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ORIGINAL INVESTIGATION

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Effects of SR48968, a selective non-peptide NK_2 receptor antagonist on emotional processes in rodents

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Abstract *Rationale:* It has been suggested that tachykinin NK_2 receptor antagonists may have therapeutic utility in anxiety and/or depressive disorders. *Objective:* The present study investigated the modulatory action of the NK_2 receptor antagonist SR48968 on emotional processes in rodents. *Methods:* The tests used include classical models of anxiety (punished lever pressing and punished drinking conflict tests, elevated plus-maze in rats), a model based on defensive behaviors of mice confronted with a natural threat (a rat), and two tests based on exposure of rats or mice to a natural predator (a cat) followed by subsequent exposure to a cat odor cue. The prototypical anxiolytic diazepam was used throughout as a positive control, the antidepressant imipramine was tested in the mouse defense test battery and in both models of predatory exposure, and the selective CRF_1 receptor antagonist antalarmin was used in the cat-exposure test in rats. *Results:* Unlike diazepam, SR48968 failed to increase rates of responding suppressed by punishment in both conflict procedures. By contrast, in the elevated plus-maze test, the NK_2 receptor antagonist (3 mg/kg, IP) elicited positive effects on traditional and ethologically derived measures of anxiety. In the mouse defense test battery, SR48968 (0.03–1 mg/kg, IP) decreased flight reactions, risk assessment behavior, defensive biting and escape attempts. While the magnitude of the effects on flight, risk assessment and escape attempts of the NK_2 receptor antagonist was less than that of diazepam, SR48968 appeared to be as effective as the BZ on defensive biting. In rats previously exposed to a cat, SR48968 (3 mg/kg, IP), antalarmin (1 mg/kg, IP), imipramine (30 mg/kg, IP), but not diazepam, reduced subsequent high levels of avoidance responses when subjects are exposed to a cat odor-saturated cue 1 h later. Similar effects of SR48968 (0.1–0.3 mg/kg, IP) were observed in mice following repeated administration (twice a day/5 days/IP). Importantly, the positive effects of the NK_2 receptor antagonist were evident at doses that did not impair general activity, unlike imipramine which displayed mainly sedative action. Moreover, the (*R*)-enantiomer of SR48968, SR48965, which was tested in the elevated plus-maze, the mouse defense test battery and the cat exposure tests, was much less active than its racemate, indicating a stereoselective action of SR48968. *Conclusion:* These data show that while SR48968 has limited or no efficacy in models or behavioral measures mainly sensitive to BZs, it shows good activity in reducing anxiety-like behaviors following traumatic stress or upon forced and unavoidable contact with a threatening stimulus. This suggests that NK_2 receptor antagonists may have a potential in the treatment of some forms of anxiety disorders.

Keywords Antalarmin · Anxiety · Cat exposure · Conflict test · Defensive behavior · Diazepam · Exploration test · Imipramine · Mice · Rat · SR48968 · Tachykinin NK_2 receptor antagonist

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Introduction

The mammalian tachykinins are a group of neuropeptides that includes substance P, neurokinin A and neurokinin B. The biological actions of tachykinins are mediated via the activation of three G protein-coupled 7-transmembrane domain receptors designated as NK_1, NK_2 and NK_3 (Regoli et al. 1994). Both NK_1 and NK_3 receptors are widely distributed in the central nervous system (CNS), while the NK_2 receptor is found in smooth muscle of the gastrointestinal, respiratory and urinary tracts, but it has also been located in discrete regions of rodent CNS (Otsuka and Yoshioka 1993; Maggi 1995; Steinberg et al. 1998; Zerari et al. 1998; Preston et al. 2000). For example, autoradiographic studies in neonate rat brain have demonstrated the existence of NK_2 receptor binding sites in the thalamus, hippocampus and cortex (Hagan et al. 1993). The neuroanatomical dis-

tribution of NK_2 receptors has prompted speculation about its functional role in anxiety disorders, and has fueled both basic research and commercial interest in this receptor, leading to numerous studies that investigated the behavioral action of NK_2 receptor ligands in animal models of anxiety.

A number of studies in rats have shown that central injection of the preferential NK_2 receptor agonist neurokinin A and the selective NK_2 receptor agonist [β-Ala^8]neurokinin A-(4-10), a fragment of neurokinin A, produce a behavioral profile that is consistent with an anxiogenic-like action (for review, see Griebel 1999). Recently, several classes of non-peptide antagonists at NK_2 receptors have been identified (Mills 1997). Studies using selective NK_2 receptor antagonists in anxiety models have reported these compounds to display anti-anxiety-like activity. For example, positive effects have been reported with the selective NK_2 receptor antagonists GR159897 and SR48968 in the light/dark exploration, social interaction, and elevated plus-maze procedures (Stratton et al. 1993a, 1994; Bernatzky and Saria 1995; De Lima et al. 1995; Walsh et al. 1995; Teixeira et al. 1996). Moreover, GR159897 and SR48968 significantly increased the time spent by marmosets at the front of the cage following confrontation with a human "threat", an effect which is consistent with an anxiolytic-like action (Walsh et al. 1995). In addition to its anxiolytic-like activity, SR48968 displayed antidepressant-like effects in several behavioral (forced swimming test in rats, maternal separation-induced vocalization in guinea-pigs) and neurochemical (CREB mRNA expression in hippocampus) assays, suggesting a broader spectrum of therapeutic activity than classical anxiolytics such as benzodiazepines (Jung et al. 2000).

The present study was undertaken to investigate further the modulatory action of SR48968 (Emonds-Alt et al. 1992; Maggi et al. 1993) on emotional processes in rodents. The tests used include classical models of anxiety (punished lever pressing and punished drinking conflict tests, elevated plus-maze in rats), a model based on defensive behaviors of mice confronted with a natural threat (a rat), and two tests based on exposure of rats or mice to a natural predator (a cat) followed by subsequent exposure to a cat odor cue. The prototypical anxiolytic diazepam was used throughout as a positive control, the antidepressant imipramine was tested in the mouse defense test battery as well as in both models of predatory exposure, and the selective CRF_1 receptor antagonist antalarmin was used in the cat exposure test in rats. Previous studies have shown the mouse defense test battery to be sensitive to both anxiolytics and antidepressants (Blanchard et al. 1997; Griebel and Sanger 1999), while predatory exposure has proved to be useful to reveal positive effects of antidepressants and CRF_1 receptor antagonists, but not of benzodiazepines (Griebel et al. 1999). Finally, the behavioral effects of the (*R*)-enantiomer of SR48968, SR48965 were examined in the mouse defense test battery, in the elevated plus-maze test and in both cat-exposure procedures.

Materials and methods

Ethics

All procedures described here fully comply with French legislation on research involving animal subjects.

