v. injection of 25 pmol neuropeptide γ (NPγ)

Pretreatment	*Treatment*	n	*Face washing* (score 30 min^{-1})	*Head scratching* (score 30 min^{-1})	*Grooming* (score 30 min^{-1})	*Wet-dog shakes* (episodes 30 min^{-1})
Vehicle	–	14	2.6±0.7	2.1±0.5	3.2±1.1	4.2±0.8
–	NPγ	8	13.9±4.2††	17.2±5.0††	16.2±3.9††	6.1±2.4
RP 67,580 (6.5 nmol)	NPγ	8	10.4±3.9††	12.1±2.6††	13.8±2.5††	8.3±4.9
–	NPγ	8	14.0±3.6†††	15.7±4.7††	23.8±5.6†††	10.4±3.3†
SR 48,968 (650 pmol)	NPγ	8	9.2±2.8††*	8.4±1.6††*	13.4±3.0††*	9.6±2.8†
–	NPγ	8	13.3±2.4††	14.2±2.8††	20.8±3.2††	9.4±2.9†
SR 48,968 (6.5 nmol)	NPγ	8	5.8±1.1†**	6.1±1.9†**	9.0±1.8†***	10.1±4.3†
–	NPγ	8	10.4±2.1†	12.8±1.6††	19.8±2.7††	15.7±6.4†
RP 67,580+SR 48,968 (6.5 nmol each)	NPγ	8	4.4±2.7*	7.2±2.0†**	9.5±2.3†***	13.0±4.0†
–	NPγ	8	14.1±3.8†††	12.0±2.5††	27.6±5.3††	13.7±3.0†
RP 67,580+SR 48,968 +R 820 (6.5 nmol each)	NPγ	8	6.7±1.3†***	5.9±1.4†**	12.9±4.1†***	6.9±3.8

Values represent the incidence of individual behaviour for 30 min and are indicated by the mean±s.e.mean of (*n*) rats. The antagonists were injected at the dose indicated 5 min prior to 25 pmol NPγ. Statistical comparison to vehicle (†) was evaluated with a Wilcoxon-Mann-Whitney (U) test, while comparison to the agonist in the absence of antagonist (*) was calculated with Student's *t* test for paired samples: *,†$P<0.05$; **,††$P<0.01$; ***,†††$P<0.001$.

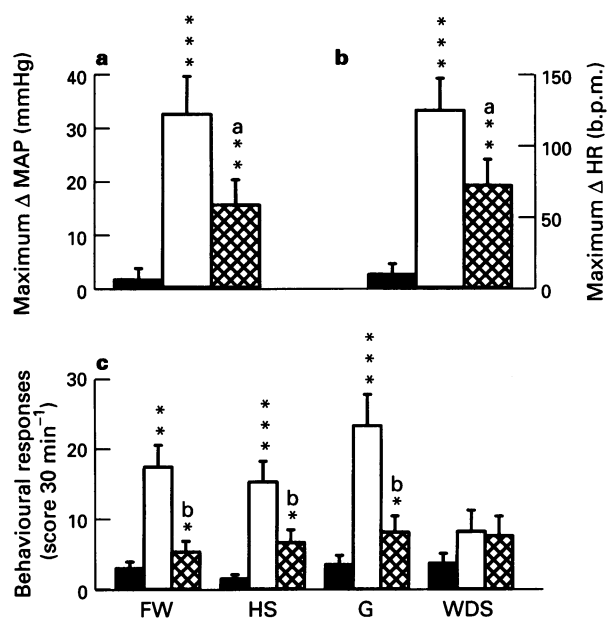

Figure 4 Cardiovascular and behavioural effects to i.c.v. injection of aCSF (solid columns, $n=22$) or 25 pmol neuropeptide γ (NPγ) on day 1 (open columns, $n=8$) and on day 2 (cross-hatched columns, $n=8$) in rats that have been previously desensitized to SP and NKA on day 2. Shown are maximal changes in (a) mean arterial blood pressure (MAP), (b) heart rate (HR) or (c) behavioural activity for a 30 min period. FW=face washing, HS=head scratching, G=grooming and WDS=wet dog shake. Each column represents the mean±s.e.mean of 6 rats. Baseline MAP and HR values are 98.5±9.2 mmHg and 346.0±19.4 b.p.m. for the day 1 group; 103.4±10.1 mmHg and 364.2±17.6 b.p.m. for the day 2 group and 102.4±11.3 mmHg and 344.6±22.4 b.p.m. for the day 3 group. Statistical comparison to aCSF (*) or to NPγ on day 1 ([a] and [b]) are indicated by [a,*]$P<0.05$; [b,**]$P<0.01$; [c,***]$P<0.001$.

