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Study of SR 142801, a new potent non-peptide NK_3 receptor antagonist on cardiovascular responses in conscious guinea-pig

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1 The cardiovascular responses to intravenous (i.v.) injection of natural tachykinins, substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and selective tachykinin (NK) receptor agonists, [Sar^9, Met(O$_2$)^{11}]SP, [βAla^8]NKA(4-10), [MePhe^7]NKB and senktide were assessed in conscious, freely moving, guinea-pigs.

2 SP and [Sar^9, Met(O$_2$)^{11}]SP (1-1000 pmol kg^{-1}) induced dose-dependent decreases in mean arterial blood pressure (MAP) accompanied by increases in heart rate (HR). NKA evoked only weak hypotensive effects at high doses (3000 pmol kg^{-1}) whereas [βAla^8]NKA(4-10) (1-3000 pmol kg^{-1}) had no effects. By contrast, NKB [MePhe^7]NKB (1-10000 pmol kg^{-1}) and senktide (1-1000 pmol kg^{-1}), produced dose-related hypertensive effects with the following rank order of potency: senktide > [MePhe^7]NKB > NKB. Bradycardia occurred simultaneously with the increases in arterial pressure.

3 The pressor response to intravenous injection of senktide (300 pmol kg^{-1}) was partially reduced by pretreatment with prazosin (0.71 μmol kg^{-1}), or clonidine (0.38 μmol kg^{-1}) and was completely inhibited by the combination of the two compounds. Atropine (1.5 μmol kg^{-1}) suppressed the decrease in HR induced by senktide without altering the blood pressure response. These findings suggest that the blood pressure response to senktide is an indirect effect mediated by noradrenaline released from sympathetic nerve endings, whereas the bradycardia is of vagal reflex origin.

4 SR 142801, ((S)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl) piperidin-3-yl) propyl)-4-phenyl-piperidin-4-yl)-N-methylacetamide), a potent and specific non-peptide NK_3 receptor antagonist dose-dependently (0.46-4.6 μmol kg^{-1}, i.v.; 4.6-46 μmol kg^{-1}, p.o.) inhibited the cardiovascular effects of senktide and displayed a long-lasting inhibitory effect after oral administration. By contrast, SR 142806 (4.6 μmol kg^{-1}, i.v.), the (R)-enantiomer of SR 142801 had no effect on the responses to senktide. SR 142801 at a high dose (15 μmol kg^{-1}, i.v.) was inactive toward the [Sar^9, Met(O$_2$)^{11}]SP-induced hypotension.

5 SR 142801 did not modify MAP in conscious guinea-pigs both after i.v. (4.6 and 15 μmol kg^{-1}) and oral (46 and 150 μmol kg^{-1}) administration, showing a lack of agonistic properties. However, a slight reduction in HR was observed only after i.v. injection.

6 In conclusion, these results show evident differences in the functional role of tachykinin receptors in the peripheral control of the cardiovascular system. Furthermore, a clear pressor effect of senktide, which was selectively blocked by SR 142801, was observed in conscious guinea-pigs. Hence, this antagonist appears suitable for investigating the functional role of NK_3 receptors.

Keywords: Conscious guinea-pigs; cardiovascular system; blood pressure; substance P; neurokinin A; neurokinin B; tachykinin receptors; SR 142801

Introduction

The tachykinins are a family of neuropeptides which includes substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). SP is a preferential endogenous ligand of NK_1 receptors while NKA and NKB preferentially interact with NK_2 and NK_3 receptors, respectively. These receptors are widely distributed in the central nervous system and peripheral tissues where they are believed to subserve several neurotransmitter functions (Otsuka & Yoshioka, 1993).

Since the discovery of the hypotensive property of SP, great interest has been focused on the peripheral cardiovascular effects of tachykinins. The use of synthetic agonists which are more selective than SP, NKA and NKB for their respective receptors (Regoli *et al.*, 1988) and the discovery of potent and selective non-peptide receptor antagonists (Garret *et al.*, 1991, Snider *et al.*, 1991; Emonds-Alt *et al.*, 1992; 1993) have widely contributed to the characterization of the peripheral action of tachykinins. However, the role of NK_3 receptors in cardiovascular regulation is not well understood at present. Moreover, the activation of this receptor seems to produce different effects according to the experimental conditions and the animal species used. In urethane-anaesthetized rats NKB and selective NK_3 receptor agonists such as senktide, [MePhe^7]NKB and [β-Asp^4, MePhe^7]NKB(4-10) produced a pronounced hypotensive effect (Couture *et al.*, 1989). By contrast, in conscious, freely moving, spontaneously hypertensive and Wistar Kyoto rats, NH_2-senktide and [MePhe^7]NKB did not significantly modify blood pressure (Pompei *et al.*, 1992). On the other hand, in pentobarbitone-anaesthetized dogs, NKB caused dose-related decreases in blood pressure but senktide elicited only weak hypotensive or hypertensive responses at high doses (Constantine *et al.*, 1991). Finally, in anaesthetized guinea-pigs NKB was potently hypotensive (Lundberg *et al.*, 1985; Aursudkij *et al.*, 1988). However, concerning the effects of NKB, it should be taken into account that this neuropeptide is not selective for the NK_3 receptor since it is also a potent agonist to NK_1 receptors (Drapeau *et al.*, 1987) and could, in this way, produce hypotension via NK_1 receptors.

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Until now, the lack of a potent and selective receptor antagonist has limited the understanding of the role played by the NK_3 receptor in the cardiovascular regulation. Recently, the discovery of SR 142801 ((S)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl) propyl)-4-phenylpiperidin-4-yl)-N-methylacetamide) (Emonds-Alt *et al.*, 1995), the first potent non-peptide NK_3 receptor antagonist, offered us the possibility to investigate further the role of NK_3 receptors. SR 142801 potently inhibited the binding of [^{125}I]-iodohistidyl-[MePhe^7]NKB to tachykinin NK_3 receptors from various species (guinea-pig and gerbil brain cortex: $K_i = 0.11 \pm 0.01$ and 0.42 ± 0.04 nM, respectively) and cloned human NK_3 receptors (CHO cells: $K_i = 0.21 \pm 0.03$ nM) but was less active on NK_3 receptors from rat brain cortex ($K_i = 15 \pm 3$ nM; [^{125}I]-eledoisin) (Emonds-Alt *et al.*, 1995).

