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Cardiovascular and behavioural effects of intracerebroventricularly administered tachykinin NK_3 receptor antagonists in the conscious rat

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1 In the conscious rat, three tachykinin NK_3 receptor antagonists, namely SR142801 ((**S**)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide), R820 (3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl) and R486 (H-Asp-Ser-Phe-Trp-β-Ala-Leu-Met-NH_2) were assessed against the intracerebroventricular (i.c.v.) effects induced by senktide, a selective NK_3 receptor agonist, on mean arterial blood pressure (MAP), heart rate (HR) and motor behaviour.

2 Senktide (10–650 pmol per animal; i.c.v; $n=4-16$) at the lowest dose caused a significant fall in MAP (-10 ± 6 mmHg), while at the highest doses (100 and 650 pmol), senktide caused a rise in MAP (9 ± 3 and 12 ± 1 mmHg, respectively) when compared to vehicle. The intermediate doses (25 and 65 pmol) had no effect on MAP. The highest two doses caused a tachycardia of 62 ± 15 and 88 ± 8 beats min^{-1}, respectively. The dose of 65 pmol had a biphasic effect on HR, an initial bradycardia of 47 ± 12 beats min^{-1} followed by a tachycardia of 46 ± 14 beats min^{-1}. The lowest doses caused either a rise of 52 ± 10 beats min^{-1} (25 pmol) or no effect (10 pmol) on HR. All doses of senktide caused similar increases in face washing, sniffing and wet dog shakes except at the dose of 100 pmol, when wet dog shakes were more than double those observed with the other doses.

3 The antagonist SR142801 (100 pmol–65 nmol per animal; i.c.v.; $n=6-8$) caused increases in MAP at the highest two doses (6.5 and 65 nmol) while HR, dose-dependently, increased (23 ± 6 to 118 ± 26 beats min^{-1}) and the onset dose-dependently decreased. The (**R**)-enantiomer, SR142806 (100 pmol–65 nmol per animal; i.c.v.; $n=6-8$) only caused rises in MAP (13 ± 2 mmHg) and HR (69 ± 11 beats min^{-1}) at the highest dose. These drugs had no apparent effect on behaviour, except for the highest dose of SR142801 which increased sniffing. The antagonist R820 (650 pmol–6.5 nmol per animal; i.c.v.; $n=6$) had no effect on MAP or HR and only increased sniffing behaviour at 6.5 nmol. At 650 pmol ($n=6$), R486 had no effect on any variable, but at 3.25 nmol, i.c.v. ($n=4$) a delayed tachycardia and a significant increase in all behavioural variables were observed.

4 The cardiovascular responses induced by 6.5 nmol SR142801 and 25 pmol senktide were inhibited by R820 (6.5 nmol, 5 min earlier i.c.v.). In contrast, R820 failed to affect the central cardiovascular and behavioural responses induced by 10 pmol [Sar^9, $Met(O_2)^{11}$]substance P, a NK_1 receptor selective agonist. The senktide-induced behavioural changes were not inhibited by R820 (6.5 nmol, i.c.v.) while R486 (650 pmol, i.c.v.) blocked both the cardiovascular and behavioural responses to 25 pmol senktide. A mixture of antagonists for NK_1 (RP67580; 6.5 nmol) and NK_2 (SR48968; 6.5 nmol) receptors injected i.c.v. did not affect the cardiovascular response to SR142801. Cross-desensitization was shown between the central responses to SR142801 and senktide, but not between SR142801 and [Sar^9, $Met(O_2)^{11}$]substance P.

5 The antagonists SR142801 and SR142806 (6.5–650 nmol kg^{-1}; $n=5-7$), given i.v., did not evoke any cardiovascular or behavioural changes, except a delayed bradycardia for SR142806 (650 nmol kg^{-1}), and also failed to inhibit the increase in MAP evoked by senktide (4 nmol kg^{-1}, i.v.). However, at the highest dose, both drugs slightly reduced the senktide-induced tachycardia.

6 Although the present data are consistent with the *in vitro* pharmacological bioassays and binding data, showing that SR142801 is a poor antagonist at rat peripheral NK_3 receptors, they suggest that SR142801 has a partial agonist action at these receptors centrally. A separation of the cardiovascular and behavioural effects mediated by central NK_3 receptor activation was achieved with SR142801 and R820 but not with R486. These results could be explained by the existence of NK_3 receptor subtypes in the rat or by the differential activation and inhibition of the same receptor protein linked to the production of different second messengers. Differences in the pharmacokinetic or pharmacodynamic properties of the antagonists cannot be excluded at this time.

Keywords: Tachykinin antagonists; substance P; NK_3 receptor; blood pressure; heart rate; behaviour

Introduction

Six mammalian tachykinins namely substance P (SP), neurokinin A (NKA), NKA(3–10), neurokinin B (NKB), neuropeptide K and neuropeptide γ (NPγ) have been identified in the periphery and in the central nervous system (Helke *et al*., 1990; Otsuka & Yoshioka, 1993). These neuropeptides exert a plethora of biological effects through the activation of three G-protein coupled receptors containing seven putative transmembrane spanning segments. These receptors have been termed neurokinin$_1$ (NK_1), NK_2 and NK_3 for which SP, NKA and NKB are the preferred endogenous ligands, respectively (Regoli *et al*., 1988; 1994a; Maggi, 1995).

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Compelling evidence suggests that the NK_3 receptor and its putative endogenous agonist NKB are involved in central cardiovascular regulation and in motor behaviour (Nagashima *et al*., 1989; Otsuka & Yoshioka, 1993). NKB, the NK_3 receptor and the mRNA for both are present in various nuclei of the brain stem and of the hypothalamus involved in cardiovascular and motor control (Mantyh *et al*., 1989; Nagashima *et al*., 1989; Dam *et al*., 1990; Mussap & Burcher, 1990; Lucas *et al*., 1992; Marksteiner *et al*., 1992; Merchenthaler *et al*., 1992; Ding *et al*., 1996; Shughrue *et al*., 1996). In the urethane-anaesthetized rat, the i.c.v. administration of senktide, a NK_3 receptor selective agonist, leads to increases in mean arterial blood pressure (MAP) and heart rate (HR) following the release of vasopressin and the activation of the sympatho-adrenal system, respectively (Nagashima *et al*., 1989; Takano *et al*., 1990). In contrast, the i.v. injection of NKB and of selective NK_3 receptor agonists leads to decreases in both MAP and HR in the urethane-anaesthetized rat (Couture *et al*., 1989). In the conscious rat, i.c.v. injection of NK_3 receptor selective agonists (senktide or [MePhe^7[NKB) elicits a slight decrease of HR and/or rises in HR and MAP (Itoi *et al*., 1992; Picard *et al*., 1994). This cardiovascular response was accompanied by behavioural responses such as face washing, sniffing and wet dog shake. Unfortunately, the study of the central role of NK_3 receptors in both the cardiovascular and motor control has been hampered by the lack of potent and selective antagonists.

The first generation of NK_3 receptor selective antagonists to be described was R486 (H-Asp-Ser-Phe-Trp-β-Ala-Leu-Met-NH_2) and R487 (H-Asp-Ser-Phe-Phe-β-Ala-Leu-Met-NH_2) (Drapeau *et al*., 1990). However, these compounds are sensitive to the action of peptidases and display agonist activity at the NK_1 and NK_2 receptors (Drapeau *et al*., 1990; Regoli *et al*., 1994a). The pseudopeptide NK_3 antagonist GR138676, on the other hand, has low affinity for the NK_2 receptor, but displays a high affinity at the NK_1 receptor (pK_B 8.26) (Stables *et al*., 1994). The most potent and selective pseudopeptide NK_3 receptor antagonist developed so far in the rat is R820 (3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl) which has a pA_2 of 7.60 on the rat portal vein (Regoli *et al*., 1994b). Nevertheless, non peptide NK_3 antagonists are needed since they possess greater bioavailability and metabolic stability than the peptide antagonists. SR142801 is the first non peptide antagonist displaying subnanomolar affinity for the human NK_3 receptor (Emonds-Alt *et al*., 1995). It also has a good affinity for the brain NK_3 receptor of the gerbil (K_i 0.42 ± 0.04 nM) and of the guinea-pig (K_i 0.11 ± 0.01 nM). Antagonist activities of SR142801 have been shown in various functional NK_3 receptor agonist-evoked responses, notably the contraction of the guinea-pig ileum and of the rat portal vein, the release of acetylcholine from guinea-pig ileum, the production of inositol monophosphate, arachidonic acid and cyclic AMP elicited by senktide or [MePhe^7]NKB in Chinese hamster ovary cells transfected with the human NK_3 receptor (Oury-Donat *et al*, 1995; Patacchini *et al*., 1995). In addition, SR142801 inhibits the turning behaviour induced by intrastriatal injection of senktide in gerbils and the rise in MAP induced by i.v. senktide in the conscious guinea-pig (Emonds-Alt *et al*., 1995; Roccon *et al*., 1996). However, SR142801 is about 100 fold more potent on human and guinea-pig than on rat NK_3 receptors *in vitro* and in binding assays (Emonds-Alt *et al*., 1995; Patacchini *et al*., 1995). This is in agreement with various studies suggesting the existence of interspecies NK_3 receptor subtypes (Petitet *et al*., 1993; Chung *et al*., 1994; Regoli *et al*., 1994a,b; Suman-Chauhan *et al*., 1994; Maggi, 1995). The (**R**)-enantiomer SR142806 is about 10 to 100 times weaker than SR142801 (Emonds-Alt *et al*., 1995; Oury-Donat *et al*, 1995; Patacchini *et al*., 1995; Roccon *et al*., 1996).

