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Effects of central administration of tachykinin receptor agonists and antagonists on plus-maze behavior in mice

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Abstract

This study assessed the effects of intracerebroventricular administration of selective agonists and antagonists for tachykinin NK_1 and NK_2 receptors on performance of mice in the elevated plus-maze, an ethological model of anxiety. Mice were treated with either vehicle (5 μl) or 1, 10, 100 or 500 pmol of substance P, neurokinin A, the selective NK_1 receptor agonist substance P methyl ester, or the selective NK_2 receptor agonist, [β-Ala^8]neurokinin A-(4–10). Other mice received similar doses of FK 888, i.e., *N*^{2}-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-y)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide, or SR 48968, i.e., (*S*)-*N*-methyl-(*N*-[4-acetylamine-4-phenylpiperidine)-2-(3,4-dichlorophenyl)buthyl]benzamide, selective antagonists of tachykinin NK_1 and NK_2 receptors, respectively. Injections of substance P, neurokinin A, substance P methyl ester or [β-Ala^8]neurokinin A-(4–10) significantly reduced the frequency of open arm entries, and [β-Ala^8]neurokinin A-(4–10) also enhanced the percentage of entries into enclosed arms. Conversely, the NK_1 antagonist FK 888 and the NK_2 antagonist SR 48968 each increased the time spent in the open arms, and SR 48968 also increased the frequency of entries into the open arms. None of the tachykinin receptor agonists or antagonists modified motor performance and coordination on the rotarod apparatus or ambulation in an activity cage. Together, these results suggest that centrally administered NK_1 and NK_2 receptor agonists and antagonists can modulate anxiety, as evaluated in the elevated plus-maze test in mice. Stimulation of either tachykinin NK_1 or NK_2 receptors induces anxiogenic-like responses, whereas the reverse occurs following their blockade. The anxiolytic-like profiles of action of both tachykinin NK_1 and NK_2 receptor antagonists suggest that central tachykinin mechanisms are tonically involved in the modulation of anxiety.

Keywords: Tachykinin NK_1 receptor; Tachykinin NK_2 receptor; Substance P; Neurokinin A; FK 888; SR 48968; Plus-maze; Anxiety; (Intracerebroventricular injection)

1. Introduction

The tachykinins, substance P, neurokinin A and neurokinin B, seem to play important roles in the central mechanisms controlling nociception (Otsuka and Yanagisawa, 1990), cardiovascular functions and defense reactions (Itoi et al., 1994, Picard et al., 1994). The biological actions of tachykinins are mediated via activation of three different G protein-coupled 7-transmembrane domain receptors, denoted as tachykinin NK_1, NK_2 and NK_3 receptors (Regoli et al., 1994). Although their selectivity is limited, substance P, neurokinin A and neurokinin B bind preferentially to NK_1, NK_2 and NK_3 receptors, respectively (for reviews see Regoli et al., 1994 and Maggi, 1995).

Both NK_1 and NK_3 receptors are widely, but unevenly, distributed in the central nervous system, whereas the occurrence of NK_2 receptors appears to be far less prevalent (for reviews see Otsuka and Yoshioka, 1993 and Maggi, 1995). Brain regions implicated in anxiety conditions and defense-rage reactions, such as the hypothalamus, amygdala, hippocampus and periaqueductal gray matter (Cuello and Kanazawa, 1978; Shaikh and Siegel, 1994), all express significant densities of tachykinin NK receptors and also substance P-like immunoreactive nerve fibers or cell bodies (for review see Otsuka and Yoshioka, 1993). Given systemically or centrally, tachykinin NK_2 receptor antagonists have been found to reduce anxiety-related be-

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haviors in three different experimental models of anxiety (Stratton et al., 1993; Walsh et al., 1995).

In the present study, we have investigated the role of tachykinin NK_1 and NK_2 receptors in the modulation of anxiety in mice submitted to the elevated plus-maze test, by means of natural tachykinins as well as of selective agonists and antagonists for both NK_1 and NK_2 receptors. The elevated plus-maze test has been proposed as a suitable and reliable ethological model for the detection of both anxiolytic and anxiogenic properties of drugs or of experimental manipulations (Rodgers and Cole, 1994).

2. Materials and methods

2.1. Animals

Experiments were conducted with male Swiss mice (25–30 g), raised under controlled ambient conditions (12-h light cycle with lights on as from 6:00 h; 22 ± 2°C). Food and water were available ad libitum, except during the experiments. All experimental observations were carried out between 7:00 and 13:00 h. Each animal was only used once.

