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Effects of RO 60 0175, a 5-HT_{2C} receptor agonist, in three animal models of anxiety

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Abstract

There is some controversy as to whether 5-HT_{2C} receptor agonists are anxiogenic or anxiolytic. The effects of the novel 5-HT_{2C} receptor agonist, (*S*)-2-chloro-5-fluoro-indol-1-yl)-1-methyl ethylamine fumarate (RO 60 0175), in three models of anxiety were therefore tested. RO 60 0175 was found to induce hypolocomotion in rats at doses greater than 0.5 mg/kg s.c., an effect reversed by the selective 5-HT_{2C} receptor antagonist, SB-242084. RO 60 0175 did not elicit anxiolytic-like responses in the social interaction test under high light unfamiliar conditions, but suppressed both time spent in social interaction and locomotion at doses of 1 and 3 mg/kg s.c., suggesting a sedative response. In the Vogel conflict test, RO 60 0175 had no significant action on the number of shocks taken. In the Geller–Seifter test, RO 60 0175 (0.3 and 1 mg/kg s.c.) simultaneously reduced both unpunished and punished lever pressing, a profile consistent with sedation. Finally, RO 60 0175 was tested in a rat social interaction test under low light familiar conditions optimal for the detection of anxiogenic-like responses. At 1 and 3 mg/kg s.c., RO 60 0175 reduced both time spent in social interaction and concurrent locomotion, a profile more consistent with sedation than anxiogenesis. In conclusion, RO 60 0175 induced sedative-like responses via 5-HT_{2C} receptor activation, but was neither anxiolytic, nor clearly anxiogenic at the doses tested.

Keywords: Anxiety; Depression; 5-HT_{2C} receptor; (Rat)

1. Introduction

5-HT_{2C} receptors have been implicated in the control of locomotor activity, food intake, temperature regulation, tumescence, adrenocortical trophic hormone (ACTH) and growth hormone (GH) secretion (Kahn and Wetzler, 1991; Kennett, 1993). 5-HT_{2C} receptors are also thought to be important in the control of emotion. Thus, in both rodents (Kennett et al., 1989) and man (Kahn and Wetzler, 1991; Kennett, 1993), the non-selective 5-HT_{2C} receptor agonist, *m*-chlorophenylpiperazine (*m*CPP), has been reported to elicit anxiogenic-like or anxiogenic responses, respectively, on administration. In rats, these effects are antagonised by 5-HT_{2C} receptor antagonists such as SB-200646-A (Kennett et al., 1994) while in man, the anxiogenic actions of *m*CPP are attenuated by the non selective 5-HT_2 receptor antagonists, ritanserin, methysergide and metergoline (see Kennett, 1993). Conversely, 5-$HT_{2C/2B}$ receptor antagonists, such as SB-200646-A (Kennett et al., 1994) and the selective 5-HT_{2C} receptor antagonist, SB-242084 (Kennett et al., 1997), have anxiolytic-like effects in a number of animal models of anxiety with different motivational and aversive bases. It is therefore of some interest that a novel 5-HT_{2C} receptor agonist, (*S*)-2-chloro-5-fluoro-indol-1-yl)-1-methyl ethylamine fumarate (RO 60 0175), has been recently characterised with full agonist efficacy at the receptor and at least 1.5 log units separation over all other sites tested with the exception of the 5-HT_{2B} receptor at which the compound reportedly has only weak partial agonist efficacy (Martin et al., 1998). On acute administration, this compound is reported to have antipanic-like properties in the rat periaqueductal gray stimulation model (Jenck et al., 1996) and anti-compulsive action in the rat schedule-induced polydipsia paradigm (Martin et al., 1998). On subchronic administration, RO 60 0175 is reported to exert antidepressant-like properties in a rat chronic mild stress model of depression (Moreau et al., 1996). It has been argued that these latter effects may be

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secondary to desensitisation of 5-HT_{2C} receptors by RO 60 0175, allowing the compound to act, in effect, as an antagonist (Moreau et al., 1996). In the present study, the actions of acute administration of RO 60 0175 in alternative models of anxiety in which selective 5-HT_{2C} receptor antagonists have been found to have anxiolytic-like properties (Kennett et al., 1997) were assessed.