Recently, it was demonstrated that SR142801 and SR142806 produced thermoanalgesia upon intrathecal injection in the rat tail-flick test (Couture & Toma, 1995). This hypoalgesic effect induced by both compounds was inhibited by R820 and therefore was ascribed to the activation of the NK_3 receptor in the rat spinal cord. Hence, the present study was undertaken to characterize further the central action of SR142801 in the conscious rat by examining its i.c.v. effects on the behaviour and the cardiovascular system. For comparison purposes, the central effects of R820 and R486, the two earlier NK_3 receptor antagonists, were characterized in the same paradigms. Part of this work has been presented previously (Cellier *et al*., 1995).

Methods

Animal preparation

The care of 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 Animal Care Committee of the Université de Montréal. Male Wistar rats (275–300 g; Charles River, St-Constant, Québec, Canada) were anaesthetized with an intraperitoneal (i.p.) injection of 65 mg kg^{-1} sodium pentobarbitone (Somnotol; M.T.C. Pharmaceuticals, Cambridge, Ontario, Canada) and a polyethylene catheter (PE-20; Intramedics, Clay Adams, NJ, U.S.A.) was inserted with a guide cannula into the right cerebral ventricle (i.c.v.) (coordinates: 0.6 mm caudal to the bregma, 1.3 mm lateral to the midline, 5 mm vertical from the skull surface) with the use of a stereotaxic apparatus (David Kopf Instrumentation, Tujunga, CA, U.S.A.), as described previously (Picard *et al*., 1994). Thereafter, the rats were allowed to recover in individual plastic cages (40 cm × 23 cm × 20 cm) and were housed in a room under a 12 h light and dark cycle (lights on 06 h 00 min–18 h 00 min) with free access to commercial food and tap water. Placement of the cannula was verified *post mortem* with Evans Blue dye.

Five days later, rats were re-anaesthetized with sodium pentobarbitone (65 mg kg^{-1}) and intravascular siliconized (Sigmacote, Sigma, St-Louis, MO, U.S.A.) PE-50 catheters, filled with physiological saline containing 50 iu ml^{-1} heparin sodium salt (Sigma, St-Louis, MO, U.S.A.), were inserted into one jugular vein and the abdominal aorta through the femoral artery for intravenous (i.v.) injections and direct blood pressure recording, respectively. The experimental protocols were performed 24 h later, on the conscious and unrestrained rat. Overall, 260 animals were used in this study.

Measurement of cardiovascular and behavioural parameters

MAP and HR were measured with a Statham pressure transducer (P23ID) and a cardiac tachometer (model 7P4) (triggered by the arterial blood pressure pulse) coupled to a Grass polygraph (model 79; Grass Instruments co., Quincy, MA, U.S.A.). The behavioural activity was measured according to a previous study (Picard *et al*., 1994). Briefly, during every consecutive period of 15 s, a score 1 or 0 was given systematically depending on whether the animal showed the specific type of behaviour or not, whatever its frequency, intensity or duration during that period. Summation of scores for the first 30 min period following the i.c.v. injection gave the behavioural scores for face washing, sniffing and digging in each experiment. The maximal theorical score was 120 (15 s intervals × 30 min). Wet dog shake behaviour was measured according to the number of episodes during the first 30 min period, whatever the intensity.

Experimental protocols

I.c.v. administration of senktide, SR142801 and SR14-2806 Rats initially received an i.c.v. injection of artificial cerebrospinal fluid (aCSF; 1 μl) followed 60 min later by a single injection of either senktide (10 pmol ($n=16$), 25 pmol ($n=16$), 65 pmol ($n=16$) or 100 pmol ($n=7$)), SR142801 (100 pmol

($n=7$), 650 pmol ($n=7$) or 6.5 nmol ($n=6$)) or SR142806 (100 pmol ($n=8$), 650 pmol ($n=8$) or 6.5 nmol ($n=6$)). Each rat was selected randomly and injected with only one of the three compounds for the remainder of the protocol. Increasing doses of senktide, SR142801 or SR142806 were given at 24 h intervals. Effects of the highest dose of senktide (650 pmol) were assessed in a separate group of 4 rats because 100 pmol of the agonist produced desensitization, as revealed in a pilot study. Likewise, effects of 65 nmol SR142801 were assessed on 6 naive rats, because desensitization occurred after rats had previously received the 6.5 nmol dose. For comparison purpose, the dose of 65 nmol SR142806 was also tested in a separate group of 5 naive rats. The doses of senktide chosen are based on the study of Itoi *et al.* (1992). Control rats ($n=13$) received only the vehicle the first day of the experiment. All i.c.v. administrations of agonists and antagonists were made in a volume of 1 μl of vehicle followed by 5 μl flush volume of aCSF which corresponds to the void volume of the catheter. Each dose was calculated per rat in 1 μl solution.

The i.c.v. effects of 65 pmol senktide on MAP, HR, face washing, sniffing and wet dog shakes were compared on three consecutive days in 6 naive rats. Similarly, the i.c.v. effects of 6.5 nmol SR142801 on MAP and HR were assessed on three consecutive days in a second group of 6 naive rats.

Effects of SR142801 and SR142806 on the behavioural responses to senktide

Four groups of 6–7 rats that had 24 h previously received 25 pmol of senktide (i.c.v.) as the control were given either SR142801 or SR142806 (650 pmol or 6.5 nmol, i.c.v.) and then 15 min later were given 25 pmol of senktide i.c.v.

Inhibition by R820 and R486 of the i.c.v. responses to senktide or SR142801

Rats that had 24 h previously received 25 pmol of senktide (the lowest dose which produced a significant tachycardia) were given i.c.v. either R820 (6.5 nmol; $n=7$) or R486 (650 pmol; $n=13$) and then 5 min later were given 25 pmol of senktide, i.c.v. Initially, the doses of antagonists were chosen to achieve a ratio antagonist/agonist of 10:1. However, in pilot experiments, 650 pmol R820 failed to block the i.c.v. effects of 25 pmol senktide while 6.5 nmol of R820 was effective. On the third day, these rats received 25 pmol of senktide i.c.v. to assess the reversibility of the inhibition. The effects of SR142801, in the absence and presence of R820 or R486, were assessed as follows: in two separate groups ($n=6-7$ each) of naive rats, R820 (6.5 nmol) or R486 (650 pmol) was injected i.c.v. and then 5 min later 6.5 nmol SR142801 was given i.c.v. The results were compared to those obtained in the control group ($n=13$) injected i.c.v. with 6.5 nmol SR142801 alone. This dose of SR142801 was chosen because it had significant cardiovascular effects and also because higher doses of antagonists would have been necessary to block the effect of 65 nmol SR142801. Thus, this allowed the use of the same doses of R820 and R486 to block the i.c.v. effects of senktide and SR142801. Four naive rats that had 24 h previously received 10 pmol [Sar^9, $Met(O_2)^{11}$]SP i.c.v. (a NK_1 selective agonist) were given 6.5 nmol R820 i.c.v. followed 5 min later by 10 pmol [Sar^9, $Met(O_2)^{11}$]SP, to test the selectivity of R820 for the NK_3 receptor.

To rule out the involvement of NK_1 and NK_2 receptors in the central action of SR142801, a mixture of selective NK_1 and NK_2 receptor antagonists, RP67580 and SR48968 at doses (6.5 nmol each) known to abolish selectively either SP or NKA mediated cardiovascular and behavioural effects (Picard *et al.*, 1994), was injected i.c.v. to another group of 6 naive rats followed 5 min later by 6.5 nmol SR142801, i.c.v.

The effects of 6.5 nmol R820, 650 pmol R486, 3.25 nmol R486 and of the mixture of RP67580 and SR48968 (6.5 nmol each) were determined on cardiovascular and behavioural parameters for a period of 60 and 30 min, respectively on four separate groups of 4–6 rats which did not receive previously any other treatment. One group of 5 rats was injected i.c.v. with 6.5 nmol RP67580 followed 5 min later by 3.25 nmol R486 i.c.v. The results were compared with the group which received 3.25 nmol R486, i.c.v., alone.

Cross-desensitization between senktide and SR142801

Naive rats received 3 repeated i.c.v. injections of either senktide (65 pmol; $n=7$) or SR142801 (6.5 nmol; $n=7$) at 2 h intervals to ensure a complete dissipation of the cardiovascular and behavioural responses. The senktide group then received, 2 h and 24 h later, 6.5 nmol of SR142801 i.c.v. while the SR142801 group received also 2 h and 24 h later 65 pmol of senktide i.c.v. In a separate group of SR142801 desensitized rats ($n=7$), 25 pmol of [Sar^9, $Met(O_2)^{11}$]SP was administered i.c.v. 2 h after the last injection of SR142801 to verify the selectivity of the NK_3 receptor desensitization. In preliminary experiments, the dose of [Sar^9, $Met(O_2)^{11}$]SP chosen produced increases in MAP and HR of similar intensities to those of 6.5 nmol SR142801. The i.c.v. effects measured with 65 pmol senktide or 25 pmol [Sar^9, $Met(O_2)^{11}$]SP 24 h before desensitization to SR142801 served as controls. Results obtained with SR142801 after desensitization to senktide were compared to the effects of SR142801 measured in a separate control group of 13 rats.