2.2. Drugs and solutions

Substance P and neurokinin A, the preferential natural ligands for NK_1 and NK_2 receptors (Regoli et al., 1994; Maggi, 1995), respectively, as well as the selective agonists of NK_1 and NK_2 receptors, substance P methyl ester (Watson et al., 1983) and [β-Ala^8]neurokinin A-(4–10) (Rovero et al., 1989), respectively, were purchased from Peninsula Laboratories (Belmont, USA). Diazepam, the reference drug for anxiolytic activity, was acquired from Laboratório Cristália (São Paulo, Brazil) and pentylenetetrazol, the anxiogenic reference drug, from Sigma Chemical Co. (St. Louis, USA). The selective NK_1 receptor antagonist, FK 888, N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-y)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide, (Fujii et al., 1992), and the NK_2 antagonist, SR 48968, (*S*)-*N*-methyl-(*N*-[4-acetylamine-4-phenylpiperidine)-2-(3,4-dichlorophenyl)buthyl]-benzamide (Emonds-Alt et al., 1992), were kindly provided by Fujisawa Pharmaceutical Co. (Osaka, Japan) and Sanofi Recherche (Montpellier, France), respectively, and the β-carboline, DMCM (methyl-6,7-dimethoxy-4-ethyl-carboline-3-carboxylate), an inverse agonist for benzodiazepine receptors, by Schering Aktiengesellschaft (Berlin, Germany). Most drugs were prepared as stock solutions (1 mM) in absolute ethanol and stored at −20°C until use, but DMCM was prepared daily by solution in dimethylsulfoxide. All drugs were then diluted daily to the desired concentrations in phosphate-buffered saline (pH 7.4). The highest final concentrations of ethanol or dimethylsulfoxide in these solutions (0.2% and 2%, respectively) were found to be ineffective to alter performance in any of the tests conducted in this study.

2.3. Intracerebroventricular injections

I.c.v. injections were given under light ether anesthesia, 'free hand' as described by Haley and McCormick (1957), and as modified by Laursen and Belknap (1986), with the bregma fissure as a reference. Substance P, substance P methyl ester, neurokinin A, FK 888 or SR 48968 were injected at doses of 1, 10, 100 or 500 pmol, whereas [β-Ala^8]neurokinin A-(4–10) was injected up to 1 nmol. The i.c.v. doses of diazepam, pentylenetetrazol and DMCM were injected at doses of 7, 300 and 130 nmol, respectively. All drugs were injected in a volume of 5 μl, given over 30 s, and the cannula remained in place for another 30 s. Control mice were similarly treated with vehicle only. After injection and recovery of the righting reflex, the animals were used for the behavioral tests, following which correct cannula placement in the brains was checked histologically. The results for animals presenting cannula misplacement or any signs of cerebral hemorrhage were discarded. To further validate the efficacy of the 'free hand' technique employed, another group of 10 animals was given an identical 5 μl i.c.v. injection of Evans' blue dye (0.5%) and a similar histological check was done 10 min later. Successful injection was obtained in 9 of these animals, in which the dye was found to have spread as far as the brainstem, as previously observed by Saija et al. (1989) for i.c.v. administration of labelled tachykinins in rats.

2.4. Anxiety evaluation

The putative anxiolytic or anxiogenic activity of the drugs was assessed using the elevated plus-maze test, as adapted for the mouse by Lister (1987). Briefly, the apparatus consisted of two opposed open arms (30 × 5 × 0.25 cm) and two opposed closed arms (30 × 5 × 15 cm), all facing a central platform (5 × 5 cm). The apparatus was made of Plexiglass and was elevated 45 cm from the floor. After i.c.v. treatment, each mouse was first placed in a Plexiglass arena (35 × 35 × 15 cm) for 5 min, to allow adaptation to the new environment and decrease basal anxiety levels (for review see Rodgers and Cole, 1994), then was transferred to the center of the plus-maze, facing an enclosed arm, and observed for a period of 5 min. The frequencies of entry into open arms and closed arms, as well as the time spent in open and closed arms were recorded. In this test, rodents tend to avoid the open arms, for fear of a novel environment associated with their inability to perform thigmotaxis on them (Treit et al., 1993). Thus, drugs with anxiolytic activity usually increase the frequency of entries into and/or time spent in open arms whereas the reverse holds true for anxiogenic drugs (Pellow et al., 1985). The frequency of total entries (i.e.,

entries into both open and closed arms) is not usually modified by either treatment.