The purpose of the present study was two fold: first, to measure the cardiovascular responses to i.v. administered tachykinins and selective tachykinin receptor agonists in conscious guinea-pigs; second, to determine the effects of SR 142801 and its less potent (**R**)-enantiomer, SR 142806 (Emonds-Alt *et al.*, 1995) on the responses to tachykinins. The effects of SR 142801 and SR 142806 on resting cardiovascular parameters were also studied. Furthermore, we were also interested in determining the origin of the cardiovascular effects induced by NK_3 receptor agonists.

Some of the results of this investigation were recently communicated to the British Pharmacological Society (Nisato *et al.*, 1995).

Methods

Preparation of the animals

Male Hartley guinea-pigs (Charles River, Saint-Aubin-lès-Elbeuf, France) weighing 250–350 g were used in this study. The animals were allowed free access to standard laboratory food and water and maintained under constant temperature (21 ± 2°C) and lighting conditions (12 h light cycle: 07 h 00 min to 19 h 00 min).

The animals were anaesthetized with an intramuscular injection of ketamine (80 mg kg^{-1}) and xylazine (10 mg kg^{-1}); the left carotid artery and the right jugular vein were cannulated for measurement of blood arterial pressure and injection of drugs, respectively. The catheters (PE 50, PE 10 at tip) were filled with physiological saline containing heparin (500 i.u. ml^{-1}), passed through a subcutaneous tunnel, and exteriorized at the back of the neck. Following surgery, the guinea-pigs were housed individually with free access to food and water and allowed to recover for at least 48 h.

Experiments were conducted in conscious guinea-pigs kept in a cylindrical plastic cage. The catheters were protected with a swivel-theter system that allowed movement in the cage. Mean arterial blood pressure (MAP) was recorded by connecting the arterial catheter to a Gould (TA 2000) polygraph via a Statham pressure transducer (P23ID). Heart rate (HR) was measured from the pulsatile arterial pressure signals triggering a cardiotachometer. Experiments were started after a resting period of about 60 min to establish baseline MAP and HR.

All the protocols involved in this study were approved by the Comité Expérimentation Animale (Animal Care and Use Committee) of Sanofi Recherche.

Experimental protocols

Cardiovascular effects of neurokinins and NK receptor selective agonists In the first series of experiments, the effects of cumulative increasing doses of SP (1–1000 pmol kg^{-1}), NKA (1–3000 pmol kg^{-1}) and NKB (1–10000 pmol kg^{-1}) and selective agonists for tachykinin receptors, [Sar^9, Met(O$_2$)^{11}]SP (1–1000 pmol kg^{-1}), [βAla^8]NKA(4–10) (1–3000 pmol kg^{-1}), [MePhe^7]NKB (1–10000 pmol kg^{-1}) and senktide (1–1000 pmol kg^{-1}) on MAP and HR were investigated following i.v. administration. Injections were started after saline administration and peptides were delivered in a volume of 1 ml kg^{-1} of body weight. The catheter was flushed with 0.1 ml saline. A subsequent injection of peptide was given only after the guinea-pig had recovered from the cardiovascular effect of the previous injection.

Mechanism of the cardiovascular response to senktide The previous experiments showed that the activation of NK_3 receptors induced hypertensive effect and bradycardia. In a second series of experiments, the possible mechanism responsible for these effects has been investigated. The participation of the sympathetic and parasympathetic components of the autonomic nervous system in the pressor response to senktide was examined using a selective α_1-receptor antagonist (prazosin), a selective α_2-presynaptic receptor agonist (clonidine) and a muscarinic receptor antagonist (atroprine). The effects of a 300 pmol kg^{-1} dose of senktide were measured before (30 and 15 min), and 15 min after i.v. administration of prazosin (0.71 μmol kg^{-1}), clonidine (0.38 μmol kg^{-1}), prazosin and clonidine (0.71 + 0.38 μmol kg^{-1}) or atropine (1.5 μmol kg^{-1}). The doses of these compounds were selected according to preliminary experiments: inhibition of hypertension (prazosin) and bradycardia (atropine) to noradrenaline and hypertension (clonidine) to senktide. The MAP and HR responses to the first two injections of senktide were averaged and the resulting means were taken to be the baseline responses.

Effects of SR 140333 and SR 48968 on the cardiovascular responses to senktide In this series of experiments, the selectivity of the cardiovascular responses to senktide against NK_1 and NK_2 receptors was investigated. This was accomplished by injecting a 300 pmol kg^{-1} dose of senktide 30 and 15 min before and then 15–180 min after i.v. administration of the selective NK_1 (SR 140333), NK_2 (SR 48968) receptor antagonists at the dose of 4.4 and 5 μmol kg^{-1}, respectively. The MAP and HR responses to the first two injections of senktide were averaged and the resulting means were taken to be the baseline responses.

Intrinsic effects of SR 142801 and SR 142806 on blood pressure and heart rate The intrinsic properties of SR 142801 and 142806 on MAP and HR were investigated. In a first experiment these compounds were injected cumulatively at a 1 h interval at doses of 4.6 and 15 μmol kg^{-1}; the vehicle (dimethylformamide (DMF) 30%) was injected in the same animals, 30 min before each dose. Another experiment consisted of testing the effect of a single dose oral administration of SR 142801 at 46 and 150 μmol kg^{-1}; MAP and HR were monitored for 3 h post-drug.