Animals

Male Wistar-Kyoto rats (Iffa Credo, L'Arbresle, France) were used in the punished lever pressing procedure. They weighed 180–200 g at the beginning of training and 350–400 g at the time of drug testing. Male Sprague-Dawley and Wistar rats (Iffa Credo and Charles River France, Saint-Aubin-lès-Elbeuf), weighing 190–230 g at time of testing, were used in the punished drinking, elevated plus-maze and staircase tests. They were housed in groups of eight. Different strains of rats were used to optimise conditions. For example, preliminary data from our laboratory have shown that, unlike Sprague-Dawley rats, Wistar rats from our provider are poor responders in the elevated plus-maze (i.e. they display weak avoidance responses of the open aversive arms), making it difficult to use them for the screening of anxiolytics. In the mouse defense test battery and in the free-exploration test, subjects were naive male Swiss mice aged 10 weeks at the time of testing (Iffa Credo). The former were housed singly in standard cages, whereas subjects used in the free-exploration test were housed in groups of five. Moreover, male Long Evans rats (400–500 g) (Iffa Credo) were used as threat stimulus in the mouse defense test battery. Rats used in the punished lever pressing procedure were restricted to the food obtained during sessions and a daily ration of 15–20 g of standard laboratory chow given at the end of each weekday and over the weekend. All animals were maintained under standard laboratory conditions (21–23°C; relative humidity: 40–60%) and kept on a 12-h light/dark cycle with light onset at 6 a.m.

Compounds

Antalarmin, diazepam, imipramine, SR48968 [(*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]-benzamide] and SR48965 (synthesized by Sanofi-Synthelabo) were dissolved or prepared as suspensions in physiological saline containing 1 or 2 drops of Tween 80. All drugs were administered intraperitoneally (IP) 30 min before experiments were carried out. In the free-exploration study, drugs were given acutely and repeatedly (twice a day, for 5 days; the last injection was performed 30 min prior to testing). All doses are expressed as the bases and were chosen on the basis of previously published behavioral studies (Stratton et al. 1993a, 1993b; Bernatzky and Saria 1995; Walsh et al. 1995).

Punished lever pressing

The procedure was a modification of that described previously (Sanger et al. 1985). Animals were tested in standard rat operant test chambers (Med Associates, Ga., USA) placed in sound-attenuated boxes with ventilation fans. Each chamber was fitted with a stainless steel grid floor. Electric shocks could be delivered to each grid by a shock generator and scrambler (Med Associates). A total of 11 rats were trained initially to press a lever for food reward (45 mg precision food pellets; P.J. Noyes, Inc., Lancaster, N.H., USA). As training progressed, schedule parameters were gradually changed to a variable interval (VI 30 s) schedule of food reinforcement during daily 15-min sessions. After several sessions of VI 30 s responding, five 60-s periods of a visual stimulus were presented during a 25-min session. Each visual stimulus consisted of three stimulus lights situated above the food pellet dispenser and to the right of the response lever, which flashed at a rate of 1 s on, 1 s off. In this component, a footshock punishment schedule

consisting of two independent VI schedules (VI 30 s for food, VI 10 s for shock) was in operation. Footshock was initially set at 0.1 mA. The first stimulus presentation started 5 min after the beginning of the session, and each following stimulus commenced 150 s after the end of the preceding stimulus. The magnitude of footshock was individually titrated for each rat (shock levels ranged from 0.3 to 0.65 mA) to obtain stable baselines of responding (i.e. an average lever pressing rate of 8±2 presses in each 60 s punished responding period). To obtain stable levels of responding, an average of approximately 30 sessions after initiation of the punishment contingency was necessary. Once stable baselines of responding were obtained, drug studies were initiated.

Drug injections were given once or twice each week with at least 2 non-drug days intervening between two drug administrations. Vehicle was injected on all non-drug days. Drugs and doses were given in a mixed order. The effects of drugs were assessed on punished and unpunished response rates. The former corresponds to those recorded during the presentation of the visual stimulus, whereas the latter were taken from the 60-s periods immediately preceding and immediately following each stimulus presentation. The mean values of punished and unpunished rates recorded during the non-drug session preceding the drug injection sessions were used as the control values. Thus, drug effects were analyzed statistically by comparing performances after drug administration with the mean values taken from appropriate control sessions using a Friedman's ANOVA.

Punished drinking

The procedure was a modification of the technique described by Vogel et al. (1971). At the beginning of the experiment, rats, deprived of water for 48 h prior to testing, were placed in cages (27×22×21 cm) with a stainless steel grid floor. Each cage contained a drinking tube connected to an external 50 ml burette filled with tap water. Trials commenced only after the animal's tongue contacted the drinking tube for the first time. An electric shock (0.6 mA/10 ms) was delivered through the drinking spout after every twenty licks, and the number of shocks received was recorded automatically during a 5-min period. Data were analyzed with the non-parametric Kruskal-Wallis test. Subsequent comparisons between treatment groups and control were carried out using the Siegel and Castellan test.

Elevated plus-maze

The test apparatus was based on that described by Pellow et al. (1985). All parts of the apparatus were made of dark polyvinylplastic with a black rubber floor. The maze was elevated to a height of 50 cm with two open (50×10 cm) and two enclosed arms (50×10×50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10×10 cm). To prevent rats falling off, a rim of Plexiglas (1 cm high) surrounded the perimeter of the open arms. The illumination in the experimental room consisted of one red neon tube fixed on the ceiling, so that experiments were performed under dim light conditions. The light intensity on the central platform was 10 lux. At the beginning of the experiment, rats were placed in the center of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms, number of open-arm entries and number of closed-arm entries (defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via videolink by an observer located in an adjacent room. This permitted the recording of the more ethologically orientated measures: (a) attempt: attempt at entry into open arms followed by avoidance responses. This includes stretched attend posture (the rat stretches forward and retracts to original position); (b) head-dipping: protruding the head over the edge of an open arm and down towards the floor (this response can occur while the animal's body is in a closed arm, central square or on an open arm). The results were expressed as mean ratio of time spent in open arms to total time spent in both open and closed arms, mean total number of both closed and open arm entries, mean total number of attempts and mean total number of head-dips. Data were analyzed by one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Mouse defense test battery

The test was conducted in an oval runway, 0.40 m wide, 0.30 m high, and 4.4 m in total length, consisting of two 2 m straight segments joined by two 0.4 m curved segments and separated by a central wall (2.0×0.30×0.06 m). The apparatus was elevated to a height of 0.80 m from the floor to enable the experimenter to easily hold the rat, while minimizing the mouse's visual contact with him. All parts of the apparatus were made of black Plexiglas. The floor was marked every 20 cm to facilitate distance measurement. Activity was recorded with video cameras mounted above the apparatus. In addition, the apparatus was equipped with infrared beams and sensors capable of measuring the velocity of the animal during the chase/flight test. The room illumination was provided by one red neon tube fixed on the ceiling and two desk lamps with red bulbs placed respectively on two tables (elevated to a height of 1 m) located 1 m away from the runway. The light intensity in the runway was 7 lux. Experiments were performed under red light between 9.30 a.m. and 3 p.m. The experimenter was unaware of treatment conditions.