In the Geller–Seifter conflict test (Geller et al., 1962), rats are trained to lever press for a food reward and subsequently to associate the onset of a light cue with the possibility that further lever pressing while the cue is shown will result in punishment. Thus, responding during the punished phase is suppressed and can be restored by anxiolytic-like treatments. These include benzodiazepines (Geller et al., 1962; Kennett et al., 1997), 5-HT_{1A} receptor agonists (Cervo and Samanin, 1995; Kennett et al., 1996) and 5-HT_{2C} receptor antagonists (Cervo and Samanin, 1995; Kennett et al., 1997). However, as configured for the present studies at least, the test was insensitive to either acute or chronically administered 5-HT reuptake inhibitors (Kennett et al., 1996).

The Vogel conflict test (Vogel et al., 1971) relies on a similar principle, where thirsty animals are motivated to lever press for water which may then be punished. In this test, however, animals are only tested once. The test is also claimed to be sensitive to benzodiazepines (Vogel et al., 1971; Shephard, 1988; Kennett et al., 1998), 5-HT_{1A} receptor agonists (Moser et al., 1990; Griebel 1995) and 5-HT_{2C} receptor antagonists (Griebel et al., 1997).

The third test employed was a social interaction test (File and Hyde, 1978) where two conspecifics are placed in a novel environment and the amount of time spent in social interaction (grooming, sniffing, following, biting, boxing, crawling over or under) is assessed. Social interaction can be suppressed by an aversive environment (unfamiliar, brightly lit) and restored by anxiolytic treatments such as benzodiazepines (File and Hyde, 1978), 5-HT_{2C} receptor antagonists (Kennett et al., 1997) and chronic administration of 5-HT reuptake inhibitors (Lightowler et al., 1994). The test has, however, often failed to detect anxiolytic-like profiles for 5-HT_{1A} agonists (Griebel, 1995). When configured for identifying anxiolytic-like treatments, none of the tests used are sensitive to anxiogenic-like effects. However, the social interaction test can detect anxiogenic-like compounds under conditions minimising aversion (low light, familiar test situation) (File and Hyde, 1978) and these conditions were therefore also employed in the present study.

2. Methods

All studies were conducted in accordance with Home Office guidelines on animal welfare and following the approval of an internal ethics committee.

2.1. Animals

Male Sprague–Dawley rats (220–250 g) were housed in groups of six under a 12-h light/dark cycle (lights on 07.00 h) at 21 ± 2°C and 65 ± 5% humidity with free access to food (CRMX, special Diet Services) and water.

2.2. Locomotion

The rats were placed in a room adjacent to the experimental room on the day of the procedure. They were dosed s.c. 30 min before the locomotion test with RO 60 0175 0.1, 0.3, 0.5, 1 or 3 mg/kg or vehicle, in groups of four. Rats were returned to their home cages after dosing. At 0 h, they were each placed in automated locomotor activity cages ($57 \times 16.6 \times 25.3$ cm) made of black perspex with a clear perspex lid and sawdust covered floor, under red light for 10 min. During this time, locomotion was recorded by means of consecutively breaking two photocell beams traversing opposite ends of the box 3.9 cm above floor level. Treatment group sizes varied from 12 (vehicle), nine (RO 60 0175, 0.1–1 mg/kg) and seven and six, respectively, for 3 and 10 mg/kg doses, but no animals were excluded from the study analysis.

Rats were placed in the experimental room on the day of the procedure. They were dosed in treatment groups of 16 s.c. 40 min before the locomotion test with SB-242084 (5 mg/kg) or vehicle, and 30 min before the test with RO 60 0175 (3 mg/kg s.c.), *m*CPP (4 mg/kg s.c.) or vehicle, in groups of 16. The two administrations were made in opposite flanks. They were returned to their home cages after dosing. At 0 h, they were each placed in automated locomotor activity cages ($48 \times 27 \times 20$ cm) AM 1052, Benwick Electronics, UK) under red light for 10 min. During this time, locomotion was scored by means of consecutively breaking two photocell beams traversing opposite ends of a box 3 cm above floor level.

