Cardiovascular and behavioural effects of centrally administered neuropeptide K in the rat: receptor characterization

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1 The cardiovascular and behavioural responses to intracerebroventricularly (i.c.v.) administered neuropeptide K (NPK) were studied in conscious rats. The central effects of NPK were characterized by pretreatment (i.c.v.) with selective antagonists for the NK₁ ((±)-CP 96345 and RP 67580), NK₂ (SR 48968) and NK₃ (R 487) receptors.

2 NPK (10–65 pmol) induced tachycardia and dose-dependent increases of mean arterial blood pressure. The cardiovascular responses reached a maximum within 3 min post-injection and lasted for more than 1 h. Concurrently, NPK produced dose-dependent increases of face washing, head scratching, grooming, walking and wet dog shakes.

3 A desensitization of most of the behavioural responses (except head scratching) but not of the cardiovascular response was shown when two consecutive injections of 25 pmol NPK were given 24 h apart.

4 Both the cardiovascular and behavioural responses (except the head scratching) to 25 pmol NPK were blocked by pre-administration (i.c.v.) of 6.5 nmol (±)-CP 96345 or RP 67580 given 5 min earlier. No inhibition of NPK responses was observed when 6.5 nmol SR 48968 or R 487 were used in a similar study. Additionally, NPK effects were significantly reduced 24 h after the prior injection of (±)-CP 96345 but not of RP 67580.

5 These results support the involvement of NK₁ receptors in the cardiovascular and behavioural effects of i.c.v. NPK. Thus, this peptide may play a putative role in central cardiovascular regulation as it is the most potent endogenous tachykinin described centrally, to date.

Keywords: Neuropeptide K; NK₁ receptors; tachykinin antagonists; central cardiovascular regulation; behaviour

Introduction

Six mammalian tachykinins namely substance P (SP), neurokinin A (NKA), NKA(3-10), neurokinin B (NKB), neuropeptide γ (NP γ) and neuropeptide K (NPK) have been identified in both the periphery and central nervous system (Helke *et al.*, 1990; Otsuka & Yoshioka, 1993). These endogenous neuropeptides bind to three receptor subtypes, termed neurokinin₁ (NK₁), NK₂ and NK₃; SP being the preferential ligand for the NK₁ receptor, NKA, NPK, NP γ and NKA(3-10) for the NK₂ receptor and NKB for the NK₃ receptor subtype (Regoli *et al.*, 1988; Guard & Watson, 1991; Maggi *et al.*, 1993).

NPK is a 36-amino acid peptide which was originally isolated from porcine brain extracts (Tatemoto *et al.*, 1985) and thought to be a precursor of NKA (Deacon *et al.*, 1987). It is postulated that β -preprotachykinin is processed to SP, NKA and NPK in neuronal cell bodies but that conversion of NPK to NKA takes place during packaging into storage vesicles for axonal transport (Deacon *et al.*, 1987). Moreover, NPK seems to be cleaved into NKA-LI in the plasma during intravenous (i.v.) infusion in the guinea-pig, suggesting as well the existence of an extraneuronal conversion of NPK to NKA (Martling *et al.*, 1987). Recent evidence suggests, however, that NPK is not only a precursor of NKA, but the most potent biologically active endogenous tachykinin *in vivo* whose effects appear to be mediated by NK₁ receptors (Takeda & Krause, 1989; Décarie & Couture, 1992; Pham & Couture, 1993). After i.v. injection in the anaesthetized rat, NPK produced sustained decreases in mean arterial blood pressure (MAP) and increases in heart rate (HR); the hypotensive effect was ascribed to a direct action of NPK on blood vessels, while the tachycardia derived from the non-

reflex peripheral activation of the sympathoadrenal system (Décarie & Couture, 1992). In conscious rats, the intrathecal (i.t.) injection of NPK at the 9th thoracic level produced dose-dependent and prolonged increases in MAP and HR which were correlated with increases in plasma levels of catecholamines and neuropeptide Y. These NPK-mediated effects were greater than those evoked by similar doses of SP (Hasséssian *et al.*, 1990; Pham *et al.*, 1993). The cardiovascular responses to intrathecal NPK and SP were antagonized by prior i.t. or i.v. pretreatment with (±)-CP 96345 (Pham & Couture, 1993) a non-peptide, selective NK₁ receptor antagonist (Snider *et al.*, 1991). Moreover, NPK was found more potent and more efficacious than SP as a stimulator of phosphatidylinositol turnover in the adult rat spinal cord; the high potency of NPK did not appear to be entirely attributable to the metabolic stability of the peptide (Prat *et al.*, 1993a). NPK is also more potent than SP as sialagogue in the rat (Takeda & Krause, 1989) and as bronchoconstrictor in the guinea-pig *in vivo* (Martling *et al.*, 1987). Furthermore, NPK can be considered as a sensory neuropeptide since it is co-localized with NKA and SP in a subpopulation of guinea-pig primary sensory C-fibres (Hua *et al.*, 1985) and is highly concentrated in the dorsal horn of the rat spinal cord (Valentino *et al.*, 1986; Chambers *et al.*, 1988).