forms of NKA which have been considered as NK_2 agonists in several biological and binding assays (Beaujouan *et al.*, 1988; Takeda & Krause, 1989; Dam *et al.*, 1990c; Van Giersbergen *et al.*, 1992). We may relate this finding to the greater metabolic stability of NPK and NPγ due to their longer N-terminal amino acid sequence (Takeda & Krause, 1989). It remains possible that NPγ is processed to a more active metabolite such as NPγ(1–9) which is the major product of post-translational processing of γ-PPT-A in rat tissues (Wang *et al.*, 1993). However, we must rule out NKA alone as a possible metabolite because this peptide is less potent than NPγ and NPK in this paradigm and unlike the latter tachykinins, the central effects of NKA are mediated entirely by NK_1 and NK_2 receptors (Couture *et al.*, 1995).

Central receptors activated by i.c.v. NPγ

Although NPγ-induced responses are qualitatively similar to those of NPK, SP and NKA, the relative involvement of NK_1 and NK_2 receptors differs substantially for each peptide. We have previously shown that i.c.v. SP activates primarily NK_1 receptors but also NK_2 receptors after NK_1 receptor blockade. Conversely, the central effects of NKA were mediated mainly through NK_2 receptors while a minor NK_1 receptor component was unmasked under NK_2 receptor inhibition (Picard *et al.*, 1994). It is noteworthy that the treatment with 6.5 nmol RP 67,580+6.5 nmol SR 48968 completely abolished the cardiovascular and behavioural responses induced by i.c.v. SP and NKA (Picard *et al.*, 1994). In contrast, the central actions of NPK were mediated solely by the NK_1 receptor as no residual responses were observed when NPK was pretreated with RP 67,580 (6.5 nmol) in a protocol similar to the present study (Prat *et al.*, 1994). Moreover, as stated earlier, the cardiovascular responses induced by either SP or NKA, thus by activation of NK_1 and NK_2 receptors, have been associated with an increased sympathoadrenal activity (Unger *et al.*, 1981; 1985; Takano *et al.*, 1990), while those induced by i.c.v. injection of NK_3 agonists would result mainly from the release of vasopressin from the hypothalamus and to a minor extent by activation of the sympathetic nervous system (Polidori *et al.*, 1989; Takano *et al.*, 1990; 1993). From these previous studies, one could reason that NPγ acts through the activation of NK_1 or NK_2 receptors or both to activate primarily the central

autonomic nervous system. Although our results suggest that NK_2 receptors are partly involved in the central effects of NPγ, they rule out the participation of NK_1 and NK_3 receptors in these effects. Important residual responses persisted after treatment with a combination of NK_1, NK_2 and NK_3 receptor antagonists at doses sufficient to abolish the central effects of SP, NKA and senktide (Couture *et al.*, 1995; Cellier *et al.*, 1995) or after NK_1 and NK_2 receptor desensitization. Hence, the presence of a new tachykinin receptor subtype or a non-related tachykinin receptor such as proposed for SP(1-7) (Hornfeldt *et al.*, 1994) displaying a high affinity for NPγ can be suggested in the rat brain.

Site of action for NPγ

A spinal site of action for NPγ after its i.c.v. injection is unlikely since the dose of NPγ necessary to elicit pressor and tachycardiac responses after i.c.v. injection (10–25 pmol) was much lower than that required intrathecally (78 pmol) (Poulat *et al.*, 1996). The hypothalamus may be the site of action of tachykinins since microinjections of SP into the anterior, ventromedial and medial preoptic parts of the hypothalamus of the awake unrestrained rat evoked cardiovascular and behavioural changes similar to those produced by i.c.v. injections of SP or NKA (Itoi *et al.*, 1991; 1994). The fast onset of the response to i.c.v. NPγ leads one to suggest that receptor sites must be localized in the circumventricular organs or in adjacent periventricular structures. A peripheral site of action is also unlikely since these peptides are vasodilators and reduce blood pressure after systemic administration (Couture *et al.*, 1989; Décarie & Couture, 1992). The cardiovascular changes elicited by i.c.v. NPγ are unlikely to be secondary to increased arousal/behavioural activity since increases in blood pressure and heart rate were also reported after i.c.v. injection of NPγ (although at higher doses) in the urethane-anaesthetized rat (Hagio *et al.*, 1991).