Effects of SR 142801 and SR 142806 on the cardiovascular responses to senktide

The ability of SR 142801 and SR 142806, to antagonize the cardiovascular responses to senktide was evaluated. When these compounds were administered intravenously, the experimental protocol was the same as that described for the study of the effects of SR 140333 and SR 48968 on the response to senktide; the same solvent-treated animals (DMF 10%, i.v.) were used in the two studies. SR 142801 was injected at doses of 0.46, 1.5 and 4.6 μmol kg^{-1} and SR 142806 at 4.6 μmol kg^{-1}. When SR 142801 was administered orally (4.6–46 μmol kg^{-1}), senktide was injected 30 and 15 min before and then 0.25–24 h after treatment. Between the 7th and 24th hour after SR 142801 administration, the guinea-pigs were disconnected from the pressure transducer and returned to the holding cage with access to food and water.

Effects of SR 142801 on the cardiovascular responses to [Sar^9, Met(O$_2$)^{11}]SP The selectivity of SR 142801 against NK_1

receptors was studied by testing this compound (15 μmol kg^{-1}, i.v.) on the cardiovascular responses to a 100 pmol kg^{-1} [Sar^9, $Met(O_2)^{11}$]SP. The protocol was the same as that previously described for the i.v. study of SR 142801 except that [Sar^9, $Met(O_2)^{11}$]SP was injected instead of senktide.

Drugs

Peptides were purchased from Bale Biochimie SARL (Voisins-le-Bretonneux, France). The non-peptide NK_1 antagonist, SR 140333, (1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-3-yl]ethyl}-4-phenyl-1-azonia-bicyclo-[2.2.2]octane,chloride), NK_2 antagonist, SR 48968 ((S)-N-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide), and NK_3 antagonists, SR 142801 and its (R)-enantiomer, SR 142806, were synthesized at Sanofi Recherche (Montpellier, France). Atropine sulphate, prazosin and clonidine were obtained from Sigma Chemical Co. SP, NKA, [Sar^9, $Met(O_2)^{11}$]SP, senktide, and [$MePhe^7$]NKB were prepared in distilled water as 1 mM stock solutions. NKB and [βAla^8]NKA(4–10) were dissolved in dimethyl sulphoxide (DMSO) and distilled water added to produce a 1 mM stock solution (final concentration of DMSO was 10%). Solutions of peptides were divided into aliquots of 20 μl each, and stored at −20°C until used. Daily dilutions were made in saline. For intravenous administration, SR 140333, SR 48968, SR 142801 and SR 142806 were dissolved in DMF and the solution made up in distilled water (final concentrations of DMF were 10% or 30% depending on the doses of the compound used). For oral administration, SR 142801 was given in suspension in 0.6% methyl cellulose solution. Atropine sulphate and clonidine were prepared in saline whereas prazosin was dissolved in distilled water containing 5% ethanol. All the compounds were delivered in a volume of 1 and 5 ml kg^{-1} for intravenous and oral administration, respectively.

Statistical analysis

The results are expressed as mean ± standard error of the mean (s.e.mean). The potency of each peptide to induce blood pressure responses was evaluated by calculating the ED_{20} which is the dose required to produce a change in MAP of 20 mmHg; this calculation was performed by use of weighted least-square fitting of experimental data to a 4 parameter logistic model (Ratkowski & Reedy, 1986) with a non-linear curve fitting RS/1 software. Confidence intervals were calculated by the Newton method. Statistical analysis of data was performed by use of a two-way analysis of variance (ANOVA) followed by a Dunnett's comparison test. A probability value of less than 0.05 was considered statistically significant.

Results

Baseline MAP and HR of conscious guinea-pigs involved in this study ($n=190$) averaged 62 ± 1 mmHg and 353 ± 3 beats min^{-1}, respectively.

Cardiovascular effects of neurokinins and NK receptor selective agonists

The responses to these peptides have been evaluated by measuring the maximal effects on MAP and HR after intravenous injections.

Cumulative injections of increasing doses of SP and [Sar^9, $Met(O_2)^{11}$]SP induced dose-related decreases in MAP accompanied by increases in HR (Figure 1). The hypotensive potencies of these two peptides were similar since the ED_{20} (pmol kg^{-1}) was 85 (confidence interval (C.I.), 58–129) and 80 (C.I., 41–178) for SP and [Sar^9, $Met(O_2)^{11}$SP], respectively. The MAP and HR responses lasted from a few seconds at the lowest doses to 4–5 min at 1000 pmol kg^{-1}. At the highest doses (from 300 pmol kg^{-1}), in most animals, the increases in HR produced by SP and [Sar^9, $Met(O_2)^{11}$]SP were followed by a bradycardia; this could explain why the HR responses reached a plateau at these doses.

NKA induced modest and non dose-related hypotensive responses (data not shown). The maximal decrease in MAP (15 ± 2 mmHg, $n=6$) was observed at 3 nmol kg^{-1}; and HR was increased by about 40 beats min^{-1}. [βAla^8]NKA(4–10) at doses up to 3000 pmol kg^{-1} ($n=6$) caused only trivial modifications of blood pressure and HR (data not shown). Because of the weak and variable MAP and HR responses to NKA and [βAla^8]NKA(4–10) these two peptides were excluded from further study.

NKB, [$MePhe^7$]NKB and senktide produced dose-related increases in blood pressure accompanied by reductions in HR (Figure 2). When senktide was injected at 3000 and 10000 pmol kg^{-1} we did not observe a further significant increase in blood pressure compared to the dose of 1000 pmol kg^{-1} (data not shown). Moreover, at these doses, some animals showed clear signs of discomfort. The ED_{20} (pmol kg^{-1}) for each compound in order of vasoconstrictor potency was as follows: senktide, 76 (C.I., 50–117) > [$MePhe^7$]NKB, 522 (C.I., 325–838) > NKB, 6577 (C.I., 1815–undefined). This rank order of potency was not observed for HR; however, the large variability of the HR responses should be mentioned. The pressor effect lasted from a few seconds to 3–4 min according to the doses. Because senktide was the most potent compound to induce MAP response it was used in the following studies at a dose of 300 pmol kg^{-1} which caused an almost maximal blood pressure increase (about 25 mmHg).

