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Comparative behavioural profile of centrally administered tachykinin NK_1, NK_2 and NK_3 receptor agonists in the guinea-pig

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1 The NK_1 tachykinin receptor agonists, septide, [Sar^9,Met$(O_2)^{11}$]SP and [Pro^9]SP produced locomotor hyperactivity (10-20 min) when injected intracerebroventricularly (i.c.v.) in the guinea-pig. The most potent in eliciting this hyperactivity was septide (from 0.63 to 5 μg), compared to [Sar^9,Met$(O_2)^{11}$]SP, which was active at 2.5 and 5 μg and [Pro^9]SP which induced a non-significant increase even at 10 μg.

2 Wet-dog shakes were elicited by septide, [Sar^9,Met$(O_2)^{11}$]SP and [Pro^9]SP injected by the i.c.v. route in the guinea-pig. [Sar^9,Met$(O_2)^{11}$]SP, active from 0.16 to 2.5 μg was more potent than septide (active at 1.25 μg) and [Pro^9]SP (active at 0.63 μg) in eliciting such behaviour. To a lesser extent, grooming was also observed after injection of these agonists.

3 The NK_2 tachykinin receptor agonist, [Lys^5, MeLeu^9, Nle^{10}]NKA(4-10), up to the dose of 10 μg i.c.v. had no effect in the guinea-pig. It neither modified locomotor activity nor induced a characteristic behavioural response. At higher doses (20 μg), some toxic effects were noted.

4 The NK_3 tachykinin receptor agonist, senktide, contrasts with the NK_1 receptor agonists in that it elicited only wet-dog shakes, at doses ranging from 0.32 to 1.25 μg. It neither modified locomotor activity (1 μg) nor induced grooming (up to 5 μg) in the guinea-pig.

5 To our knowledge, these results are the first demonstration that the guinea-pig could be useful to differentiate tachykinin agonists on the basis of their behavioural profile, distinct from those obtained in mice and rats.

Keywords: Guinea-pig; tachykinin receptor agonists; locomotion; wet-dog shakes; grooming; intracerebroventricular

Introduction

The tachykinins, substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are widely distributed within the mammalian peripheral and central nervous system (Glowinski *et al.*, 1987). So far, three tachykinin receptors termed NK_1, NK_2 and NK_3 have been cloned (Nakanishi, 1991) and pharmacologically characterized with SP, NKA and NKB as the preferred endogenous agonists, respectively (Buck & Burcher, 1986; Guard & Watson, 1991; Mussap *et al.*, 1993).

Various tachykinin receptor antagonists have been used to characterize the receptor subtypes which mediate the cardiovascular and behavioural effects of the tachykinins in rats (Picard *et al.*, 1994). Interestingly, the use of a number of these selective NK_1 receptor antagonists has demonstrated that species differences in NK_1 receptors exist (Beresford *et al.*, 1991; Fardin & Garret, 1991). For example, CP-96,345 (Snider *et al.*, 1991) displays high affinity for NK_1 receptors found in human subjects, guinea-pigs, rabbits and hamsters and a lower affinity for those found in rat, mouse and chicken tissues while the converse is true for RP 67580 (Garret *et al.*, 1991; Fardin *et al.*, 1993). Thus NK_1 tachykinin receptor binding sites in the guinea-pig brain could be more representative of human receptors than those in rat and mouse (Gitter *et al.*, 1991; McLean *et al.*, 1993; Petitet *et al.*, 1993a).

SP or related compounds, which possess a high affinity for tachykinin NK_1 sites, lead to enhanced locomotor activity, awareness, scratching, grooming and face-washing behaviour when injected intracerebroventricularly (i.c.v.) in conscious freely moving rats (Jolicoeur *et al.*, 1980; Elliott & Iversen, 1986; Itoi *et al.*, 1992; Tschöpe *et al.*, 1992). In mice, the NK_1 receptor agonists have been shown to increase locomotor activity (Naranjo & Del Rio, 1984; Elliott *et al.*, 1991) and to induce scratching and grooming (Ravard *et al.*, 1994). In guinea-pigs, a locomotor hyperactivity, wet-dog shakes, face-washing and lachrymation have been reported (Brent *et al.*, 1988; Elliott *et al.*, 1991; Johnston & Chahl, 1991; Chahl & Johnston, 1993).

A controversy exists concerning the existence of NK_2 tachykinin receptors in the central nervous system (Quirion & Dam, 1988; Saffroy *et al.*, 1988; Dam *et al.*, 1990; Hagan *et al.*, 1993; Maggi *et al.*, 1993a). NKA is widely distributed in the spinal cord and central nervous system of mammals, but the presence of the NK_2 receptor mRNA in rodent brain has not yet been demonstrated (Glowinski *et al.*, 1993; Humpel & Saria, 1993). Maggi *et al.* (1991) have not been able to demonstrate any specific binding in all investigated brain membrane preparations by using the new selective NK_2 receptor ligand, MEN 10,376. They have thus suggested that the density of the NK_2 tachykinin binding sites could be too low to be detected, or that these sites are not present in the brain. Behavioural responses similar to those observed with SP, such as grooming, have been observed after an i.c.v. injection of NKA in the rat (Elliott & Iversen, 1986). An increase in locomotor activity after infusion of NKA or GR 64349 into the median raphe nucleus of the rat (Paris & Lorens, 1989; Mason & Elliott, 1992) and contralateral rotational responses after unilateral activation of the nigrostriatal dopaminergic pathway by GR 51667 or GR 64349 (Elliott *et al.*, 1991) have also been observed.

The existence of two different NK_3 tachykinin receptors has been recently reported, on the basis of studies with antagonists (Nguyen *et al.*, 1994). However, as for NK_1 receptors, this could correspond to species variants since the binding profile of the guinea-pig receptor was different from that of the rat receptor (Merchenthaler *et al.*, 1992; Petitet *et al.*, 1993b). Behavioural studies performed in rats have shown that the administration of the NK_3 receptor-selective agonist, senktide, either subcutaneously or intracisternally (Stoessil *et al.*, 1988a) or into the lateral ventricle evokes only wet-dog shakes (Itoi *et al.*, 1992).