2.5. Locomotor activity

To evaluate the influence of the various treatments on spontaneous locomotor activity, mice were placed individually, immediately after i.c.v. injection, in activity cages ($40 \times 12 \times 20$ cm) divided into 4 equal sections by 3 parallel light beams, each focused on a photocell. Locomotor activity was recorded cumulatively, as the number of light beam interruptions, over 3 consecutive 5-min periods (Geyer et al., 1986).

2.6. Motor coordination

The possible influence of the different drugs on motor coordination was evaluated, 5 min after the i.c.v. injection, by placing the mice on the revolving bar (diameter 2.5 cm, 12 r.p.m.) of a rotarod apparatus for 1 min. Only animals which had successfully remained on the apparatus during a 1-min session carried out on the previous day, without treatment, were selected for this test (Duham and Miya, 1957). During the test session itself, i.e. after i.c.v. treatment, both the latency to fall from the revolving bar and the number of falls were recorded.

2.7. Statistical analysis

All data presented are expressed as the means ± S.E.M., and each value reflects the mean of either 10 (plus-maze or locomotor activity tests) or 8 (rotarod test) animals per group. The means were compared in a one-way analysis of variance (ANOVA) (independent variable = drug treatment), followed either by Dunnett's test or by a two-tailed Student's t-test for unpaired samples, where appropriate. Differences were considered significant when $P \leq 0.05$.

3. Results

3.1. Effects of the tachykinin receptor agonists and antagonists in the elevated plus-maze test

As shown in Fig. 1A, i.c.v. administration of substance P at 1 or 10 pmol, but not at 100 or 500 pmol, induced significant decreases in the frequency of entries into open arms, $F(5,54) = 2.844$, $P < 0.01$ and < 0.05, respectively. Similarly, significant decreases in this parameter were also observed in mice treated with the selective tachykinin NK_1 receptor agonist, substance P methyl ester, at 1 and 10 pmol $F(4,45) = 2.822$ (both $P < 0.05$), but not at 100 or 500 pmol (Fig. 1B). Neither of the agonists (up to 500 pmol) modified in a significant way the time spent in open arms (Fig. 1A,B), or the frequencies of entries into closed arms or of total entries (results not shown).

Mice injected with 10 or 100 pmol of the endogenous tachykinin NK_2 receptor agonist, neurokinin A, displayed significantly fewer entries into the open arms, $F(5,62) = 5.570$ ($P < 0.01$) and spent less time in these arms, $F(5,62) = 4.534$ ($P < 0.01$), whereas 1 or 500 pmol was ineffective (Fig. 2A). Other parameters measured in the