I.v. administration of senktide, SR142801 and SR142806

A group of naive rats received increasing doses of senktide (0.4 ($n=10$), 4 ($n=10$) and 25 ($n=5$) nmol kg^{-1}, i.v.). The first two doses were given at 1 h intervals while the third was given 24 h later to avoid the problem of desensitization that was observed in preliminary experiments after injection of 4 nmol kg^{-1} senktide. The potency of SR142801 and its (**R**)-enantiomer, SR142806, against the effects of i.v. senktide was assessed as follows: naive rats ($n=35$) received, on the first day, an i.v. injection of 0.4 ml of the vehicle saline (0.9% NaCl) followed 30 min later by an i.v. injection of 4 nmol kg^{-1} senktide (in a volume of 0.15 ml followed by 0.25 ml of flush volume of saline corresponding to the void volume of the catheter). Twenty four hours later, these rats were divided in six groups which were given 4 nmol kg^{-1} senktide i.v. either 5 min (group 1 (65 nmol kg^{-1} SR142801, $n=6$); group 2 (65 nmol kg^{-1} SR142806; $n=6$); group 3 (650 nmol kg^{-1} SR142801; $n=5$); group 4 (650 nmol kg^{-1} SR142806; $n=6$)) or 30 min later (group 5 (650 nmol kg^{-1} SR142801; $n=7$); group 6 (650 nmol kg^{-1} SR142806; $n=5$)). The response to senktide was compared to that obtained on day 1. On day 3, SR142801 ($n=7$) and SR142806 ($n=5$) were injected i.v. at 650 nmol kg^{-1} and their effects on MAP and HR were measured for 1 h. Only one compound was given to each rat.

Statistical analysis of data

Results are expressed as means $\pm$ s.e.mean. Pairwise comparisons were made with a Student's t test for paired samples. For comparisons of parametric values, statistical differences were evaluated with a one-way or a two-way (time-dependent effects) analysis of variance (ANOVA) followed by Dunnett's test (multiple comparisons) or by Student's t test (simple comparison). Statistical differences between the non-parametric episodes of behaviour were evaluated with a Kruskal-Wallis test and a *post-hoc* Wilcoxon Mann-Whitney test. The uniformity of the variances between the different groups was verified a priori with the Bartlett test. Only probability values (P) less than 0.05 were considered to be statistically significant.

Drugs and solutions

The composition of aCSF was, in mM: NaCl 128.6, KCl 2.6, $MgCl_2$ 2.0 and $CaCl_2$ 1.4 (pH adjusted to 7.2). Senktide (Suc-Asp-Phe-MePhe-Gly-Leu-Met-NH_2) (MW: 842.0),

[Sar^9, Met(O$_2$)^{11}]SP (MW: 1392.9), R820 (3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl) (MW: 624.6) and R486 (H-Asp-Ser-Phe-Trp-β-Ala-Leu-Met-NH$_2$) (MW: 868) were synthesized in the laboratory of D. Regoli at the Université de Sherbrooke (Sherbrooke, Québec, Canada). The non peptide antagonist SR142801 ((**S**)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide) (MW: 652.1), its (**R**) enantiomer SR142806 and SR48968 ((**S**)-N-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (MW: 552.5) were given by Dr J.C. Brelière of Sanofi Research (Montpellier, France). RP67580 (racemic form of 7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl] perhydroisoindol-4-one (3a**R**, 7a**R**)) (MW: 438.6) was provided by Dr C. Garret (Rhone Poulenc, Paris, France). Heparin sodium salt (porcine, grade 1-A) was purchased from Sigma company (St-Louis, Missouri, U.S.A.). Agonists and antagonists were solubilized in dimethyl sulphoxide (DMSO; Fisher Scientific, Montréal, Québec, Canada). The stock solutions (10 mg ml^{-1}) were stored in aliquots of 100 μl at -20°C until used. Senktide and the antagonists were injected either in saline (i.v.) or in aCSF (i.c.v.) containing less than 1% DMSO except for the doses of 6.5 (18% DMSO) and 65 nmol (60% DMSO) of SR142801 and SR142806. In all experiments, vehicle was tested as control and no significant changes were seen on any parameters when compared to aCSF (i.c.v) or saline (i.v.) values.

Results

Cardiovascular effects of senktide, [Sar^9, Met(O$_2$)^{11}]SP and non peptide antagonists

Senktide administered i.c.v. at the dose of 100 ($n=7$) and 650 ($n=4$) pmol caused significant rises in MAP of 9 ± 3 and 12 ± 1 mmHg, when compared with vehicle values, within the first 15 min (Figure 1a). Rise in MAP elicited by 650 pmol senktide was associated with a tachycardia reaching a maximum again within the first 15 min of 88 ± 8 beats min^{-1}. The dose of 100 pmol senktide caused a delayed tachycardia after 20 min and this was maintained for the period of the experiment (60 min). Doses of 25 and 65 pmol of senktide i.c.v. ($n=16$) failed to have any effect on MAP while they caused a significant increase of HR from 20 min (25 pmol) and 30 min (65 pmol) which was again maintained over the experimental period (maximum was 52 ± 10 and 46 ± 14 beats min^{-1}, respectively) when compared with vehicle (Figure 1a). In addition, 65 pmol senktide elicited a initial bradycardia of 47 ± 12 beats min^{-1} at between 5 and 10 min. The lower dose of 10 pmol of senktide ($n=16$) evoked a significant fall in MAP (-10 ± 6 mmHg) within the first 15 min, but had no effect on HR (Figure 1a). [Sar^9, Met(O$_2$)^{11}]SP 25 pmol given i.c.v. ($n=7$) had a similar effect to the highest dose of senktide causing a rapid and significant increase in MAP (13 ± 1 mmHg) and HR (96 ± 8 beats min^{-1}) (Figure 2a).

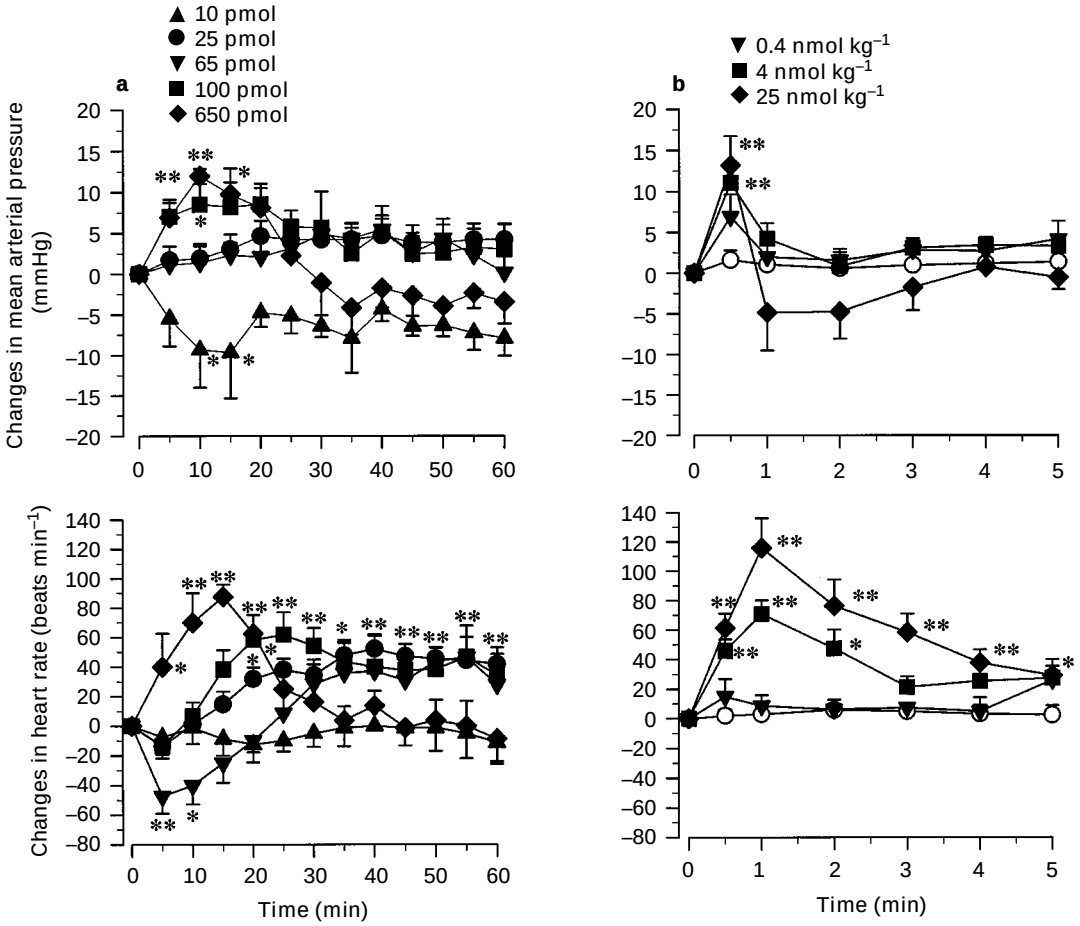

Figure 1 Time-course effects of senktide injected (a) i.c.v. at 10 ($n=16$), 25 ($n=16$), 65 ($n=16$), 100 ($n=7$) and 650 ($n=4$) pmol per animal for a period of 60 min and (b) i.v. at 0.4 ($n=10$), 4 ($n=10$) and 25 ($n=5$) nmol kg^{-1} for a period of 5 min, on changes in mean arterial blood pressure (MAP) and heart rate (HR) in conscious rats. Control data with i.c.v. vehicle were omitted for the sake of clarity and are presented in Figure 5a. Basal values were: MAP: 105 ± 3 mmHg; HR: 389 ± 6 beats min^{-1} in (a) and MAP: 109 ± 3 mmHg; HR: 389 ± 8 beats min^{-1} in (b). Statistical significance of differences between senktide and vehicle ($n=13$; i.c.v.) or between senktide and saline ($n=35$; i.v.) values were calculated with a two-way ANOVA followed by a Dunnett's test and are indicated by: $^*P<0.05$; $^{**}P<0.01$.

