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Anxiolytic Action of a Neurokinin$_1$ Receptor Antagonist in the Social Interaction Test

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FILE, S. E. *Anxiolytic action of a neurokinin$_1$ receptor antagonist in the social interaction test.* PHARMACOL BIOCHEM BEHAV **58**(3) 747–752, 1997.—CGP 49823, a substance P antagonist acting at NK_1 receptors, had significant anxiolytic effects at 3, 10 and 30 mg/kg orally in the high-light unfamiliar and low-light unfamiliar conditions of the social interaction test but was without effect in the low-light familiar condition. The effects were less marked after 3 and 6 weeks of treatment (10 mg/kg/day), indicating that some tolerance had developed, but a significant anxiolytic effect still remained. After 3 weeks of diazepam treatment (2 mg/kg/day, intraperitoneally), tolerance developed to the anxiolytic effects, and there was an anxiogenic response 24 h after withdrawal. In contrast, there were no anxiogenic withdrawal effects 24 h after 3 weeks or 24, 48 or 72 h after 6 weeks treatment with CGP 49823 (10 mg/kg/day). These results suggest that the compound may prove to be a useful anxiolytic and that substance P may play a role in mediating states of anxiety. © 1997 Elsevier Science Inc.

Substance P NK_1 receptors CGP 49823 Anxiety Social interaction Tolerance Withdrawal

SUBSTANCE P is widely distributed in the central and peripheral nervous systems, where it functions as a neurotransmitter or neuromodulator (16,22,25). It is released from sensory neurones in the spinal cord in response to noxious stimuli (24) and from neurones in the midbrain in response to stressors such as mild foot shock or exposure to an unfamiliar environment (2,6). Unger et al. (28) reported that centrally administered substance P induces a cardiovascular defence reaction in rats. Receptors for substance P are widely distributed in the brain, with high densities in the amygdala and hippocampus (23), areas traditionally implicated in the control of fear and anxiety. Direct evidence for a role for substance P in anxiety comes from recent reports that administration of picomolar concentrations of substance P into the lateral ventricles, the bed nucleus of the stria terminalis or the basolateral nucleus of the amygdala had anxiogenic effects in the elevated plus-maze (27,5). However, the effects of substance P may be dependent on both dose and specific brain region. Thus, after intraperitoneal (IP) administration, the 50 μg/kg dose was anxiolytic, whereas the 500 μg/kg dose was anxiogenic, and after administration to the nucleus basalis magnocellularis the 1 ng dose was anxiolytic (18).

The preferred receptor for substance P is the NK_1 receptor (20); thus, it is of interest that NK_1 receptor antagonists have been reported to have anxiolytic effects in animal tests. In one experiment, FK 888 had anxiolytic effects in the elevated plus-maze after intracerebroventricular administration to the mouse (27), but this effect was not found in a subsequent experiment in rats (5). CGP 49823 (2R, 4S)-2-benzyl-1-(3,5-dimethylbenzoyl-*N*-[4-quinolinyl)methyl]-4-piperidineamine (19) has been reported to have anxiolytic effects in the rat social interaction test (29) and to increase social investigation in gerbils (4). In both these cases, testing was conducted in a brightly lit, unfamiliar environment. Thus, the purpose of Experiment 1 was to determine the effects of CGP 49823 in a range of test conditions of the social interaction test of anxiety. This test manipulates both the light level and unfamiliarity of the test arena and benzodiazepines increase social interaction that has been suppressed by both these factors; other compounds have selective effects acting only when behaviour is suppressed by light or novelty (7,8). The doses of CGP 49823 (3, 10 and 30 mg/kg) were selected on the basis of the minimum effective dose (MED) of 10 mg/kg reported by Vassout et al. (29).

Vassout et al. (29) reported anxiolytic effects after both acute and subchronic treatment, and Cutler (4) reported an enhanced effect after 10 days of treatment, although this may have been an artefact of the increased handling. Handling produces marked changes in the GABA acid and 5-hydroxytryptamine systems (3,15) and in peptides such as cholecystokinin and corticotropin-releasing factor (21,1), and it is possi-

