Effects of the tachykinin NK₁ receptor antagonist, RP 67580, on central cardiovascular and behavioural effects of substance P, neurokinin A and neurokinin B

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1 We have investigated the effects of the non-peptide NK₁ tachykinin receptor antagonist, RP 67580, and its inactive enantiomer, RP 68651, on the cardiovascular and behavioural responses to substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) injected intracerebroventricularly (i.c.v.) in conscious rats.

2 The SP and NKA (25 pmol)-induced increases in blood pressure (BP) and heart rate (HR) were of the same magnitude. The cardiovascular responses to both peptides were associated with excessive grooming behaviour and wet dog shakes (WDS). Relative to SP, NKA was weaker in inducing hindquarter grooming (HG), but more effective in eliciting WDS. The cardiovascular response to NKB (50 pmol) comprised an increase in BP and HR, while the behavioural response was weak.

3 RP 67580 (100 pmol), injected 10 or 30 min prior to SP, effectively inhibited the cardiovascular and behavioural responses to the peptide whereas lower doses were ineffective. Pretreatment with 500 pmol of RP 67580, 10 or 30 min prior to SP, reduced the BP response. Of the behavioural manifestations, only face washing was attenuated when the antagonist was injected 10 min before SP. At 2500 pmol, the antagonist exaggerated the BP response to the peptide without affecting the behavioural response. RP 68651 (100 or 2500 pmol) did not modify the central responses to SP.

4 Neither RP 67580 nor RP 68651 (100 pmol), affected the cardiovascular and behavioural responses to NKA or NKB.

5 Our results indicate that RP 67580 is a selective and high affinity antagonist at central NK₁ tachykinin receptors in the rat.

Keywords: Substance P; neurokinin A; neurokinin B; RP 67580; blood pressure; heart rate; behaviour; central actions

Introduction

Substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) represent the principal tachykinins in mammals and are widely distributed in the central nervous system (CNS) and peripheral tissues (Maggio, 1988). The physiological actions exerted by tachykinins are mediated by three types of receptors referred to as NK₁, NK₂ and NK₃. SP binds preferentially to NK₁ receptors, whereas NKA and NKB display highest affinity for NK₂ and NK₃ receptors, respectively (for review see Helke *et al.*, 1990; Guard & Watson, 1991; Nakanishi, 1991; Maggi *et al.*, 1993).

In recent years, several non-peptide antagonists for tachykinin receptors have been developed. CP-96,345 was the first selective and high affinity non-peptide antagonist described for NK₁ receptors (Snider *et al.*, 1991). Functional and binding studies revealed that CP-96,345 displayed a higher affinity for NK₁ receptors in man and guinea-pig than in mouse and rat (Snider *et al.*, 1991; Beresford *et al.*, 1991; Barr & Watson, 1993; Fardin *et al.*, 1993). In addition to possessing high affinity for the NK₁ receptors, CP-96,345 binds to L-type calcium channels and displays a variety of non-specific effects that are not stereoselective (Wang & Hakanson, 1992; Griesbacher *et al.*, 1992; Donnerer *et al.*, 1992; Guard *et al.*, 1993).

Recently, another specific non-peptide NK₁ receptor antagonist, RP 67580, (3aR, 7aR)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl] perhydroisoindol-4-one, has been described which is selective for the rodent NK₁ receptor type (Garret *et al.*, 1991). This antagonist was shown to inhibit binding of [³H]-SP to NK₁ receptors in a competitive manner in membranes or crude synaptosomes prepared from rat

brain (Garret *et al.*, 1991; Beaujoan *et al.*, 1993). RP 67580 also inhibited the plasma extravasation induced by SP in the urinary bladder or by antidromic electrical stimulation of the saphenous nerve in the hind paw skin of the rat. When injected peripherally, the antagonist induced a dose-dependent inhibition of nociceptive responses to formalin in the rat paw as well as the facilitation of the spinal reflex by various conditioning stimuli in the rat. In contrast, the 3aS, 7aS enantiomer of RP 67580, RP 68651, was inactive in these assays (Garret *et al.*, 1991, 1993; Laird *et al.*, 1993). Non-peptide antagonists for NK₁ receptors such as RP 67580 may thus represent a novel group of drugs for the treatment of neurogenic inflammation and pain (Garret *et al.*, 1991; Moussaoui *et al.*, 1993).