2.3. Social interaction

Rats were housed singly in a room adjacent to the testing room on day 1. On day 5, they were dosed in groups of six s.c. 20 min before the test with RO 60 0175 0.1, 0.3, 1 or 3 mg/kg or vehicle in treatment and weight (± 5 g) matched pairs unfamiliar to each other and returned to their home cages. Rats were then placed in a white perspex test box ($54 \times 37 \times 26$ cm) for 15 min under bright white light (150 lux) in an adjacent darkened room containing a fan to generate white noise. Alternatively, under low light familiar conditions, rats were individually habituated to a black perspex test box of the same dimensions for 10 min under red light on both days 3 and 4. On day 5, rats were administered treatments as above or with FG 7142 orally, 1 h pre-test in groups of 12–16 and tested in the same black perspex apparatus under red light.

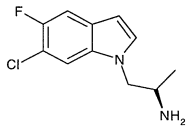

Fig. 1. Structure of RO 60 0175, a 5-HT_{2C} receptor agonist.

In both procedures, active social interaction (sniffing, following, grooming, biting, boxing and crawling over or under) was scored by an observer blinded with respect to treatment by remote video monitoring and a computerised score pad. At the end of each test the box was thoroughly wiped with moistened tissue paper. However, in the low light familiar study, two identical experiments were conducted to provide a higher number of subjects per treatment group. No subjects were excluded from the final analysis.

2.4. Vogel conflict test

Rats were water deprived for 20 h on day 1, prior to being placed in a uniformly lit operant conditioning chamber ($45 \times 25 \times 25$ cm) with a metal barred floor, into which a water bottle spout protruded. Rats were allowed to explore the chamber freely and drink for 3 min, timed from the first lick of the spout which was automatically recorded by computer. The rat was then returned to the home cage and allowed access to water for 4 h and then again water deprived for 20 h. On the test day, rats were administered vehicle, chlordiazepoxide (5 mg/kg) or RO 60 0175 0.1, 0.3, 1 or 3 mg/kg s.c. 30 min pre-test in groups of 13. At test time, the animals were placed in the conditioning chamber with free access to the water spout. After 30 s of continuous drinking, each subsequent 5-s cumulative drinking was punished by an electric shock (0.25 mA for 0.2 s) delivered through the water spout and the latency to begin licking and the number of shocks accepted over 3 min was recorded. Many rats that were given RO 60 0175 at doses of 3 (3/6) and 10 mg/kg (3/4) did not complete the 30 s of continuous drinking required to initiate the test and hence could not be included in the study. When this had been ascertained, no further animals were administered with these doses.

2.5. Geller–Seifter test

Forty male Sprague–Dawley CFY rats (Interfauna 400–600 g) were housed in pairs under a 12-h light/dark cycle (lights on 0700 h) and fed a restricted diet to maintain their body weight at 80% of a free-feeding animal. Rats were trained initially in a typical operant box (Campden Instruments) to associate pressing of a lever with a food pellet reinforcement. As training progressed, the rats were introduced to a multiple schedule of reinforcement, i.e., five 3-min variable interval components [one reinforcement every 10–50 s (mean 30), VI30] alternating with five 3-min fixed ratio [one reinforcement every five lever presses; FR5] components. The FR component was signalled to the rat by a light above the lever and, in this component, reinforcement was contingent with a footshock of pulse width 15 ms at intervals of 200 ms for 1 s. The magnitude of footshock was individually titrated for each rat up to a maximum of 0.75 mA, to give a lever pressing rate of between two and seven reinforcements during each of the five 3-min punished responding periods. Fully trained rats also had a high level of responding in the VI phases (typically 180 presses in 3 min) to detect non-specific effects such as sedation or stimulant properties. Before use, all rats met specific performance criteria. Thus, the number of shocks taken on consecutive days did not vary by more than three shocks and that the rat had been on the same shock level for at least 3 days. Rats whose level of punished responses exceeded 49 lever presses during pre-test day trials were also excluded, as were those whose unpunished responses during VI periods varied by > 3 after square root transformation on the three consecutive days before an experimental drug. Finally, before use, all animals had shown a positive response to a reference anxiolytic drug (e.g., chlordiazepoxide). A period of at least 7 days was left between subsequent tests. No animal received two consecutive doses of the same drug or type of drug and no animal received more than five treatments. Rats were given RO 60 0175, 0.1, 0.3 or 1 mg/kg s.c. or chlordiazepoxide 5 mg/kg 30 min pre-test in groups of six.