Effects on spontaneous locomotor activity: the pre-test

Subjects were placed into the runway for a 3-min familiarization period during which line crossings and wall rears were recorded.

Effects on flight responses: the rat avoidance test

This test was run immediately after the 3-min familiarization period and, to ensure an initial separation of 2 m between the threatening stimulus and subject, commenced only when the mouse was at one end of the apparatus. At this point, a hand-held dead rat (killed by CO_2 inhalation) was introduced at the opposite end of the apparatus and brought up to the subject at an approximate speed of 0.5 m/s. Approach was initiated only if the subject was at a standstill with its head oriented towards the hand-held rat. Consequently, intervals between trials were variable but never exceeded 15 s. Approach was terminated when contact with the subject was made or the subject ran away from the approaching rat. If the subject fled, avoidance distance (the distance from the rat to the subject at the point of flight) was recorded. The rat was removed from the apparatus between each trial so that there was no visual contact between the predatory stimulus and the subject. This procedure was repeated five times, with mean avoidance distance (cm) and number of avoidances calculated for each subject.

Effects on risk assessment: the chase and the straight alley tests

The hand-held rat was brought up to the subject at a speed of approximately 2.0 m/s. As was the case in the rat avoidance test, a constant distance of 2 m separated the rat and the subject when the former was introduced in the runway. Chase was initiated only when the subject was at a standstill with its head oriented toward the hand-held rat, and was completed when the subject had traveled a distance of 15 m. During the chase, a constant distance of 20 cm was maintained between the two animals. Consequently, if the subject stopped fleeing before traveling the full 15 m, the chase was stopped in order to avoid actual stimulus contact. The experimenter then moved the hand-held rat quickly from left to right in front of the subject to elicit flight. During the chase, flight speed (measured when the subject is running straight) and the number of stops (pause in movement) were recorded. The rat was removed after the chase was completed. By the closing of two doors (60 cm distant

from each other), the runway was then converted to a straight alley in which the subject was constrained. The rat was introduced in one end of the straight alley. This phase was initiated only when the subject faced the rat and at a stimulus-subject distance of 40 cm. During 30 s, the number of approaches/withdrawals (subject must move more than 20 cm forward from the closed door, then return to it) and immobility time were recorded. The hand-held rat remained at the place it was introduced for the full 30 s, after which it was removed from the straight alley.

Effects on defensive threat and attack responses: the forced contact test

In this test, the experimenter brought the rat up to contact the subject in the straight alley. Approaches were directed quickly (within 1 s) to the subject's head. For each such contact, bites and upright postures by the subjects were noted. If no defensive threat and/or attack responses were elicited within 15 s, the rat was removed from the apparatus. This was repeated three times. The time interval between each trial was approximately 5±1 s. The results were expressed as mean number of bites.

Effects on contextual defense: the post-test

Immediately after the forced contact test, the rat was removed and the door opened. Escape attempts including wall rears, wall climbs, and jump escapes were recorded during a 3-min session. Data were analyzed by one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Staircase test following cat exposure

Rats were first confronted during a 10-min session with a cat in a cage (82×56×62 cm) subdivided into two compartments, one containing the cat, the other the rat. Separation consisted of a transparent PVC wall with holes allowing the cat to reach the other side with its paws. One hour after cat exposure, rats were placed at the bottom of the staircase test (Thiébot et al. 1973) containing a cat odor-saturated brush on its top stair. The test started after a 1-min familiarization period. The following behavioral measures were recorded during a 5-min session under red light via a closed circuit TV camera by an observer located in an adjacent room: time spent in contact with the brush, rearings against the wall of the apparatus and stairs climbed. The results were expressed as mean percentage of time spent in contact with the brush, mean total number of rears and mean total number of stairs climbed. Data were analyzed by one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Free-exploration test following cat exposure

The familiarization procedure in the free-exploration box was a modification of the technique described in a previous paper (Griebel et al. 1993). The apparatus consisted of a PVC box (30×20×20 cm) covered with Plexiglas and subdivided into six equal square exploratory units, which were all interconnected by small entries. It could be divided in half lengthwise by closing three temporary partitions. Approximately 20 h before testing, each subject was placed in one half of the apparatus with the temporary partitions in place, in order to be familiarized with it. The floor of this half was covered with fresh sawdust and the animal was given unlimited access to food and water. On the test day, subjects were removed from the free-exploration box and confronted individually with a cat during a 5-min session (same apparatus as above). The mouse was then put back in the free-exploration apparatus and was exposed 1 h later to both familiar and novel compartments by removal of the temporary partitions. The novel compartment contained cat feces in each unit. The behavior of the mouse was then observed, under red light, for 5 min via a closed circuit TV camera by an observer located in an adjacent room. The following parameters were recorded: (a) time spent in the novel compartment; (b) total unit entries. The results were expressed as mean percentage of time spent in the novel compartment and mean total number of novel unit changes. Data were analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using Dunnett's *t*-test.

Results

Punished lever pressing in rats

Table 1 shows that the rates of responding decreased by the punishment contingency were significantly increased by diazepam (χ^2=20.5, P<0.001) from 1.2 mg/kg. In contrast, SR48968 failed to produce any statistically significant increases in rates of punished responding. Diazepam, but not SR48968 produced disinhibitory effects as it increased significantly unpunished responding at 2.5 mg/kg (χ^2=19.6, P<0.01).

Punished drinking test in rats

Table 2 shows that diazepam (3–10 mg/kg) significantly increased the number of punished licks (K=16.5,

Table 1 Effects in the punished lever pressing test in rats. Drugs were administered IP 30 min before the beginning of the test. Data represent mean±SEM. n=8

Drugs	Dose mg/kg	Punished responding	Unpunished responding
Diazepam	0	10.0±1.1	72.5±6.6
	0.3	17.7±3.4	78.7±8.9
	0.6	19.2±3.3	84.2±7.5
	1.2	36.7±7.1*	93.6±11.5
	2.5	32.0±7.4*	95.5±8.0*
	5	28.0±4.6*	57.6±6.8
SR48968	0	6.0±0.7	96.8±8.5
	0.1	6.9±0.8	96.8±9.2
	0.3	7.8±1.2	98.0±10.6
	1	6.6±1.1	99.3±7.4
	3	4.4±0.6	90.6±10.3

*P<0.05 (Friedman)

Table 2 Effects in the punished drinking test in rats. Drugs were administered IP 30 min before the beginning of the test. Data represent mean±SEM. n=11–14