Since the pharmacology of tachykinin receptors in the

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guinea-pig brain may more closely resemble that of the receptor found in the human brain, compared to the rat or mouse receptors, the purpose of the present study was to determine the behavioural profile of a number of tachykinin agonists administered by the i.c.v. route in guinea-pigs. The agonists used were septide ([pGlu^6,Pro^9]SP(6–11), Wormser *et al.*, 1986), [Sar^9,Met(O$_2$)^{11}]SP (Regoli *et al.*, 1988) and [Pro^9]SP (Lavielle *et al.*, 1986) for the NK_1 tachykinin receptor, [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10) (Chassaing *et al.*, 1991) for the NK_2 tachykinin receptor and senktide (succinyl-[Asp^6,Me-Phe^8]SP(6–11), Laufer *et al.*, 1986) for the NK_3 tachykinin receptor. It has been demonstrated in mice that behavioural responses induced by intrathecal or i.c.v. injection of SP or NKA were rapid in onset and short-lasting, due to rapid enzymatic cleavage of the peptides (Hooper *et al.*, 1985; Matsas *et al.*, 1983; 1984). Sakurada *et al.* (1990) have demonstrated that these responses could be potentiated by the endopeptidase-24,11 inhibitor, phosphoramidon. Therefore, in our experiments the peptides were co-administered with phosphoramidon in order to elicit reproducible, sustained responses.

Methods

Animals

Male Dunkin-Hartley guinea-pigs (Charles River Laboratories, France) weighing 145–215 g were used. The animals were allowed free access to food and water and maintained on a 12 h light/dark cycle (lights on 06 h 00 min–18 h 00 min) with constant temperature (22±2°C) and humidity (55±20%).

Experimental procedure

Animals were anaesthetized with a gaseous mixture of 3% isoflurane (or 2.5% halothane), 1 l min^{-1} nitrous oxide and 0.5 l min^{-1} oxygen, before the surgery required for the i.c.v. injection. After incision of the skin on the skull, two holes were made (2.5 mm lateral to the midline and 2.5 mm posterior to the bregma) with a Monitor drill. Peptides were then infused bilaterally in a volume of 7.5 µl/side, through a needle (0.45 × 12 mm) which descended 5 mm vertically from the skull into the cerebral ventricle. The incision was finally glued with cyanoacrylate adhesive.

The motor activity was measured in an automated apparatus (99.5 × 60 × 161.5 cm) with 8 compartments. Each compartment (48 × 58 × 36 cm) was equipped with an activity cage (43 × 28 × 19 cm) and fitted with infra-red emitters and receivers situated on the long axis of the compartment, 3 cm above the cage floor. Interruptions of the light beams were registered on-line by a computer (Imetronic, Bordeaux, France). Since we tested for locomotor stimulation, guinea-pigs were placed in the motor cages and allowed to habituate for a period of 30 min before testing. The activity of the control group was thus quite low, which allowed us satisfactorily to detect an increase in locomotor activity. After the peptide injection, guinea-pigs were replaced in their original photocell cage and the measurement began 10 min after the animals were fully awake for a 40- min test session.

For the behavioural assessment, *i.e.* wet-dog shakes and grooming, guinea-pigs were placed individually in boxes (17 × 35 × 25 cm) immediately after the peptide injection, and the observation began 10 min after the i.c.v. administration and lasted 50 min.

Drugs

Septide, [Sar^9,Met(O$_2$)^{11}]SP and senktide were obtained from Bâle Biochimie SARL (Bubendorf, Switzerland). [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10) was obtained from Neosystem (Strasbourg, France) and [Pro^9]SP was a generous gift from Dr. S. Lavielle (Paris VI University, Paris, France). The metallopeptidase inhibitor phosphoramidon was obtained from Sigma (Sigma Chemical, St Louis, U.S.A.).

All the peptides were first dissolved in distilled water, except septide and senktide, for which addition of dimethylsulphoxide (8%) was necessary before they were dissolved in distilled water. These compounds were stored frozen (−30°C) at the concentration of 2 µg µl^{-1}. On the test-day, phosphoramidon was added to peptides at the concentration of 0.023% (0.35 µg/animal) and dilutions were made in saline (0.9% NaCl aqueous solution) from the thawed aliquots. They were stored at +4°C and used within the day. For the comparisons, absolute control groups were treated by i.c.v. route with saline containing phosphoramidon.

Statistical analysis

The data are expressed as mean ± standard error of mean (s.e.mean) and analysed by the non-parametric Kruskal-Wallis test. Subsequent *post-hoc* comparisons were made by the Dunn test. A significance level of $P \leqslant 0.05$ was accepted.

Results

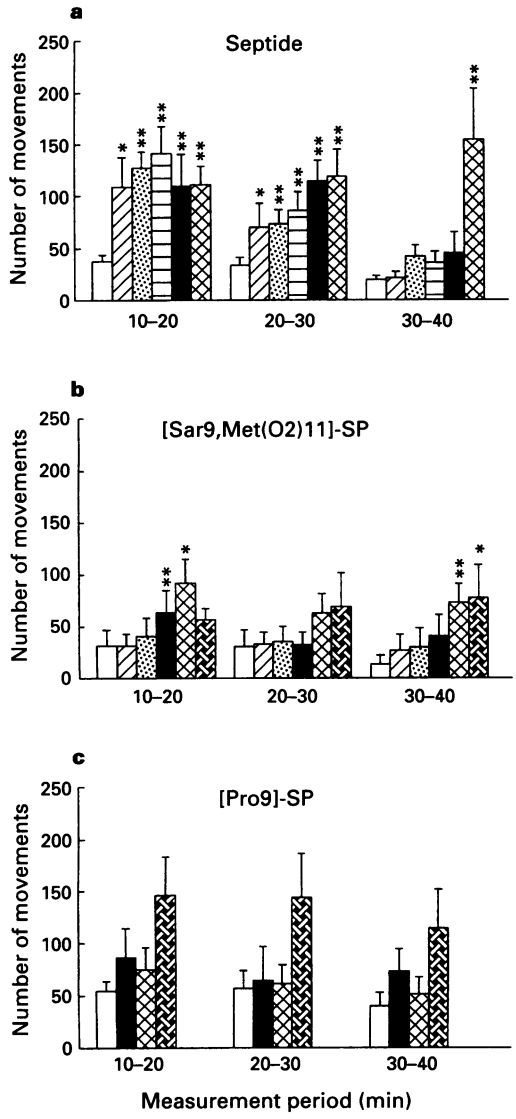
Effects of NK_1 tachykinin receptor agonists