Tachykinins in the brain are thought to be involved in the regulation of a variety of physiological processes (Helke *et al.*, 1990; Otsuka & Yoshioka, 1993). Peripherally administered non-peptide antagonists generally cross the blood-brain-barrier and may therefore modify central tachykinin actions.

Of the biological effects induced by stimulation of central tachykinin receptors, the cardiovascular and behavioural responses appear to be the most prominent (Unger *et al.*, 1988; Itoi *et al.*, 1992).

In the present study, we have investigated the effects of RP 67580 on central cardiovascular and behavioural responses evoked by endogenous mammalian tachykinins. SP, NKA and NKB were used because these tachykinin neuropeptides represent the natural agonists for tachykinin receptors. The peptides and the non-peptide antagonist were injected intracerebroventricularly (i.c.v.) in conscious rats. The effects of RP 67580 were compared with those of its inactive enantiomer, RP 68651.

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Methods

Implantation of cannulae and catheters and measurement of cardiovascular and behavioural parameters

Male Wistar rats (Dr Karl Thomae, Biberach, Germany) weighing 320–350 g were used. The animals were allowed free access to food and water and maintained on a 12 h light/dark cycle.

For i.c.v. injections, chronic polyethylene cannulae (PP 20) were implanted into the left lateral brain ventricle 7 days before experiments. Five days were allowed for recovery, and then femoral artery catheters (PP 50) were implanted. The surgical procedures have been described previously (Itoi *et al.*, 1992). Experiments were performed 48 h after the femoral artery cannulation.

All experiments were carried out in conscious, freely moving rats. On each test day, rats were placed in the test cages which were of the same size as the home cages and habituated to the new environment at least for 1 h. Then, the femoral artery catheter was connected to the transducer. The experiments were started when the animals were resting and when basal mean arterial pressure (MAP) and heart rate (HR) were stable. SP and NKA, dissolved in isotonic saline, were injected i.c.v. in 1 µl and flushed with 4 µl of saline. NKB, dissolved in acidic saline (approximate pH 3.5), was injected i.c.v. in 1 µl together with 4 µl of phosphate buffered saline (pH 7.4). I.c.v. injection procedure for RP 67580 and for RP 68651 was the same as for NKB (see Materials).

Measurements of MAP and HR were performed via the arterial catheters using a Statham P23Db pressure transducer connected to a Gould Brush 2400 recorder. Analogue output signals of MAP and HR from the blood pressure computer were digitalized and then processed using a computerized programme (Stauss *et al.*, 1990). The MAP and HR are expressed as the area under the curve (AUC) (MAP: mmHg × min; HR: b.p.m. × min). These values represent the sum of MAP and HR changes integrated in time, i.e. one number quantitatively characterizes the whole response. The values have been calculated for a period of 20 min starting at the time point of the i.c.v. injection using a computerized calculation programme developed in our laboratory (Stauss *et al.*, 1990; Itoi *et al.*, 1992).

Behavioural responses were recorded in test cages with grid cage tops removed over a 20 min period starting immediately following the i.c.v. injections. The frequency of the following behavioural manifestations: face washing/head scratching (FW) (movement of forepaws over the nose, eyes and ears or scratching the head with the forepaws), hindquarter grooming/biting (HG) (licking or biting the caudal part of the body including the hindlimbs) and wet dog shakes (WDS) (whole body shaking) was determined according to the 15 s sampling procedure of Gispen *et al.* (1975). During every consecutive period of 15 s, a score of 1 or 0 was given depending on whether the animals showed the specific type of behavioural manifestation or not, regardless of the intensity or the duration of the response. Summation of scores for 20 min following the i.c.v. injection gave the total behavioural score. The maximum theoretical score was 80.

Experimental protocols

Effects of RP 67580 and of its inactive enantiomer, RP 68651, on the cardiovascular and behavioural responses induced by i.c.v. SP The central responses to the same dose of tachykinins may vary from animal to animal. The cardiovascular and behavioural responses to SP obtained after pretreatment with RP 67580 or RP 68651 were, therefore, compared to the responses induced by the same dose of the peptide after vehicle pretreatment in each animal.

Seven groups of rats were used in the first set of experiments. On the first day, rats were pretreated i.c.v. with vehicle (1 µl of acidic saline, approximate pH 4, injected

together with 4 µl phosphate buffered saline, pH 7.4). Ten min thereafter, SP (25 pmol) was injected i.c.v. and the cardiovascular and behavioural responses were recorded. Two days later, rats were pretreated i.c.v. with RP 67580 (5 groups of rats) or RP 68651 (2 groups of rats). Ten min later, SP (25 pmol) was injected i.c.v. and the cardiovascular and behavioural responses were recorded. Each rat received only one dose of RP 67580 or RP 68651. Five doses of RP 67580 (25, 50, 100, 500 and 2500 pmol) and two doses of its inactive enantiomer, RP 68651 (100 and 2500 pmol) were used.