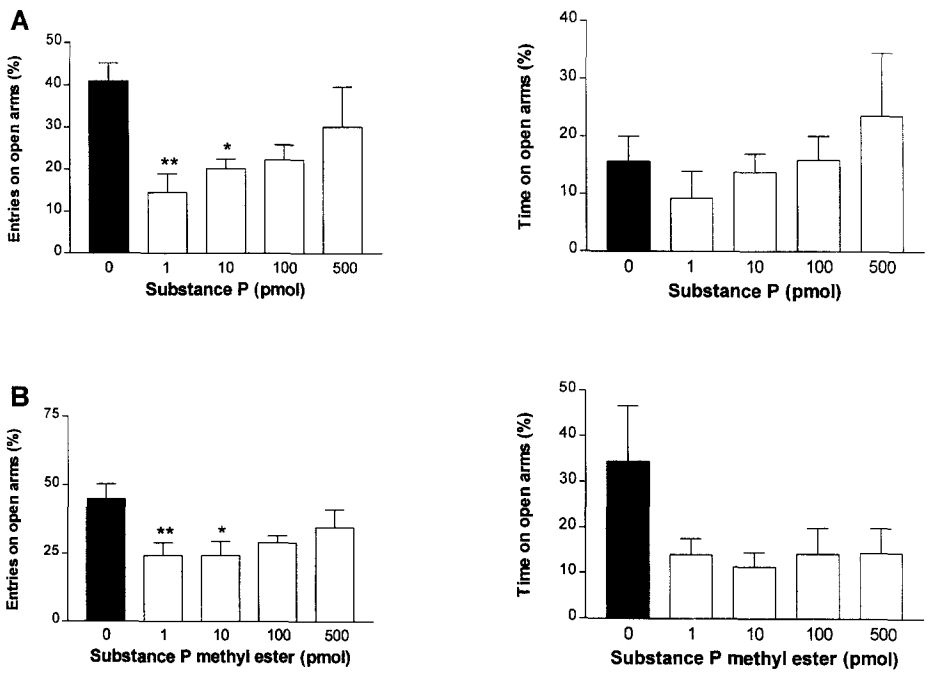

Fig. 1. Effects of substance P (A) and substance P methyl ester (B) on the behavior of mice in the elevated plus-maze test. The percentage of entries into open arms and time spent in these arms were recorded for 5 min, starting 5 min after the i.c.v. injection. Each value represents the mean ± S.E.M. of 10 observations. * $P < 0.05$ as compared to control (one-way ANOVA followed by Dunnett's test).

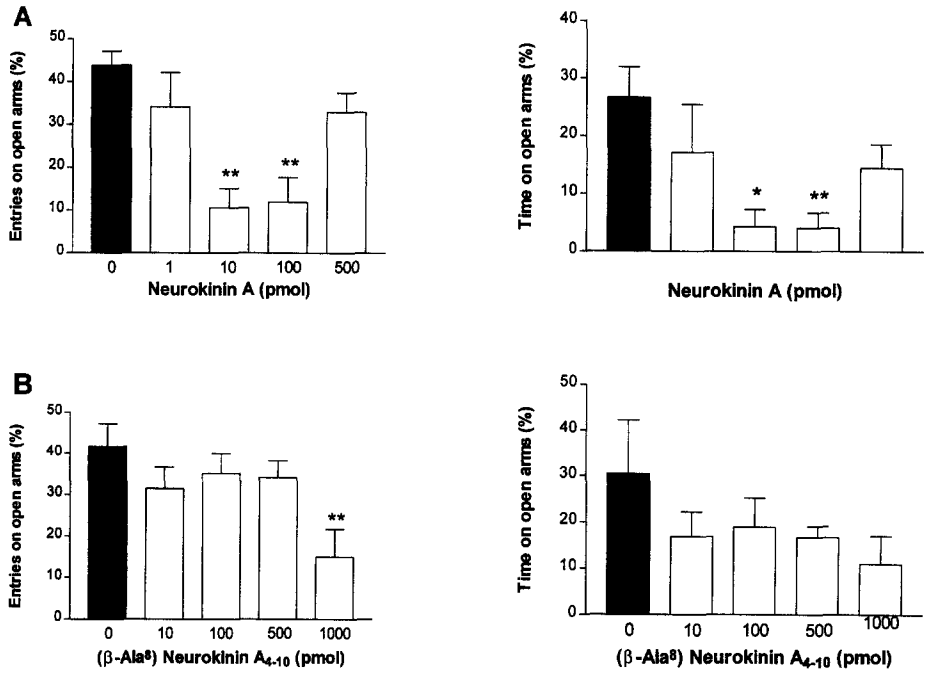

Fig. 2. Effects of neurokinin A (A) and [β-Ala^8]neurokinin A-(4–10) (B) on the behavior of mice in the elevated plus-maze test. The percentage of entries into open arms and time spent in these arms were recorded for 5 min, starting 5 min after the i.c.v. injection. Each value represents the mean ± S.E.M. of 10 observations. * $P < 0.05$, ** $P < 0.01$ as compared to control (one-way ANOVA followed by Dunnett's test).

plus-maze test were not significantly altered by neurokinin A up to 500 pmol (results not shown). Although the selective tachykinin NK_2 receptor agonist, [β-Ala^8]neurokinin A-(4–10) failed to affect the frequency of entries and time spent in open arms up to 500 pmol, it strongly reduced the frequency of open-arm entries when injected at 1 nmol, $F(5,54) = 2.916$ ($P < 0.05$, Fig. 2B). Concerning the other parameters analysed, [β-Ala^8]neurokinin A-(4–10) at 500 pmol enhanced the frequencies of entries into enclosed arms (control = 3.1 ± 0.7; drug-treated = 9.3