Drugs	Dose mg/kg	Number of shocks
Diazepam	0	8.4±1.3
	1	14.2±4.1
	3	30.5±4.4*
	10	23.6±4.6*
SR48968	0	6.8±1.3
	0.1	11.4±2.8
	0.3	7.8±1.4
	1	7.1±1.9
	3	9.2±3.0

*P<0.05 (Siegel and Castellan non-parametric test)

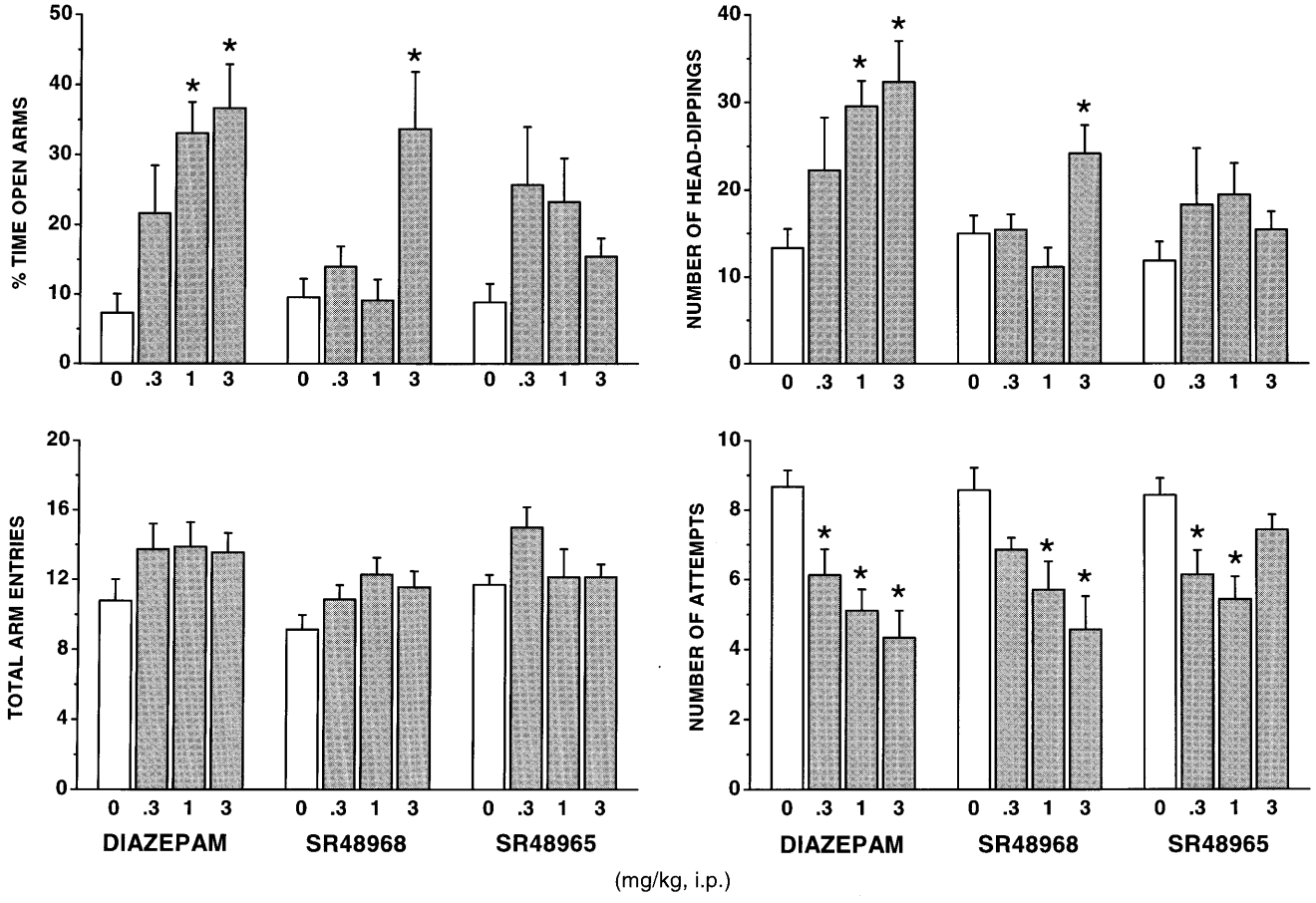

Fig. 1 Effects in the elevated plus-maze test in rats. Drugs were administered 30 min before the beginning of the test. Data represent mean±SEM. *n*=7–9. **P*<0.05 (Dunnett's *t*-test)

P<0.001). By contrast, SR48968 failed to modify significantly punished responding in this test.

Elevated plus-maze test in rats

Figure 1 shows that diazepam (1 and 3 mg/kg), SR48968 (3 mg/kg), but not SR48965 significantly increased the percentage of time spent into open arms [$F(3,31)=6.6$, $P<0.001$; $F(3,24)=5.9$, $P<0.01$, respectively]. With respect to the ethologically derived measures, diazepam (0.3–3 mg/kg) and SR48968 (1 and 3 mg/kg) reduced the number of attempts at entry into open arms followed by avoidance responses [$F(3,31)=8.4$, $P<0.001$; $F(3,24)=5.6$, $P<0.01$, respectively], and increased directed exploration (head-dipping) at their highest doses [$F(3,31)=4.4$, $P<0.01$; $F(3,24)=5.3$, $P<0.01$, respectively]. The latter measure remained unaffected by SR48965, but the (*R*)-enantiomer of SR48968 significantly reduced attempts at 0.3 and 1 mg/kg [$F(3,24)=5.4$, $P<0.01$]. Total arm entries remained unchanged in all groups.

Mouse defense test battery

Effects on spontaneous locomotor activity: the pre-test

Table 3 shows that prior to confrontation with the rat, imipramine (from 10 mg/kg), but none of the other drugs significantly decreased the number of line crossings [$F(3,44)=19.7$, $P<0.001$] and wall rears [$F(3,44)=4.4$, $P<0.001$].

Effects on flight responses: the rat avoidance test

Table 4 shows that diazepam and SR48968, but not the other compounds significantly modified avoidance distance [diazepam: $F(3,29)=12.1$, $P<0.001$; SR48968: $F(5,66)=2.8$, $P<0.05$] and frequency [diazepam: $F(3,32)=11.6$, $P<0.001$; SR48968: $F(5,66)=2.6$, $P<0.05$]. Post-hoc analysis showed that diazepam and SR48968 significantly reduced these measures at the highest doses.