Tachykinins have been implicated in central cardiovascular regulation and in motor behaviour (Unger *et al.*, 1985; Itoi *et al.*, 1988). In conscious rats, SP and NKA injected intracerebroventricularly (i.c.v.) increase MAP and HR through the activation of NK₁ and NK₂ receptors, respectively (Tschöpe *et al.*, 1992). A role for NPK in central cardiovascular regulation remains a possibility as this neuropeptide is present in a high concentration in the brain (Tatemoto *et al.*, 1985; Arai & Emson, 1986; Valentino *et al.*, 1986).

The goals of this study were: (a) to examine the central

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(i.c.v.) action of NPK on the rat cardiovascular system and on behavioural activity and (b) to determine the tachykinin receptor subtype(s) involved in these effects. For that purpose, selective antagonists of the NK₁ ((±)-CP 96345 (Snider *et al.*, 1991) and RP 67580 (Garret *et al.*, 1992)), NK₂ (SR 48968) (Emonds-Alt *et al.*, 1992) and NK₃ (R 487) (Drapeau *et al.*, 1990) receptors were used. Some of these results have been described in a preliminary communication (Prat *et al.*, 1993b).

Methods

Animal preparation

Male Wistar rats (Charles River, St-Constant, Québec, Canada) weighing 300–350 g were anaesthetized with an intraperitoneal (i.p.) injection of 65 mg kg⁻¹ sodium pentobarbitone (Somnotol, MTC. Pharmaceuticals, Cambridge, Ont., Canada). By use of a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.), an i.c.v. polyethylene cannula (PE-20; Intramedic, Clay Adams, NJ, U.S.A.) was inserted into the right lateral ventricle (coordinates: 0.6 mm caudal to bregma, 1.3 mm lateral to midline and 5.0 mm vertical to the skull surface) and fixed to the skull with dental cement. The angle of the head was adjusted according to the horizontal plan with respect to bregma and lambda reference points. Five days later, the animals were anaesthetized again and a polyethylene cannula (PE-60) filled with physiological saline containing heparin (100 iu ml⁻¹) was inserted into one femoral artery, passed through a subcutaneous tunnel and emerged at the back of the neck. Following surgery, animals were housed individually in a plastic cage (43 × 23 × 20 cm) with free access to food and water and maintained in a room with a 12 h light/dark cycle.

Measurement of cardiovascular and behavioural parameters

The experiments were conducted on freely moving rats 24 h after the intra-arterial surgery. The arterial blood pressure was monitored through the intra-femoral catheter with a Statham pressure transducer (P231D), while the HR was measured with a cardiac tachometer (model 7P4) and both parameters were displayed on a Grass Polygraph model 79D (Grass instruments Co., Quincy, MA, U.S.A.).

The behavioural activity was recorded in their resident plastic cage (43 × 23 × 20 cm) over a 30 min period at 15 s interval according to the procedure of Gispen *et al.* (1975). During the course of these experiments, the grid cage top was removed. During every consecutive period of 15 s, a score of 1 or 0 was given depending on the presence (1) or absence (0) of each behaviour (face washing, head scratching, grooming, walking and/or ipsilateral turning). The intensity, duration and frequency of each behaviour during the 15 s period were not taken into account. Summation of scores recorded over 30 min gave the behavioural score in each experiment. The maximal theoretical score was 120 in each behaviour (15 s intervals × 30 min). The wet dog shake was measured according to the number of episodes (duration of less than 1 s each) during the 30 min period whatever the intensity.

Experimental protocols

In the first series of experiments, rats received a single i.c.v. injection of artificial cerebrospinal fluid (CSF) (composition in mM: NaCl 128.6, KCl 2.6, MgCl₂ 2.0 and CaCl₂ 1.4; pH adjusted to 7.2) in a volume of 1 µl. The catheter was flushed in 4 µl of CSF over a period of 10–15 s and the cardiovascular and behavioural responses were measured. One hour later, the animal received 1, 10, 25 or 65 pmol of NPK dissolved in 1 µl of CSF and flushed with 4 µl of CSF. Only

one injection of NPK was given to a rat to construct the dose-response curve or to test the antagonists.