NK_1 and NK_3 receptors have been found in moderate to high density in the rat hypothalamus (Dam *et al.*, 1990a,b; Larsen *et al.*, 1992; Maeno *et al.*, 1993). In contrast, the presence of NK_2 receptors in the rat brain remains controversial (Mantyh *et al.*, 1989; Quirion *et al.*, 1991; Takeda & Krause 1991; Mussap *et al.*, 1993) most likely because these receptors, present in small amount in very discrete areas, cannot be measured adequately with currently available NK_2 receptor radioligands ([^{3}H]-NKA, [^{125}I]-NKA and Bolton Hunter-labelled NKA) which exhibit poor selectivity at NK_2 receptors (Buck *et al.*, 1986; Bergstrom *et al.*, 1987; Buck & Krstenansky, 1987; Foster & Tridgett, 1988; Burcher *et al.*, 1989; Geraghty *et al.*, 1992). High-affinity NK_2 binding sites were found using the radioligand [^{125}I]-NPγ in the rat CNS (Dam *et al.*, 1990c; Takeda & Krause, 1991). NPγ was the most potent binding competitor of [^{125}I]-NPγ in the CNS (NPγ > NKA > eledoisin > SP), but appears equipotent with NPK and NKA in the periphery, suggesting that [^{125}I]-NPγ can interact with more than one receptor. Whereas [^{125}I]-NPγ binding sites in the CNS appear to correlate well with the potent antidipsogenic and antinatriorexic effects of NKA and related tachykinins in the rat (Massi *et al.*, 1990; 1991), other investigators (Badgery-Parker *et al.*, 1993) have reported weak specific binding for a new NK_2 radioligand [^{125}I]-[Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]-NKA (4-10) in adult rat brain, suggesting a low level of central NK_2 receptors. Consistent with the relative abundance of γ-PPT mRNA in rat brain (Krause *et al.*, 1987; Marchand *et al.*, 1993) and [^{125}I]-NPγ in the rat CNS (Dam *et al.*, 1990c) in comparison with NK_2-like binding sites, distinct NPγ-preferring receptors may exist.

Conclusion

In summary, i.c.v. NPγ induces marked increases in MAP, HR as well as grooming and motor behaviour. Pharmacological evidence suggests that these effects are secondary to NK_2 receptor activation but may also involve another yet unidentified NPγ-preferring site in the rat brain. Molecular characterization of distinct intra-species NK_2 receptor subtypes and the development of new highly selective radioligands and antagonists for NPγ-binding sites will be necessary to confirm this hypothesis.

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References

BADGERY-PARKER, T., ZENG, X.-P., LAVIELLE, S. & BURCHER, E. (1993). A novel, selective radioligand, [^{125}I]-[Lys^5,Tyr(I$_2$)^7,MeLeu^9,Nle^{10}]-NKA(4-10), for the NK-2 receptor. *Regul. Pept.*, **46**, 455–457.

BEAUJOUAN, J.-C., SAFFROY, M., PETITET, F., TORRENS, Y. & GLOWINSKI, J. (1988). Neuropeptide K, scyliorhinin I and II: new tools in the tachykinin receptor field. *Eur. J. Pharmacol.*, **151**, 353–354.

BERGSTROM, L., BEAUJOUAN, J.-C., TORRENS, Y., SAFFROY, M., GLOWINSKI, J., LAVIELLE, S., CHASSAING, G., MARQUET, A., D'ORLÉANS-JUSTE, P., DION, S. & REGOLI, D. (1987). ^{3}H-Neurokinin A labels a specific tachykinin-binding site in the rat duodenal smooth muscle. *Mol. Pharmacol.*, **32**, 764–771.

BUCK, SH., HELKE, C.J., BURCHER, E., SHULTS, C.W. & O'DONOHUE, T.L. (1986). Pharmacologic characterization and autoradiographic distribution of binding sites for iodinated tachykinins in rat central nervous system. *Peptides*, **7**, 1109–1120.

BUCK, S.H. & KRSTENANSKY, J.L. (1987). The dogfish peptides scyliorhinin I and scyliorhinin II bind with differential selectivity to mammalian tachykinin receptors. *Eur. J. Pharmacol.*, **144**, 109–111.