On the basis of the results obtained in the first set of experiments, it appeared possible that RP 67580, especially in higher doses, displayed some intrinsic activity. Therefore, in the second set of experiments, the time interval between the i.c.v. pretreatment with the antagonist and the i.c.v. SP injection was increased to 30 min. These experiments were conducted to investigate the intrinsic activity of the antagonist by measuring the cardiovascular and behavioural effects induced by the antagonist itself and to dissociate the cardiovascular effects induced by SP from those induced by the antagonist.

Three experimental groups of rats were used. On the first day of experiment, all rats received SP (25 pmol, i.c.v.) 30 min after i.c.v. pretreatment with vehicle (see above). Two days later, the first group was pretreated i.c.v. with 25 pmol, the second group with 100 pmol and the third group with 500 pmol of RP 67580. BP and HR were monitored and the behavioural manifestations were recorded starting immediately following the antagonist injection. SP (25 pmol) was injected i.c.v. 30 min after the antagonist, and the cardiovascular and behavioural responses to the peptide were recorded.

The intrinsic activity of the highest dose of RP 67580 (2500 pmol) and the intrinsic activity of its inactive enantiomer, RP 68651 were examined in separate experiments.

Effects of RP 67580 and of its inactive enantiomer, RP 68651, on the cardiovascular and behavioural responses induced by i.c.v. NKA and NKB The first group of rats was pretreated i.c.v. with vehicle (see above), the second with RP 67580 (100 pmol, i.c.v.) and the third with RP 68651 (100 pmol, i.c.v.). Ten min thereafter, NKA (25 pmol) was injected i.c.v. and the cardiovascular and behavioural responses to the peptide were recorded.

In a further protocol, rats received an i.c.v. injection of NKB (50 pmol) instead of NKA (25 pmol).

Materials

RP 67580 (3aR, 7aR)-7,7-diphenyl-2[1-imino-2-(2-methoxyphenyl)-ethyl] perhydroisoindol-4-one and its (3aS, 7aS) enantiomer, RP 68651, were kind gifts from Dr C. Garret, Rhône-Poulenc Rorer, France. RP 67580 and RP 68651 were dissolved in a small volume of 0.1 M HCl (1/10 of the final volume of the stock solution). Saline was added to obtain the final volume in the stock solution (5000 pmol/1 µl). On the day of experiment, the stock solution was further diluted with saline to obtain the desired concentration of the compounds. One µl of the solution containing either RP 67580 or RP 68651 (approximate pH 3.5) was injected i.c.v., together with 4 µl of phosphate buffered saline, pH 7.4.

SP, NKA and NKB were purchased from Bachem, Biochemica GmbH, Heidelberg, Germany. SP and NKA were dissolved in isotonic saline. NKB was dissolved in a minimal volume of 50% acetic acid. After a short (15–20 s) sonication of the solution in an ultrasound bath, saline was added to obtain the stock solution (500 pmol/1 µl).

The stock solutions of the peptides (500 pmol/1 µl) were divided in aliquots and stored at –20°C no longer than two weeks. On the day of experiment, the stock solution was further diluted with saline to obtain the desired concentration of the peptide.

Statistical analysis of data

All values are expressed as means $\pm$ s.e.mean. The data obtained in experiments employing SP were analysed by Student's *t* test for paired samples since the cardiovascular and behavioural responses to SP after pretreatment with the vehicle and those after pretreatment with RP 67580 or RP 68651 were always obtained from the same animal. When more than one comparison was made, the data were subjected to a one-way analysis of variance (ANOVA) followed by a *post hoc* Bonferroni test. A significance level of $P < 0.05$ was accepted.

Results

I.c.v. injections of RP 67580 (25, 100, 500 and 2500 pmol, i.c.v.) and RP 68651 (25, 100 and 2500 pmol, i.c.v.) were without appreciable effects on cardiovascular parameters (Table 1). Similarly, when administered i.c.v. at the above doses, the behavioural responses to both drugs did not differ from vehicle (data not shown).