The second series of experiments was designed to evaluate possible tachyphylaxis after two consecutive injections of NPK to the same rats. On day 1, 8 rats received 1 µl of CSF and 8 rats received NPK (25 pmol, i.c.v.), then the cannula was flushed with 4 µl of CSF. The second day (24 h later), all animals received a single i.c.v. injection of 25 pmol NPK and the cardiovascular and behavioural effects were measured. The cardiovascular and behavioural responses obtained with the two groups were then compared.

In the third series of experiments, one of the four antagonists ((±)-CP 96345, RP 67580, SR 48968 or R 487) was given i.c.v. in a volume of 1 µl, 5 min or 24 h prior to the injection of 25 pmol NPK. An animal received only one antagonist at a single dose. The intrinsic activity of receptor antagonists was tested in separate experiments. The changes in MAP (ΔMAP) and in HR (ΔHR) at a given time were calculated from baseline values taken 1 min before injection.

At the end of each experiment, 25 pmol of angiotensin II was injected to ascertain the position of the i.c.v. cannula. Most rats (90–100%) displayed an immediate dipsogenic behaviour, characterized by a water intake of 10–15 ml during the first 15 min post-injection, as well as an increase in MAP. Only results from these rats which responded to this test were retained.

Peptides and non-peptides

(±)-CP 93645 (racemic mixture of *cis*-3-(2-methoxybenzylamino)-2-benzhydryl-quinuclidine (2R,3R) and (2S,3S); mol. wt.: 412.6) was generously provided by Drs B. Gitter and J. Howbert at Eli Lilly, Indianapolis, U.S.A. while RP 67580 (racemic form of 7,7-diphenyl-2-[1-imino-2-(2-methoxy-phenyl)-ethyl]perhydroisoindol-4-one (3aR, 7aR); mol. wt.: 475.0 for the hydrochloride salt) was a gift from Dr C. Garret, Rhône-Poulenc Rorer, Paris, France. SR 48968 ((S)-N-methyl-N-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)-butyl]benzamide; mol. wt.: 570.0) was a kind gift from Dr J.C. Brelière, Sanofi, Montpellier, France. R 487 (Asp-Ser-Phe-Phe-β-Ala-Leu-Met-NH₂; mol. wt.: 829.1) was provided by Dr D. Regoli at Sherbrooke University, Sherbrooke, Canada. NPK (porcine) and angiotensin II were purchased from Hükabel Scientific Ltd. (Montréal, Québec, Canada) and prepared in CSF solution. Antagonists were made in dimethyl sulphoxide (DMSO; Fisher, Montreal, Canada) and CSF was added to obtain the desired dilution (the final concentration contained a maximum of 10% DMSO). Stock solutions (1–10 mg ml⁻¹) of peptides and non-peptides were divided in 100 µl aliquots and stored at –20°C until used.

Statistical analysis of data

The results are expressed as mean ± s.e.mean. Statistical differences (time × effects) were evaluated with a two-way analysis of variance (ANOVA) and a Dunnett test *a posteriori*. Multiple comparisons with a single variable were evaluated with a one-way ANOVA followed by an unpaired Student's *t* test modified by Dunnett. Behavioural responses were evaluated by a one-way ANOVA and a U-Wilcoxon Mann Whitney test. Only probability values (*P*) less than 0.05 were considered to be statistically significant.

Results

Effects of NPK on MAP and HR

The time course of changes on MAP and HR elicited by the i.c.v. injection of NPK are illustrated in Figure 1. While 1 pmol NPK (data not shown) and CSF failed to cause cardiovascular changes, 10, 25 and 65 pmol of NPK elicited dose-dependent and significant increases in MAP. The pres-

sor response reached a maximum within 3 min and persisted for more than 1 h. However, the increase in HR was not dose-dependent since 25 pmol NPK elicited a greater increase in HR than 65 pmol.

Effect of NPK on behaviour

The cardiovascular responses to i.c.v. NPK were simultaneously accompanied by a marked increase in behavioural activity. As shown in Table 1, face washing, head scratching, grooming, walking and/or ipsilateral turning as well as wet dog shakes increased dose-dependently following injection of 10, 25 and 65 pmol NPK. These changes in behaviour were

significantly different from those elicited by CSF, which had little effect on behaviour.