Mechanism of the cardiovascular response to senktide

The effects of prazosin, clonidine, combined treatment with prazosin and clonidine and atropine, on the cardiovascular responses to senktide are illustrated in Figure 3.

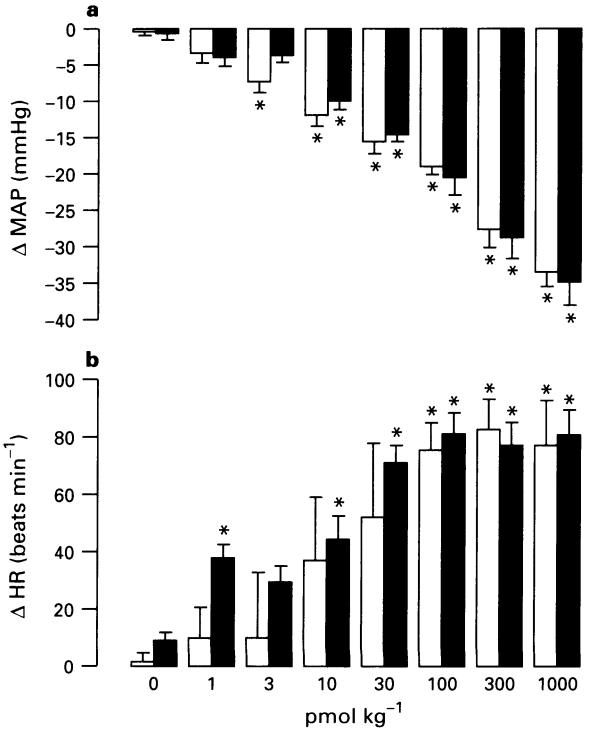

Figure 1 Maximal effects of cumulative intravenous injections of substance P (SP) (open columns, $n=6$), and [Sar^9, $Met(O_2)^{11}$]SP (solid columns, $n=8$) on (a) mean arterial pressure (MAP) and (b) heart rate (HR) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines. *$P<0.05$ compared to vehicle (analysis of variance followed by Dunnett's test).

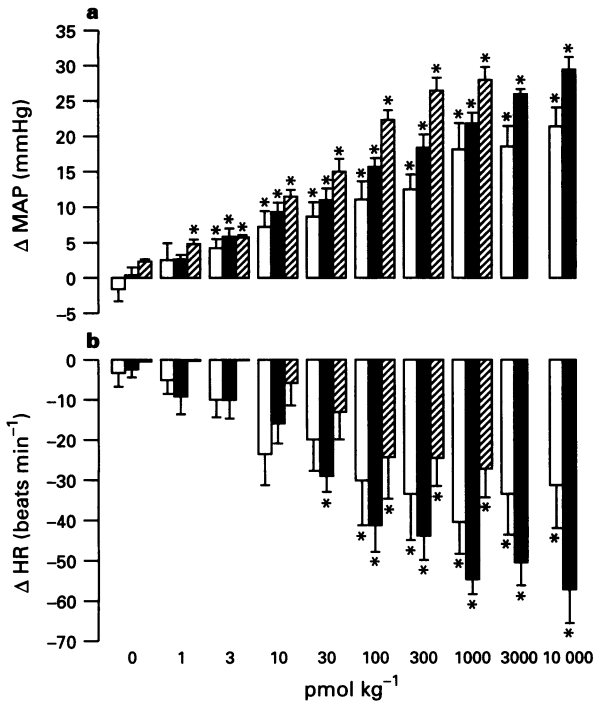

Figure 2 Maximal effects of cumulative intravenous injections of neurokinin B (NKB) (open columns, $n=6$), [MePhe^7]NKB (solid columns, $n=9$) and senktide (hatched columns, $n=7$) on (a) mean arterial pressure (MAP) and (b) heart rate (HR) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines. *$P<0.05$ compared to vehicle (analysis of variance followed by Dunnett's test).

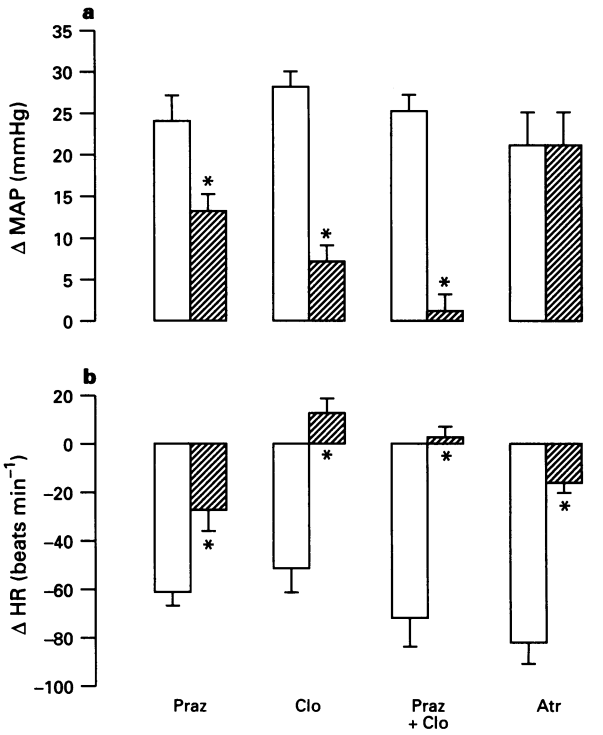

Figure 3 (a) Mean arterial pressure (MAP) and (b) heart rate (HR) responses to senktide (300 pmol kg^{-1}, i.v.) before (open columns) and 15 min after (hatched columns) pretreatment with prazosin (Praz, 0.71 μmol kg^{-1}), clonidine (Clo, 0.38 μmol kg^{-1}), prazosin + clonidine (0.71 + 0.38 μmol kg^{-1}) and atropine (Atr, 1.5 μmol kg^{-1}) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines ($n=6-8$ in each group). *$P<0.05$ compared to pretreatment responses (analysis of variance followed by Dunnett's test).