Enhanced locomotor activity was observed in guinea-pigs treated with the NK_1 receptor agonists, whereas there were no significant changes in the locomotor activity of the guinea-pig due to the i.c.v. injection of vehicle (dimethylsulphoxide and phosphoramidon in saline for septide and only phosphoramidon in saline for [Sar^9,Met(O$_2$)^{11}]SP and [Pro^9]SP at any time). Septide, injected i.c.v. at doses ranging from 0.63 µg to 5 µg, increased for at least 30 min the number of transitions [exploration; data not shown] and the number of small movements [general activity] in the guinea-pig ($Chi^2 = 50.94$, d.f. = 7, $P = 0.0001$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 1a). Septide was the most potent agonist in eliciting such hyperactivity. This effect was observed only after treatment with doses of 2.5 and 5 µg of [Sar^9,Met(O$_2$)^{11}]SP ($Chi^2 = 14.37$, d.f. = 6, $P = 0.0258$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 1b). [Pro^9]SP also induced hyperactivity at 10 µg but this increase was not statistically significant ($Chi^2 = 2.83$, d.f. = 3, $P = 0.4183$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 1c).

Furthermore, a quantitative behavioural assessment has shown that NK_1 receptor agonists injected i.c.v. elicited wet-dog shakes and grooming in the guinea-pig. Septide induced these behaviours from doses of 1.25 µg ($Chi^2 = 26.84$, d.f. = 6, $P = 0.0002$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 2a) and 2.5 µg ($Chi^2 = 21.43$, d.f. = 6, $P = 0.0015$, Kruskal-Wallis test; data not shown), respectively. [Sar^9,Met(O$_2$)^{11}]SP induced wet-dog shakes at doses ranging from 0.16 to 2.5 µg ($Chi^2 = 44.07$, d.f. = 7, $P = 0.0001$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 2b). The time spent grooming was increased at similar doses ($Chi^2 = 31.09$, d.f. = 7, $P = 0.0001$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; data not shown). [Pro^9]SP elicited wet-dog shakes at doses ranging from 0.63 µg to at least, 10 µg ($Chi^2 = 49.33$, d.f. = 9, $P = 0.0001$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 2c). Relative to septide, [Sar^9,Met(O$_2$)^{11}]SP and [Pro^9]SP were less potent in inducing locomotor hyperactivity, but more effective in eliciting wet-dog shakes.

Effect of a NK_2 tachykinin receptor agonist

When injected by the i.c.v. route at doses of 2.5, 5, 10 and 20 µg/animal, in the presence of phosphoramidon, [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10), a highly selective NK_2 receptor agonist, neither modified the spontaneous locomotor activity



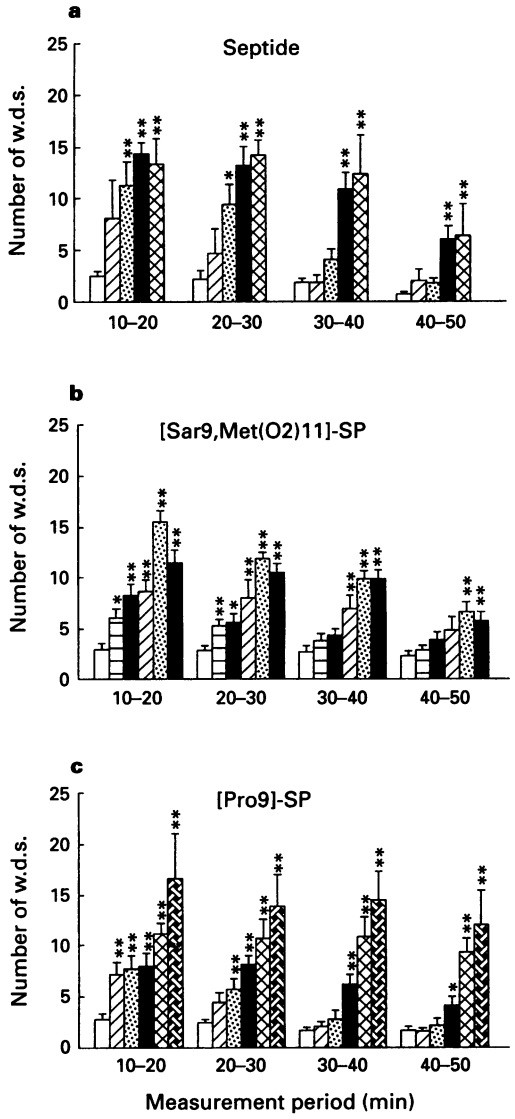


Figure 1 Effects on locomotor activity of several doses of NK_1 receptor agonists injected by the i.c.v. route (7.5 μl/side) in the guinea-pig. Open columns represent the control group. Septide ($n=10-39$ animals per group; (a)) was studied at doses of 0.63, 1.25, 2, 2.5 and 5 μg, [Sar^9,$Met(O_2)^{11}$]SP ($n=8-16$ animals per group; (b)) at doses of 0.63, 1.25, 2.5, 5 and 10 μg and [Pro^9]SP ($n=8$ animals per group; (c)) at doses of 2.5, 5 and 10 μg. Septide: 0.63 (hatched columns), 1.25 (stippled columns), 2 (horizontally hatched columns), 2.5 (solid columns) and 5 μg (cross-hatched columns); [Sar^9,$Met(O_2)^{11}$]SP: see septide plus 10 μg (plaited columns), [Pro^9]SP: 2.5 (solid columns), 5 (cross-hatched columns) and 10 μg (plaited columns). Each value represents the mean ± s.e.mean of small movements observed in each group. A statistically significant difference compared to the respective control group is indicated by $*P\leqslant 0.05$ and $**P\leqslant 0.01$ (non-parametric Kruskal-Wallis test).