Effects on risk assessment

Chase test. Table 4 shows that, except for SR48965, the drugs significantly modified the number of stops [diazepam: $F(3,35)=54.2$, $P<0.001$; imipramine: $F(3,44)=2.9$, $P<0.05$; SR48968: $F(5,66)=4.6$, $P<0.001$]. Dunnett comparisons indicated that stops were significantly reduced by diazepam at all doses (0.5–3 mg/kg), by imipramine

Table 3 Measures of activity in the mouse defense test battery before (line crossings and rears) and during (flight speed and immobility time) confrontation with the rat. Drugs were administered 30 min before the beginning of the test. Data represent mean±SEM. *n*=9–12

Drugs	Dose mg/kg IP	Line crossings	Rears	Flight speed (s)	Immobility time (s)
Diazepam	0	142.8±8.3	14.4±4.2	0.62±0.07	14.2±1.0
	0.5	125.2±8.7	12.8±3.7	0.66±0.06	15.8±1.3
	1	158.8±7.7	15.3±4.4	0.59±0.04	15.1±1.3
	3	114.6±10.1	9.2±2.7	0.58±0.07	13.9±1.8
Imipramine	0	134.8±9.8	8.3±2.3	0.89±0.11	9.7±2.2
	3	109.4±10.8	7.6±1.6	0.66±0.06	11.1±1.8
	10	91.6±7.4*	4.4±1.2	0.52±0.05*	8.4±1.4
	30	41.0±7.2*	1.6±0.9*	0.45±0.07*	9.8±2.3
SR48968	0	149.8±7.8	14.8±1.8	0.71±0.04	17.8±1.1
	0.01	142.2±7.7	10.2±2	0.72±0.05	18.8±1.2
	0.03	144.3±9.3	9.5±1.5	0.71±0.07	17.8±1.4
	0.1	136.8±9.7	10.8±2.4	0.58±0.05	18.6±1.3
	0.3	146.0±9.3	9.3±2.2	0.72±0.06	17.8±0.9
	1	137.8±8.2	13.3±2.5	0.8±0.77	16.8±0.2
SR48965	0	129.1±7.9	12.2±2.4	0.7±0.11	23.2±0.9
	0.03	119.7±9.2	7.7±1.5	0.62±0.12	23.1±1.4
	0.1	127.3±12.3	9.2±2.5	0.64±0.06	21.7±1.4
	0.3	113.2±10.0	9.2±2.3	0.73±0.11	18.4±2.1
	1	129.0±10.6	13.3±2.5	0.65±0.10	19.4±1.5

*P<0.05 (Dunnett's *t*-test)

Table 4 Effects on measures of defensive behaviors in the mouse defense test battery. Drugs were administered 30 min before the beginning of the test. Data represent mean±SEM

Drugs	Dose mg/kg IP	Avoidance distance (cm)	Avoidance frequency	Number of stops	Approaches-withdrawals	Escape attempts
Diazepam	0	147.5±4.2	3.4±0.3	9.7±0.2	0±0	44±14.7
	0.5	135.4±12.9	3.4±0.3	8.3±0.3*	0.3±0.2	35.1±11.7
	1	91.2±5.8*	2.6±0.5	7.1±0.1*	0.8±0.3*	29.6±9.9*
	3	77.9±5.9*	0.7±0.3*	4.6±0.5*	1.6±0.4*	16.3±5.4*
Imipramine	0	156.0±17.5	1.8±0.3	9.3±0.5	1.2±0.4	32.1±9.3
	3	102.0±20.0	1.8±0.4	7.8±0.8	0.8±0.3	25.0±7.3
	10	150.9±17.9	1.3±0.3	7.0±1.0*	0.7±0.3	26.6±7.7
	30	102.7±16.9	1.1±0.3	6.3±0.7*	1.3±0.3	4.9±1.4*
SR48968	0	164.1±14.2	2.7±0.2	9.3±0.2	0.0±0.0	40.7±11.7
	0.01	181.7±1.9	2.7±0.2	9.0±0.1	0.0±0.0	36.0±10.4
	0.03	142.2±9.7	2.3±0.2	8.9±0.2	0.1±0.1	31.0±9.0*
	0.1	153.3±14.6	2.0±0.3	8.8±0.2	0.0±0.0	34.2±9.9*
	0.3	172.9±15.2	2.3±0.2	8.6±0.2*	0.0±0.0	31.0±9.0*
	1	120.9±12.2*	1.8±0.3*	8.0±0.3*	0.3±0.2	29.6±8.5*
SR48965	0	164.2±10.3	2.3±0.5	8.0±0.9	0.0±0.0	32.7±10.3
	0.03	143.0±10.5	2.4±0.6	7.1±1.2	0.0±0.0	31.2±10.4
	0.1	129.5±13.9	2.9±0.5	9.9±1.1	0.2±0.2	29.9±10.0
	0.3	142.6±7.1	3.4±0.3	8.3±1.3	0.8±0.3	30.8±9.7
	1	161.3±8.0	2.3±0.5	10.1±1.4	0.3±0.2	32.4±10.3

*P<0.05 (Dunnett's *t*-test)

at 10 and 30 mg/kg and by SR48968 from 0.3 mg/kg. In addition, Table 3 shows that imipramine, but none of the other drugs, significantly decreased flight speed at 10 and 30 mg/kg.

Straight alley test. Diazepam [$F(3,32)=7.3$, $P<0.001$] but not the other drugs significantly increased the number of approaches followed by withdrawal responses at 1 and 3 mg/kg. Moreover, none of the drugs significantly modified immobility time during this phase.

Effects on defensive attack responses: the forced contact test

Figure 2 shows that diazepam [$F(3,32)=43.8$, $P<0.001$], imipramine [$F(3,44)=4.2$, $P<0.01$], SR48968 [$F(5,66)=5.6$, $P<0.001$], but not SR48965 significantly affected defensive biting. Further comparisons indicated that diazepam at all doses, imipramine at 10 and 30 mg/kg, and SR48968 from 0.03 mg/kg significantly decreased this defense reaction.

Effects on contextual defense: the post-test

Data are summarized in Table 4. ANOVA indicated that diazepam [$F(3,32)=10.2$, $P<0.001$], imipramine [$F(3,44)=30.2$, $P<0.001$] and SR48968 [$F(5,66)=2.4$, $P<0.05$], but not SR48965 modified escape attempts from the runway cage following the removal of the rat. Post-hoc comparisons revealed that diazepam decreased significantly this behavior from 1 mg/kg, whereas imipramine reduced it

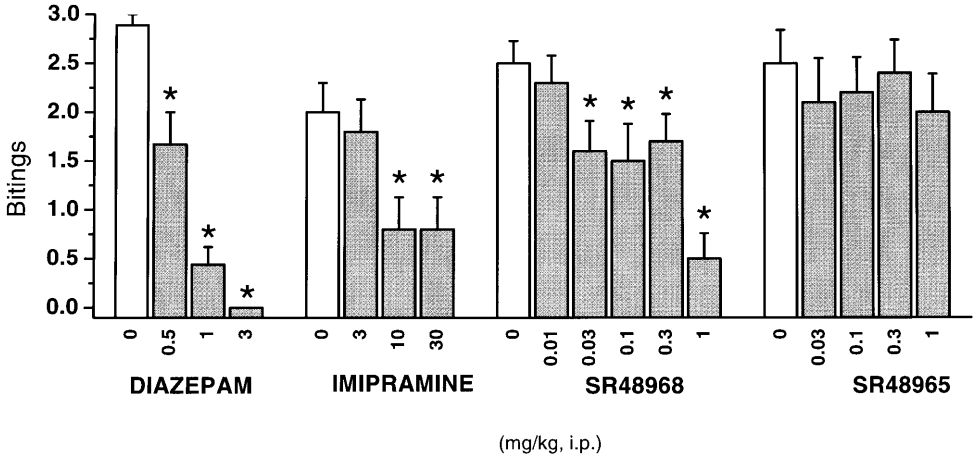

Fig. 2 Effects on defensive biting in the mouse defense test battery. Drugs were administered 30 min before the beginning of the test. Data represent mean±SEM. *n*=9–12. **P*<0.05 (Dunnett's *t*-test)