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ble that it could also alter the level of substance P release. Thus, in Experiment 1, animals were left unhandled (apart from that needed to house them in single cages) before testing so that the stress from handling would be maximal. In Experiment 2, the effects of an acute administration were compared with the effects after 6 weeks of treatment, and in this experiment all the rats were handled and injected daily for 6 weeks so that they were all thoroughly habituated to the handling stress. Finally, because tolerance to the anxiolytic effects of CGP 49823 was found after 6 weeks of treatment, Experiment 3 investigated the effects after 3 weeks of treatment and compared these effects with those of diazepam. In addition to testing for the development of tolerance to anxiolytic effects, rats were tested for possible anxiogenic withdrawal responses 24 h after withdrawal from 3 weeks of treatment and 24, 48 and 72 h after withdrawal from 6 weeks of treatment. The highlight, unfamiliar test condition, was selected for testing for tolerance because this is most sensitive to anxiolytic effects, and the low light, familiar condition was used to test for withdrawal because this is the most sensitive to anxiogenic effects.

METHOD

Animals

In all experiments, male, hooded Lister rats (Harlan, Bicester, U.K.) were housed singly for 5 days before testing. At testing, they weighed 200–230 g (Experiment 1) and 280–350 g (Experiments 2 and 3). They were housed in a dimly lit room and maintained at 22°C, with food and water freely available. In Experiments 2 and 3, apart from the 5 days before test, the rats were housed in groups.

Apparatus

The social interaction test arena was a wooden box (60 × 60 × 35 cm); the illuminance on the floor of the box was 30 or 300 scotopic lux, for the low and high light test conditions, respectively. Infrared photocells were located in the walls, 4.5 cm and 12.5 cm from the floor, and the interruption of these beams provided automated measures of locomotor activity and rearing, respectively. A camera was mounted above the arena for observing the behaviour of the rats from a video monitor in the adjacent room. The scorer, blind to the drug treatment of the animal, scored the total time spent by each pair of rats in active social interaction. Investigatory (sniffing and grooming the partner) and aggressive (boxing, wrestling, submitting, biting) behaviours were scored separately.

Drugs

CGP 49823 (CIBA, Basle, Switzerland) was prepared by making a stock suspension of 3 mg/ml in methylcellulose (0.5%); this concentration was used for the 30 mg/kg doses, and further dilutions with methylcellulose were made for the lower doses, so that all rats, including the control group, received the same volume of injection (10 ml/kg). The drug suspensions were freshly prepared each day and shaken vigorously prior to oral administration, which was always 90 min before the test. In Experiment 2, all rats received 6 weeks of daily oral injections of vehicle or CGP 49823 (10 mg/kg), as appropriate. In Experiment 3, rats received 3 or 6 weeks of daily oral treatment with vehicle (half of the control group) or CGP 49823 (10 mg/kg), as appropriate or 3 weeks of daily IP injection with vehicle (half of the control group) or diazepam (2 mg/kg). Diazepam (Roche Products Ltd, Welwyn Garden City, U.K.) was suspended in a water/Tween solution to a concentration of 1 mg/ml; the water/Tween solution was used as the vehicle for the IP injections to the control group. The IP injections were given 30 min before social interaction testing.

Procedure

Experiment 1. Thirty pairs of rats were randomly allocated to each of these drug groups: control or CGP 49823 (3, 10 and 30 mg/kg). In each drug group, 10 pairs were then randomly allocated to each of the following 3 test conditions: high light unfamiliar test arena; high light, familiar arena; low light familiar arena. Rats allocated to the familiar test arena conditions received a 10 min familiarisation period in the arena on each of the 2 days before social interaction testing. On the test day, pairs of rats were placed in the test arena, under the appropriate light level and their behaviour scored for 10 min. At the end of this period, the rats and any faecal boluses were removed and the arena wiped with a damp cloth. The rats were tested in an order randomised for drug treatment, between 0900 and 1300 h.

On the test day, both members of each pair received the same drug treatment and all injections were oral 90 min prior to testing. After injection, the rats were returned to their home cages and left in a dimly lit, quiet area adjacent to the test room.

Experiment 2. Pairs of rats were randomly allocated (n = 10/group) to these groups: control or CGP 49823 (10 mg/kg acute and 6 weeks chronic). All rats were tested for 10 min in the high light, unfamiliar test condition in an order randomised for drug treatment, between 0900 and 1300 h.