Prazosin (0.71 μmol kg^{-1}, i.v.) caused a pronounced decrease in blood pressure (43 ± 1 from 62 ± 2 mmHg, $P<0.05$) without significantly affecting HR. Pretreatment with prazosin significantly reduced both the pressor response and the decrease in HR induced by senktide by about 50% (Figure 3).

The administration of clonidine (0.38 μmol kg^{-1}, i.v.) produced a marked increase in MAP (about 40 mmHg from a baseline of 63 ± 2 mmHg) and the recovery of baseline blood pressure was observed after 10 min. This effect was accompanied by a sustained bradycardia (265 ± 6 from 350 ± 10 beats min^{-1}, $P<0.05$). The injection of senktide, 15 min after clonidine, evoked only a weak pressor effect (7 ± 2 mmHg) which corresponds to a 75% inhibition compared to the control response (Figure 3). The injection of senktide showed a tendency to reverse the bradycardia elicited by clonidine.

The combination of prazosin and clonidine (0.71 + 0.38 μmol kg^{-1}) produced first a pressor response (about 20 mmHg) which lasted for about 2–3 min followed by a sustained fall in blood pressure (55 ± 4 mmHg compared to a starting value of 65 ± 4 mmHg, $P<0.05$). At the same time, a decrease in HR was observed (268 ± 11 beats min^{-1} compared to 357 ± 11 beats min^{-1}, $P<0.05$). Pretreatment with the combination of prazosin and clonidine completely abolished both the MAP and HR responses to senktide (Figure 3).

Pretreatment with atropine (1.5 μmol kg^{-1}, i.v.) reduced baseline MAP to 49 ± 4 from 59 ± 3 mmHg ($P<0.05$) whereas HR was slightly but not significantly increased (382 ± 7 from 360 ± 15 beats min^{-1}). The pressor response to senktide (300 pmol kg^{-1}, i.v.) injected 15 min after atropine was not modified whereas the bradycardia which accompanied this hypertensive effect was reduced by about 80% ($P<0.05$) (Figure 3).

Effects of SR 140333 and SR 48968 on the cardiovascular responses to senktide

Figure 4 illustrates the kinetics of the effects of the NK_1 (SR 140333) and NK_2 SR 48968) receptor antagonists, 4.4 and

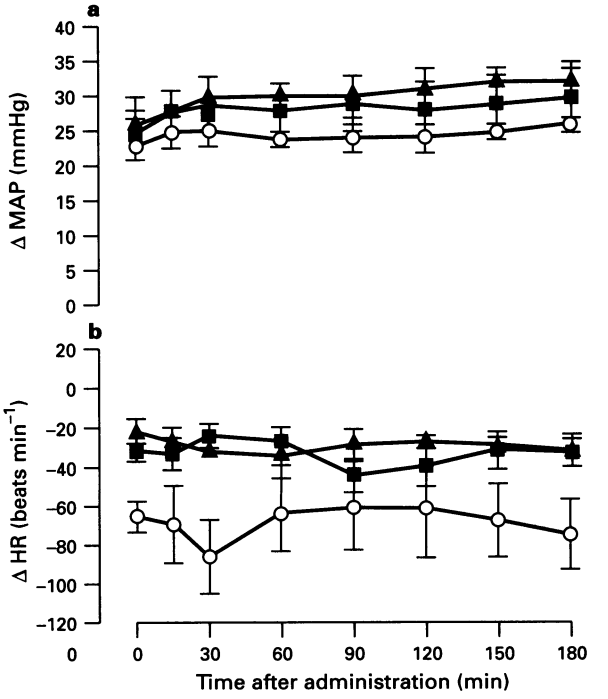

Figure 4 Kinetics of the effects of intravenous administration of SR 140333 4.4 μmol kg^{-1} (■), SR 48698 5 μmol kg^{-1} (▲) and vehicle (○) on (a) mean arterial pressure (MAP) and (b) heart rate (HR) responses to senktide (300 pmol kg^{-1}, i.v.) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines ($n=6$ in each group).

5 μmol kg^{-1} i.v. respectively, on the cardiovascular responses to 300 pmol kg^{-1} senktide. The doses of SR 140333 and 48968 are about 75 times higher than the ID_{50} of the [Sar^9, Met $(O_2)^{11}$]SP and [Nle^{10}]-NKA(4–10)-induced bronchoconstriction in anaesthetized guinea-pigs, respectively (Emonds-Alt *et al.*, 1992; 1993).

The administration of solvent (DMF 10%) did not significantly modify the MAP response to senktide over the 3 h post drug period. A reliable and reproducible response to senktide was observed after repeated injections (7 injections, 30 min apart). SR 140333 and SR 48968 had no significant effect on MAP and HR responses throughout the observation period. Despite a large variability of the HR decreases to senktide among the 3 groups (basal responses: 23, 33 and 66 beats min^{-1}) these effects were not modified by either the vehicle or the compounds.

Intrinsic effects of SR 142801 and SR 142806 on blood pressure and heart rate

Cumulative i.v. doses of SR 142801 and SR 142806 The effects of cumulative intravenous doses of SR 142801 or SR 142806 are presented in Figure 5. The administration of vehicle (DMF 30%, i.v.) caused a slight hypotension (4 to 6 mmHg) accompanied by a transient decrease in HR (10 to 20 beats min^{-1}) in both groups. This effect on MAP was not significantly modified by cumulative injections of 4.6 and 15 μmol kg^{-1} SR 142801. SR 142806, 4.6 μmol kg^{-1}, did not show any effect on MAP whereas at 15 μmol kg^{-1} this compound seemed to induce a further decrease in MAP (about 11 mmHg, $P<0.05$). By contrast, the administration of these two compounds at a dose of 4.6 μmol kg^{-1} produced decreases in HR (50 to 70 beats min^{-1}) which were statistically significant compared to the respective preinjection values. HR had not completely returned to baseline when the additional dose of 15 μmol kg^{-1} was administered. This last dose caused a further decrease in HR (about 70 beats min^{-1}) which persisted throughout the observation period.