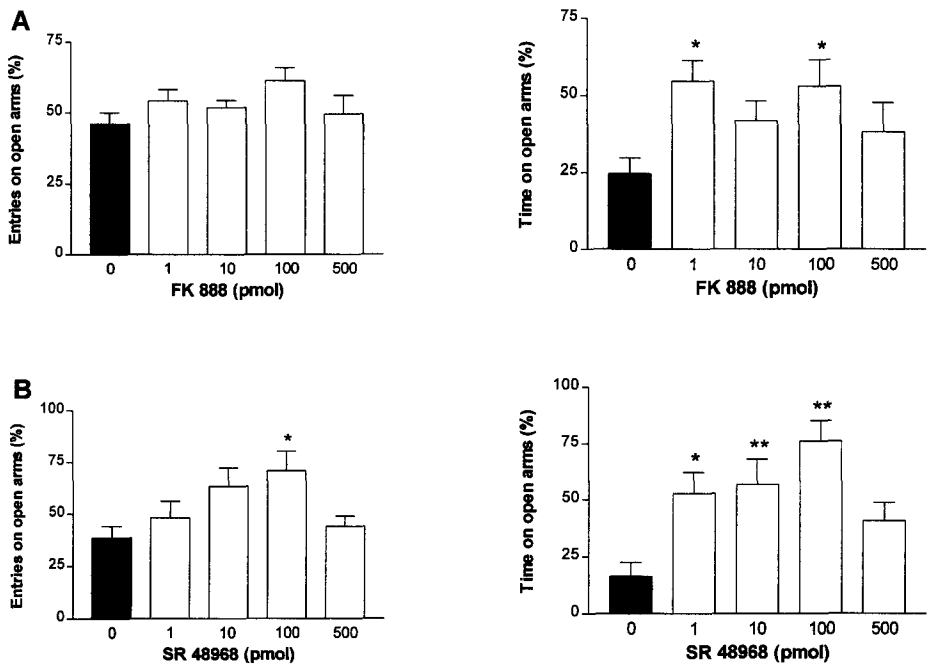

Fig. 3. Effect of FK888 (A) and SR 48968 (B) on the behavior of mice in the elevated plus-maze test. The percentage of entries into open arms and time spent in these arms were recorded for 5 min, starting 5 min after the i.c.v. injection. Each value represents the mean ± S.E.M. of 10 observations. * $P < 0.05$ as compared to control (one-way ANOVA followed by Dunnett's test).

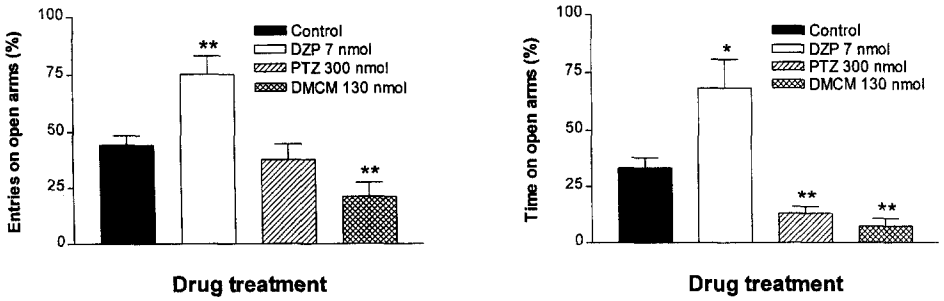

Fig. 4. Effects of the anxiolytic drug, diazepam (DZP, 7 nmol), and the anxiogenic drugs, pentylenotetrazol (PTZ, 300 nmol) and DMCM (130 nmol), on the behavior of mice in the elevated plus-maze test. The percentage of entries into open arms and time spent in these arms were recorded for 5 min, starting 5 min after the i.c.v. injection. Each value represents the mean ± S.E.M. of 10 observations. * $P < 0.05$ as compared to control (one-way ANOVA followed by Dunnett's test).

± 1.7), $F(5,54) = 4.244$ ($P < 0.01$), and of total entries (control = 5.5 ± 1.2; drug-treated = 13.0 ± 2.0), $F(5,54) = 4.151$ ($P < 0.05$).

On the other hand, i.c.v. injections of the selective tachykinin NK_1 receptor antagonist, FK 888, produced statistically significant increases in the time spent in the open arms of the plus-maze at 1 and 100 pmol (Fig. 3A), $F(4,45) = 2.674$ (both $P < 0.05$), but not at 10 or 500 pmol. None of the doses of FK 888 employed modified the frequencies of entries into open (Fig. 3A) or closed arms or of total entries (results not shown). Furthermore, central administration of the selective tachykinin NK_2 antagonist, SR 48968, augmented the frequency of open-arm entries (100 pmol; Fig. 3B), $F(4,45) = 3.141$ ($P < 0.05$), and the time spent in the open arms (1, 10 and 100 pmol; Fig. 3B), $F(4,45) = 6.060$ (each $P < 0.01$), without modifying the other parameters recorded in the plus-maze test (results not shown).