Figure 2 Effects of several doses of the NK_1 receptor agonists injected by the i.c.v. route (7.5 μl/side) in eliciting wet-dog shakes (w.d.s.) in the guinea-pig. Open columns represent the control group ($n=12$). Septide ($n=6-12$ animals per group; (a)) was studied at doses of 0.63, 1.25, 2.5 and 5 μg, [Sar^9,$Met(O_2)^{11}$]SP ($n=6-12$; (b)) at doses of 0.16, 0.32, 0.63, 1.25 and 2.5 μg and, [Pro^9]SP ($n=6-18$ animals per group; (c)) at doses ranging from 0.63 μg to 10 μg. Septide: 0.63 (hatched columns), 1.25 (stippled columns), 2.5 (solid columns) and 5 μg (cross-hatched columns); [Sar^9,$Met(O_2)^{11}$]SP: 0.16 (horizontally hatched columns), 0.32 (grey columns) and see septide; [Pro^9]SP: see septide plus 10 μg (plaited columns). Each value represents the mean ± s.e.mean number of wet-dog shakes in each group. A statistically significant difference compared to the respective control group is indicated by $*P\leqslant 0.05$ and $**P\leqslant 0.01$ (non-parametric Kruskal-Wallis test).

nor elicited any characteristic or reproducible behavioural responses in the guinea-pig (general activity: $Chi^2=4.3$, d.f. = 3, $P=0.2305$; Figure 3a; wet-dog shakes: $Chi^2=0.99$, d.f. = 3, $P=0.8032$; Figure 3b). At the dose of 20 μg, dyspnoea and cyanosis were observed for at least 20 min (data not shown). No significant behaviour was noted after this period.

Effect of a NK_3 tachykinin receptor agonist

The most pronounced response to the injection of senktide in the guinea-pig was wet-dog shake behaviour ($Chi^2=24$, d.f. = 6, $P=0.0005$, Kruskal-Wallis test, drug-treatment vs. vehicle-treatment; Figure 4b); senktide did not induce any variation in spontaneous motor activity at the dose of 1 μg (general activity: $Chi^2=0.16$, d.f. = 1, $P=0.6852$; Figure 4a). The number of wet-dog shakes was increased from the dose of 0.32 μg with a maximal response reached at 1.25 μg i.c.v.. The higher doses of 2.5 and 5 μg were devoid of significant activity (Figure 4b).

Discussion

The present study shows that i.c.v. injection of selective NK_1 tachykinin receptor agonists in freely-moving guinea-pigs caused a significant increase in locomotor hyperactivity. This

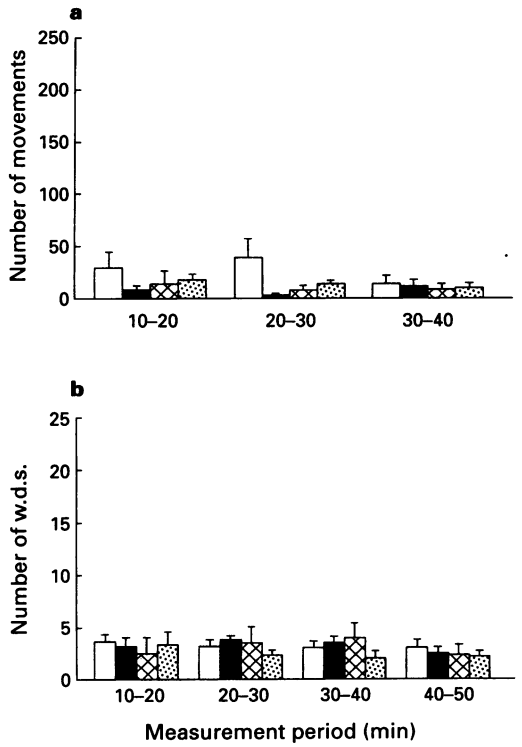


Figure 3 Effects of several doses of the NK_2 receptor agonist, [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10) injected by the i.c.v. route on locomotor activity (a) and in eliciting wet-dog shakes (w.d.s.) (b) in the guinea-pig. Open columns represent the control group ($n=6-8$). [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10) ($n=6-8$ animals per group) was studied at doses of 2.5 (solid columns), 5 (cross-hatched columns) and 10 μg (stippled columns). Each value represents the mean ± s.e.mean number of movements (a) and number of wet-dog shakes (b) in each group.

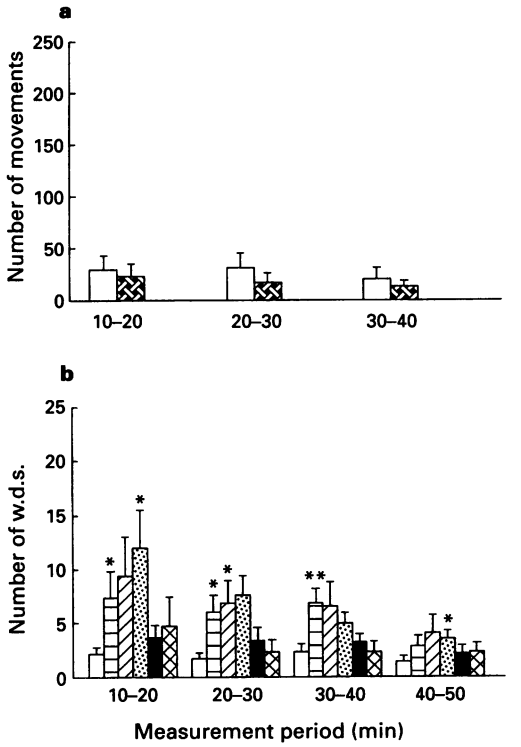


Figure 4 Effects of the NK_3 receptor agonist, senktide, injected by the i.c.v. route on locomotor activity and in eliciting wet-dog shakes (w.d.s.) in the guinea-pig. Open columns represent the control group ($n=6-8$). For the motor activity measurement (a), a dose of 1 μg (plaited columns, $n=8$) was used. For the behavioural assessment (b), senktide ($n=6-12$) was studied at doses of 0.32 μg (horizontally hatched columns), 0.63, 1.25, 2.5 and 5 μg (cross-hatched columns). Each value represents the mean ± s.e.mean number of movements (a) and number of wet-dog shakes (b) in each group. A statistically significant difference compared to the respective control group is indicated by $*P\leqslant 0.05$ and $**P\leqslant 0.01$ (non-parametric Kruskal-Wallis test).