Tachyphylaxis to NPK

This series of experiments was undertaken to evaluate the possible desensitization of the cardiovascular and behavioural effects to two successive injections of 25 pmol NPK, given one day apart. The changes in MAP and HR produced by 25 pmol NPK were not significantly different between rats which received either an i.c.v. injection of CSF or NPK 24 h earlier. On the other hand, all behaviours, except head scratching, elicited by 25 pmol NPK were significantly reduced in

Figure 1 Time course of cardiovascular responses to the intracerebroventricular injection of CSF (●, $n=9$), or neuropeptide K (NPK) at the dose of 10 pmol (○, $n=6$), 25 pmol (□, $n=9$) and 65 pmol (△, $n=6$) in conscious rats. Changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) are shown. Each point represents the mean $\pm$ s.e.mean of (n) rats in each group. The statistical comparison of CSF values (0–60 min) is given as follows: 10 pmol NPK [$F(1,14)=16.4$; $P<0.01$ for Δ MAP and $F(1,14)=21.4$; $P<0.01$ for Δ HR], 25 pmol NPK [$F(1,17)=54.3$; $P<0.001$ for Δ MAP and $F(1,17)=47.8$; $P<0.001$ for Δ HR] and 65 pmol NPK [$F(1,14)=70.2$; $P<0.001$ for Δ MAP and $F(1,14)=44.5$; $P<0.001$ for Δ HR]. The comparison between the three doses of NPK and CSF is highly significant: [$F(3,29)=11.2$; $P<0.001$ for Δ MAP and $F(3,29)=13.8$; $P<0.001$ for Δ HR].

Figure 2 Cardiovascular and behavioural effects induced by the intracerebroventricular injection of 25 pmol neuropeptide K (NPK) at 24 h pretreated with CSF at 0 h (open columns, $n=8$) or 25 pmol NPK at 24 h pretreated with 25 pmol NPK at 0 h (hatched columns, $n=8$). Maximal changes (a) in mean arterial pressure (MAP) and (b) heart rate (HR) or (c) behavioural activity for a 30 min period are shown. FW = face washing, HS = head scratching; Gr = grooming, W & T, walking and turning, WDS = wet dog shake. Each point represents the mean $\pm$ s.e.mean of (n) rats. Differences in MAP and HR between the two groups were non-significant. Differences in behaviour between the two groups are indicated by * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 1 Behavioural responses elicited by the i.c.v. injection of neuropeptide K (NPK) to conscious rats

Agonist	n	Face washing (score 30 min ⁻¹)	Head scratching (score 30 min ⁻¹)	Grooming (score 30 min ⁻¹)	Walking and/or ipsilateral turning (score 30 min ⁻¹)	Wet dog shakes (episodes 30 min ⁻¹)
CSF	9	3.3 $\pm$ 1.6	0.2 $\pm$ 0.1	2.6 $\pm$ 1.3	3.3 $\pm$ 1.9	1.1 $\pm$ 0.7
NPK 10 pmol	6	6.5 $\pm$ 1.9	1.8 $\pm$ 1.4	12.2 $\pm$ 3.8*	11.8 $\pm$ 5.1*	7.2 $\pm$ 3.8*
NPK 25 pmol	10	40.0 $\pm$ 4.5***	14.1 $\pm$ 3.4***	42.9 $\pm$ 4.5***	42.2 $\pm$ 3.4***	16.8 $\pm$ 2.7***
NPK 65 pmol	6	54.0 $\pm$ 11.7***	34.4 $\pm$ 8.7***	85.2 $\pm$ 18.3***	60.4 $\pm$ 18.0***	19.8 $\pm$ 4.7***

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

rats pre-injected, 24 h earlier, with the same dose of NPK when compared with rats pre-injected with CSF (Figure 2).

Effects of (±)-CP 96345 on NPK

The i.c.v. injection of (±)-CP 96345 (6.5 nmol) 5 min before NPK, significantly blocked the pressor ($F(1,16) = 74.5$; $P < 0.001$) and HR ($F(1,16) = 48.9$; $P < 0.001$) responses induced by 25 pmol NPK (Figure 3). The cardiovascular response (0–60 min) to 25 pmol NPK in the presence of 6.5 nmol (±)-CP 96345 was not statistically different from CSF values ($F(1,16) = 1.5$; $P > 0.05$ for Δ MAP and $F(1,16) = 1.4$; $P > 0.05$ for Δ HR). Furthermore, (±)-CP 96345, reduced significantly all behaviours (except the head scratching) elicited by 25 pmol NPK (Table 2). Both the cardiovascular ($F(1,16) = 44.7$; $P < 0.001$ for Δ MAP and $F(1,16) = 22.8$; $P < 0.001$ for Δ HR) and behavioural responses to NPK were also significantly attenuated when the antagonist was given 24 h earlier (Figure 3 and Table 2). The residual cardiovascular response to 25 pmol NPK in rats pretreated 24 h earlier with (±)-CP 96345 was significantly different from CSF values and therefore was not completely abolished ($F(1,16) = 4.7$; $P < 0.05$ for Δ MAP and $F(1,16) = 4.9$; $P < 0.05$ for Δ HR). At 650 pmol, (±)-CP 96345 failed to alter the cardiovascular changes elicited by 25 pmol NPK (maximal Δ MAP and Δ HR were 24 ± 2 mmHg and 180 ± 17 beats min^{-1} in the presence of antagonist ($n = 8$) versus 27 ± 3 mmHg and 215 ± 19 beats min^{-1} in the absence of antagonist ($n = 8$); $P > 0.05$).