BURCHER, E., ALOUAN, L.A., JOHNSON, P.R.A. & BLACK, J.L. (1991). Neuropeptide gamma, the most potent contractile tachykinin in human isolated bronchus, acts via a 'non-classical' NK2 receptor. *Neuropept.*, **20**, 79–82.

BURCHER, E., WATKINS, D.J. & O'FLYNN, N.M. (1989). Both neurokinin A and substance P bind to NK1 receptors in guinea-pig lung. *Pulm. Pharmacol.*, **1**, 201–203.

CARTER, M.S. & KRAUSE, J.E. (1990). Structure, expression, and some regulatory mechanisms of the rat preprotachykinin gene encoding substance P, neurokinin A, neuropeptide K, and neuropeptide γ. *J. Neurosci.*, **10**, 2203–2214.

CELLIER, E., PICARD, P. & COUTURE, R. (1995). Mécanisme d'action des effets cardiovasculaires et comportementaux d'un agoniste du récepteur NK-3 des tachykinines chez le rat éveillé. *Médecine/Sciences*, **11** (Suppl. 1), 4, (abstract).

COUTURE, R., LANEUVILLE, O., CUIMOND, C., DRAPEAU, G. & REGOLI, D. (1989). Characterization of the peripheral action of neurokinins and neurokinin receptor selective agonists on the rat cardiovascular system. *Naunyn-Schmied. Arch. Pharmacol.*, **340**, 547–557.

COUTURE, R., PICARD, P., POULAT, P. & PRAT, A. (1995). Characterization of the tachykinin receptors involved in spinal and supraspinal cardiovascular regulation. *Can. J. Physiol. Pharmacol.*, **73,** 892–902.

DAM, T.V., ESCHER, E. & QUIRION, R. (1990a). Visualization of neurokinin-3 receptor sites in the rat brain using the highly selective ligand [^{3}H]senktide. *Brain Res.*, **506,** 175–179.

DAM, T.V., MARTINELLI, B. & QUIRION, R. (1990b). Autoradiographic distribution of brain neurokinin-1/substance P receptors using a highly selective ligand [^{3}H]-[Sar^9,Met(O$_2$)^{11}]-substance P. *Brain Res.*, **531,** 333–337.

DAM, T.V., TAKEDA, Y., KRAUSE, J.E., ESCHER, E. & QUIRION, R. (1990c). γ-Preprotachykinin-(72-92)-peptide amide: an endogenous preprotachykinin I gene-derived peptide that preferentially binds to neurokinin-2 receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **87,** 246–250.

DÉCARIE, A. & COUTURE, R. (1992). Characterization of the peripheral action of neuropeptide K on the rat cardiovascular system. *Eur. J. Pharmacol.*, **213,** 125–131.

FOSTER, A.C. & TRIDGETT, R. (1988). Comparison of the binding of radiolabelled neurokinin A and eledoisin in rat cortex synaptic membranes. *Br. J. Pharmacol.*, **94,** 602–608.

GERAGHTY, D.P., MUSSAP, C.J. & BURCHER, E. (1992). Radioiodinated substance P, neurokinin A, and eledoisin bind predominantly to NK1 receptors in guinea-pig lung. *Mol. Pharmacol.*, **41,** 147–153.

GISPEN, W.H., WIEGANT, V.M., GREVEN, H.M. & DE WIED, D. (1975). The induction of excessive grooming in the rat by intraventricular application of peptides derived from ACTH: structure-activity studies. *Life Sci.*, **17,** 645–652.

GUARD, S. & WATSON, S.P. (1991). Tachykinin receptor types: classification and membrane signalling mechanisms. *Neurochem. Int.*, **18,** 149–165.

HAGIO, T., TAKANO, Y., NAGASHIMA, A., NAKAYAMA, Y., TATEISHI, K. & KAMIYA, H. (1991). The central pressor actions of a novel tachykinin peptide, γ-preprotachykinin (72-79)-peptide amide. *Eur. J. Pharmacol.*, **192,** 173–176.

HELKE, C.J., KRAUSE, J.E., MANTYH, P.W., COUTURE, R. & BANNON, M.J. (1990). Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. *FASEB J.*, **4,** 1606–1615.

HORNFELDT, C.S., SUN, X. & LARSON, A.A. (1994). The NH_2-terminus of substance P modulates NMDA-induced activity in the mouse spinal cord. *J. Neurosci.*, **14,** 3364–3369.

ITOI, K., JOST, N., BADOER, E., TSCHÖPE, C., CULMAN, J. & UNGER, TH. (1991). Localization of the substance P-induced cardiovascular responses in the rat hypothalamus. *Brain Res.*, **558,** 123–126.