The cardiovascular effects of SR142801 and SR142806, injected i.c.v., are shown in Figure 3. SR142801 (100 pmol–65 nmol; $n=6-8$) evoked dose- and time-dependent increases in HR (ranging from 23 ± 6 to 118 ± 26 beats min^{-1}). The doses of 100 pmol ($n=6$) and 650 pmol ($n=6$) of SR142801 did not cause significant changes in MAP. The dose of 6.5 nmol ($n=8$) caused a very delayed (40 min), and significant, increase in MAP (10 ± 2 mmHg) while 65 nmol ($n=8$) produced a significant pressor response (17 ± 2 mmHg) from 5 min onwards. The initial increase in MAP and HR induced by 65 nmol SR142801 at 5 min was followed by a transient drop in MAP and HR between 10 and 20 min. On the other hand, SR142806 increased MAP (from 5 to 60 min) and HR (from 35 to 60 min) only at the highest dose of 65 nmol (13 ± 2 mmHg and 69 ± 11 beats min^{-1}; $n=8$). However, it should be noted that 7 out of the 53 rats treated with SR142801 (6.5 nmol–65 nmol) (13% of the animals) did not exhibit any increases of HR and MAP. Since the i.c.v. effects of senktide were also blunted in those rats, they were therefore classified as non-responding and excluded from the study.

The tachycardiac and behavioural (see section below) effects of three i.c.v. injections of senktide (65 pmol) given 24 h apart were not significantly different (Table 1). At this dose, senktide had no significant effect on MAP (see also Figure 1a). In contrast, the cardiovascular changes elicited by 6.5 nmol SR142801 were not significantly different from vehicle values on day 2 and day 3. A partial recovery of the cardiovascular response to SR142801 occurred on day 3 since significant differences were seen only between day 1 and day 2 and not between day 1 and day 3. Basal HR for rats treated with SR142801 on day 1 was significantly higher ($P<0.01$) than that measured in the vehicle group.

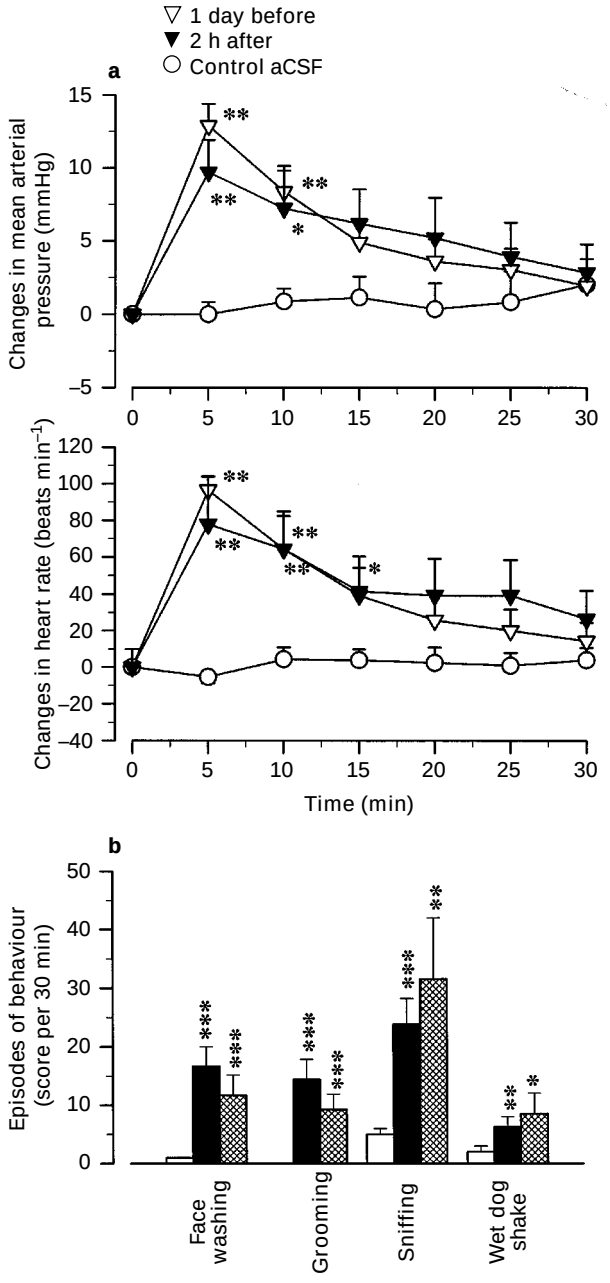

Figure 2 Effects of [Sar^9, $Met(O_2)^{11}$]SP (25 pmol per animal; i.c.v.) on changes in mean arterial pressure (MAP) and heart rate (HR) (a) or behaviour (b) one day before (solid columns) or 2 h after desensitization to SR142801 (cross-hatched columns) in 7 conscious rats. Changes in behaviour were measured during the first 30 min post-injection. Basal values were: MAP (mmHg): 108 ± 6 (before) and 122 ± 5 (after); HR (beats min^{-1}): 396 ± 13 (before) and 339 ± 11 (after). Statistical significance of differences between [Sar^9, $Met(O_2)^{11}$]SP and aCSF (control, open columns; $n=13$) values were calculated with a two-way ANOVA followed by a Dunnett's test (a) or Kruskall-Wallis and a *post-hoc* Wilcoxon Mann-Whitney test (b) and are indicated by: $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

Behavioural effects of senktide, [Sar^9, $Met(O_2)^{11}$]SP and non peptide antagonists

The i.c.v. administration of senktide (10 to 650 pmol) elicited increases of behaviour during the first 30 min post-injection (wet dog shake > sniffing > face washing) while digging episodes were only slightly increased (Figure 4). The behavioural responses to 25 pmol [Sar^9, $Met(O_2)^{11}$]SP were mainly composed of sniffing, face washing and grooming behaviours. At the same dose (25 pmol), the NK_1 agonist produced a significantly reduced effect compared to that of senktide on wet dog shake (Figures 2 and 4). SR142801 but not SR142806 produced a significant increase of sniffing episodes at the highest dose of 65 nmol (Table 2). Neither SR142801 nor SR142806, at doses up to 6.5 nmol, inhibited behavioural responses to 25 pmol senktide. In contrast, 6.5 nmol SR142801 enhanced face washing and wet dog shake episodes elicited by 25 pmol senktide (Table 2).

Characterization of the receptor involved in the i.c.v. effects of senktide and SR142801

The HR response evoked by i.c.v. senktide (25 pmol) was antagonized by the NK_3 receptor antagonists R820 (6.5 nmol; 2 ± 20 vs 63 ± 9 beats min^{-1}; $P<0.05$) and R486 (650 pmol; 1 ± 13 vs 63 ± 9 beats min^{-1}; $P<0.05$), as shown in Figure 5. Whereas 6.5 nmol R820 failed to modify the behavioural effects of 25 pmol senktide, 650 pmol R486 did significantly reduce the sniffing and wet dog shake elicited by 25 pmol senktide (Table 2). The inhibitory action of R820 and R486 on the i.c.v. effects of 25 pmol senktide was no longer seen after 24 h (data not shown). A lower dose of R820 (650 pmol) was ineffective against both the HR and behavioural responses to 25 pmol senktide (data not shown). Whereas R820 (6.5 nmol) failed to modify the increases in MAP (12 ± 2 vs 16 ± 1 mmHg) and in HR (98 ± 11 vs 121 ± 17 beats min^{-1}) as well as behavioural activity elicited by 10 pmol [Sar^9, $Met(O_2)^{11}$]SP (Figure 6), the MAP and HR responses to 6.5 nmol SR142801 were completely antagonized (-3 ± 3 vs 10 ± 2 mmHg ($P<0.01$) and -13 ± 10 vs 67 ± 8 beats min^{-1} ($P<0.001$)) by the same dose of R820 (Figure 5). R820 was devoid of any cardiovascular effects (Table 3) although it did increase the sniffing behaviour (Table 2). R486 (650 pmol) had no effect on both cardiovascular and behavioural parameters (Tables 2 and 3) and did not block the central cardiovascular response induced by SR142801 (data not shown). Higher doses of R486 (3.25 nmol) increased HR at 30 min post-injection (Table 3), as well as face washing, sniffing and wet dog shakes (Table

2). Therefore, this dose of R486 could not be tested against SR142801. The HR response and wet dog shake (but not face washing and sniffing) elicited by 3.25 nmol R486 were prevented by RP67,580 (6.5 nmol i.c.v., 5 min earlier) (Tables 2 and 3).

A co-pretreatment with both NK_1 (RP67580) and NK_2 (SR48968) receptor antagonists (6.5 nmol each i.c.v.) had no effect on the pressor (8 ± 1 vs 10 ± 2 mmHg) and HR (60 ± 20 vs 67 ± 8 beats min^{-1}) responses to 6.5 nmol SR142801 (Figure 5). Upon i.c.v. co-administration, RP67580 and SR48968 (6.5 nmol each) had no direct effects on MAP and HR (Table 3) or on behaviour (data not shown). The basal values of MAP and HR in all these experimental groups were not significantly different (Figure 5).