Experiment 3. In this experiment, the rats that had received 3 weeks of chronic vehicle or drug treatment, as appropriate, were randomly allocated among the different groups. The following were tested for anxiolytic effects in the high light, unfamiliar test condition: control (oral, n = 5), CGP 49823 (10 mg/kg acute and 3 weeks chronic, n = 10/group); control (IP, n = 5), diazepam (2 mg/kg, 3 weeks chronic, n = 12). The following were tested for anxiogenic withdrawal responses in the low light, familiar test condition: control (n = 10), CGP 49823 (24 h withdrawal from 10 mg/kg, n = 10) and diazepam (24 h withdrawal from 2 mg/kg, n = 12).

Pairs of rats from the 6-week treatments were randomly allocated to the following groups: control (n = 10 tested in the high light, unfamiliar arena, n = 10 tested in the low light, familiar arena), CGP 49823 (10 mg/kg tested in high-light unfamiliar arena, n = 10) and CGP 49823 (24, 48 and 72 h withdrawal from 10 mg/kg tested in the low light, familiar arena, n = 10/group).

In Experiment 3, the test duration was 4.5 min, and rats were tested in an order randomised for drug treatment, between 0900 and 1300 h.

Statistics

The data for each test condition were analysed by one-way analyses of variance, with Duncan's post-hoc tests between individual groups. Significant results are shown in figures and tables.

RESULTS

Experiment 1

In the high light unfamiliar and low light unfamiliar test conditions, there were significant drug effects on social inter-

action [$F(3, 36) = 21.0$ and 7.7, respectively, $p < 0.0005$] and in both conditions all the doses were significantly higher than the control group (Fig. 1). There were no changes in motor activity in these two conditions [for both locomotor activity and rears, $F(3, 36) < 1.0$; see Table 1]. In the low light, familiar test condition, there was no drug effect on social interaction [$F(3, 36) = 1.3$] or locomotor activity [$F(3, 36) = 0.8$], but there was an increase in rears [$F(3, 36) = 2.9$, $p < 0.05$] that reached significance for the 3 and 30 mg/kg doses (Table 1).

Experiment 2

Acute administration of CGP 49823 (10 mg/kg) significantly increased social interaction but had no effect on locomotor activity or rearing (Table 2), indicating a specific anxiolytic effect. However, after 6 weeks of chronic treatment, tolerance had developed to this effect and the level of social interaction was significantly lower than in the acute group; although the level was higher than in the control group, this no longer reached significance (Table 2).

When the scores for the first 6 min of the test were analysed, the group treated for 6 weeks with CGP 49823 showed a significant anxiolytic effect [control = 22.5 ± 4.2, CGP = 45.4 ± 8.5; $F(1, 17) = 5.4$, $p < 0.05$].

Experiment 3

Tolerance after 3 weeks of treatment. CGP 49823 significantly increased social interaction [$F(2, 27) = 12.1$, $p < 0.0005$], indicating an anxiolytic action that was significant ($p < 0.01$) for both the acute and the 3-week treatment groups. However, the effect in the 3-week treatment group was significantly less than in the acute group (Fig. 2). Locomotor activity was unaffected by CGP 49823 [$F(2, 27) = 1.2$], but the 3-week treatment group did make fewer rears than did the controls (Table 3). After 3 weeks of treatment, diazepam was without significant effect on social interaction, motor activity or rears ($F \leq 1.0$ in all cases; Fig. 2).

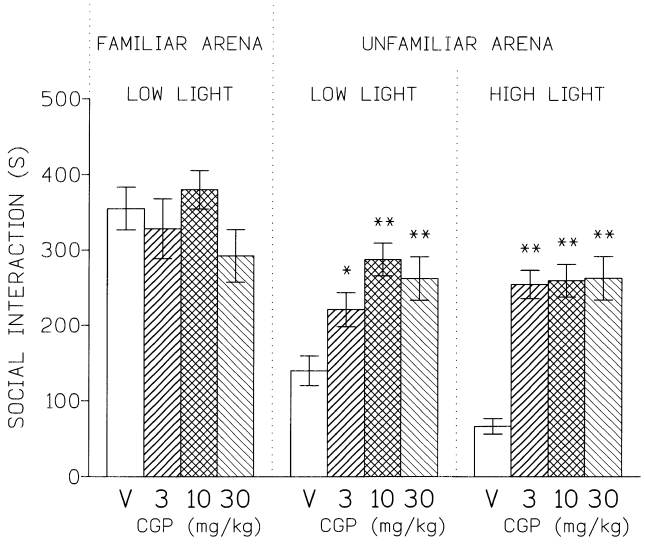

FIG. 1. Mean (± SEM) time (s) spent in a 10-min social interaction test by rats tested 90 min after oral administration of vehicle (V) or CGP 49823 (3, 10 or 30 mg/kg). $p < 0.05$, $^{*}p < 0.01$ vs. vehicle control, Duncan's tests after analysis of variance.