ITOI, K., JOST, N., CULMAN, J., TSCHÖPE, C. & UNGER, TH. (1994). Further localization of cardiovascular and behavioural actions of substance P in the rat brain. *Brain Res.*, **668,** 100–106.

ITOI, K., TSCHÖPE, C., JOST, N., CULMAN, J., LEBRUN, C., STAUSS, B. & UNGER, TH. (1992). Identification of the central tachykinin receptor subclass involved in substance P-induced cardiovascular and behavioral responses in conscious rats. *Eur. J. Pharmacol.*, **219,** 435–444.

KAGE, R., MCGREGOR, G.P., THIM, L. & CONLON, J.M. (1988). Neuropeptide-γ a peptide isolated from rabbit intestine that is derived from γ-preprotachykinin. *J. Neurochem.*, **50,** 1412–1417.

KIRBY, R.F., THUNHORST, R.L. & JOHNSON, A.K. (1992). Effects of a non-peptide angiotensin receptor antagonist on drinking and blood pressure responses to centrally administered angiotensins in the rat. *Brain Res.*, **576,** 348–350.

KRAUSE, J.E., CHIRGWIN, J.M., CARTER, M.S., XU, Z.S. & HERSHEY, A.D. (1987). Three rat preprotachykinin mRNAs encode the neuropeptide substance P and neurokinin A. *Proc. Natl. Acad. Sci. U.S.A.*, **84,** 881–885.

LARSEN, P.J., JESSOP, D.S., CHOWDREY, H.S., MIKKELSEN, J.D. & LIGHTMAN, S.L. (1992). Osmotic regulation of substance P and neurokinin A peptide content and substance P binding sites in distinct hypothalamic nuclei of the rat. *Peptides*, **13,** 705–712.

MAENO, H., KIYAMA, H. & TOHYAMA, M. (1993). Distribution of the substance P receptor (NK-1 receptor) in the central nervous system. *Mol. Brain Res.*, **18,** 43–58.

MANTYH, P.W., GATES, T., MANTYH, C.R. & MAGGIO, J.E. (1989). Autoradiographic localization and characterization of tachykinin receptor binding sites in the rat brain and peripheral tissues. *J. Neurosci.*, **9,** 258–279.

MARCHAND, J.E., ZACCHEO, T.S., CONNELLY, C.S. & KREAM, R.M. (1993). Selective in situ hybridization histochemical analyses of alternatively spliced mRNAs encoding β and γ-preprotachykinins in rat central nervous system. *Mol. Brain Res.*, **17,** 83–94.

MASSI, M., GENTILI, L., PERFUMI, M., DE CARO, G & SCHULKIN, J. (1990). Inhibition of salt appetite in the rat following injection of tachykinins into the medial amygdala. *Brain Res.*, **513,** 1–7.

MASSI, M., POLIDORI, C., PERFUMI, M., GENTILI, L. & DE CARO, G. (1991). Tachykinin receptor subtypes involved in the central effects of tachykinins on water and salt intake. *Brain Res. Bull.*, **26,** 155–160.

MUSSAP, C.J., GERAGHTY, D.P. & BURCHER, E. (1993). Tachykinin receptors: a radioligand binding perspective. *J. Neurochem.*, **60,** 1987–2009.

PICARD, P., REGOLI, D. & COUTURE, R. (1994). Cardiovascular and behavioural effects of centrally administered tachykinins in the rat: characterization of receptors with selective antagonists. *Br. J. Pharmacol.*, **112,** 240–249.

POLIDORI, C., SAIJA, A., PERFUMI, M., COSTA, G., DE CARO, G. & MASSI, M. (1989). Vasopressin release induced by intracranial injection of tachykinins is due to activation of central neurokinin-3 receptors. *Neurosci. Lett.*, **103,** 320–325.

POULAT, P., DE CHAMPLAIN, J. & COUTURE, R. (1996). Cardiovascular effects induced by the intrathecal administration of neuropeptide gamma in conscious rats: receptor characterization and mechanism of action. *Br. J. Pharmacol.*, **117,** 260–267.

PRAT, A., PICARD, P. & COUTURE, R. (1994). Cardiovascular and behavioural effects of centrally administered neuropeptide K in the rat: receptor characterization. *Br. J. Pharmacol.*, **112,** 250–256.