Cross-desensitization between the i.c.v. response to senktide and SR142801

Both HR and behavioural responses to senktide were completely desensitized after 3 consecutive i.c.v. injections of 65 pmol given 2 h apart. The desensitization of the HR response occurred earlier, being complete after the second injection (ΔHR: 49 ± 10, -11 ± 13 and -3 ± 7 beats min^{-1}) while a third injection was required in the majority of the animals (4 out of 7) to obtain a complete desensitization of the behavioural response (face washing: 12 ± 3, 6 ± 2, 5 ± 3; sniffing: 71 ± 9, 39 ± 5, 15 ± 8; wet dog shake: 79 ± 20, 38 ± 14, 16 ± 9 episodes per 30 min). Under these conditions, the pressor (0 ± 1 vs 10 ± 2 mmHg; $P<0.05$) and HR (-20 ± 10 vs

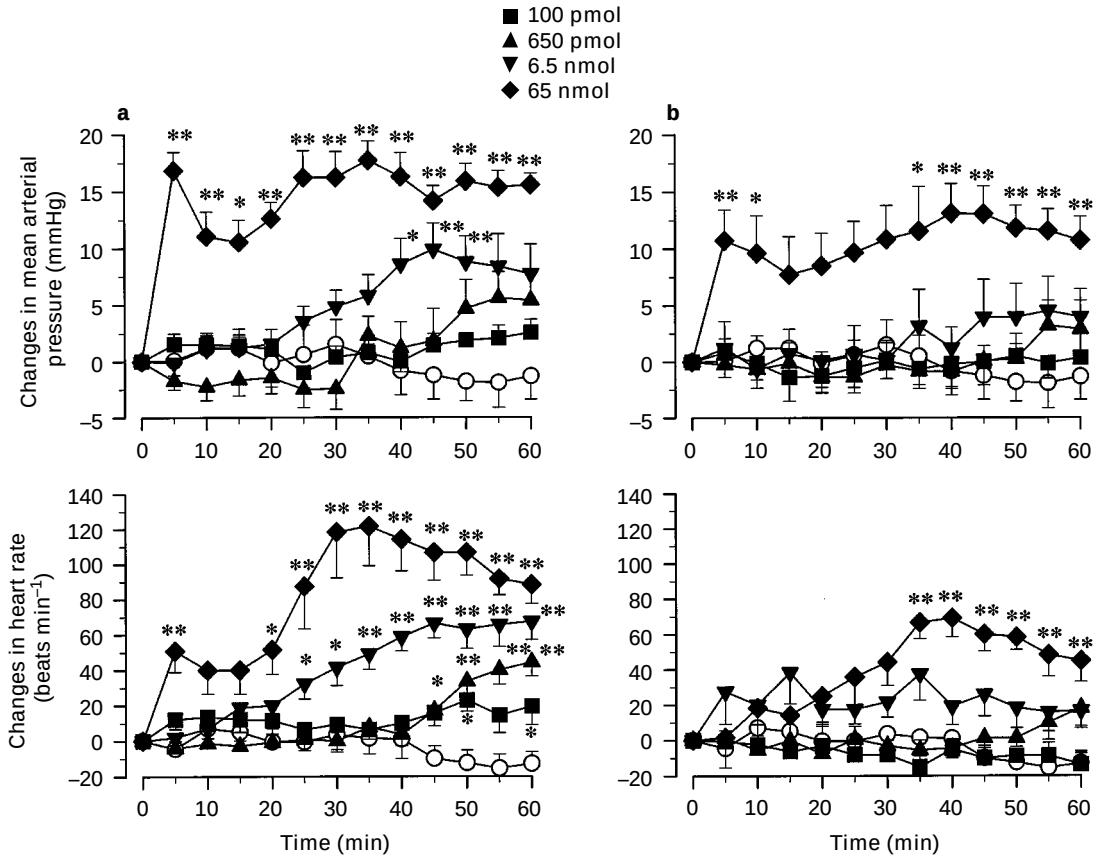

Figure 3 Time-course effects of (a) SR142801 and (b) SR142806 injected i.c.v. at 100 pmol, 650 pmol, 6.5 nmol and 65 nmol per animal on changes in mean arterial blood pressure (MAP) and heart rate (HR) in 5–8 conscious rats. Basal values were: MAP (mmHg): 107 ± 7 (SR142801), and 110 ± 2 (SR142806); HR (beats min^{-1}): 380 ± 4 (SR142801) and 359 ± 6 (SR142806). Statistical significance of differences between the compound and vehicle (○; $n=13$) values were calculated with a two-way ANOVA followed by a Dunnett's test and are indicated by: $^{*}P<0.05$; $^{**}P<0.01$.

Table 1 A comparison of the cardiovascular and behavioural responses of senktide and SR142801 administered i.c.v. repeatedly, once a day, over a 3 day period

Treatment	*Basal MAP* (mmHg)	Δ*MAP* (mmHg)	*Basal HR* (beats min^{-1})	Δ*HR* (beats min^{-1})	*Face washing*	*Sniffing*	*Wet dog shake*	n
Vehicle	106 ± 2	0 ± 2	378 ± 7	-9 ± 6	1 ± 0	5 ± 1	2 ± 1	13
Senktide; day 1	104 ± 8	2 ± 4	410 ± 13	74 ± 7**	5 ± 1*	69 ± 7***	55 ± 19***	6
Senktide; day 2	107 ± 5	10 ± 6	391 ± 22	79 ± 20**	6 ± 2*	55 ± 22***	50 ± 20***	6
Senktide; day 3	85 ± 3	4 ± 3	391 ± 18	76 ± 22**	6 ± 1*	52 ± 14***	67 ± 16***	6
SR142801; day 1	107 ± 3	9 ± 0*	422 ± 11**	40 ± 5**	0	0	0	6
SR142801; day 2	105 ± 5	2 ± 2†	399 ± 15	-7 ± 12††	0	0	0	6
SR142801; day 3	105 ± 3	5 ± 2	380 ± 16††	15 ± 27	0	0	0	6

Data are means$\pm$s.e.mean of (n) rats. Behavioural responses are expressed as score for the first 30 min period. Maximal cardiovascular changes were measured at 55 min for senktide (65 pmol) or 45 min for SR142801 (6.5 nmol). Statistical comparison with vehicle: $^{*}P<0.05$, $^{**}P<0.01$; $^{***}P<0.001$ or with SR142801 on day 1: †$P<0.05$; ††$P<0.01$.

67 ± 8 beats min^{-1}; $P < 0.01$) responses to 6.5 nmol SR142801, injected 2 h after the last injection of senktide, were completely abolished (Figure 7). One day after the desensitization to senktide, the MAP and HR responses to SR142801 were not significantly different from those measured in intact rats (11 ± 2 mmHg and 121 ± 40 beats min^{-1}) (Figure 7).

SR142801 (6.5 nmol) evoked increases in MAP (11 ± 2 mmHg) and HR (63 ± 10 beats min^{-1}) which were absent after the second and third injections given 2 h apart (3 ± 2, 1 ± 1 mmHg and -6 ± 15, -7 ± 7 beats min^{-1}, respectively). Two h after the last injection of SR142801, senktide (65 pmol) failed to produce HR changes (-2 ± 4 vs 48 ± 11 beats min^{-1}) (Figure 8a). The inhibition of the senktide - induced tachycardia was entirely reversible after 24 h (54 ± 23 beats min^{-1}). In addition, the sniffing and wet dog shakes elicited by senktide were diminished after desensitization to SR142801; these inhibitions were no longer observed after 24 h (Figure 8b). Conversely, the increases in MAP (10 ± 2 vs 13 ± 1 mmHg), in HR (78 ± 26 vs 96 ± 8 beats min^{-1}) and behaviour (face washing, grooming, sniffing and wet dog shakes) induced by 25 pmol [Sar^9, $Met(O_2)^{11}$]SP were not significantly affected after desensitization with SR142801 (Figure 2).

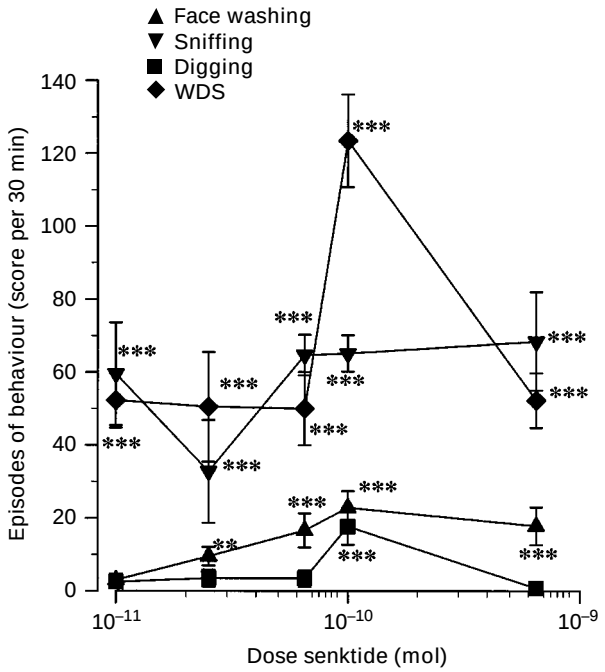

Figure 4 Increases of face washing, sniffing, digging and wet dog shakes (WDS) elicited by i.c.v. injection of senktide (10–650 pmol per animal; same rats as in Figure 1a) during the first 30 min period in conscious rats. The vehicle score for each behaviour was as follows: 1 ± 1 (face washing); 5 ± 1 (sniffing); 1 ± 1 (digging); 2 ± 1 (wet dog shake). Statistical significance of differences between senktide and vehicle ($n = 13$) values were calculated with a Kruskall-Wallis test and a *post-hoc* Wilcoxon Mann-Whitney test and are indicated by: $^{**}P < 0.01$; $^{***}P < 0.001$.

Intravenous effects of senktide, SR142801 and SR142806

As illustrated in Figure 1b, the i.v. injection of 4 and 25 nmol kg^{-1} senktide induced dose-dependent and transient increases in MAP (11 ± 2 and 13 ± 4 mmHg) and HR (71 ± 9 and 116 ± 20 beats min^{-1}) which returned to baseline after 1 min and 3–5 min, respectively. The dose of 0.4 nmol kg^{-1} senktide had no effect on MAP and HR. The i.v. injection of SR142801 (650 nmol kg^{-1}) had no effect on cardiovascular parameters whereas the (**R**)-enantiomer SR142806 elicited a slow developing decrease in HR (25 min post-injection) (Table 4). Neither senktide (0.4–25 nmol kg^{-1}) nor SR142801 and SR142806 (65–650 nmol kg^{-1}) had significant effects on the behaviour (results not shown). Pretreatment with either antagonist (650 nmol kg^{-1}) produced a slight reduction of the HR effect of 4 nmol kg^{-1} senktide (Table 4). This inhibition was observed when SR142801 was given 30 min but not 5 min before senktide. Conversely, SR142806 was effective when given 5 min before senktide. Both compounds were inactive against the pressor response to senktide.