2.6. Materials

RO 60 0175 (Fig. 1) was synthesised by SmithKline Beecham Pharmaceuticals Medicinal Chemistry Department and was dissolved and administered subcutaneously in 0.9% saline in a 1 ml/kg volume, 20 or 30 min pre-test

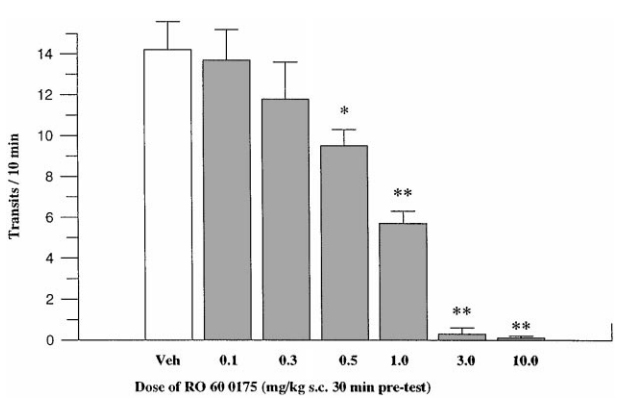

Fig. 2. Effect of RO 60 0175 on locomotor activity over 10 min. All data shown as means ± SEM, $n = 6–12$. Significantly different from vehicle treated group $^{*}P < 0.05$, $^{**}P < 0.01$ by Dunnett's test and one-way ANOVA. Estimated ID_{50} dose = 0.75 ± 0.04 mg/kg s.c. by the iterative curve fitting program ''Allfit''.

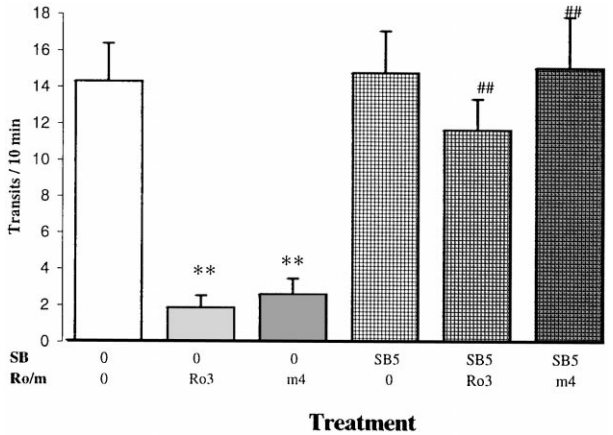

Fig. 3. Effect of SB-242084 on RO 60 0175 and *m*CPP-induced hypolocomotion. 0 — veh, SB5 — SB-242084, 5 mg/kg s.c. 40 min pretest, Ro3 — RO 60 0175, 3 mg/kg s.c. 30 min pretest, *m*4 — *m*CPP, 4 mg/kg s.c. 30 min pretest. All data shown as means ± SEM, $n = 16$. Significantly different from veh/veh group $^{**}P < 0.01$, and corresponding veh/Ro3 or m4 group $^{\#\#}P < 0.01$ by Tukey's test and two-way ANOVA.

as indicated. Chlordiazepoxide (synthesised by Courtin and Warner, Lewes, Sussex) was given in a similar manner. FG 7142 was also synthesised by SmithKline Beecham Pharmaceuticals Medicinal Chemistry Department and given orally in a 2 ml/kg, 1% methyl cellulose vehicle 1 h pre-test. In the social interaction test, drug and vehicle treatments were independently coded prior to experiments to establish blind conditions.