Effects of RP 67580 and of its inactive enantiomer, RP 68651, on the cardiovascular and behavioural responses induced by i.c.v. SP

I.c.v. administration of SP resulted in increases in MAP and HR which reached a maximum at 2–5 min and then returned gradually to basal values within 15–20 min. The cardiovascular responses to the peptide were associated with increased behavioural activity. In agreement with our previous findings (Culman *et al.*, 1993), FW and HG were the most dominant behavioural manifestations.

I.c.v. pretreatment with 25 or 50 pmol RP 67580, 10 min prior to SP, did not modify the cardiovascular and behavioural responses to the peptide (Figure 1, Table 2). The dose of 100 pmol RP 67580, injected 10 min prior to SP, significantly reduced the BP and HR responses (by about 60%–75%) to values which did not differ significantly from values obtained in i.c.v. saline-treated control rats (Figure 1). FW and HG, which represent the most prominent behavioural manifestations evoked by SP, as well as WDS, were also antagonized (Table 2). I.c.v. pretreatment with 500 pmol RP 67580 markedly reduced only the BP response to SP while the HR response remained unaffected (Figure 1). Of all behavioural manifestations, only FW was inhibited (Table 2). The highest dose of the antagonist, 2500 pmol injected 10 min prior to SP, increased the BP responses to the

peptide (Figure 1), whereas the HR responses and the behavioural effects were not altered (Figure 1, Table 2).

Similar results were obtained when RP 67580 was injected i.c.v. 30 min prior to SP. The dose of 25 pmol of the antagonist did not affect the cardiovascular and the behavioural responses to SP. RP 67580 injected i.c.v. at a dose of 100 pmol, 30 min prior to SP, reduced the BP and HR response to the peptide to values that were not significantly different from those obtained in i.c.v. saline-treated controls (Figure 2). This dose of antagonist also effectively inhibited all behavioural manifestations elicited by SP (Table 2). I.c.v. pretreatment with 500 pmol RP 67580 resulted solely in a reduction of the BP response to the peptide, whereas the behavioural effects were not altered (Figure 2, Table 2).

A dose of 100 pmol RP 67580 most effectively inhibited the central cardiovascular and behavioural responses to SP. The same dose of its inactive enantiomer, RP 68651, injected i.c.v. 10 min before SP, failed to affect these responses. Similarly, the dose of 2500 pmol RP 68651 did not alter the central effects of SP (Figure 3, Table 3).

Effects of RP 67580 and RP 68651 on the central cardiovascular and behavioural responses induced by i.c.v. NKA and NKB

The cardiovascular and behavioural effects observed following i.c.v. NKA and NKB administration are thought to be mediated by NK₂ and NK₃ receptors, respectively (Takano *et al.*, 1990; Tschöpe *et al.*, 1992). To test the selectivity of RP 67580 for the NK₁ receptor *in vivo*, the effects of pretreat-

Table 1 Effects of RP 67580 and RP 68651 injected i.c.v. on mean arterial pressure (MAP) and heart rate (HR)

Treatment (i.c.v.)	n	MAP (AUC)	HR (AUC)
Vehicle	9	15.7 $\pm$ 4.4	66 $\pm$ 18
RP 67580 (25 pmol)	7	11.4 $\pm$ 6.3	85 $\pm$ 51
RP 67580 (100 pmol)	7	22.7 $\pm$ 7.2	133 $\pm$ 38
RP 67580 (500 pmol)	8	20.4 $\pm$ 7.5	144 $\pm$ 42
RP 67580 (2500 pmol)	7	15.9 $\pm$ 7.4	71 $\pm$ 19
RP 68651 (25 pmol)	7	12.5 $\pm$ 13	166 $\pm$ 80
RP 68651 (100 pmol)	8	13.2 $\pm$ 5.2	102 $\pm$ 39
RP 68651 (2500 pmol)	6	28.0 $\pm$ 4.5	157 $\pm$ 82

Values represent the means $\pm$ s.e.mean of (*n*) rats. MAP and HR are expressed as area under the curve (AUC) (MAP: mmHg $\times$ min; HR: b.p.m. $\times$ min). RP 67580 and RP 68651 were injected i.c.v. at indicated doses. AUC was calculated for a period of 20 min starting at the time point of the i.c.v. injection. Statistical comparison to vehicle treatment was made with ANOVA. No statistically significant differences were observed.