Discussion

Effects of tachykinin receptor agonists and SR142801 given i.c.v.

The NK_3 receptor selective agonist senktide injected i.c.v. induced either decreases (10 pmol), increases (100 and 650 pmol)

Table 2 Effects of senktide and non peptide NK_3 receptor compounds injected i.c.v. on behaviour in conscious rats

Antagonist	*Agonist*	*Face washing*	*Sniffing*	*Wet dog shake*	n
	Vehicle	1 ± 1	5 ± 1	2 ± 1	13
SR142801, 6.5 nmol		3 ± 2	11 ± 4	3 ± 1	6
SR142801, 65 nmol		3 ± 2	34 ± 5*	3 ± 1	6
SR142806, 6.5 nmol		3 ± 2	11 ± 4	4 ± 1	6
SR142806, 65 nmol		1 ± 1	15 ± 4	2 ± 2	5
	Senktide	13 ± 3***	51 ± 14***	60 ± 13***	6
SR142801, 650 pmol	Senktide	17 ± 4***	51 ± 16***	72 ± 15***	
	Senktide	10 ± 3*	63 ± 11***	65 ± 3***	6
SR142801, 6.5 nmol	Senktide	27 ± 5***†	75 ± 5***	108 ± 12***†	
	Senktide	22 ± 4***	37 ± 11***	68 ± 15***	6
SR142806, 650 pmol	Senktide	14 ± 4***	31 ± 9***	53 ± 10***	
	Senktide	14 ± 6***	43 ± 10***	61 ± 9***	7
SR142806, 6.5 nmol	Senktide	10 ± 3***	29 ± 10***	42 ± 8***	
	Senktide	14 ± 5***	53 ± 7***	38 ± 9***	7
R820, 6.5 nmol	Senktide	11 ± 2***	86 ± 10***	73 ± 15***	
R820, 6.5 nmol		0 ± 0	41 ± 4***	3 ± 2	6
	Senktide	5 ± 2**	56 ± 7***	46 ± 6***	13
R486, 650 pmol	Senktide	3 ± 1**	35 ± 6***†	23 ± 5***††	
R486, 650 pmol		1 ± 1	6 ± 4	0 ± 0	6
R486, 3.25 nmol		8 ± 4**	37 ± 12***	$26 + 9$***	4
R486, 3.25 nmol + RP67,580, 6.5 nmol		5 ± 2**	31 ± 6***	6 ± 3††	5

Data are means $\pm$ s.e.mean of (n) rats. Behavioural responses are expressed as score for the first 30 min post-injection. Statistical comparison with vehicle ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$) or with 25 pmol senktide or 3.25 nmol R486 alone ($^{\dagger}P < 0.05$; $^{\dagger\dagger}P < 0.01$).

or no effect (25 and 65 pmol) on MAP during the first 15 min post-injection. The effect of senktide on HR was also dependent on the dose as it induced either no effect (10, 25 and 100 pmol), a decrease (65 pmol) or an increase (650 pmol) during the first 10–15 min or a more sustained increase (25, 65 and 100 pmol) from 20–60 min. These cardiovascular responses were accompanied by face washing, sniffing and wet dog shakes during the first 30 min period of measurement. These effects are similar to those observed by Itoi *et al*. (1992) with senktide but differ from those obtained by Picard *et al*. (1994) who found transient increases of MAP and HR with [MePhe^7]NKB. These discrepancies may be due to the use of different agonists, although behavioural responses were similar in the three studies. Similar to the high doses of senktide, the NK_1 receptor selective agonist [Sar^9, Met$(O_2)^{11}$]SP induces rapid increases of MAP and HR that are accompanied by intensive episodes of face washing, grooming and sniffing. These effects are consistent with earlier findings with SP (Itoi *et al*., 1992; Picard *et al*., 1994). The wet dog shake behaviour was ascribed to the activation of NK_3 receptor on the basis of experiments with endogenous and selective agonists and antagonists (Picard *et al*., 1994).

Surprisingly, the NK_3 receptor antagonist SR142801 induced dose-dependent, slow-developing and long-lasting increases in MAP and HR when injected i.c.v. The slow onset of these cardiovascular responses is difficult to explain at this

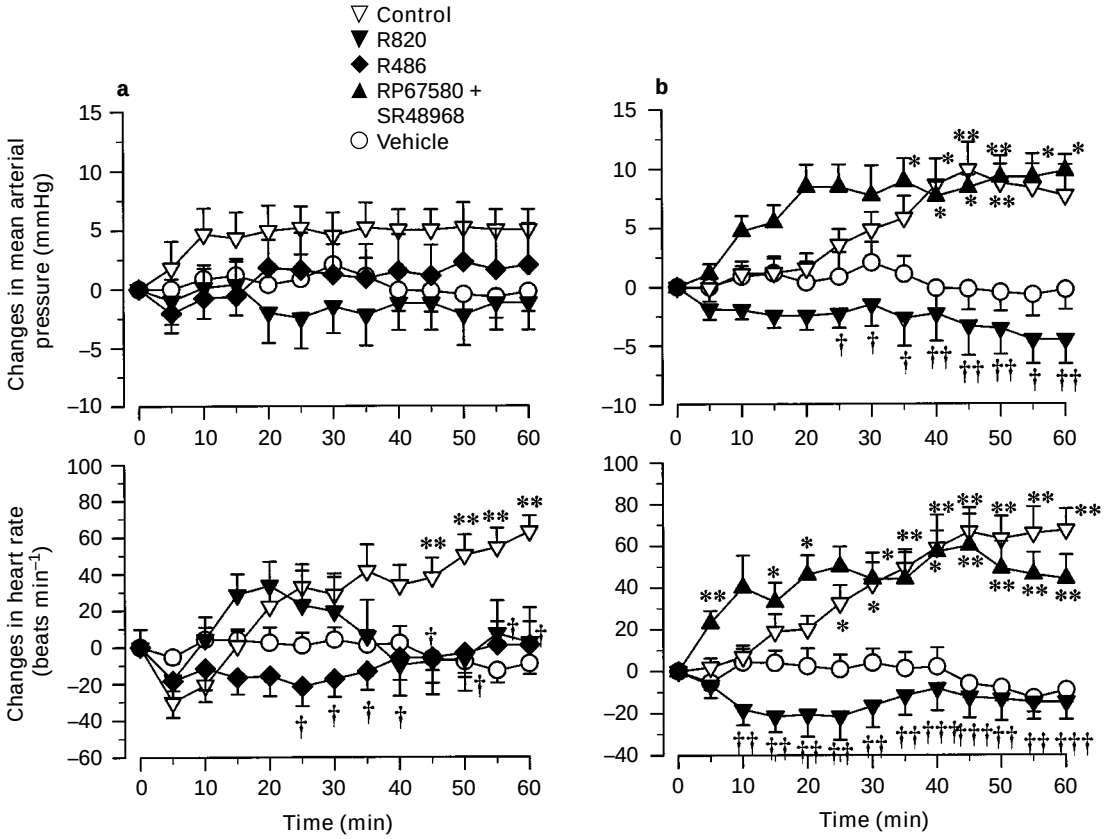

Figure 5 Inhibition of the changes in mean arterial pressure (MAP) and heart rate (HR) evoked by i.c.v. injection of (a) senktide (25 pmol) by R820 (6.5 nmol; $n=7$) or R486 (650 pmol; $n=13$) and of (b) SR142801 (6.5 nmol) by R820 (6.5 nmol; $n=7$) or RP67580 plus SR48968 (6.5 nmol each; $n=6$) in conscious rats (doses are per animal). Antagonists were injected i.c.v. 5 min beforehand. Shown are effects of senktide or SR142801 without (control) or with R820, R486 or RP65780 plus SR48968. Vehicle values are also indicated. Basal values were: MAP (mmHg): 106±2 (vehicle), 109±5 (senktide), 104±9 (senktide+R820), 98±3 (senktide+R486), 100±3 (SR142801), 107±1 (SR142801+R820) and 99±4 (SR142801+RP67,580+SR48,968); HR (beats min^{-1}): 378±7 (vehicle), 403±10 (senktide), 399±20 (senktide+R820), 371±16 (senktide+R486), 386±6 (SR142801), 403±7 (SR142801+R820) and 388±12 (SR142801+RP67,580+SR48,968). Statistical comparisons with vehicle (*; $n=13$) and to senktide or SR142801 (†; $n=13$) alone were calculated with a two-way ANOVA followed by a Dunnett's test and are indicated by: *·†$P<0.05$; **·††$P<0.01$; †††$P<0.001$.