TABLE 1

MEAN (±SEM) LOCOMOTOR ACTIVITY (L.A. BEAM BREAKS) AND NUMBER OF REARS MADE BY PAIRS OF RATS TREATED WITH VEHICLE, CGP 49823 (3, 10 OR 30 mg/kg) IN A 10 MIN SOCIAL INTERACTION TEST

	High light unfamiliar		Low light unfamiliar		Low light familiar	
	L.A.	Rears	L.A.	Rears	L.A.	Rears
Vehicle	1155.8	37.0	1208.8	30.6	1186.2	26.5
	±61.1	±2.1	±42.1	±1.6	±53.3	±2.8
CGP 3 mg/kg	1167.4	41.0	1191.9	35.3	1132.0	34.5*
	±38.0	±2.5	±48.2	±1.0	±56.6	±1.9
CGP 10 mg/kg	1061.4	38.6	1263.3	31.6	1218.3	29.8
	±58.0	±2.9	±39.2	±0.9	±34.5	±1.6
CGP 30 mg/kg	1137.9	34.9	1212.7	29.6	1112.0	35.8*
	±54.1	±3.4	±60.2	±3.0	±71.9	±3.6

$^{*}p < 0.05$ compared with vehicle, Duncan's tests after analysis of variance.

Withdrawal 24 h after 3 weeks of treatment. Rats tested 24 h after the last of 21 daily injections with CGP 49823 (10 mg/kg) had significantly higher social interaction scores than did the control rats when tested in the low light familiar test condition [$F(1, 18) = 5.3$, $p < 0.05$; Fig. 3, right side]; this increase was due primarily to increased aggression [$F(1, 18) = 11.0$, $p < 0.005$] and social investigation was unchanged ($F < 1.0$). This group also showed increased locomotor activity [$F(1, 18) = 4.3$, $p = 0.05$], but a reduced number of rears [$F(1, 18) = 7.3$, $p = 0.01$; Table 3]. Thus, analyses of covariance were performed to determine to what extent these changes were independent of each other. The increase in social interaction remained significant ($p = 0.05$) when the increased locomotor activity was accounted for, whereas the change in locomotor activity lost significance when the increased social interaction was accounted for. Rats tested 24 h after the last of 3 weeks of daily injections with diazepam showed a significant reduction in social interaction [$F(1, 20) = 6.5$, $p < 0.05$] but no change in motor activity or rears [$F(1, 20) \leq 1.0$ in both cases], thus indicating a specific anxiogenic withdrawal response (Fig. 2, Table 3).

Tolerance after 6 weeks of treatment. Rats tested with CGP 49823 (10 mg/kg) after 6 weeks of treatment with this dose

TABLE 2

MEAN (±SEM) TIME (s) SPENT IN SOCIAL INTERACTION, LOCOMOTOR ACTIVITY (BEAM BREAKS) AND NUMBER OF REARS MADE BY VEHICLE-TREATED RATS, THOSE TESTED AFTER AN ACUTE DOSE OF CGP 49823 (10 mg/kg) AND THOSE TESTED AFTER 6 WEEKS OF TREATMENT WITH CGP 49823 (10 mg/kg) IN A 10 MIN TEST

	Vehicle	Acute	6 weeks
Social interaction	56.9 ± 12.1	256.2** ± 21.3	90.4^{++} ± 19.4
Locomotor activity	736.1 ± 29.8	798.4 ± 44.8	660.4^{+} ± 25.6
Rears	42.7 ± 2.0	44.3 ± 2.3	48.5 ± 2.3

$^{**}p < 0.01$ compared with control, $^{+}p < 0.05$, $^{++}p < 0.01$ compared with acute group, Duncan's test after analysis of variance.