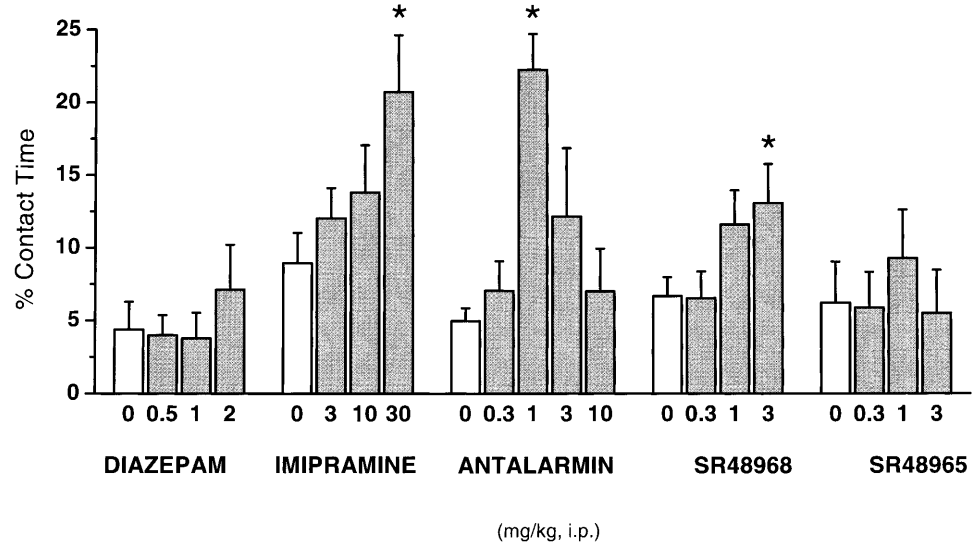

Fig. 3 Percentage of time spent in contact with a cat odor-saturated brush in the staircase test, 1 h after unavoidable exposure to a cat. Drugs were administered 30 min before the beginning of the test. Data represent mean±SEM. *n*=6–15. **P*<0.05 (Dunnett's *t*-test)

at the highest dose only (30 mg/kg), and SR48968 from 0.03 mg/kg.

Staircase test following cat exposure in rats

Figure 3 shows that imipramine [$F(3,44)=2.9$, $P<0.05$], antalarmin [$F(4,40)=5.9$, $P<0.01$] and SR48968 [$F(3,56)=2.5$, $P<0.05$], but not diazepam or SR48965 significantly modified the percentage of time subjects spent in contact with the cat odor-saturated brush. Post-hoc analysis revealed that this parameter was increased by imipramine at 30 mg/kg, by antalarmin at 1 mg/kg, and by SR48968 at 3 mg/kg. Activity in the staircase apparatus was not significantly affected by diazepam, antalarmin and SR48968, but imipramine [$F(3,44)=6.3$, $P<0.01$] significantly decreased rearing at 30 mg/kg (Table 5).

Free-exploration test following cat exposure in mice

Figure 4 shows that none of the acute treatments significantly modified any of the behavioral measures in

Table 5 Effects on measures of activity in the staircase test following cat exposure. Drugs were administered IP 30 min before the beginning of the test. Data represent mean±SEM

Drugs	Dose mg/kg	Rears	Steps climbed
Diazepam	0	11.7±1.7	10.2±2.2
	0.5	13.5±1.9	10.8±2.0
	1	13.8±3.7	10.5±3.2
	2	5.0±2.3	7.2±2.5
Imipramine	0	21.6±2.4	14.1±2.1
	3	20.5±1.7	15.9±1.8
	10	16.8±1.8	10.8±1.9
	30	10.8±1.7*	9.4±1.8
Antalarmin	0	22.7±1.5	16.7±2.5
	0.3	16.3±1.8	10.2±2.0
	1	20.6±2.1	15.2±1.7
	3	23.1±2.9	12.7±2.7
	10	19.7±2.1	9.6±3.5
SR48968	0	17.8±1.6	15.8±1.8
	0.3	17.3±1.6	11.9±1.8
	1	20.9±1.6	14.7±2.3
	3	19.8±1.4	14.5±1.3