Table 3 Effects of NK_3 receptor antagonists injected i.c.v. on mean arterial pressure (MAP) and heart rate (HR) of the conscious rat

	ΔMAP (mmHg) *Time* (min) *after injection*			ΔHR (beats min^{-1}) *Time* (min) *after injection*			
Treatment	*5*	*30*	*60*	*5*	*30*	*60*	n
Vehicle	0±1	2±2	0±2	−5±4	4±7	−9±6	13
R820 (6.5 nmol)	0±2	0±2	−1±4	−8±7	−8±11	−8±12	6
R486 (650 pmol)	0±1	2±1	1±2	1±3	−4±4	−23±6	6
R486 (3.25 nmol)	−2±2	−1±2	−3±3	−20±13	68±20**	−14±15	4
R486 (3.25 nmol)+ RP67,580 (6.5 nmol)	−1±1	−1±2	1±3	−12±11	2±19†	18±18	5
RP67,580+SR48,968 (6.5 nmol each)	−3±1	−1±1	−2±1	−1±8	−6±11	−18±10	6

Data are means±s.e.mean of (n) rats. Statistical comparison with vehicle (**$P<0.01$) or to 3.25 nmol R486 alone (†$P<0.05$).

time, although it is consistent with the long period of preincubation (120–140 min) that SR142801 needed to inhibit the senktide-induced contraction of the guinea-pig isolated ileum (Patacchini *et al*., 1995). However, in conscious guinea-pigs, the activity of SR142801 against the pressor effect of senktide is maximal 15 min after either i.v. or oral administration (Roccon *et al*., 1996). The increases of MAP and HR elicited by SR142801 were stereoselective since the (**R**)-enantiomer SR142806 elicited cardiovascular responses only at the highest dose of 65 nmol. Hence, the present study reveals a dissociation between the effects of SR142801 in the central nervous system and the periphery where SR142801 has no agonist-like activity on the rat cardiovascular system. The latter compound was also inactive on the cardiovascular system of the conscious guinea-pig when administered i.v., except at μmol doses where it decreased HR (Roccon *et al*., 1996).. When injected i.c.v., SR142801 produced only a weak effect on the sniffing behaviour at the highest dose. This suggests that the cardiovascular and behavioural effects induced by central activation of NK_3 receptors are mediated by independent mechanisms. This finding could be related either to the existence of intra-species NK_3 receptor subtypes or to the production of different second messengers induced by different NK_3 agonists, in accord with the data obtained by Krause *et al*. (1996). These authors have shown, in Chinese hamster ovary cells transfected with human NK_3 receptors, that NKB and the selective agonists, [Me-Phe^7]NKB and senktide, differentially activate phosphoinositide hydrolysis, arachidonic acid release and cyclic AMP production. *In vivo* differences could also be explained by the pharmacokinetic properties of SR142801 which may selectively access to some brain areas involved in cardiovascular but not in motor regulation. However, SR142801 appeared to reach the same sites as senktide, as illustrated by its ability to potentiate the senktide-induced behaviour changes.

Identification of the receptor involved in the i.c.v. actions of senktide and SR142801

The NK_3 receptor selective antagonists R820 and R486 inhibited in a reversible manner the central HR effect of senktide while only R486 inhibited both the cardiovascular and behavioural responses to senktide (present study) and to

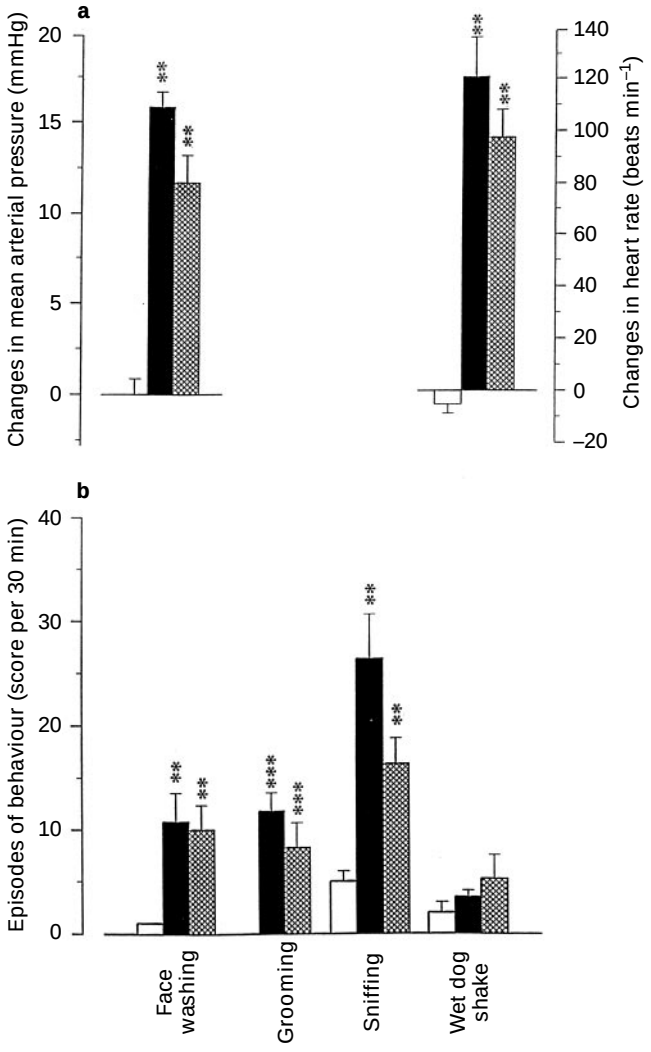

Figure 6 Effects of [Sar^9, Met$(O_2)^{11}$]SP (10 pmol per animal; i.c.v.) on changes in mean arterial pressure (MAP) and heart rate (HR) (a) or behaviour (b) one day before (solid columns) or 5 min after injection of 6.5 nmol R820 (cross-hatched columns) in 4 conscious rats. Maximal changes in MAP and HR were taken at 5 min post-injection while changes in behaviour were measured during the first period of 30 min. Basal values were: MAP (mmHg): 104 ± 5 (before) and 103 ± 7 (after); HR (beats min^{-1}): 413 ± 8 (before) and 400 ± 15 (after). Statistical significance of differences between [Sar^9, Met$(O_2)^{11}$]SP and aCSF (open columns; $n=13$) values were calculated with a one-way ANOVA followed by a Dunnett's test (a) or a Kruskall-Wallis test and a *post-hoc* Wilcoxon Mann-Whitney test (b) and are indicated by: $^{**}P<0.01$; $^{***}P<0.001$.

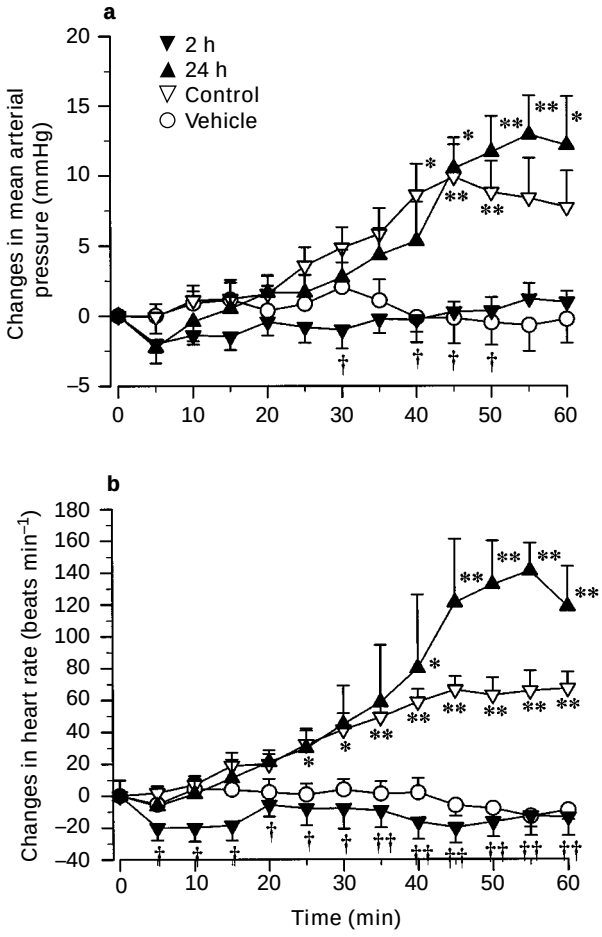

Figure 7 Effects of SR142801 (6.5 nmol per animal; i.c.v.) on changes in (a) mean arterial pressure (MAP) and (b) heart rate (HR), 2 h or 24 h after desensitization to senktide in 7 conscious rats. The intact group which received 6.5 nmol SR142801 alone (control; $n=13$) was the one used in experiments described in Figure 5. Basal values were: MAP (mmHg): 100 ± 3 (control), 100 ± 3 (2 h after) and 105 ± 3 (24 h after); HR (beats min^{-1}); 386 ± 6 (control), 388 ± 15 (2 h after) and 341 ± 13** (24 h after). Statistical comparisons to vehicle ($n=13$) were calculated with a two-way ANOVA followed by a Dunnett's test ($^*P<0.05$; $^{**}P<0.01$) and those to SR142801 in intact rats were calculated with a two-way ANOVA followed by a Student's t test ($\dagger P<0.05$; $\dagger\dagger P<0.01$).

[MePhe^7]NKB (Picard 1994). This finding again suggests that centrally injected NK_3 receptor agonists/antagonists alter the cardiovascular system and the motor behaviour through different mechanisms. This may not be due to the pharmacokinetic features of R820 because this compound displayed a direct effect on the sniffing despite its lack of agonist-like effect on MAP and HR. Thus, R820 has probably access to both behavioural and cardiovascular functional sites. Whereas R486 did not discriminate between the rat and guinea-pig NK_3 receptor, R820 was inactive on the guinea-pig NK_3 receptor and exhibited a high affinity on the rat NK_3 receptor (Regoli *et al*., 1994b). By inference, this indicates that the NK_3 receptor which mediates the i.c.v. action of senktide on the behaviour has pharmacological characteristics similar to the NK_3 receptor present in the guinea-pig. Therefore, we cannot exclude the possible existence of intraspecies NK_3 receptors in the rat central nervous system to explain the separation between the cardiovascular (rat NK_3 receptor subtype) and behavioural (guinea-pig NK_3 receptor subtype) responses to senktide.