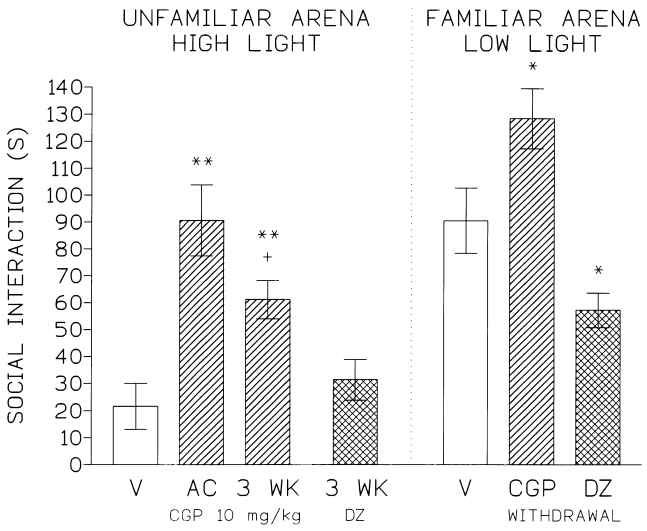

FIG. 2. Mean (± SEM) time (s) spent in a 4.5-min social interaction test by rats tested in the high-light unfamiliar test arena, after vehicle (V), CGP 49823 [10 mg/kg: acute (AC) or after 3 weeks of treatment] or diazepam [DZ, 2 mg/kg: acute (AC) or after 3 weeks] or tested in the low-light familiar test arena after 24 h withdrawal from 3 weeks of treatment with vehicle (V), CGP 49823 (CGP, 10 mg/kg) or diazepam (DZ, 2 mg/kg IP). $*p < 0.05$, $*p < 0.01$ vs. vehicle; $^{+}p < 0.05$ vs. acute group, Duncan's tests after analysis of variance.

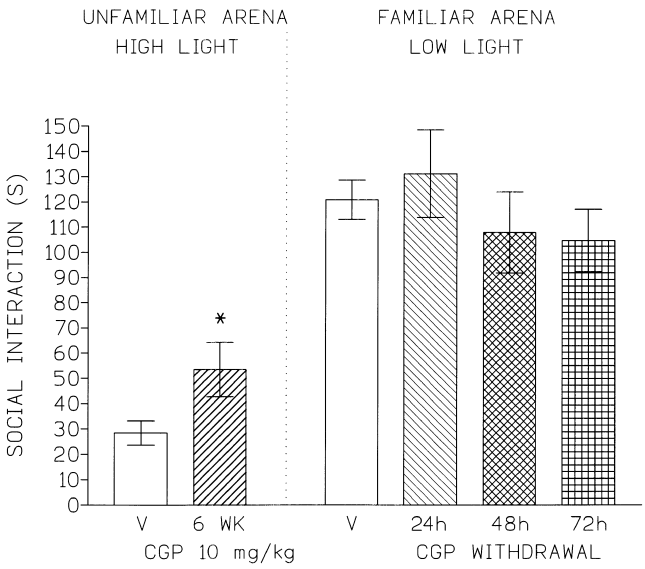

FIG. 3. Mean (± SEM) time (s) spent in a 4.5-min social interaction test by rats tested in the high-light unfamiliar test arena, after vehicle (V) or CGP 49823 (10 mg/kg for 6 weeks) or tested in the low-light familiar test arena after 24, 48 or 72 h withdrawal from CGP 49823 (CGP 10 mg/kg for 6 weeks).$*p < 0.05$ vs. vehicle, Duncan's tests after analysis of variance.

still had significantly increased levels of social interaction [$F(1, 18) = 4.3$, $p = 0.05$; Fig. 3]. There were no significant changes in locomotor activity or rears ($F \leq 1.1$ in both cases).

Withdrawal after 6 weeks of treatment. There were no significant withdrawal effects at any of the time points tested for CGP 49823 (Fig. 3).

DISCUSSION

The pattern of results seen in the social interaction test after acute administration of CGP 49823 most closely resembles that previously found for the benzodiazepines, i.e., it prevents the decline in social interaction seen in the control rats when tested in an unfamiliar or brightly lit test arena. The increased social interaction seen under these conditions was indicative of a strong anxiolytic effect because it was independent of any change in motor activity. As is found with the benzodiazepines (7,8), in the least threatening test condition (low light, familiar arena), when the control scores are highest, CGP 49823 did not change social interaction. This result suggests that, rather than enhancing social interaction per se, CGP 49823 is most effective when this interaction is inhibited by threatening circumstances. However, because there was no evidence of a greater anxiolytic effect in Experiment 1, when unhandled rats were tested in the high light, unfamiliar test arena, than in Experiment 2, when the rats were extremely well handled, there was no evidence that the anxiolytic effects of CGP were affected in a major way by handling. Furthermore, if handling influenced the release of substance P and this in turn had an anxiogenic effect, then CGP 49823 would have had an anxiolytic effect in Experiment 1, even in the low-light familiar test condition. Thus, if the anxiolytic effects of CGP 49823 are due to antagonism of centrally released substance P, then high light and unfamiliar environments would seem to be more important releasers of this peptide than the stress from handling.