The reference anxiolytic drug, diazepam (7 nmol), also increased the frequency of entries into and time spent in the open arms. In contrast, the reference anxiogenic drugs, pentylenetetrazol (300 nmol) and DMCM (130 nmol), both decreased the time spent in open arms and the latter also reduced the frequency of open-arm entries, $F(3,42) = 9.918$ and $F(3,42) = 13.379$, respectively ($P < 0.01$; Fig. 4). Other parameters measured were not influenced by diazepam, pentylenetetrazol or DMCM (results not shown).

3.2. Effect of the tachykinin receptor agonists and antagonists on locomotor activity and motor coordination

Mice treated i.c.v. with substance P, substance P methyl ester, neurokinin A, [β-Ala^8]neurokinin A-(4–10), FK888 or SR48968 (each 100 pmol), or with the reference drugs, diazepam (7 nmol), pentylenetetrazol (300 nmol) or DMCM (130 nmol), failed to display any detectable alterations in locomotor activity, when compared to the cumulative values recorded from control vehicle-treated animals over 5, 10 and 15 min, $F(8,63) = 0.6324$, 0.6934 and 0.8504, respectively ($P > 0.05$; Table 1).

Likewise, as shown in Table 1, none of the tachykinin receptor agonists or antagonists (100 pmol) or the reference drugs, diazepam (7 nmol), pentylenetetrazol (300 nmol) or DMCM (130 nmol), altered the number of falls

Table 1
Effect of anxiolytic and anxiogenic compounds on locomotor activity and on motor performance in the rotarod test

Drug	Dose	Locomotor activity			Rotarod	
		5 min	10 min	15 min	Number of falls	Time on the rotating bar(s)
Control	–	25.8 ± 6.8	52.1 ± 12.6	76.5 ± 24.0	0.1 ± 0.1	54.2 ± 5.8
SP	100 pmol	31.3 ± 6.5	46.4 ± 11.5	65.9 ± 14.0	0.5 ± 0.4	52.5 + 5.6
SPME	100 pmol	34.3 ± 7.5	59.4 ± 13.0	86.8 ± 23.3	0.1 ± 0.1	57.0 ± 3.0
NKA	100 pmol	45.9 ± 13.0	55.4 ± 15.0	62.8 ± 14.0	0.0 ± 0.0	60.0 ± 0.0
NKA-(4–10)	100 pmol	31.6 ± 9.6	58.8 ± 17.2	94.5 ± 25.6	0.2 ± 0.1	49.6 ± 6.9
FK 888	100 pmol	23.9 ± 5.3	46.4 ± 15.3	66.0 ± 15.3	0.8 ± 0.4	42.4 ± 8.0
SR 48968	100 pmol	26.6 ± 6.2	40.7 ± 10.3	49.0 ± 13.0	0.0 ± 0.0	60.0 ± 0.0
DZP	7 nmol	18.3 ± 5.4	32.0 ± 9.6	48.0 ± 14.4	0.2 ± 0.1	53.0 ± 5.2
PTZ	300 nmol	17.9 ± 5.3	30.4 ± 8.1	50.0 ± 12.4	0.1 ± 0.1	56.0 ± 3.5
DMCM	130 nmol	46.7 ± 13.0	52.4 ± 14.0	62.1 ± 14.0	0.5 ± 0.3	48.0 ± 6.7

Data represent the means ± S.E.M. of the number of photobeam crossings in a cumulative way or of the number of falls and the total time spent on the rotating bar during a 1-min test ($n = 8–10$). Drugs were injected i.c.v., immediately before the test. SP = substance P; SPME = substance P methyl ester; NKA = neurokinin A; NKA-(4–10) = [β-Ala^8]neurokinin A-(4–10); DZP = diazepam; PTZ = pentylenetetrazol. $P > 0.05$ against PBS-treated animals (one-way ANOVA followed by Dunnett's test).

from ($F(7,72) = 1.05$, $P > 0.05$), or the time spent on ($F(7,72) = 0.65$, $P > 0.05$), the revolving bar in the rotarod test.