Effects of RP 67580 on NPK

The NK₁ receptor antagonist, RP 67580 (6.5 nmol, 5 min earlier) was found to reduce significantly ($F(1,16) = 45.8$; $P < 0.001$ for Δ MAP and $F(1,16) = 49.5$; $P < 0.001$ for Δ HR) both cardiovascular and behavioural responses (except the head scratching) to 25 pmol NPK (Figure 4 and Table 2). The cardiovascular response to 25 pmol NPK (0–60 min) in the presence of 6.5 nmol RP 67580 (5 min treatment) was not statistically different from CSF values ($F(1,16) = 1.5$; $P > 0.05$ for Δ MAP and $F(1,16) = 3.9$; $P > 0.05$ for Δ HR). However, RP 67580 injected i.c.v. 24 h before, had no significant effect on the cardiovascular ($F(1,16) = 1.9$; $P > 0.05$ for Δ MAP and $F(1,16) = 2.8$; $P > 0.05$ for Δ HR) and behavioural effects to

25 pmol NPK (Figure 4 and Table 2). Moreover, 650 pmol RP 67580 had no significant effect on the cardiovascular changes induced by 25 pmol NPK (maximal Δ MAP and Δ HR were 23 ± 4 mmHg and 200 ± 14 beats min^{-1} in the

Figure 3 Time course of cardiovascular effects to the intracerebroventricular injection of 25 pmol neuropeptide K (NPK) in the absence (□, $n = 9$) and presence of 6.5 nmol (±)-CP 96345 given 5 min (○, $n = 8$) or 24 h (●, $n = 8$) beforehand to conscious rats. Changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) are shown. Each point represents the mean $\pm$ s.e.mean of (n) rats in each group. When compared to CSF values (given in Table 3), the effects of NPK in the absence of antagonist were highly significant ($F(1,17) = 70.2$; $P < 0.001$ for Δ MAP and $F(1,17) = 47.8$; $P < 0.001$ for Δ HR).

Table 2 Effects of tachykinin receptor antagonists on behavioural responses elicited by the i.c.v. injection of 25 pmol neuropeptide K (NPK) to conscious rats

Agonist	Antagonist	n	Face washing (score 30 min ⁻¹)	Head scratching (score 30 min ⁻¹)	Grooming (score 30 min ⁻¹)	Walking and/or ipsilateral turning (score 30 min ⁻¹)	Wet dog shakes (episodes 30 min ⁻¹)
CSF	–	9	3.3 $\pm$ 1.6	0.2 $\pm$ 0.1	2.6 $\pm$ 1.3	3.3 $\pm$ 1.9	1.1 $\pm$ 0.7
NPK	–	10	40.0 $\pm$ 4.5***	14.1 $\pm$ 3.4***	42.9 $\pm$ 4.5***	42.2 $\pm$ 3.4***	16.8 $\pm$ 2.7***
NPK	(±)-CP96345 6.5 nmol 5 min before	8	18.4 $\pm$ 5.5†††*	5.4 $\pm$ 1.9**	14.2 $\pm$ 3.8†††***	6.6 $\pm$ 3.1†††*	2.2 $\pm$ 0.9†††
NPK	(±)-CP96345 6.5 nmol 24 h before	8	19.0 $\pm$ 4.9††*	9.6 $\pm$ 3.8**	12.6 $\pm$ 3.3†††***	22.9 $\pm$ 4.8††***	4.3 $\pm$ 2.7††*
NPK	RP 67580 6.5 nmol 5 min before	8	16.6 $\pm$ 4.2††***	7.9 $\pm$ 0.9**	20.1 $\pm$ 1.8†††***	10.4 $\pm$ 5.1††*	4.1 $\pm$ 2.1††*
NPK	RP 67580 6.5 nmol 24 h before	8	34.5 $\pm$ 4.0***	19.0 $\pm$ 2.7***	45.8 $\pm$ 5.4***	38.0 $\pm$ 6.2***	14.8 $\pm$ 1.9***
NPK	SR 48968 6.5 nmol 5 min before	8	31.6 $\pm$ 6.7***	15.6 $\pm$ 5.3***	36.9 $\pm$ 10.8***	44.3 $\pm$ 9.4***	16.6 $\pm$ 1.6***
NPK	R 487 6.5 nmol 5 min before	6	45.5 $\pm$ 3.1***	46.3 $\pm$ 6.9†††***	45.6 $\pm$ 3.8***	55.1 $\pm$ 5.2***	18.2 $\pm$ 4.1***