There was evidence that tolerance developed to the anxiolytic effects of CGP 49823 with chronic administration, but the rate of development was slower than that for the benzodiazepines, and some anxiolytic activity persisted even after 6 weeks. This result was significant when the 4.5 min test period was used and for the first 6 min of the 10 min test, but significance was lost with the full 10 min test period because the test arena is gradually becoming familiar during the test and, hence, the influence of unfamiliarity is reduced. For this rea-

TABLE 3

MEAN (±SEM) LOCOMOTOR ACTIVITY (BEAM BREAKS) AND NUMBER OF REARS MADE BY RATS TREATED ACUTELY OR FOR 3 WEEKS WITH CGP 49823 (10 mg/kg) AND TESTED FOR 4.5 MIN IN THE HIGH-LIGHT UNFAMILIAR (HU) CONDITION AND THOSE TESTED 24 H AFTER WITHDRAWAL FROM 3 WEEKS TREATMENT IN THE LOW-LIGHT FAMILIAR CONDITION

	Locomotor Activity	Rears
HU test condition		
Control	445.6 ± 18.1	23.2 ± 1.0
CGP 49823 (acute)	454.4 ± 17.4	21.6 ± 0.7
CGP 49823 (3 wks)	483.1 ± 18.0	19.1* ± 1.7
LF test condition		
Control, withdrawal	474.7 ± 26.4	16.6 ± 1.5
CGP 49823 (3 weeks to withdrawal)	548.1* ± 23.8	11.6** ± 1.1
Diazepam (3 weeks to withdrawal)	487.3 ± 21.6	15.3 ± 4.4

$*p < 0.05$ compared with control, Duncan's tests after after analysis of variance.

son, it has generally been more useful to use test periods of 7.5 or 4.5 min (11,12,14). Six weeks is twice as long as the time usually found for tolerance to develop to the anxiolytic effects of the benzodiazepines (9). The mechanism underlying the development of tolerance to the anxiolytic effects of CGP 49823 and that of the benzodiazepines is probably different. An oppositional mechanism of tolerance (31) operates with the anxiolytic effects of the benzodiazepines and, thus, anxiogenic effects are seen on drug withdrawal [Experiment 3; see 9]. However, no such effects were seen after withdrawal from either 3 or 6 weeks of treatment with CGP 49823. Although it is impossible to exclude the possibility that such effects might be found after even longer periods of treatment or at different time intervals, the results of Experiment 3 show no indication of changes in the anxiogenic direction. Indeed, there was still an increase in social interaction 24 h after the last of 21 daily doses, which took the form of increased time in aggressive episodes. This effect was not seen in the rats withdrawn from 6 weeks of treatment, and at present it is difficult to assess the importance of this finding. Although an oppositional mechanism can probably be excluded, the present data do not allow a distinction between a pharmacodynamic decremental mechanism and pharmacokinetic tolerance. The potential of CGP 49823 to reverse the increased anxiety during benzodiazepine and alcohol withdrawal would be well worth investigating.

In conclusion, the results of the present experiments extend and support the previous reports (4,28) that CGP 49823 has anxiolytic potential. Given that there is growing evidence that different animal tests of anxiety reflect different types of anxiety (10) and activate different neurochemical pathways (13,17), it will be of great interest to compare more completely the profile of CGP 49823 with other NK_1 receptor antagonists in a broad range of animal tests of anxiety and related disorders. In addition to a role for NK_1 receptors in anxiety, there is also evidence that other tachykinin receptors may be important. Thus, neurokinin A and the selective NK_2 receptor agonist, [β-Ala^8]neurokinin A (4–10), had anxiogenic effects in the mouse elevated plus-maze and NK_1 receptor antagonists have been reported to have anxiolytic effects in several animal tests (26,27,30). Anxiolytic effects after administration of receptor antagonists suggest a physiological role for substance P in the modulation of anxiety and that this peptide is released by the test conditions. Certainly, the possibility that tachykinin release from brain regions, such as substance P release from limbic areas, could play a role in mediating anxiety is exciting enough to warrant further preclinical and clinical research.

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