Single oral dose of SR 142801

Single oral administration of SR 142801 at 46 and 150 μmol kg^{-1} did not cause any effects on MAP and HR. Only a slight hypotension (5 mmHg) was observed 2 h after the administration of the highest dose ($P<0.05$ compared with the vehicle control group).

Effects of SR 142801 and SR 142806 on the cardiovascular responses to senktide

Effect of intravenous administration of SR 142801 and SR 142806

Figure 6 illustrates the kinetics of the effects of intravenous administration of SR 142801 and SR 142806 on the cardiovascular responses to 300 pmol kg^{-1} senktide. At a dose of 0.46 μmol kg^{-1} SR 142801 did not cause any significant modification of the MAP response to senktide. By contrast, the two highest doses induced inhibitions which were about 60 and 80% at 1.5 and 4.6 μmol kg^{-1}, respectively. These inhibitory effects were maximal 15 min post drug and lasted about 30 min after 1.5 μmol kg^{-1} and 1 h after 4.6 μmol kg^{-1} (Figure 6). Inhibition of the decrease in HR induced by senktide was also observed at these two highest doses. Furthermore, this inhibitory effect was statistically significant ($P<0.05$ compared with the vehicle control group) throughout

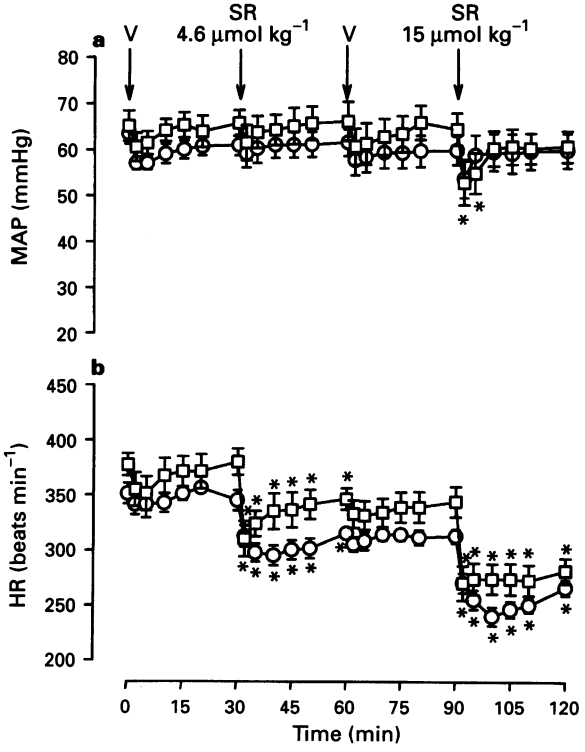

Figure 5 Effects of cumulative intravenous administration of SR 142801 (○) or SR 142806 (□), 4.6+15 μmol kg^{-1}, on (a) mean arterial pressure (MAP) and (b) heart rate (HR) in conscious guinea-pigs. Vehicle (V) was administered 30 min before each dose. Values are mean with s.e.mean shown by vertical lines ($n=8$ per group). *$P<0.05$ versus corresponding last preinjection value (analysis of variance followed by Dunnett's test).

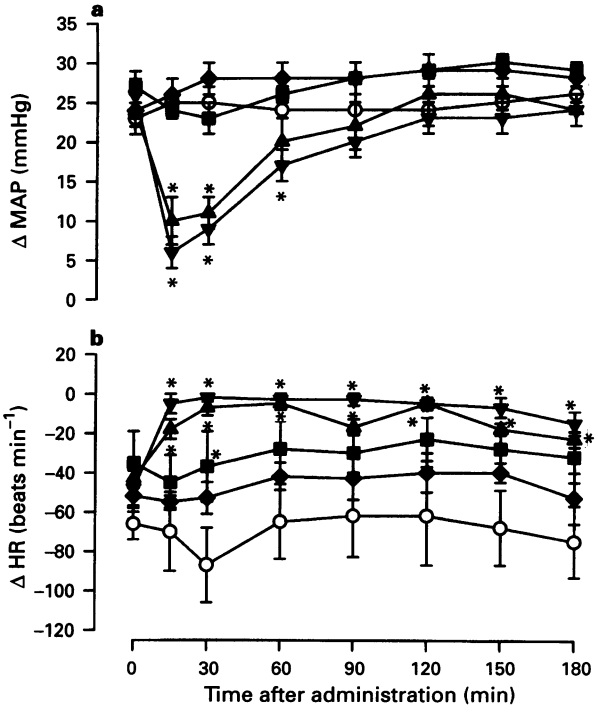

Figure 6 Kinetics of the effects of intravenous administration of SR 142801 0.46 (■), 1.5 (▲) and 4.6 μmol kg^{-1} (▼), SR 142806 4.6 μmol kg^{-1} (◆) and vehicle (○) on (a) mean arterial pressure (MAP) and (b) heart rate (HR) responses to senktide (300 pmol kg^{-1}, i.v.) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines ($n=6$ in each group). *$P<0.05$ versus vehicle-treated group (analysis of variance followed by Dunnett's test).

the 3 h post drug period. In contrast to the effects of SR 142801, the cardiovascular response to senktide was not affected by SR 142806 (Figure 6).

Effects of oral administration of SR 142801 The kinetics of the effects of oral administration of SR 142801 on the cardiovascular responses to 300 pmol kg^{-1} senktide are presented in Figure 7. In the vehicle control group, the MAP and HR responses to senktide were not affected either throughout the 7 h post drug period or 24 h later. SR 142801 4.6, 15 and 46 μmol kg^{-1} caused dose-related inhibition of the pressor response to senktide. The onset of the inhibitory effect was within 15 min suggesting a rapid absorption in the intestinal tract. The inhibition reached about 80% at 46 μmol kg^{-1} and, at the two highest doses, it was still present 7 h post drug ($P<0.05$ compared with the vehicle control group). In a similar manner, SR 142801 also inhibited the decrease in HR induced by senktide (Figure 7). The MAP and HR responses to senktide had completely returned to baseline 24 h after the administration of these two antagonists.