observation is in agreement with previous studies that reported an enhanced locomotion in the guinea-pig (Brent *et al.*, 1988; Seymour *et al.*, 1991) and in the rat (Jolicoeur *et al.*, 1980; Naranjo & Del Rio, 1984). Interestingly, the potency of each agonist in eliciting behavioural responses did not reflect the differences of affinities that each displays for NK_1 tachykinin receptors in radioligand binding assays. In inducing locomotor hyperactivity, septide, active from 0.63 μg (0.79 nmol) to 5 μg (6.39 nmol), was more potent than [Sar^9,Met(O$_2$)^{11}]SP, active at 2.5 and 5 μg (1.79 and 3.58 nmol, respectively) and, especially more potent than [Pro^9]SP, which was not active even at doses as high as 10 μg (6.37 nmol). However, septide was about 1000 fold less active than [Sar^9,Met(O$_2$)^{11}]SP and [Pro^9]SP at displacing [^{3}H]-SP from NK_1 binding sites in the rodent brain (Drapeau *et al.*, 1987; Lew *et al.*, 1990; Petitet *et al.*, 1991) and in the guinea-pig brain ($K_i=142$ nM vs. 0.19 and 0.20 nM, respectively; Fardin *et al.*, 1993). As has been previously suggested, this discrepancy between functional and binding assays observed with septide and other NK_1 receptor agonists could be indicative of the existence of NK_1 tachykinin receptor subtypes (Petitet *et al.*, 1992; Geraghty *et al.*, 1993; Maggi *et al.*, 1993b; Pradier *et al.*, 1994) or could be due to a differential peptide degradation. The number of wet-dog shakes and the time spent grooming, (even if more regularly) were also significantly increased by the NK_1 receptor agonists. These results partially differ from those reported in rats by Unger *et al.* (1988) and by Itoi *et al.* (1992), which indicated face-washing, scratching and grooming as the most prominent behaviours in conscious rats after i.c.v. SP. Moreover, the relative potency of these agonists at inducing wet-dog shakes was quite different compared to their efficacy on locomotion, since [Sar^9,Met(O$_2$)^{11}]SP and [Pro^9]SP were more potent (minimum effective doses = 0.11 and 0.50 nmol, respectively) than septide (1.59 nmol).

The selective NK_2 tachykinin receptor agonist [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10) did not induce, up to the dose of 20 μg, any behavioural responses in the guinea-pig in our experimental design. The data obtained in this species appeared quite different from those reported at the same doses in mice and rats, in which excessive grooming and washing are seen when given various NK_2 receptor agonists [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10), NKA and D-septide (Elliott & Iversen, 1986; Sakurada *et al.*, 1989; Tschöpe *et al.*, 1992; Culman *et al.*, 1993; Picard *et al.*, 1994; Ravard *et al.*, 1994). Moreover, rotational responses to intranigral infusion of selective NK_2 receptor agonists in rats have been described (Elliott *et al.*, 1991). A pharmacological heterogeneity of this receptor has been also demonstrated: the NK_2 tachykinin receptor expressed in guinea-pig, rabbit and human smooth muscle is distinct from that expressed in rat or hamster smooth muscle (Maggi *et al.*, 1991; 1992). Thus the apparent discrepancy between our results and those previously reported in rodents may be species-related (Hall *et al.*, 1993). Even if Stratton *et al.* (1993) have observed anxiolytic-like properties of different tachykinin NK_2 receptor antagonists in the mouse light/dark box test, and despite some evidence suggesting the presence of discrete NK_2 tachykinin binding sites in the mammalian brain (Dam *et al.*, 1990), the involvement of NK_2 tachykinin receptors in centrally-related behaviours still remains to be confirmed. In our study, the lack of effects of [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10) was noted in the presence of 0.6 nM of phosphoramidon; it is possible that this concentration of the neutral endopeptidase 24.11 inhibitor was not able to prevent the degradation of [Lys^5, MeLeu^9, Nle^{10}]NKA(4–10).

When injected by the i.c.v. route into the guinea-pig, the

NK_3 tachykinin receptor agonist, senktide, elicited wet-dog shakes occurring over a period of at least 30 min following i.c.v. injection. Whereas the dose-response relationship for the NK_1 receptor agonists seemed to be sigmoid, that for senktide was bell-shaped. The maximum response rate was seen at 1.25 μg (1.48 nmol), while the higher doses of 2.5 and 5 μg were without effect in eliciting this behaviour. Neither locomotor activity (1 μg) nor an increase of the time spent grooming, at doses ranging from 0.16 to 5 μg, was seen with senktide. Our results are partially in agreement with those reported in rodents, indicating that NK_3 receptor stimulation induced mainly wet-dog shakes behaviour (Stoessl *et al.*, 1988a; 1990; Picard *et al.*, 1994). However, intracisternal or subcutaneous administration of senktide in the rat has been also reported to produce a syndrome mediated by endogenous 5-HT including forepaw treading and hindlimb splaying (Wormser *et al.*, 1986; Stoessl *et al.*, 1987) and acetylcholine-mediated behaviours, yawning, chewing mouth movements and sexual arousal (Stoessl *et al.*, 1988b). Petitet *et al.* (1993b) have shown by using the NK_2 tachykinin receptor antagonist SR 48968, that a species difference also exists between NK_3 tachykinin binding sites, this compound eliciting NK_3 receptor antagonist properties in the guinea-pig but not in the rat. In this context, only the development of selective NK_3 receptor antagonists could confirm the inter-species difference (Boden & Woodruff, 1994).