Figure 1 Cardiovascular responses to substance P (SP, 25 pmol, i.c.v.) injected 10 min after i.c.v. pretreatment with RP 67580 (solid columns; 25 pmol: *n* = 9; 50 pmol: *n* = 8; 100 pmol: *n* = 8; 500 pmol: *n* = 7; 2500 pmol: *n* = 10) or vehicle (open columns); saline (hatched columns). MAP (a) and HR (b) are expressed as area under the curve (AUC). AUC was calculated for a period of 20 min starting at the time point of the i.c.v. injection of SP or saline. Values are given as means $\pm$ s.e.mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ statistical comparison to saline, calculated with one-way ANOVA followed by a *post-hoc* Bonferroni test. • $P < 0.05$, •• $P < 0.01$, ••• $P < 0.001$ statistical comparison to the respective vehicle-pretreated group, calculated with Student's *t* test for paired samples.

Table 2 Effect of RP 67580 (i.c.v.) on behavioural responses induced by substance P (SP, 25 pmol, i.c.v.)

Pretreatment (i.c.v.)	Agonist (i.c.v.)	Time interval (min) between i.c.v.-injections	n	Face washing	Hind-quarter grooming	Wet-dog shakes
–	Saline	10	9	0.8 ± 0.3	0.5 ± 0.5	0.6 ± 0.3
Vehicle	SP	10	9	8.7 ± 1.4**	6.0 ± 1.1*	3.9 ± 1.4
RP 67580 (25 pmol)	SP	10	9	6.7 ± 1.0*	5.8 ± 1.8*	3.9 ± 1.0
Vehicle	SP	10	8	5.9 ± 1.0*	6.1 ± 1.3*	2.5 ± 0.7
RP 67580 (50 pmol)	SP	10	8	5.6 ± 2.1	5.3 ± 2.2	1.6 ± 0.8
Vehicle	SP	10	8	10.3 ± 1.5***	9.3 ± 2.1**	3.2 ± 0.8
RP 67580 (100 pmol)	SP	10	8	3.3 ± 0.8 ^b	2.3 ± 1.4*	0.8 ± 0.4*
Vehicle	SP	10	7	9.5 ± 1.1**	8.3 ± 1.0**	3.3 ± 1.3
RP 67580 (500 pmol)	SP	10	7	5.4 ± 1.4*	8.1 ± 2.7*	1.3 ± 0.7
Vehicle	SP	10	10	7.7 ± 1.4**	7.6 ± 2.0**	1.7 ± 0.7
RP 67580 (2500 pmol)	SP	10	10	8.5 ± 1.8	9.9 ± 2.1**	2.0 ± 0.7
Vehicle	SP	30	10	7.4 ± 1.4**	6.1 ± 1.3*	4.0 ± 1.2
RP 67580 (25 pmol)	SP	30	10	6.0 ± 1.5*	6.8 ± 2.0*	3.1 ± 1.2
Vehicle	SP	30	7	7.7 ± 1.8**	8.1 ± 2.8**	4.3 ± 0.9
RP 67580 (100 pmol)	SP	30	7	1.9 ± 0.6*	2.6 ± 1.3*	1.0 ± 0.3 ^b
Vehicle	SP	30	10	8.1 ± 2.0**	6.6 ± 1.6*	3.6 ± 1.3
RP 67580 (500 pmol)	SP	30	10	4.1 ± 1.2	3.5 ± 1.0	3.9 ± 1.0

Values represent the frequency of individual behavioural manifestations for 20 min and are indicated by the means ± s.e.mean of (n) rats. RP 67580 was injected i.c.v. at indicated doses 10 or 30 min prior to SP (25 pmol, i.c.v.)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, statistical comparison to saline, calculated with one-way ANOVA followed by a *post hoc* Bonferroni test.

^a $P < 0.05$, ^b $P < 0.01$, statistical comparison to the respective, vehicle-pretreated group, calculated with Student's *t* test for paired samples.

Figure 2 Cardiovascular responses to substance P (SP, 25 pmol, i.c.v.) injected 30 min after i.c.v. pretreatment with RP 67580 (solid columns; 25 pmol: $n = 10$; 100 pmol: $n = 7$; 500 pmol: $n = 10$) or vehicle (open columns); saline (hatched columns). For further details see the legend to Figure 1.

ment with RP 67580 (100 pmol) on the cardiovascular and behavioural effects induced by i.c.v. NKA and NKB were investigated. The cardiovascular responses to NKA were practically identical with those of an equimolar dose of SP (Figure 1, Figure 4). In agreement with our previous findings (Culman *et al.*, 1993), NKA was as potent as SP in eliciting FW. Relative to SP, NKA was weaker in inducing HG behaviour, but more effective in eliciting WDS (Table 2,

Table 4). The cardiovascular response to i.c.v. NKB (50 pmol) comprised an increase in BP and HR, while the behavioural response to NKB was less prominent compared to behavioural effects induced by SP or NKA. The NK₁ receptor antagonist, RP 67580, and its inactive enantiomer, RP 68651, did not significantly alter the cardiovascular and behavioural responses either to NKA or NKB (Figure 4, Table 4).