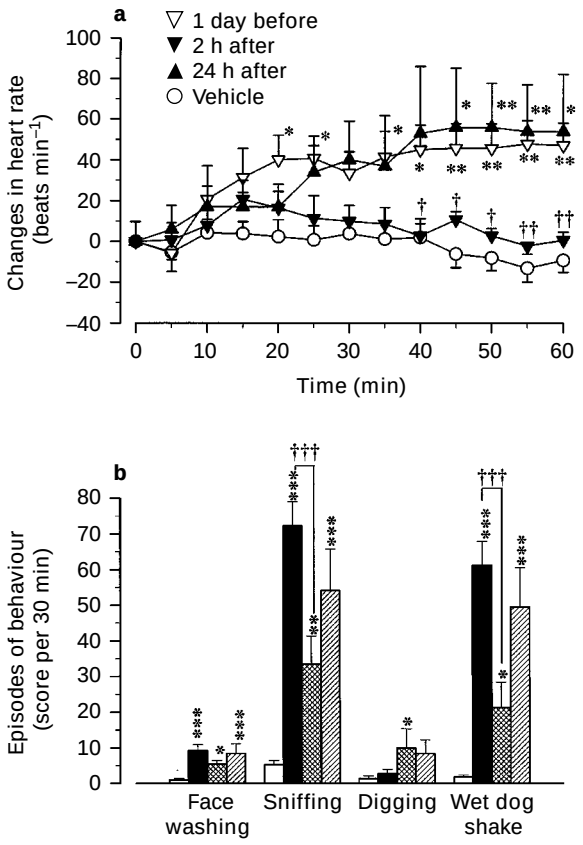

Figure 8 Effects of senktide (65 pmol per animal; i.c.v.) on changes in heart rate (HR) (a) or behaviour (b) one day before (solid columns) or 2 h (cross-hatched columns) and 24 h (hatched columns) after desensitization to SR142801 in 7 conscious rats. Changes in behaviour were measured during the first 30 min post-injection. Basal values were: MAP (mmHg): 102±3 (before), 111±5 (2 h after) and 105±5 (24 h after); HR (beats min^{-1}): 384±11 (before), 367±14 (2 h after) and 344±10 (24 h after). Statistical comparisons to vehicle (open columns; $n=13$) were calculated with a two-way ANOVA followed by a Dunnett's test (a) or a Kruskall-Wallis test and a *post-hoc* Wilcoxon Mann-Whitney test (b) and are indicated by: $*P<0.05$; $**P<0.01$; $***P<0.001$. Statistical comparisons to senktide before desensitization were calculated with a two-way ANOVA followed by a Student's t test (a) or a Kruskall-Wallis test and a *post-hoc* Wilcoxon Mann-Whitney test (b) and are indicated by: $\dagger P<0.05$; $\dagger\dagger P<0.01$; $\dagger\dagger\dagger P<0.001$.

In the present study, R820 completely abolished the pressor and tachycardiac responses to SR142801 which is in agreement with the blockade by R820 of the hypoalgesia induced by intrathecally administered senktide and SR142801 in the rat tail-flick test (Couture & Toma, 1995). In the rat isolated portal vein, R820 is a potent NK_3 receptor antagonist ($pA_2=7.60$) with low affinity for the NK_1 and NK_2 receptors (Regoli *et al*., 1994b). Indeed, R820 did not block the central cardiovascular and behavioural effects induced by the NK_1 receptor selective agonist [Sar^9, Met $(O_2)^{11}$]SP (this study) and by NPγ which acts partly through the activation of central NK_2 receptors (Picard & Couture, 1996). SR142801 seems to interact selectively with NK_3 receptors since pretreatment with a cocktail of antagonists for both NK_1 (RP67580) and NK_2 (SR48968) receptors failed to inhibit its cardiovascular effects. Both antagonists prevented the central effects of SP and NKA in the same paradigm (Picard *et al*., 1994). Thus, the results obtained with R820 together with the cross-desensitization between senktide and SR142801 strongly suggest that senktide and SR142801 act via a similar mechanism. The centrally mediated responses to [Sar^9, Met$(O_2)^{11}$]SP were not affected following desensitization to SR142801, which suggests that desensitization may be specific for the NK_3 receptor. Surprisingly, the desensitization to SR142801 partially reduced in a reversible manner the sniffing and wet dog shake behaviours induced by senktide. Although SR142801 has no direct behavioural effect, it enhanced the senktide-mediated behaviours. These observations could be explained by differential interaction of SR142801 with the NK_3 receptor, as underlined for other non peptide tachykinin receptor antagonists. Thus, SR142801 and the agonist senktide may interact with different epitopes of the receptor as is the case for the non peptide NK_1 receptor antagonists (Gether *et al*., 1994). In addition, SR142801 may stabilize the NK_3 receptor in its active conformation.

Table 4 Cardiovascular effects of senktide and non peptide NK_3 receptor compounds given i.v. to conscious rats

Antagonist	*Agonist*	*Basal MAP* (mmHg)	*ΔMAP* (mmHg)	*Basal HR* (beats min^{-1})	*ΔHR* (beats min^{-1})	n
	Saline	105±12	2±1	371±36	−9±5	35
SR142801		107±12	−1±2	396±11	−8±7	7
SR142806		105±5	−6±0	400±13	−43±12*	5
	Senktide	104±5	14±3*	390±11	71±10**	5
SR142801(5 min earlier)	Senktide	114±7	18±3**	354±22†	65±10**	
	Senktide	118±6	9±1**	389±7	84±11*	7
SR142801 (30 min earlier)	Senktide	114±4	9±1**	394±12	61±12*††	
	Senktide	110±5	9±1**	393±17	89±11**	6
SR142806 (5 min earlier)	Senktide	95±4	9±1**	388±10	61±11**†	
	Senktide	104±4	8±2*	400±13	70±8*	5
SR142806 (30 min earlier)	Senktide	98±5	11±3*	352±17†	60±3*	

Data are means±s.e.mean of (n) rats. Statistical comparison with saline ($*P<0.05$, $**P<0.01$) or senktide alone ($\dagger P<0.05$, $\dagger\dagger P<0.01$). Maximal MAP and HR changes with senktide (4 nmol kg^{-1}) were recorded at 0.5 min (MAP) or 1 min (HR) while those of NK_3 antagonists (650 nmol kg^{-1}) were recorded at 25 min.

Effects of an NK_3 receptor agonist and SR142801 injected i.v.

When injected intravenously, senktide induced transient and dose-dependent increases of MAP and HR without affecting behaviour, while when given i.c.v., senktide evoked dose-dependent increases of face washing, sniffing and wet dog shakes. This suggests that the NK_3 selective agonist may not pass through the blood brain barrier. These peripheral effects of senktide are in apparent disagreement with the finding by Pompei *et al.* (1993) who showed that NH_2-senktide and [MePhe^7]NKB are inactive on the cardiovascular system of non-anaesthetized rats. This discrepancy is likely due to the dose administered. Indeed, Pompei *et al.* (1993) used doses lower than 4 nmol kg^{-1} which were also ineffective on MAP and HR in our study. On the other hand, in the urethane-anaesthetized rats, Couture *et al.* (1989) and Cellier *et al.* (1996) have observed that senktide and other NK_3 receptor agonists ([MePhe^7]NKB, [β-Asp^4, MePhe^7]NKB(4–10) and NKB) exert opposite effects, namely hypotension and bradycardia. These effects were ascribed to a von Bezold-Jarisch reflex (Couture *et al.*, 1989). In the conscious guinea-pig, NKB, [MePhe^7]NKB and senktide elicited an increase in MAP along with a decrease in HR (Roccon *et al.*, 1996). The hypertension was ascribed to activation of the sympathetic nervous system and not to vasopressin, while the bradycardia was due to a baroreflex. NKB has an hypotensive effect in anaesthetized guinea-pigs (Lundberg *et al.*, 1985) and in anaesthetized dogs (Constantine *et al.*, 1991). Discrepancies between the various studies appear to be due to several factors, notably anaesthesia, species, doses and selectivity of the agonist.

In the periphery, SR142801 and its enantiomer SR142806 were found to be almost inactive in inhibiting the cardiovascular response to senktide (ratio antagonist: agonist = 160:1). These results contrast with those obtained by Roccon *et al.* (1996) who demonstrated that SR142801 is a potent antagonist of the pressor and bradycardiac responses to i.v. senktide in the conscious guinea-pig. These discrepancies may be due to the greater affinity of SR142801 for NK_3 receptors of human and guinea-pig versus the rat NK_3 receptor, as underscored by *in vitro* binding and functional assays (Emonds-Alt *et al.*, 1995; Patacchini *et al.*, 1995). Thus, our results confirm the existence of inter-species NK_3 receptors subtypes (Petitet *et al.*, 1993; Chung *et al.*, 1994; Regoli *et al.*, 1994b; Suman-Chauhan *et al.*, 1994; Maggi, 1995). Finally, SR142801 and SR142806 do not act as agonists at peripheral NK_3 receptors as shown by their lack of direct effects on blood pressure and heart rate. This is in accord with data from *in vitro* studies conducted on the rat portal vein, the classical preparation for studying the NK_3 receptor (Regoli *et al.*, 1994b; Patacchini *et al.*, 1995).

Conclusion

SR142801 is inactive on its own and is almost ineffective as an NK_3 receptor antagonist on the rat cardiovascular system in the periphery and on central behaviour. However, SR142801 is a centrally active agonist on the cardiovascular system. The agonistic effect of SR142801 is due to central NK_3 receptor activation since it is blocked by R820, an antagonist which also inhibits senktide. This is also supported by the cross-desensitization between the central effects of senktide and SR142801. Moreover, the central cardiovascular and behavioural effects mediated by senktide were dissociated by the selective inhibition of the former effects with R820. Taken together, these results suggest the existence of either intra-species heterogeneity for the NK_3 receptor or the differential activation and inhibition of the same receptor protein linked to the production of different second messengers. However, the present results may reflect differences in the pharmacokinetic properties of the non natural compounds. Finally, because R486 acts as an agonist at NK_1 and NK_2 receptors, R820 appears to be the most suitable NK_3 receptor antagonist currently available for studies in the rat central nervous system.

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