Effects of SR 142801 on the cardiovascular responses to [Sar^9, Met$(O_2)^{11}$]SP

Figure 8 illustrates the kinetics of the effects of intravenous administration of SR 142801 on the cardiovascular responses to 100 pmol kg^{-1} [Sar^9, Met$(O_2)^{11}$]SP. No statistically significant differences in the MAP and HR responses to [Sar^9, Met$(O_2)^{11}$]SP between vehicle and SR-treated groups was observed throughout the study (Figure 8). The decrease of about 35% of the [Sar^9, Met$(O_2)^{11}$]SP-induced hypotension which was observed 15 min post treatment both in the vehicle and in the SR-treated group could be due to the lower baseline blood pressure induced by the high concentration of solvent (DMF 30%) and that had already been observed in previous experiments (Figure 5). Moreover, the MAP response to [Sar^9, Met$(O_2)^{11}$]SP showed a tendency to decrease with time in the two groups.

Discussion

The cardiovascular effects of tachykinins and selective tachykinins receptor agonists have been investigated in conscious guinea-pigs. SP and the selective NK_1 receptor agonist [Sar^9, Met$(O_2)^{11}$]SP were equipotent in inducing a vasodepressor response. Previous studies have suggested that the hypotensive effect of SP is mediated by a direct action on endothelial NK_1 receptors of arterial blood vessels via the release of an endothelium-derived relaxing factor (EDRF) (D'Orléans-Juste *et al.*, 1985). The hypotensive effect was accompanied by an increase in HR followed by bradycardia at the highest doses (from 300 pmol kg^{-1}). Since tachycardia followed the onset of the hypotension, and taking into account that SP has no direct positive chronotropic effect on guinea-pig isolated heart (Burcher *et al.*, 1977; Lundberg *et al.*, 1985), the observed tachycardia was suggested to be mainly a vagal reflex response. Some investigators have shown that bolus administration of SP can elicit bradycardia and a decrease in perfusion pressure in guinea-pig isolated perfused heart (Hoover & Hancock, 1988). These authors showed that specific SP binding sites were present in cardiac parasympathetic ganglia and suggested that SP caused bradycardia by stimulating intrinsic cholinergic neurones. Furthermore, Hoover (1990) found that this negative chronotropic response to SP was blocked by atropine. Consequently, these findings support the speculation that the bradycardia observed at high doses of SP and [Sar^9, Met$(O_2)^{11}$]SP in conscious guinea-pigs may be due to a facilitatory action of NK_1 receptors in neurotransmission in cardiac parasympathetic nerve endings.

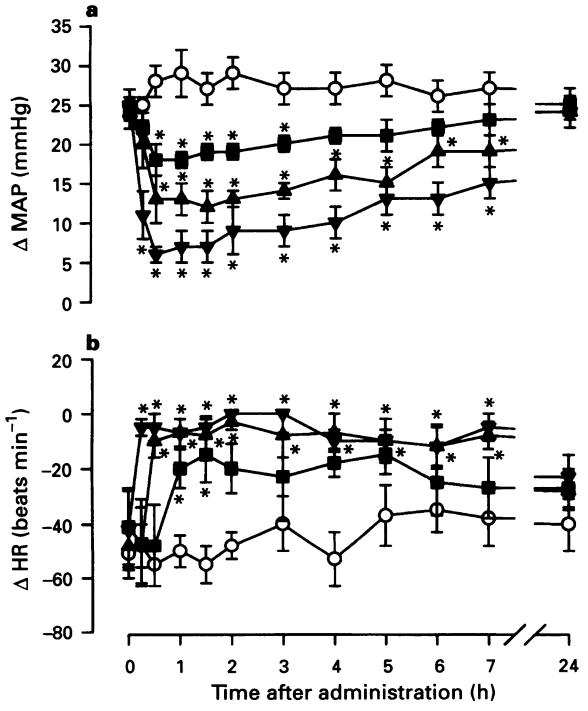

Figure 7 Kinetics of the effects of oral administration of SR 142801 4.6 (■), 15 (▲) and 46 μmol kg^{-1} (▼) and vehicle (○) on (a) mean arterial pressure (MAP) and (b) heart rate (HR) responses to senktide (300 pmol kg^{-1}, i.v.) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines ($n=6$ in each group). *$P<0.05$ versus vehicle-treated group (analysis of variance followed by Dunnett's test).

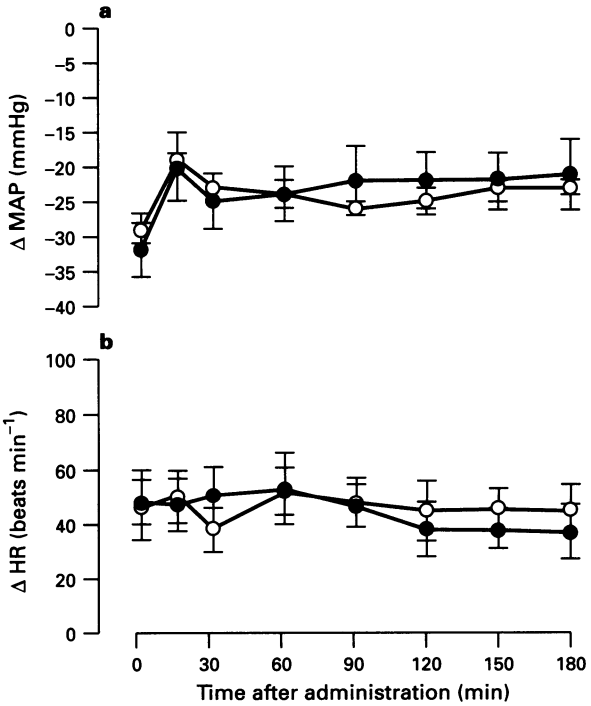

Figure 8 Kinetics of the effects of intravenous administration of SR 142801 15 μmol kg^{-1} (●), and vehicle (○) on (a) mean arterial pressure (MAP) and (b) heart rate (HR) responses to [Sar^9, Met$(O_2)^{11}$]SP (100 pmol kg^{-1}, i.v.) in conscious guinea-pigs. Values are mean with s.e.mean shown by vertical lines ($n=6$ per group).