Anatomical and pharmacological studies in rodents have demonstrated the potential for both tachykinin NK_1 and NK_3 receptors to modify locomotion by influencing dopaminergic systems (Dam & Quirion, 1986; Stoessl *et al.*, 1991). Surprisingly, in our experiments, only the NK_1 receptor agonists, when administered centrally to conscious freely moving guinea-pigs, lead to enhanced locomotion, the NK_3 receptor agonist senktide being inactive. It may be useful in further studies to re-examine the involvement of dopamine in the response elicited in the guinea-pig by the NK_1 receptor agonists. On the other hand, both tachykinin NK_1 and NK_3 receptor agonists evoked wet-dog shakes in the guinea-pig in our experiments. This behaviour, that is only a part of the behavioural syndrome elicited by senktide in other species (Stoessl *et al.*, 1988a; 1990), is mediated by 5-HT_2 receptor stimulation (Arnt *et al.*, 1984). Thus, the possibility that both specific tachykinin receptors may be involved in the regulation of the 5-hydroxytryptaminergic system also require further attention.

Taken together, these data indicate that selective tachykinin agonists can be differentiated on the basis of their behavioural profile in the guinea-pig. Thus, if receptors in this species are more representative of receptors in man for the tachykinin antagonists, the pharmacological studies performed in the guinea-pig could be very useful, because more predictive, for characterizing the central effects of compounds interacting with the different tachykinin receptor subtypes.

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References

ARNT, J., HYTTEL, J. & LARSEN, J.J. (1984). The citalopram/5-HTP-induced head shake syndrome is correlated to 5-HT_2 receptor affinity and also influenced by other transmitters. *Acta Pharmacol. Toxicol.*, **55**, 363–372.

BERESFORD, I.J.M., BIRCH, P.J., HAGAN, R.M. & IRELAND, S.J. (1991). Investigation into species variants in tachykinin NK_1 receptors by use of the non-peptide antagonist, CP 96345. *Br. J. Pharmacol.*, **104**, 292–293.

BODEN, P. & WOODRUFF, G.N. (1994). Presence of NK_3-sensitive neurones in different proportions in the medial habenula of guinea-pig, rat and gerbil. *Br. J. Pharmacol.*, **112**, 717–719.

BRENT, P.J., JOHNSTON, P.A. & CHAHL, L.A. (1988). Increased plasma catecholamines and locomotor activity induced by centrally administered substance P in guinea-pigs. *Neuropharmacology*, **27**, 743–748.

BUCK, S.H. & BURCHER, E. (1986). Neurokinin binding site nomenclature definition. *Trends Pharmacol. Sci.*, **7**, 437–439.

CHAHL, L.A. & JOHNSTON, P.A. (1993). Effect of the nonpeptide NK_1 receptor antagonist CP 96345 on the morphine withdrawal response of guinea-pigs. *Regul. Pept.*, **46**, 373–375.

CHASSAING, G., LAVIELLE, S., LOEUILLET, D., ROBILLIARD, P., CARRUETTE, A., GARRET, C., BEAUJOUAN, J.C., SAFFROY, M., PETITET, F., TORRENS, Y. & GLOWINSKI, J. (1991). Selective agonists of NK_2 binding sites, highly active on rat portal vein (NK_3 bioassay). *Neuropeptides*, **1**, 91–95.

CULMAN, J., TSCHOPE, C., JOST, N., ITOI, K. & UNGER, T. (1993). Substance P and neurokinin A induced desensitization to vascular and behavioural effects: evidence for the involvement of different tachykinin receptors. *Brain Res.*, **625**, 75–83.

DAM, T.V. & QUIRIION, R. (1986). Pharmacological characterization and autoradiographic localization of substance P receptors in guinea-pig brain. *Peptides*, **7**, 855–864.

DAM, T.V., TAKEDA, Y., KRAUSE, J.E., ESCHER, E. & QUIRION, R. (1990). γ-preprotachykinin-(72-92)-peptide amide: an endogenous preprotachykinin 1 gene-derived peptide that preferentially binds to neurokinin-2 receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **87**, 240–250.

DRAPEAU, G., D'ORLEANS-JUSTE, P., DION, S., RHALEB, N.E., ROUISSI, N.E. & REGOLI, D. (1987). Selective agonists for substance P and neurokinin receptors. *Neuropeptides*, **10**, 43–54.

ELLIOTT, P.J. & IVERSEN, S.D. (1986). Behavioural effects of tachykinins and related peptides. *Brain Res.*, **381**, 68–76.

ELLIOTT, P.J., MASON, G.S., STEPHENS-SMITH, M. & HAGAN, R.M. (1991). Behavioral and biochemical responses following activation of midbrain dopamine pathways by receptor selective neurokinin agonists. *Neuropeptides*, **19**, 119–126.

FARDIN, V., FOUCAULT, F., BLOCK, M.D., JOLY, A., FLAMAND, O., CLERC, F. & GARRET, C. (1993). Variations in affinities for the NK_1 receptor: differences between the non-peptide substance P antagonists RP 67580 and CP 96345 and the agonist septide. *Regul. Pept.*, **46**, 300–303.

FARDIN, V. & GARRET, C. (1991). Species differences between ^{3}H-substance P binding in rat and guinea-pig shown by the use of peptide agonists and antagonists. *Eur. J. Pharmacol.*, **201**, 231–234.

GARRET, C., CARRUETTE, A., FARDIN, V., MOUSSAOUI, S., PEYRONEL, J.F., BLANCHARD, J.C. & LADURON, P. (1991). Pharmacological properties of a potent and selective non-peptide substance P antagonist. *Proc. Natl. Acad. Sci. U.S.A.*, **88**, 10208–10212.

GERAGHTY, D.P. & BURCHER, E. (1993). Two classes of binding sites for [^{3}H]SP in rat cerebral cortex. *Brain Res.*, **601**, 34–40.

GITTER, B.D., WATERS, D.C., BRUNS, R.F., MASON, N.R., NIXON, J.A. & HOWBERT, J.J. (1991). Species differences in affinities of non-peptide antagonists for substance P receptors. *Eur. J. Pharmacol.*, **197**, 237–238.

GLOWINSKI, J., KEMEL, M.L., DESBAN, M., GAUCHY, C., LAVIELLE, S., CHASSAING, G., BEAUJOUAN, J.C. & TREMBLAY, L. (1993). Distinct presynaptic control of dopamine release in striosomal- and matrix-enriched areas of the rat striatum by selective agonists of NK_1, NK_2 and NK_3 tachykinin receptors. *Regul. Pept.*, **46**, 124–128.