QIAN, Y., ADVENIER, C., NALINE, E., BELLAMY, J.G. & EMONDSALT, X. (1994). Effects of SR 48968 on the neuropeptide gamma-induced contraction of the human isolated bronchus. *Fundam. Clin. Pharmacol.*, **8,** 71–75.

QUIRION, R., DAM, T.V. & GUARD, S. (1991). Selective neurokinin receptor radioligands. *Ann. N.Y. Acad. Sci.*, **632,** 137–144.

REGOLI, D., BOUDON, A. & FAUCHÈRE, J.-L. (1994). Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.*, **46,** 551–599.

REGOLI, D., DRAPEAU, G., DION, S. & D'ORLÉANS-JUSTE, P. (1987). Pharmacological receptors for substance P and neurokinins. *Life Sci.*, **40,** 109–117.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1988). The NK_3 tachykinin receptor agonist senktide elicits 5-HT-mediated behaviour following central or peripheral administration in mice and rats. *Br. J. Pharmacol.*, **94,** 285–287.

TAKANO, Y., NAGASHIMA, A., HAGIO, T., TATEISHI, K. & KAMIYA, H.O. (1990). Role of central tachykinin peptides in cardiovascular regulation in rats. *Brain Res.*, **528,** 231–237.

TAKANO, Y., NAKAYAMA, Y., MATSUMOTO, T., SAITO, R. & KAMIYA, H.-O. (1993). The mechanism of central pressor actions of tachykinin NK-3 receptor in the paraventricular nucleus of the hypothalamus in rats. *Regul. Pept.*, **46,** 360–363.

TAKEDA, Y. & KRAUSE, J.E. (1989). Neuropeptide K potently stimulates salivary gland secretion and potentiates substance P-induced salivation. *Proc. Natl. Acad. Sci. U.S.A.*, **86,** 392–396.

TAKEDA, Y. & KRAUSE, J.E. (1991). Pharmacological and molecular biological studies on the diversity of rat tachykinin NK-2 receptor subtypes in rat CNS, duodenum, vas deferens, and urinary bladder. *Ann. New York Acad. Sci.*, **632,** 479–482.

TAKEDA, Y., TAKEDA, J., SMART, B.M. & KRAUSE, J.E. (1990). Regional distribution of neuropeptide γ and other tachykinin peptides derived from the substance P gene in the rat. *Regul. Pept.*, **28,** 323–333.

TSCHÖPE, C., PICARD, P., CULMAN, J., PRAT, A., ITOI, K., REGOLI, D., UNGER, TH. & COUTURE, R. (1992). Use of selective antagonists to dissociate the central cardiovascular and behavioural effects of tachykinins on NK_1 and NK_2 receptors in the rat. *Br. J. Pharmacol.*, **107,** 750–755.

UNGER, TH., BECKER, H., PETTY, M., DEMMERT, G., SCHNEIDER, B., GANTEN, D. & LANG, R.E. (1985). Differential effects of central angiotensin II and substance P on sympathetic nerve activity in conscious rats. *Circ. Res.*, **56,** 563–575.

UNGER, TH., RASCHER, W., SCHUSTER, C., PAVLOVITCH, R., SCHÖMIG, A., DIETZ, R. & GANTEN, D. (1981). Central blood pressure effects of substance P and angiotensin II: role of the sympathetic nervous system and vasopressin. *Eur. J. Pharmacol.*, **71,** 33–42.

VAN GIERSBERGEN, P.L.M., SHATZER, S.A., BURCHER, E. & BUCK, S.H. (1992). Comparison of the effects of neuropeptide K and neuropeptide γ with neurokinin A at NK_2 receptors in the Hamster urinary bladder. *Naunyn-Schmied. Arch. Pharmacol.*, **345,** 51–56.

WANG, Y., BOCKMAN, C.S., LOVAS, S., ABEL, P.W., MURPHY, R.F. & CONLON, J.M. (1993). Neuropeptide γ-(1-9)-peptide: a major product of the post-translational processing of γ-preprotachykinin in rat tissues. *J. Neurochem.*, **61,** 1231–1235.

YUAN, Y. (1995). Central actions of neurokinins and their receptors on renal function in the conscious rat. *Master Thesis in Physiology, Université de Montréal.*

YUAN, L., BURCHER, E. & NAIL, B.S. (1994). Use of selective agonists and antagonists to characterize tachykinin receptors mediating airway responsiveness in anesthetized guinea-pigs. *Pulm. Pharmacol.*, **7,** 169–178.

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