Discussion

In the present study, we examined the effects of RP 67580 on central cardiovascular and behavioural responses induced by the endogenous mammalian tachykinin peptides, SP, NKA and NKB. These peptides represent the preferred endogenous ligands for NK₁, NK₂ and NK₃ receptors, respectively (Guard & Watson, 1991; Maggi *et al.*, 1993). Since it is likely that most areas of the CNS contain more than one type of tachykinin receptor, it is difficult to characterize exactly the effects mediated by specific tachykinin receptor types in the CNS. In the present study, peptides and antagonist were administered i.c.v. Recent studies have demonstrated that all three types of tachykinin receptor are present in periventricular areas of rat brain (Takano *et al.*, 1990; Itoi *et al.*, 1992; Tschöpe *et al.*, 1992; Culman *et al.*, 1993). It has been suggested that, at low concentrations, SP, NKA and NKB, are selective for particular tachykinin receptor binding sites (Helke *et al.*, 1990). This idea is consistent with our recent finding demonstrating that patterns of the behavioural responses induced by low doses of SP and NKA differ and that the observed differences may be ascribed to the activation of different types of tachykinin receptors (Culman *et al.*, 1993). Low doses of SP, NKA and NKB were also used in the present study.

The cardiovascular and behavioural responses induced by i.c.v. injections of SP, NKA and NKB were evaluated for a period of time of 20 min since we have shown previously that increased BP and HR after i.c.v. injections of 25 pmol SP or NKA returned to baseline within 15 min (Tschöpe *et al.*, 1992).

Table 3 Effect of RP 68651 (i.c.v.) on behavioural responses induced by substance P (SP, 25 pmol, i.c.v.)

Pretreatment (i.c.v.)	Agonist (i.c.v.)	Time interval (min) between i.c.v.-injections	n	Face washing	Hind-quarter grooming	Wet-dog shakes
–	Saline	10	9	0.8 ± 0.3	0.5 ± 0.5	0.6 ± 0.3
Vehicle	SP	10	6	7.5 ± 1.3**	6.2 ± 1.8*	3.5 ± 1.0
RP 68651 (100 pmol)	SP	10	6	12.0 ± 2.8***	9.8 ± 2.1***	6.8 ± 2.3*
Vehicle	SP	10	8	7.6 ± 0.9**	6.1 ± 1.2*	2.8 ± 0.8
RP 68651 (2500 pmol)	SP	10	8	6.4 ± 1.0*	5.9 ± 1.3*	3.6 ± 0.6

Values represent the frequency of individual behavioural manifestations for 20 min and are indicated by the means ± s.e.mean of (*n*) rats. RP 68651 was injected i.c.v. at indicated doses 10 min prior to SP (25 pmol, i.c.v.)

P* < 0.05; *P* < 0.01; ****P* < 0.001, statistical comparison to saline, calculated with one-way ANOVA followed by a *post hoc* Bonferroni test. Statistical significance of the differences between RP 68651-pretreated groups and their respective, vehicle-pretreated controls was evaluated with Student's *t* test for paired samples. No significant differences were observed.

Figure 3 Cardiovascular responses to substance P (SP, 25 pmol, i.c.v.) injected 10 min after i.c.v. pretreatment with RP 68651 (solid columns; 100 pmol: *n* = 6; 2500 pmol: *n* = 8) or vehicle (open columns; saline (hatched columns)). ***P* < 0.01, ****P* < 0.001 statistical comparison to saline calculated with one-way ANOVA followed by a *post-hoc* Bonferroni test. No statistical differences were found between RP 68651-pretreated groups and their respective vehicle-pretreated controls (Student's *t* test for paired samples). For further details see the legend to Figure 1.