NKA and the selective NK_2 receptor agonist [β-Ala^8]NKA(4-10) were also tested. NKA induced only weak hypotensive effects at high doses (3 nmol kg^{-1}) whereas [β-Ala^8]NKA(4-10) did not evoke significant changes in arterial blood pressure. These observations strongly suggest that the hypotension observed after NKA administration is probably not due to activation of NK_2 receptors. Similar findings have been described in both conscious and anaesthetized rats (Couture *et al.*, 1989; Pompei *et al.*, 1992).

By contrast, NKB and the two selective NK_3 receptor agonists, [MePhe^7]NKB and senktide, produced dose-related hypertensive effects with the following rank order of potency: senktide > [MePhe^7]NKB > NKB. Bradycardia occurred simultaneously with the increases in arterial pressure. When administered in urethane-anaesthetized guinea-pigs all NK_3 receptor agonists also induced hypertensive effects (data not shown); these findings are in sharp contrast to the marked hypotensive effect of these three peptides described by Couture *et al.* (1989) in urethane-anaesthetized rats. Taken together these results may suggest a different functional role for NK_3 receptors in the control of the cardiovascular system in different species. In conscious guinea-pigs, the hypertensive response to senktide was not affected by selective NK_1 and NK_2 receptor antagonists, indicating that this agonist elicits specific responses via activation of NK_3 recpeptors. Furthermore, reliable and reproducible responses were obtained after repeated injections indicating an absence of tachyphylaxis.

The mechanism whereby the activation of NK_3 receptors induces a hypertensive effect and bradycardia has been investigated. The pressor response to intravenous injection of senktide was partially reduced by pretreatment with prazosin or clonidine and was completely inhibited by the combination of these two compounds. Based on these results, the mechanism of hypertension produced by senktide appears to be due to the activation of the sympathetic nervous system and these findings support the notion that the blood pressure response to senktide is an indirect effect probably mediated by noradrenaline released from sympathetic nerve endings. The mechanism by which the activation of NK_3 receptors induces an increase of sympathetic activity remains to be elucidated. On the other hand, we showed that atropine suppressed the decrease in HR induced by senktide without altering the blood pressure response which suggests that the bradycardia is a reflex response mediated by vagal stimulation.

The cardiovascular responses induced by senktide were dose-dependently inhibited by SR 142801, whereas SR 142806 the less potent (**R**)-enantiomer was without effect. These findings provide further evidence that the pressor response to senktide is selectively mediated by NK_3 receptors. Moreover, after oral administration, the onset of the inhibitory effect was within 15 min which suggests a rapid absorption from the gastrointestinal tract. It is noteworthy that the rapid inhibitory effect of SR 142801 occurring after either i.v. or oral administration contrasts with the long incubation time required by this compound (120-140 min) to exert full activity *in vitro* (Emonds-Alt *et al.*, 1995; Patacchini *et al.*, 1995). Such discrepancies were also observed for SR 140333 and SR 48968, two antagonists bearing a chemical structure similar to SR 142801 (Emonds-Alt *et al.*, 1992; 1993). Interestingly, Maggi *et al.* (1993) observed that such a long incubation was not necessary when the effects of high concentrations (up to 100 nM) of SR 48968, were evaluated *in vitro* (rabbit pulmonary artery and hamster trachea). As suggested by these authors, it may be that low concentrations of this compound require a longer time to equilibrate than higher ones. Although an extrapolation between *in vitro* and *in vivo* results is open to criticism, it is possible that the rapid onset of the inhibitory effect observed *in vivo* after either i.v. or oral administration of SR 142801, is a consequence of the higher drug concentrations than those used *in vitro*.

The failure of SR 142801 to reduce the hypotension induced by [Sar^9, Met(O$_2$)^{11}]SP, a selective NK_1 receptor agonist, in conscious guinea-pigs, supports the view that it is a specific antagonist at the NK_3 receptors. Because of the non significant and reproducible cardiovascular effects of NKA and the selective NK_2 receptor agonist, the interaction of SR 142801 with NK_2 receptors could not be tested in this model. However, this compound was completely inactive in the bronchoconstriction induced by (Nle^{10})NKA-(4-10) in anaesthetized guinea-pigs, where the role of NK_2 receptors has been well established (Emonds-Alt *et al.*, 1995).

The study of the intrinsic cardiovascular effects of SR 142801 in conscious guinea-pigs showed a lack of any agonistic property of this compound since it did not increase arterial blood pressure at doses up to 15 μmol kg^{-1} i.v., or 150 μmol kg^{-1} p.o. However, after i.v. administration at doses of 4.6 and 15 μmol kg^{-1}, SR 142801 elicited decreases in heart rate. The fact that SR 142806, the (**R**)-enantiomer which was inactive against senktide-induced hypertension, reduced heart rate in a similar manner may suggest that this bradycardia is not related to the blockade of NK_3 receptors. SR 142801 has been shown to interact with verapamil-sensitive calcium channels at μM concentration (Emonds-Alt *et al.*, 1995). This seems the most likely source of the bradycardia caused by i.v. doses of this compound and also by SR 142806. Nevertheless, when SR 142801 was administered orally at high doses (up to 150 μmol kg^{-1}), no reduction of baseline HR was observed. A high plasma drug concentration after i.v. injection could be responsible for the discrepancy between i.v. and oral administration.

In conclusion, this study revealed evident differences in the functional role for tachykinins in the peripheral control of the cardiovascular system in conscious guinea-pigs. The most surprising finding was the clear pressor effect induced by NKB and specific NK_3 receptor agonists. Since SR 142801, the first potent orally active non-peptide NK_3 receptor antagonist selectively blocked these effects, this antagonist appears to be suitable for investigating the functional role of NK_3 receptors.

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