GLOWINSKI, J., TORRENS, Y., SAFFROY, M., BERGSTROM, L., BEAUJOUAN, J.C., LAVIELLE, S., PLOUX, O., CHASSAING, G. & MARQUET, A. (1987). Tachykinin receptors in the CNS. *Prog. Brain Res.*, **72**, 197–203.

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

HAGAN, R.M., BERESFORD, I.J.M., STABLES, J., DUPERE, J., STUBBS, C.M., ELLIOTT, P.J., SHELDRICK, R.L.G., CHOLLET, A., KAWASHIMA, E., McELROY, A.B. & WARD, P. (1993). Characterization, CNS distribution and function of NK_2 receptors studied using potent NK_2 receptor antagonists. *Regul. Pept.*, **46**, 9–19.

HALL, J.M., CAULFIELD, M.P., WATSON, S.P. & GUARD, S. (1993). Receptor subtypes or species homologues: relevance to drug discovery. *Trends Pharmacol. Sci.*, **14**, 376–383.

HOOPER, N.M., KENNY, A.J. & TURNER, A.J. (1985). The metabolism of neuropeptides neurokinin A (substance K) is a substrate for endopeptidase-24.11 but not for peptidyl dipeptidase A (angiotensin-converting enzyme). *Biochem. J.*, **231**, 357–361.

HUMPEL, C. & SARIA, A. (1993). Characterization of neurokinin binding sites in rat brain membranes using highly selective ligands. *Neuropeptides*, **25**, 65–71.

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

JOHNSTON, P.A. & CHAHL, L.A. (1991). Tachykinin antagonists inhibit the morphine withdrawal response in guinea-pigs. *Naunyn Schmied. Arch. Pharmacol.*, **343**, 283–288.

JOLICOEUR, F.B., RONDEAU, D.B., BELANGER, F., FOURIEZOS, G. & BARBEAU, A. (1980). Influence of substance P on the behavioural changes induced by haloperidol in rats. *Peptides*, **1**, 103–107.

LAVIELLE, S., CHASSAING, G., JULIEN, S., MARQUET, A., BERGSTROM, L., BEAUJOUAN, J.C., TORRENS, Y. & GLOWINSKI, J. (1986). Specific recognition of SP and NKB receptors by analogues of SP substituted at positions 8 and 9. *Eur. J. Pharmacol.*, **125**, 461–462.

LAUFER, R., GILON, C., CHOREV, M. & SELINGER, Z. (1986). Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. *J. Biol. Med.*, **261**, 10257–10263.

LEW, R., GERAGHTY, D.P., DRAPEAU, G., REGOLI, D. & BURCHER, E. (1990). Binding characteristics of [^{125}I]Bolton-Hunter [Sar^{9} Met(O$_2$)^{11}]substance P, a new selective radioligand for the NK$_1$ receptor. *Eur. J. Pharmacol.*, **184**, 97–108.

MAGGI, C.A., EGKEZOS, A., QUARTARA, L., PATACCHINI, R. & GIACHETTI, A. (1992). Heterogeneity of NK$_2$ tachykinin receptors in hamster and rabbit smooth muscles. *Regul. Pept.*, **37**, 85–93.

MAGGI, C.A., PATACCHINI, R., ASTOLFI, M., ROVERO, P., GIULIANI, S. & GIACHETTI, A. (1991). NK$_2$ receptor agonists and antagonists. *Ann. New York Acad. Sci.*, **632**, 184–191.

MAGGI, C.A., PATACCHINI, R., ROVERO, P. & GIACHETTI, A. (1993a). Tachykinin receptors and tachykinin receptor antagonists. *J. Autonom. Pharmacol.*, **13**, 23–93.

MAGGI, C.A., PATACCHINI, R., MEINI, S. & GIULIANI, S. (1993b). Evidence for the presence of a septide-sensitive tachykinin receptor in the circular muscle of the guinea-pig ileum. *Eur. J. Pharmacol.*, **235**, 309–311.

MASON, G.S. & ELLIOTT, P.J. (1992). Behavioural consequences following infusion of selective neurokinin agonists into the median raphe nucleus of the rat. *Neuropharmacology*, **31**, 757–760.

MATSAS, R., FULCHER, I.S., KENNY, A.J. & TURNER, A.J. (1983). Substance P and [Leu]enkephalin are hydrolysed by an enzyme in pig caudate synaptic membranes that is identical with the endopeptidase of kidney microvilli. *Proc. Nat. Acad. Sci. U.S.A.*, **80**, 3111–3115.

MATSAS, R., KENNY, A.J. & TURNER, A.J. (1984). The metabolism of neuropeptides. The hydrolysis of peptides, including enkephalins: tachykinins and their analogues, by endopeptidase-24.11. *Biochem. J.*, **223**, 433–440.

MCLEAN, S., GANONG, A., SEYMOUR, P., SNIDER, R.M., DESAI, M.C., ROSEN, T., BRYCE, D.K., LONGO, K.P., REYNOLDS, L.S., ROBINSON, G., SCHMIDT, A.W., SIOK, C. & HEYM, J. (1993). Pharmacology of CP-99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. *J. Pharmacol. Exp. Ther.*, **267**, 472–479.

MERCHENTHALER, I., MADERDRUT, J.L., O'HARTE, F. & CONLON, J.M. (1992). Localization of Neurokinin B in the central nervous system of the rat. *Peptides*, **13**, 815–819.

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

NAKANISHI, S. (1991). Mammalian tachykinin receptors. *Annu. Rev. Neurosci.*, **14**, 123–136.

NARANJO, J.R. & DEL RIO, J. (1984). Locomotor activation induced in rodents by substance P and analogues. Blockade of the effect of substance P by met-enkephalin antiserum. *Neuropharmacology*, **23**, 1167–1171.

NGUYEN, Q.T., JUKIC, D., CHRETIEN, L., GOBEIL, F., BOUSSOUGOU, M. & REGOLI, D. (1994). Two NK$_3$ receptor subtypes: demonstration by biological and binding assays. *Neuropeptides*, **27**, 157–161.

PARIS, J.M. & LORENS, S.A. (1989). A dose-response analysis of intra-raphe tachykinin-induced hyperactivity. *J. Pharmacol. Exp. Ther.*, **251**, 388–393.