Figure 4 Cardiovascular responses to neurokinin A (NKA, 25 pmol, i.c.v.) and NKB (50 pmol, i.c.v.) after i.c.v. pretreatment with vehicle (open columns; NKA: *n* = 8; NKB: *n* = 8), RP 67580 (horizontally lined columns; 100 pmol, i.c.v., NKA: *n* = 8; NKB: *n* = 7) and RP 68651 (solid columns; 100 pmol, i.c.v., NKA: *n* = 7; NKB: *n* = 7). MAP (a) and HR (b) are expressed as area under the curve (AUC). Statistical comparison was made with one-way ANOVA followed by *post-hoc* Bonferroni test. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 as compared to saline (hatched columns) (NKA) or to vehicle (stippled columns) (NKB). For both peptides, the values in vehicle-pretreated, RP 67580-pretreated and RP 68651-pretreated groups did not differ significantly.

In the present study, we observed that pretreatment with a dose of 100 pmol of RP 67580 effectively inhibited the cardiovascular responses and the behavioural manifestations induced by 25 pmol of SP. Previous studies have shown that a 50 times higher dose of (±)-CP-96,345 was necessary to achieve an inhibition of the cardiovascular responses to the same dose of the peptide. Of the behavioural manifestations, only FW was abolished by (±)-CP-96,345 (Tschöpe *et al.*, 1992). These findings are consistent with recent data demonstrating that RP 67580 is more potent than CP-96,345 in inhibiting the specific binding of [³H]-SP to NK₁ receptors in rat brain tissue or in antagonizing NK₁ receptor-stimulated hydrolysis of inositol phospholipids in rat glial cells

(Fardin *et al.*, 1993; Barr & Watson, 1993; Beaujouan *et al.*, 1993).

RP 67580 has been reported to inhibit the nociceptive response to formalin injection into the hind paw or plasma extravasation in rats after various stimuli in a dose-dependent manner (Garret *et al.*, 1993; Moussaoui *et al.*, 1993). In contrast to these studies, in our experiments RP 67580 did not inhibit the central responses to SP in a dose-dependent manner. Regardless of whether RP 67580 was injected 10 or 30 min before SP, 100 pmol RP 67580 very potently inhibited both the cardiovascular and behavioural responses to the peptide, while lower doses were ineffective. A higher dose of RP 67580 (500 pmol) was less potent in

Table 4 Effect of RP 67580 and RP 68651 (i.c.v.) on behavioural responses induced by neurokinin A (NKA, 25 pmol, i.c.v.) and NKB (50 pmol, i.c.v.)

Pretreatment (i.c.v.)	Agonist (i.c.v.)	Time interval (min) between i.c.v.-injections	n	Face washing	Hind-quarter grooming	Wet-dog shakes
–	Saline	10	6	0.5 ± 0.3	0.7 ± 0.3	0.7 ± 0.3
Vehicle	NKA	10	8	6.8 ± 0.9**	3.6 ± 0.8	6.1 ± 1.4*
RP 67580 (100 pmol)	NKA	10	8	5.9 ± 0.9*	6.0 ± 1.7	3.8 ± 0.9
RP 68651 (100 pmol)	NKA	10	7	6.3 ± 2.1*	3.2 ± 0.9	4.9 ± 1.6
–	Vehicle	10	8	0.3 ± 0.2	0.5 ± 0.3	0.4 ± 0.2
Vehicle	NKB	10	8	3.4 ± 1.1	1.6 ± 0.5	1.8 ± 0.6
RP 67580 (100 pmol)	NKB	10	7	2.7 ± 1.1	2.8 ± 1.2	3.0 ± 0.8*
RP 68651 (100 pmol)	NKB	10	7	2.0 ± 0.7	0.3 ± 0.3	1.6 ± 0.6

Values represent the frequency of individual behavioural manifestations for 20 min and are shown as means ± s.e.mean of (n) rats. RP 67580 (100 pmol) and RP 68651 (100 pmol) were injected i.c.v. 10 min prior to NKA (25 pmol) or NKB (50 pmol).

Statistical comparison was made with ANOVA followed by a post-hoc Bonferroni test, separately for each peptide. * $P < 0.05$; ** $P < 0.01$ as compared to saline (NKA) or vehicle (NKB). For both peptides, the values in vehicle-pretreated, RP 67580-pretreated and RP 68651-pretreated groups did not differ significantly.

antagonizing the SP responses than 100 pmol, whereas the highest dose of the antagonist used in the present study (2500 pmol) even potentiated the SP-induced increase in BP without affecting the behavioural responses to the peptide.