Values represent the mean $\pm$ s.e.mean of (n) rats for a period of 30 min. Statistical comparison to NPK alone (†) or CSF (*) was evaluated with a one-way ANOVA and a Wilcoxon-Mann-Whitney (U) test *a posteriori*: *† $P < 0.05$; ***†† $P < 0.01$; ***††† $P < 0.001$.

presence of antagonist (*n* = 6) versus 29 ± 5 mmHg and 220 ± 14 beats min⁻¹ in the absence of antagonist (*n* = 6); *P* > 0.05).

Effects of SR 48968 or R 487 on NPK

Pre-administration (i.c.v., 5 min earlier) of 6.5 nmol of the selective NK₂ (SR 48968) or NK₃ (R 487) receptor antagonists did not significantly affect the MAP or HR increases and most behavioural activity elicited by 25 pmol NPK (Tables 2 and 3). However, 6.5 nmol R 487 enhanced head

scratching, suggesting a possible agonist activity of the compound. Indeed, R 487 caused a transient increase in MAP at 1 min post-injection (Table 4). Otherwise, the three antagonists ((±)-CP 96345, RP 67580 and SR 48968) were devoid of direct effects on MAP and HR when given alone for a period of 15 min (Table 4). No apparent motor deficit nor respiratory distress were seen with any of the four antagonists.

Discussion

The i.c.v. injection of NPK to the conscious rat produced rapid, prolonged and dose-dependent increases in MAP and behavioural activity. The increase in HR, however, did not appear to be dose-dependent. It is very unlikely that these central effects are produced by a leakage of NPK into the systemic circulation as intravenous injection of NPK caused a vasodepressor response (Dcarie & Couture, 1992). A spinal site of action is also unlikely as the dose of NPK necessary to elicit pressor and tachycardiac responses after i.c.v. injection (10 pmol) was much lower than that required intrathecally (650 pmol) (Pham *et al.*, 1993). The higher efficacy of NPK in activating the cardiovascular system when injected i.c.v. is still unexplained, but it is consistent with a similar observation made with SP. While 25 pmol SP i.c.v. produced significant increases in MAP and HR (Tschpe *et al.*, 1992), 6.5 nmol of SP was required to produce a significant pressor effect when injected i.t. in conscious rats (Hassssian *et al.*, 1988). The pressor and tachycardiac effects of i.c.v. or i.t. NPK are more prolonged and greater in intensity than those evoked by any other endogenous tachykinins namely SP, NKA and NKB (Hassssian *et al.*, 1988; Tschpe *et al.*, 1992; Pham *et al.*, 1993).

This study shows that the central effects elicited by i.c.v. NPK are blocked by i.c.v. pretreatment with ()-CP 96345 or RP 67580, two selective NK₁ receptor antagonists (Snider *et al.*, 1991; Emonds-Alt *et al.*, 1992; Garret *et al.*, 1992). In previous studies, ()-CP 96345 (Tschpe *et al.*, 1992) and RP 67580 (Picard *et al.*, 1994; preceding paper) were found to be good NK₁ antagonists that prevented by 40–50% the cardiovascular action of centrally administered SP. These antagonists did not affect the cardiovascular effect of i.c.v. NKA (NK₂ agonist) or [MePhe⁷]NKB (NK₃ receptor agonist). Furthermore, the NK₂ (SR 48968) and NK₃ (R 487) receptor antagonists, did not affect the cardiovascular or the behavioural changes elicited by i.c.v. administered NPK, while they were found to block the central effects of NKA and [MePhe⁷]NKB, respectively (Picard *et al.*, 1994). This pharmacological evidence suggests that NPK acts on NK₁

Figure 4 Time course of cardiovascular effects to the intracerebroventricular injection of 25 pmol neuropeptide K (NPK) in the absence (□, *n* = 9) and presence of 6.5 nmol RP 67580 given 5 min (○, *n* = 8) or 24 h (●, *n* = 8) beforehand to conscious rats. Changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR) are shown. Each point represents the mean ± s.e.mean of (*n*) rats in each group. When compared to CSF values (given in Table 3), the effects of NPK in the absence of antagonist were highly significant (*F*(1,17) = 54.3; *P* < 0.001 for ΔMAP and *F*(1,17) = 47.8; *P* < 0.001 for ΔHR).