PETITET, F., BEAUJOUAN, J.C., SAFFROY, M., TORRENS, Y., CHASSAING, G., LAVIELLE, S., BESSEYRE, J., GARRET, C., CARRUETTE, A. & GLOWINSKI, J. (1991). Further demonstration that Pro^{9}-substance P is a potent and selective ligand of NK$_1$ tachykinin receptors. *J. Neurochem.*, **56**, 879–889.

PETITET, F., BEAUJOUAN, J.C., SAFFROY, M., TORRENS, Y., FARDIN, V. & GLOWINSKI, J. (1993a). NK$_1$ tachykinin receptor in rat and guinea-pig brains: pharmacological and autoradiographical evidence for a species difference. *Peptides*, **14**, 551–559.

PETITET, F., BEAUJOUAN, J.C., SAFFROY, M., TORRENS, Y. & GLOWINSKI, J. (1993b). The non-peptide NK$_2$ antagonist SR48968 is also a NK$_3$ antagonist in the guinea-pig but not in the rat. *Biochem. Biophys. Res. Commun.*, **191**, 180–187.

PETITET, F., SAFFROY, M., TORRENS, Y., LAVIELLE, S., CHASSAING, G., LOEUILLET, D., GLOWINSKI, J. & BEAUJOUAN, J.C. (1992). Possible existence of a new tachykinin receptor subtype in the guinea-pig ileum. *Peptides*, **13**, 383–388.

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

PRADIER, L., MENAGER, J., LE GUERN, J., BOCK, M.D., HEUILLET, E., FARDIN, V. & MAYAUX, J.F. (1994). Septide: an agonist for the NK$_1$ receptor acting at a site distinct from substance P. *Mol. Pharmacol.*, **45**, 287–293.

QUIRION, R. & DAM, T.V. (1988). Multiple neurokinin receptors: recent developments. *Regul. Pept.*, **22**, 18–24.

RAVARD, S., BETSCHART, J., FARDIN, V., FLAMAND, O. & BLANCHARD, J.C. (1994). Differential ability of tachykinin NK$_1$ and NK$_2$ agonists to produce scratching and grooming behaviours in mice. *Brain Res.*, **651**, 199–208.

REGOLI, D., DRAPEAU, G., DION, S. & COUTURE, R. (1988). New selective agonists for neurokinin receptors: pharmacological tools for receptor characterization. *Trends Pharmacol. Sci.*, **9**, 290–295.

SAFFROY, M., BEAUJOUAN, J.C., TORRENS, Y., BESSEYRE, J., BERGSTROM, L. & GLOWINSKI, J. (1988). Localization of tachykinin binding sites (NK$_1$, NK$_2$, NK$_3$ ligands) in the rat brain. *Peptides*, **9**, 227–241.

SAKURADA, T., TAN-NO, K., YAMADA, T., SAKURADA, S. & KISARA, K. (1990). Phosphoramidon potentiates mammalian tachykinin-induced biting, licking and scratching behaviour in mice. *Pharmacol. Biochem. Behav.*, **37**, 779–783.

SAKURADA, T., YAMADA, T., SAKURADA, S., KISARA, K. & OHBA, M. (1989). Substance P analogues containing D-histidine antagonize the behavioural effects of intrathecally co-administered SP in mice. *Eur. J. Pharmacol.*, **174**, 153–160.

SEYMOUR, P.A., ROBINSON, G.L. & BRYCE, D.K. (1991). Hyperlocomotion induced by i.c.v. [Sar^{9},Met(O$_2$)^{11}]SP is reversed by the selective NK$_1$ antagonist, CP 96345. *Soc. Neurosci.*, **17**, 804.

SNIDER, R.M., CONSTANTINE, J.W., LOWE III, J.A., LONGO, K.P., LEBEL, W.S., WOODY, H.A., DROZDA, S.E., DESAI, M.C., VINICK, J.F., SPENCER, R.W. & HESS, H.J. (1991). A potent non-peptide antagonist of the substance P (NK$_1$) receptor. *Science*, **251**, 435–437.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1988a). The NK$_3$ tachykinin receptor agonist senktide elicits 5-HT-mediated behaviour following central and peripheral administration in mice and rats. *Br. J. Pharmacol.*, **94**, 285–287.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1988b). The NK$_3$ tachykinin agonist senktide elicits yawning and chewing mouth movements following subcutaneous administration in the rat. Evidence for cholinergic mediation. *Psychopharmacology*, **95**, 502–506.

STOESSL, A.J., DOURISH, C.T. & IVERSEN, S.D. (1990). Pharmacological characterization of the behavioural syndrome induced by the NK$_3$ tachykinin agonist senktide in rodents: evidence for mediation by endogenous 5-HT. *Brain Res.*, **517**, 111–116.

STOESSL, A.J., DOURISH, C.T., YOUNG, S.C., WILLIAMS, B.J., IVERSEN, S.D. & IVERSEN, L.L. (1987). Senktide, a selective neurokinin B-like agonist, elicits serotonin-mediated behaviour following intracisternal administration in the mouse. *Neurosci. Lett.*, **80**, 321–326.

STOESSL, A.J., SZCZUTKOWSKI, E., GLENN, B. & WATSON, I. (1991). Behavioural effects of selective tachykinin agonists in midbrain dopamine regions. *Brain Res.*, **565**, 254–262.

STRATTON, S.C., BERESFORD, I.J.M., HARVEY, F.J., TURPIN, M.P., HAGAN, R.M. & TYERS, M.B. (1993). Anxiolytic activity of tachykinin NK_2 receptor antagonists in the mouse light-dark box test. *Eur. J. Pharmacol.*, **250**, R11–R12.

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

UNGER, TH., CAROLUS, S., DEMMERT, G., GANTEN, D., LANG, R.E., MASER-GLUTH, C., STEINBERG, H. & VEELKEN, R. (1988). Substance P induces a cardiovascular defence reaction in the rat: pharmacological characterization. *Circ. Res.*, **63**, 812–820.

WORMSER, U., LAUFER, R., HART, Y., CHOREV, M., GILON, C. & SELINGER, Z. (1986). Highly selective agonists for substance P receptor subtypes. *EMBO J.*, **5**, 2805–2808.

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