**P*<0.05 (Dunnett's *t*-test)

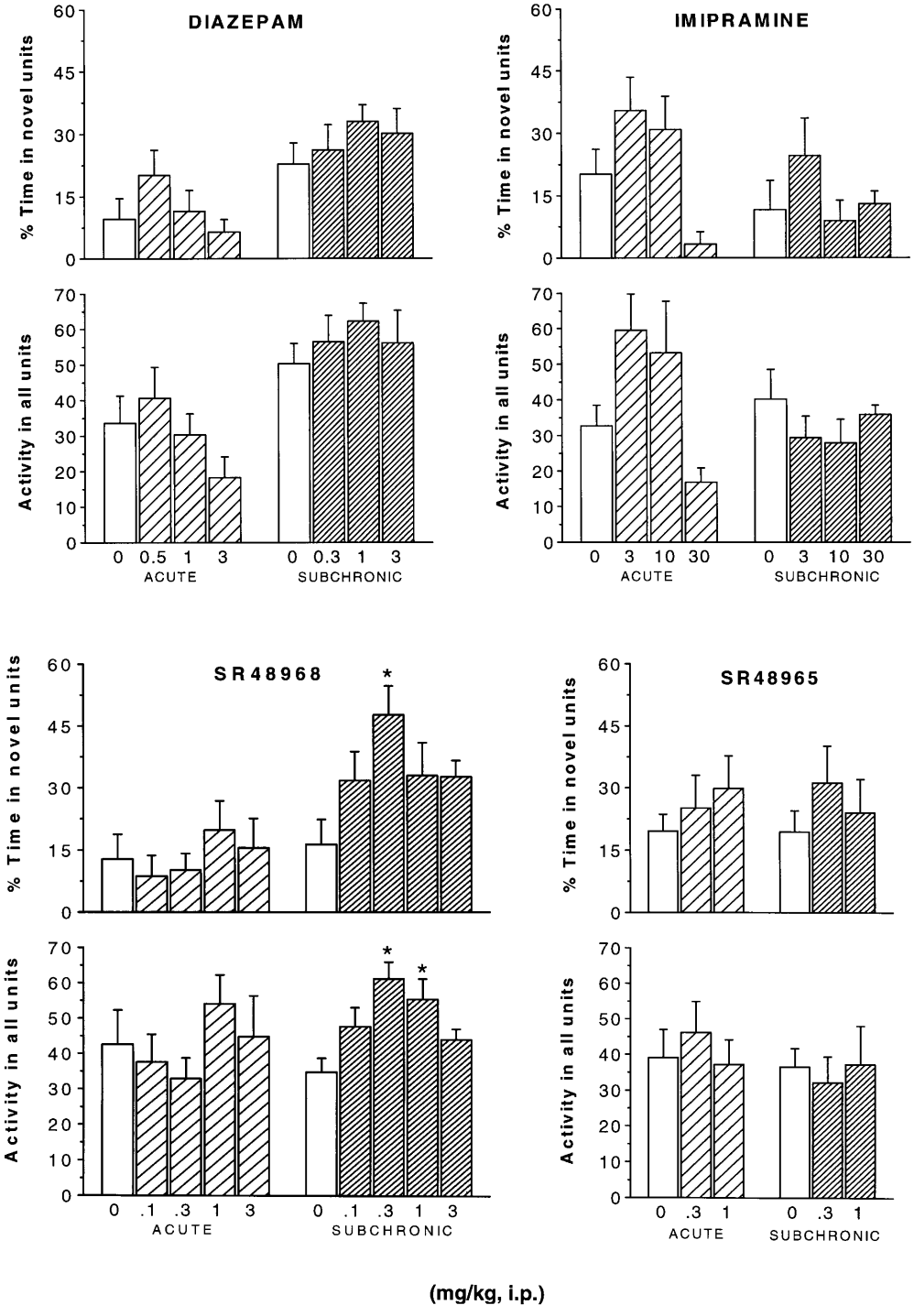

Fig. 4 Percentage of time spent by mice in areas containing cat feces and general activity recorded in the free-exploration test 1 h after unavoidable cat exposure. Drugs were administered 30 min before the beginning of the test. Data represent mean±SEM. n=6–10. *P<0.05 (Dunnett's t-test)

the free-exploration apparatus following cat exposure. After repeated administrations, only SR48968 significantly changed the percentage of time spent in the novel area containing cat feces [$F(4,30)=2.9$, $P<0.05$] and total number of unit changes [$F(4,30)=4.5$, $P<0.01$]. Post-test analysis indicated that the NK_2 receptor antagonist increased activity in all units at 0.3 and 1 mg/kg, and percentage of time spent in novel units at 0.3 mg/kg.

Discussion

The results of the present series of experiments showed that the selective non-peptide NK_2 receptor antagonist SR48968 failed to elicit anxiolytic-like effects in both conflict procedures in rats. By contrast, the drug produced some evidence of reduced anxiety-related responses in the elevated plus-maze, the mouse defense test battery, and in both models based on predatory stress. Overall, these behavioral effects of SR48968 in

anxiety/stress models differed from those observed with the BZ diazepam and the tricyclic antidepressant imipramine.

This is the first report on the behavioral action of an NK_2 receptor antagonist in traditional conflict procedures. These tests have been successfully used to screen compounds that interact with the GABA/BZ receptor complex, while atypical anxiolytics such as 5-HT_{1A} receptor ligands or selective 5-HT reuptake inhibitors have produced unconvincing results in these models (Treit 1985; Griebel 1995; Rodgers 1997). In the present study, diazepam produced an increase in rates of responding suppressed by punishment in the punished lever pressing and the punished drinking tests in rats, whereas the NK_2 receptor antagonist was inactive in both tests. It is unlikely that this negative finding can be attributed to dose range as doses used in this study overlapped with those from previous studies in which SR48968 produced behavioral changes following peripheral administration (Stratton et al. 1993a; Bernatzky and Saria 1995; Walsh et al. 1995). It therefore seems that the lack of significant action of SR48968 on both punished and unpunished responses suggests that conflict models are of limited utility in the study of the behavioral action of NK_2 receptor antagonists.

Most previous studies of NK_2 receptor antagonists in models of anxiety used exploratory-based procedures, such as the elevated plus-maze and the light/dark tests in mice. These studies invariably showed NK_2 receptor blockers (e.g. GR100679, GR159897, SR48968) to elicit anxiolytic-like activity in these tests (Stratton et al. 1993b, 1994; De Lima et al. 1995; Teixeira et al. 1996). The present experiments with the elevated plus-maze is in agreement with these studies. Like diazepam, SR48968 elicited positive effects on all behavioral measures of anxiety. Thus, on a traditional behavioral index, it increased percentage of time spent in open arms, while on the ethologically derived measures, SR48968 markedly decreased attempts and increased head-dippings, two parameters that are viewed as valid indexes of anxiety (Cruz et al. 1994; Rodgers 1997). Moreover, the NK_2 receptor antagonist did not modify significantly total arm entries (a presumed reliable measure of general activity), suggesting that its anxiolytic-like effects have not been contaminated by behavioral suppression. It is noteworthy that SR48968 was active at much higher doses (0.1–3 mg/kg) than those necessary to elicit anxiolytic-like activity in the light/dark test in previous studies (0.00005–5 µg/kg). The reason for this difference is unclear, but may at least partially be due to variation in routes of administration (subcutaneous versus intraperitoneal), species or tests of anxiety (mouse light/dark versus rat elevated plus-maze). In addition, there is no evidence that SR48968 enters the brain at doses lower than 0.1 mg/kg (Jung et al. 2000; Steinberg et al. 2000), suggesting that the positive effects observed previously with the drug in the light/dark test may not be mediated centrally and involve mechanisms unrelated to emotional processes.

The (*R*)-enantiomer of SR48968, SR48965, was much less active in the elevated plus-maze as it modified attempts significantly, while leaving unchanged the two other anxiety parameters. This finding fits well with radioligand binding experiments where SR48965 was shown to display weaker affinity for the NK_2 receptor than SR48968 (K_i of 945 and 0.51 nM, respectively). Moreover, SR48968, but not its (*R*)-enantiomer, was found to block turning behavior in mice induced by the neurokinin A receptor agonist [Nle^{10}]-NKA(4-10) (Poncelet et al. 1993). Similarly, in a model of NKA-induced bronchoconstriction in guinea-pigs, SR48965 was completely inactive at doses up to 200 µg/kg IV, whereas SR48968 produced dose-dependent inhibition of bronchoconstriction with an ID_{50} value of 37 µg/kg, IV (Emonds-Alt et al. 1992).