This response pattern could occur if RP 67580 acts as a partial agonist at the NK₁ receptor. However, RP 67580, in addition to blocking NK₁ receptors, has been reported to exert action unrelated to the inhibition of NK₁ receptors including an interaction with calcium channels and non-specific inhibitory effects on neurotransmission by a mechanism similar to that of local anaesthetics (Wang *et al.*, 1994; Lombet & Spedding, 1994). The sensitization phenomenon of the highest dose of RP 67580 on SP-induced increase in BP may therefore result from such non-specific effects of the compound on neurotransmission or neuronal excitability in neural circuits related to the central BP regulation. The fact that the potentiation of the BP responses to SP after pretreatment with the highest dose of RP 67580 was not accompanied by a corresponding exaggeration of the behavioural responses would argue in favour of such a non-specific interaction.

On the other hand, the inactive enantiomer, RP 68651, at both doses tested, did not affect the central responses to SP. The decreased ability of higher doses of RP 67580 to inhibit the central responses to SP and the potentiation of the BP response to the peptide by the highest dose of the antagonist therefore appears to be enantioselective and may result from specific interactions of RP 67580 with central NK₁ receptors. In contrast to CP-96,345, the molecular interactions between RP 67580 and the NK₁ receptor have not yet been described. However, it has been suggested that RP 67580 and CP-96,345 bind to overlapping but not identical sites at the NK₁ receptor (Gether *et al.*, 1993b; Fong *et al.*, 1993; Fardin *et al.*, 1993). Both non-peptide antagonists have been considered to act as allosteric inhibitors which alter the formation of the high affinity binding site for the natural agonist (Gether *et al.*, 1993a). If the potentiation of the blood pressure response results from a specific interaction of RP 67580 with central NK₁ receptors, our data suggest that the molecular interaction of RP 67580 with the brain NK₁ receptors *in vivo* may at least partly depend on the concentration of the agonist. RP 67580 at lower doses behaves as a full antagonist for central NK₁ receptors, whereas higher concentrations of this compound seem to sensitize these receptors to SP.

The dose of 100 pmol RP 67580 was most effective in inhibiting the cardiovascular and behavioural responses to SP. To test the selectivity of RP 67580 at central tachykinin receptors *in vivo* we investigated the effects of the same dose of RP 67580 and of its inactive enantiomer, RP 68651, on the cardiovascular and behavioural responses induced by the

natural agonists for NK₂ and NK₃ receptors, NKA and NKB, respectively.

Compared to senktide, a specific agonist for NK₃ receptors, the cardiovascular response to an equimolar dose of NKB was weaker. The cardiovascular response induced by stimulation of central NK₃ receptors is brought about by increased release of vasopressin into the circulation rather than by stimulation of sympathetic activity (Takano *et al.*, 1990). As with senktide, the initial increase in BP was accompanied by a slight bradycardia followed by tachycardia. The behavioural response to NKB was weak and, in contrast to senktide, WDS did not represent the prominent behavioural manifestation (Itoi *et al.*, 1992). Compared to senktide, the faster degradation of NKB in the ventricular system or in periventricular tissues may explain the weaker potency of the peptide in inducing the cardiovascular and behavioural effects. Nevertheless, NKB was employed in the present study because we intended to investigate the effect of RP 67580 on central effects induced by the natural agonists for tachykinin receptors. The failure of RP 67580 to affect the central effects of NKB is not surprising since among the three tachykinin receptors, NK₁ and NK₃ receptors show the most distinct differences in their peptide binding profile (Regoli *et al.*, 1987; Helke *et al.*, 1990).

However, the same is not true for central NK₁ and NK₂ receptors. The central effects of SP are predominantly mediated by NK₁ receptors and those of NKA solely by NK₂ receptors (Tschöpe *et al.*, 1992). However, recent studies have demonstrated that SP can also interact with NK₂ receptors and that NKA, in turn, may desensitize one of the two proposed subtypes of central NK₁ receptors (Culman *et al.*, 1993). In the present study, the central responses to NKA remained unaffected after pretreatment with either RP 67580 or with RP 68651, suggesting that RP 67580 does not interact with central NK₂ receptors.

In conclusion, we have shown that RP 67580 but not its inactive enantiomer, RP 68651, potently inhibits in a relatively narrow range of effective doses the cardiovascular and behavioural effects induced by SP without affecting those induced by NKA or NKB. Hence, our data demonstrate that RP 67580 is a selective high affinity antagonist at central NK₁ tachykinin receptors in the rat. High doses of RP 67580 stereoselectively potentiated the blood pressure response to SP. The present data do not allow any definite conclusion regarding the nature of this phenomenon.

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