Table 3 Changes in mean arterial pressure (MAP) and heart rate (HR) induced by the i.c.v. injection of 25 pmol neuropeptide (NPK) following the pre-injection of tachykinin receptor antagonists

Agonist	Antagonist	n		Baseline MAP or HR	Time (min) after NPK injection (ΔMAP or ΔHR)				
					1	5	10	30	60
CSF	–	8	MAP	99 ± 7	0 ± 1	1 ± 1	2 ± 1	3 ± 2	1 ± 1
			HR	350 ± 13	7 ± 8	5 ± 7	8 ± 5	3 ± 5	4 ± 4
NPK	–	9	MAP	110 ± 7	17 ± 2***	26 ± 2***	23 ± 2***	15 ± 2***	8 ± 1***
			HR	371 ± 12	118 ± 19***	222 ± 30***	146 ± 13***	113 ± 17***	55 ± 7***
NPK	SR 48968 6.5 nmol 5 min before	8	MAP	113 ± 7	16 ± 2***	23 ± 3***	24 ± 4***	23 ± 4***	12 ± 2***
			HR	391 ± 18	82 ± 8***	131 ± 13***	117 ± 14***	108 ± 17***	65 ± 15***
NPK	R 487 6.5 nmol 5 min before	6	MAP	117 ± 8	15 ± 2***	21 ± 3***	21 ± 3***	16 ± 4***	8 ± 2***
			HR	389 ± 11	80 ± 5***	140 ± 9***	124 ± 10***	121 ± 9***	81 ± 21***

Values represent the mean ± s.e.mean from (*n*) rats. Statistical comparison to CSF (***) or NPK without antagonist was evaluated with a two-way ANOVA. No significant differences were observed to NPK.

Table 4 Changes in mean arterial pressure (MAP) and heart rate (HR) induced by the i.c.v. injection of tachykinin receptor antagonists

Antagonist	n		Baseline MAP or HR	Time (min) after antagonist injection (Δ MAP or Δ HR)			
				1	5	10	15
CSF	8	MAP	113 $\pm$ 10	2 $\pm$ 2	4 $\pm$ 3	2 $\pm$ 2	0 $\pm$ 2
		HR	323 $\pm$ 21	12 $\pm$ 7	6 $\pm$ 10	5 $\pm$ 7	9 $\pm$ 8
(±)-CP 96345 (6.5 nmol)	6	MAP	115 $\pm$ 8	-4 $\pm$ 3	0 $\pm$ 2	-2 $\pm$ 5	3 $\pm$ 5
		HR	384 $\pm$ 16	25 $\pm$ 6	20 $\pm$ 4	21 $\pm$ 8	12 $\pm$ 10
RP 67580 (6.5 nmol)	8	MAP	117 $\pm$ 7	5 $\pm$ 3	3 $\pm$ 4	2 $\pm$ 2	2 $\pm$ 1
		HR	321 $\pm$ 24	24 $\pm$ 18	11 $\pm$ 12	5 $\pm$ 5	1 $\pm$ 7
SR 48968 (6.5 nmol)	6	MAP	120 $\pm$ 5	4 $\pm$ 5	2 $\pm$ 2	-3 $\pm$ 6	-3 $\pm$ 5
		HR	371 $\pm$ 13	10 $\pm$ 5	16 $\pm$ 7	9 $\pm$ 7	11 $\pm$ 8
R 487 (6.5 nmol)	6	MAP	112 $\pm$ 9	15 $\pm$ 4*	10 $\pm$ 3	3 $\pm$ 2	2 $\pm$ 4
		HR	379 $\pm$ 10	19 $\pm$ 10	13 $\pm$ 14	9 $\pm$ 5	9 $\pm$ 8

Values represent the mean $\pm$ s.e.mean from (*n*) rats. Statistical comparison to CSF was evaluated with a two-way ANOVA: **P* < 0.05.

but not on NK₂ or NK₃ receptors to produce its effects in the rat brain.