4. Discussion

The plus-maze test is one of the most reliable animal models for assessing the influence of drugs on anxiety. It is very sensitive to anxiogenic drugs such as pentylenetetrazol and the inverse benzodiazepine receptor agonist, DMCM, or to anxiolytic drugs, such as benzodiazepines, serotoninergic compounds and cholecystokinin CCK_B receptor antagonists (Harro et al., 1993, Zangrossi and Graeff, 1994; Pokk and Zharkovsky, 1995). The current results demonstrate that i.c.v. injection of substance P and neurokinin A, as well as of selective agonists of the tachykinin NK_1 receptor, substance P methyl ester, and of the tachykinin NK_2 receptor, [β-Ala^8]neurokinin A-(4–10), each induced anxiogenic-like effects in the mouse plus-maze test, similar to those elicited by much higher doses of pentylenetetrazol or DMCM. Conversely, i.c.v. injections of selective antagonists of NK_1 (FK888) or NK_2 receptors (SR48968) caused anxiolytic-like effects, like those induced by a higher dose of diazepam. These findings strongly suggest that anxiety levels in the mouse can be modulated via manipulation of central tachykinin mechanisms.

The natural (substance P) and selective (substance P methyl ester) NK_1 receptor agonists only reduced the frequency of open-arm entries, whereas NK_2 receptor agonists (neurokinin A and [β-Ala^8]neurokinin A-(4–10), respectively) reduced both the frequency of open-arm entries and the time spent in open arms. Conversely, similar distinctions were detected between the anxiolytic-like actions of NK_1 and NK_2 antagonists. Although it might be argued that these differences could be used to discriminate between the anxiogenic-like profiles of action of the agonists or anxiolytic-like actions of the antagonists, we believe that the data obtained thus far are still insufficient to justify this possibility. Further studies, comparing the time courses of action of NK_1 and NK_2 receptor agonists and antagonists in the plus-maze test and in other models of anxiety, may help to elucidate this point. Alternatively, it would be interesting to analyse the possible occurrence of synergistic interactions between NK_1 and NK_2 receptor agonists or antagonists in these tests, as reported by Couture et al. (1993) concerning the roles played by central NK_1 and NK_2 receptors in nociception in rats.

Another important aspect to be considered is the specificity of anxiolytic- and anxiogenic-like actions of tachykinin NK_1 and NK_2 receptor agonists and antagonists, respectively. Several central effects of substance P have been suggested to be mediated by actions of either N-terminal or C-terminal metabolites of the peptide (Larson and Sun, 1994; Huston and Hasenöhrl, 1995, for review see Regoli et al., 1994). However, the fact that FK 888 and SR 48968 themselves each caused effects opposite to those induced by the NK_1 (substance P and substance P methyl ester) and NK_2 agonists (neurokinin A and [β-Ala^8]neurokinin A-(4–10)), respectively, suggests that the present findings truly reflect actions on specific tachykinin receptors. However, confirmation of this view must await further studies analysing the influence of antagonist pretreatment and the anxiogenic-like actions of NK_1 and NK_2 agonists in the plus-maze test, even though such data may be difficult to interpret due to the fact that the antagonists themselves modify behavior.

Substance P, substance P methyl ester and neurokinin A each decreased, whereas FK 888 and SR 48968 increased, the frequency of entries into the open arms of the plus-maze at doses which did not modify the frequency of total entries (i.e. into both open and closed arms). The only exception was [β-Ala^8]neurokinin A-(4–10), which at 500 pmol enhanced the frequency of closed arm entries and of total entries. However, at a higher dose (1 nmol), even this peptide effectively decreased open-arm entries without modifying the former parameters. Thus, the anxiogenic- and anxiolytic-like effects of NK_1 and NK_2 receptor agonists and antagonists were unrelated to actions on locomotor activity or exploratory behavior. The fact that neither of these drugs, at doses modifying behavior in the plus-maze, affected either spontaneous locomotor activity in the activity cage or motor coordination in the rotarod test further substantiates this view.

Central administration of tachykinin NK_1 and NK_2 receptor agonists has been reported to induce scratching, biting, licking, hind paw tapping, grooming, 'wet dog shakes' and increased locomotion in rodents (Ravard et al., 1994; Rupniak and Williams, 1994). In the current study, only neurokinin A caused tail-tapping in about 30% of the mice treated with the 1-nmol dose (results not shown). Perhaps the remarkably small doses we employed (largely in the pmol range) were insufficient to trigger these behaviors.