In the mouse defense test battery, diazepam, SR48968, but not imipramine or SR48965 decreased flight reactions after the rat was introduced into the runway, although the magnitude of the effects of the NK_2 receptor antagonist was less than that of the BZ. Previous studies with the mouse defense test battery have demonstrated that clinically effective anti-panic compounds specifically decrease animals' flight responses (e.g. alprazolam, chronic imipramine, fluoxetine) (Blanchard et al. 1997). Notably, these studies showed that avoidance distance appears to be particularly sensitive to panic-modulating drug treatment. The results obtained with SR48968 on flight suggest that NK_2 receptor antagonists may possess potency as a therapeutic agent for panic disorder.

During the chase test, diazepam, imipramine and SR48968 reduced risk assessment activities (i.e. stops), whereas in the straight alley situation, only the BZ increased risk assessment behavior (i.e. approaches followed by withdrawals displayed when subjects were constrained in one part of the runway). Because of a potential isomorphism between risk assessment activities and certain key features of generalized anxiety disorder (e.g. hypervigilance, apprehensive expectation), it has been suggested that they may represent a pattern of responses particularly sensitive to anxiolytic drug challenge (Blanchard et al. 1991). This was subsequently confirmed by extensive pharmacological investigations showing that BZs affected these responses (Blanchard et al. 1993, 1997). Thus, the actions of SR48968 and imipramine may be consistent with an anxiolytic-like effect. However, the finding that they modified only one risk assessment measure indicates only partial efficacy in affecting these behaviors, and therefore suggests that NK_2 receptor antagonists have a different anxiety-reducing potential as compared to classical anxiolytics. In addition, the positive effects of imipramine on risk assessment were observed at doses that did also produce behavioral impairment as was shown by activity measures (Table 3), thereby suggesting a non-specific anxiolytic-like action.

When contact was forced between threat stimulus and subject, diazepam, imipramine and SR48968 markedly

reduced bites to the rat. This behavioral profile is very similar to that observed in previous studies with classical (i.e. BZs) and atypical (i.e. 5-HT_{1A} receptor agonists/antagonists and 5-HT reuptake inhibitors) anxiolytics in the mouse defense test battery, thereby confirming that these terminal defense reactions are reliable indices of anxiety (Blanchard et al. 1997, 1998). In addition, the effects of SR48968 on defensive biting fit well with the ability of the drug to reduce defensiveness in the isolation-induced aggressive procedure in mice (J. Simiand, unpublished data). Following the removal of the rat from the runway, diazepam, SR48968 and, to a lesser extent, imipramine, decreased escape attempts from the test apparatus. Marked reductions in these behaviors during the post-rat period have been observed with a variety of anxiolytics and antidepressants, including BZ receptor ligands, 5-HT_{1A} receptor agonists, and 5-HT reuptake inhibitors (Blanchard et al. 1997). Overall, the behavioral profile displayed by the NK_2 receptor antagonist in the mouse defense test battery is consistent with an anxiolytic-like action. However, while the drug is much less active than diazepam on responses that include cognitive aspects of defensive behaviors (risk assessment), it appears to be as effective as the BZ on defensive attack, a more "affective"-orientated defense.

In the present study, rats and mice previously exposed to a cat show subsequent high levels of avoidance responses when exposed to a cat odor-saturated cue one hour later. SR48968, but not its (*R*)-enantiomer, reduced avoidance behavior of the anxiogenic stimulus in the staircase after a single injection, and in the free-exploration test following repeated treatment. Previous studies have shown that anxiogenic response to cat odors in rats is relatively insensitive to the effects of BZs (Zangrossi and File 1992a, 1992b, 1994), while the clinically effective antidepressant fluoxetine and the potential anxiolyic and/or antidepressant CRF_1 receptor antagonist CP-154,526 completely abolished avoidance of a cat odor (Griebel et al. 1999, manuscript in preparation). In line with these pharmacological studies, the present findings showed that diazepam was inactive in both the free-exploration and the staircase tests, while the CRF_1 receptor antagonist antalarmin displayed positive effects in the latter procedure. Imipramine was active in the staircase test, but only at a sedative dose, and it failed to modify the behavior of animals in the free-exploration test following both treatments. It has been argued that long-lasting effects of cat exposure on anxiety-related behaviors in rodents may model aspects of anxiety disorder associated with posttraumatic stress disorder (PTSD) (Adamec 1997). The model has both face and ethological validity. It produces many effects that mimic symptoms of PTSD. The finding that NK_2 receptor blockade after cat exposure may prevent later increases in anxiety-related responses has interesting clinical implications. However, the suggestion that NK_2 antagonists may be effective against PTSD cannot be made since the cat-exposure procedures used in this study do not assess long-lasting behavioral changes. Unlike the model described by Adamec and colleagues, they assess only short-term effects of cat-exposure, which does not fit well with the clinical profile of PTSD, involving chronic symptomatology. Thus, the idea that NK_2 receptor antagonists may have a potential to provide post-stressor pharmacological prophylaxis against development of anxiety disorder following traumatic stress requires further investigation.

The site of the anxiolytic-like action of SR48968 remains to be determined. The lack of significant effects of the (*R*)-enantiomer of SR48968, SR48965, which shows only weak affinity for the NK_2 site, indicates that NK_2 receptor blockade may be necessary to produce such effects. It has been suggested that tachykinin NK_2 receptor antagonists may produce their anxiolytic-like effects within the dorsal raphe nucleus (DRN) by interaction with other neurotransmitters such as 5-HT or GABA (Walsh et al. 1995). This suggestion is based notably on the finding that intra-DRN infusion of the NK_2 receptor antagonists GR100679, GR115211 and GR159897 produced anxiolytic-like effects in the social interaction and elevated plus-maze tests in rats (Stratton et al. 1993b, 1994). Moreover, the NK_2 receptor agonist GR64349 was found to increase anxiety-like behavior in the rat social interaction test following infusion into the DRN (Stratton et al. 1993b). However, additional brain areas may participate in the action of SR48968 on emotional processes, especially cortical or septo-hippocampal structures where NK_2 receptors have been mainly identified. In line with this idea are recent findings showing that SR48968 reduced the maternal separation-induced increase in the number of neurons displaying NK_1 receptor internalization in the amygdala of guinea-pig pups (Jung et al. 2000). Furthermore, the drug counteracted stress (tail-pinch, icv CRF infusion)-induced increase in locus coeruleus firing or norepinephrine release in the prefrontal cortex (Steinberg et al. 2000).

In conclusion, this study demonstrated that the potent selective non-peptide NK_2 receptor antagonist, SR48968 is able to reduce anxiety-related responses in several animal models of emotional behavior. While the drug has limited or no efficacy in classical animal models of anxiety, it shows good activity in reducing anxiety-like behaviors following traumatic stress or upon forced and unavoidable contact with a threatful stimulus. Together, these data suggest that NK_2 receptor antagonists may have anti-anxiety properties that are different from those displayed by existing anxiolytic agents.

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