It is worthwhile to note here that the central cardiovascular effect induced by NPK was entirely blocked by either (±)-CP 96345 or RP 67580, while the same antagonists reduced by only 40–50% the central cardiovascular changes induced by SP. The residual response to SP measured after treatment with RP 67580 was, however, blocked after the addition of SR 48968, indicating that SP activates both NK₁ and NK₂ receptors. It seems therefore that NPK is a better NK₁ receptor agonist than SP in the rat central nervous system.

Since the discovery of NPK by Tatemoto *et al.* (1985), results of numerous pharmacological experiments conducted *in vivo* suggest that NPK and SP may act on the same receptor which may be of the NK₁ subtype (Takeda & Krause, 1989; Décarie & Couture, 1992; Pham & Couture, 1993). Nevertheless, binding or radioligand assays identified NPK as a potent NK₂ receptor agonist in the rat duodenum (Beaujouan *et al.*, 1988) and hamster urinary bladder (Van Giersbergen *et al.*, 1992) with a high selectivity for NK₂ binding sites and very weak binding affinity for NK₁ and NK₃ binding sites on rat brain synaptosomes (Beaujouan *et al.*, 1988). In submandibular gland membranes, a tissue rich in NK₁ receptors, both NPK and NKA appear to interact with the NK₁ receptor with 100 times lower affinity than that of SP (Takeda & Krause, 1989). The reason for the discrepancy between binding assays and functional studies is still unknown. One major difference between functional and binding studies is the fact that no peptidase inhibitors have been used to prevent the metabolic transformation of NPK *in vivo*. Thus, one cannot exclude the possibility that a metabolite of NPK produced by enzymatic degradation *in vivo* has higher affinity for the NK₁ receptor, while the entire molecule may behave as an NK₂ receptor agonist in binding assays. The C-terminal part of NPK contains the sequence of NKA; however, the present results do not support the view that NKA is the biologically active metabolite of NPK as suggested earlier (Martling *et al.*, 1987) since NKA is much less potent (magnitude and duration) than NPK when given i.c.v., and the central effects of NKA are mediated primarily by NK₂ receptors (Tschöpe *et al.*, 1992; Picard *et al.*, 1994). A similar conclusion was reached on ingestive behaviour induced by i.c.v. NPK in the rat; the spectrum of antidiapogenic activity of NPK was markedly different from that of NKA (Achapu *et al.*, 1992).

The two NK₁ receptor antagonists had dissimilar time course effects. While RP 67580 failed to block NPK when administered 24 h earlier, (±)-CP 96345 did significantly reduce the central effects of NPK. This finding is consistent with a previous study from our laboratory where it was

shown that (±)-CP 96345 given either by the i.t. (6.5 nmol) or i.v. (0.13 mg kg⁻¹) route, blocks for several days the cardiovascular effects induced by i.t. injection of NPK (3.25 nmol) (Pham & Couture, 1993). Hence, RP 67580 shows a different pharmacological profile from CP 96345 on central NK₁ receptors.

The relation between tachykinins and behaviour has been studied previously (Itoi *et al.*, 1988; Stoessl *et al.*, 1988), but our paper is the first to show that NPK enhances several behaviours dose-dependently. The most prominent are face washing, grooming and locomotor activity (walking and/or ipsilateral turning) while head scratching and wet dog shakes are observed with lower frequency. The use of selective receptor antagonists showed that nearly all behaviours elicited by NPK, except for head scratching, are probably mediated by NK₁ receptors. We do not have any valid explanation to explain why head scratching behaviour remains resistant to NK₁, NK₂ and NK₃ receptor antagonists. Interestingly, head scratching was the only behaviour resistant to desensitization induced by two injections of NPK. Although the cardiovascular and behavioural responses to i.c.v. NPK occurred concurrently and via the NK₁ receptor, they appear to derive from different mechanisms. This assumption is based on the observation that the cardiovascular effects mediated by NPK were not altered when the behavioural responses were desensitized upon the second injection of NPK.

The increasing effect of R 487 on the head scratching behaviour induced by NPK may be related to a residual agonist activity of this compound on NK₁ and NK₂ receptors (Regoli *et al.*, 1991). The inhibitory effect of (±)-CP 96345 and RP 67580 was not related to motor deficits nor to changes of baseline parameters.

In summary, selective and potent antagonists of NK₁, NK₂ and NK₃ receptors have been used to characterize the receptor subtype which is responsible for the central cardiovascular and behavioural effects of NPK in the conscious rat. Our findings suggest that the response to NPK is mediated primarily by NK₁ receptors, unlike SP which has the ability to stimulate NK₂ receptors when NK₁ receptors are occupied by NK₁ antagonists. It is suggested that NPK, or one of its metabolites (that is not NKA), may play a role in central cardiovascular regulation.

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