To our knowledge, this is the first study to demonstrate that stimulation of central NK_1 receptors elicits anxiogenic-like effects. Also, the anxiolytic-like action of the tachykinin NK_1 receptor antagonist, FK 888, in the plus-maze test corroborates previous findings that systemic injection of the NK_1 receptor antagonist, CP 96345, delayes the entrance of mice into the dark compartment of a light-dark box and increased the time spent in the bright compartment (Zernig et al., 1992). However, as CP 96345, at the same doses, also decreased motor activity and rearing in both dark and bright compartments, the authors concluded that it acted mainly to cause sedation and motor impairment. On the other hand, our results would seem to be at variance with the reported inverse relationships found between substance P levels in the lumbar cerebrospinal fluid and the intensity of 'inner tension' and 'psychic

anxiety' in humans (Almay et al., 1988). However, a large proportion of the subjects analysed in that study were patients with chronic back pain, and it is known that painful stimuli increase the release of met-enkephalin heterosegmentally in the rat spinal cord (Le Bars et al., 1987), which in turn inhibits substance P release in the spinal dorsal horn (Yaksh et al., 1980).

Despite the relative paucity of tachykinin NK_2 receptors in the brain (for review see Quartara et al., 1995), we have found that i.c.v. injection of NK_2 receptor agonists also elicits anxiogenic-like effects, and that a selective antagonist of these receptors, SR 48968, markedly attenuates anxiety-related behaviors. These observations are in good agreement with reports showing that systemic treatment with SR 48968 or another NK_2 receptor antagonist, GR 159897, induces anxiolytic-like effects in mice in the light-dark box test, and in rats in a social interaction test or the elevated plus-maze test (Stratton et al., 1993; Walsh et al., 1995). The anxiolytic-like profiles of action of both NK_1 and NK_2 receptor antagonists per se emphasize an important tonic modulatory role for central tachykinins in the expression of anxiety-related behaviors, mediated via activation of either NK_1 or NK_2 receptors.

The central sites responsible for the effects of i.c.v. injected NK_1 or NK_2 receptor ligands on anxiety-related behaviors remain to be defined. Saija et al. (1989) reported that radiolabelled substance P, neurokinin A and eledoisin injected i.c.v. in rats were detected within 2–5 min of injection in areas as far away as the brainstem. Thus, it may be safe to speculate that, in the present study, the drugs reached one or more of the several periventricular areas controlling anxiety-related or defense behaviors such as septum, hippocampus, amygdala, raphe nuclei, medial hypothalamus or periaqueductal gray matter (Graeff, 1994; Shaikh and Siegel, 1994). Many of these areas exhibit significant substance P-like immunoreactive nerve fibers and/or cell bodies and tachykinin NK receptors (for review see Otsuka and Yoshioka, 1993). We are currently attempting to clarify this aspect by selective microinjection studies in rats. These studies will also include NK_3 receptor ligands, as we have found that the selective tachykinin NK_3 receptor agonist, senktide, induces a pronounced anxiolytic-like effect in the mouse plus-maze test, when given i.c.v. (De Lima et al., unpublished observation).

As both GABA-ergic and serotoninergic mechanisms are intimately implicated in the control of anxiety states (Shephard, 1986; Davis, 1992; Griebel et al., 1993), it seems plausible to suggest that central tachykinins modulate anxiety by interfering with one or both of these classical neurotransmitter systems. In this respect, there is ample evidence for the co-existence of substance P with either 5-HT or GABA in central neurones and showing that substance P can modify central levels of these transmitters (Johansson et al., 1981; Rosen et al., 1995). However, this and other possible cellular mechanisms of modulation of anxiety by tachykinins remains to be elucidated.

In conclusion, we have shown that central mechanisms mediated via tachykinin NK_1 and NK_2 receptors modulate anxiety substantially, as evaluated in the plus-maze test in mice. Agonists for these receptors are extremely potent stimulators of anxiogenic-related behaviors, whereas the reverse is observed with selective antagonists of these receptors. The current results raise the exciting possibility that several potent non-peptide NK_1 and NK_2 receptor antagonists developed in recent years (Hagan et al., 1991; Emonds-Alt et al., 1992; Fujii et al., 1992) may constitute a potentially effective alternative treatment for anxiety-related disorders. However, to fulfill this expectation, considerable effort must now be directed to characterize the sites and modes of cellular action underlying the potent anxiolytic effects of tachykinin NK_1 and NK_2 receptor antagonists, as well as the various other roles played by the central tachykinin systems.

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