2.7. Data analysis and statistics

The effect of RO 60 0175 on locomotor activity, and behaviour in the social interaction and Vogel conflict tests were determined by one-way analysis of variance (ANOVA) and Dunnett's or Newman–Keuls test as indicated. The effect of SB-242084 in combination with RO 60 0175 on locomotor activity was determined by 2-way ANOVA and Tukey's test. Geller–Seifter test data were analysed by 2-way ANOVA (treatment × subjects) of the number of lever presses on 2 consecutive days before the test day (2 scores per subject), and on the test day itself (1 score per subject). Both control day scores were included in one treatment group for the purposes of this analysis and these were compared with the relevant test day scores for each subject. The dose of RO 60 0175 producing 50% inhibition of locomotion (ID_{50}) was estimated by the four parameter–logistic function using the iterative curve fitting program ''Allfit'' (DeLean et al., 1978). All data are cited as the mean ± SEM unless otherwise indicated. In the Vogel conflict test, data from rats that failed to drink, and

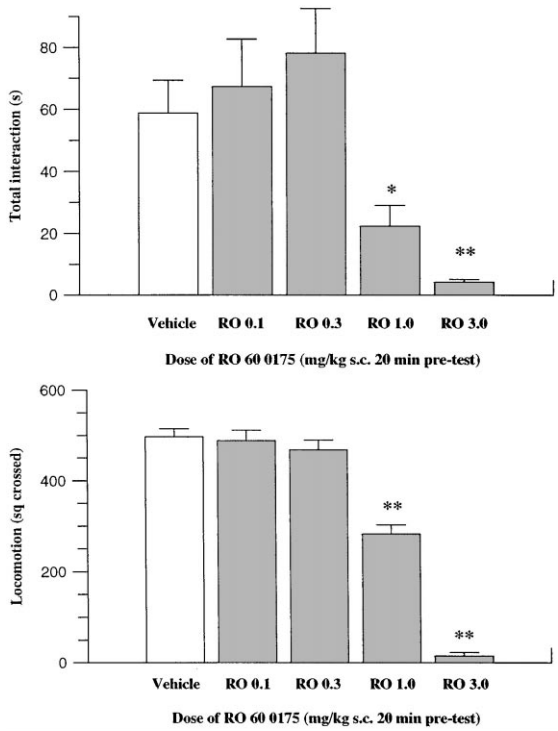

Fig. 4. Effect of RO 60 0175 on time spent in social interaction (upper histogram) and concurrent locomotion (lower histogram) in a 15 min social interaction test under high light unfamiliar conditions. All data cited as means ± SEM, $n = 6$, Significantly different from vehicle treated group $^{*}P < 0.05$, $^{**}P < 0.01$ by Dunnett's test and one-way ANOVA.

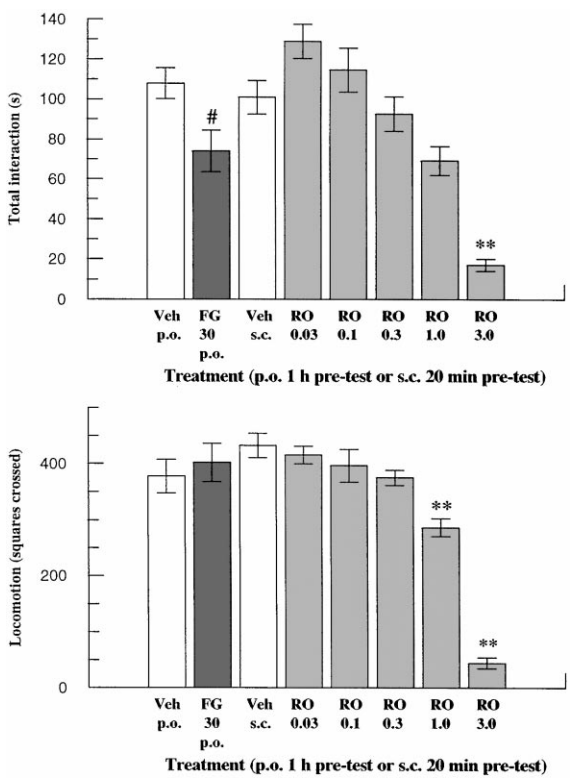

Fig. 5. Effect of RO 60 0175 on time spent in social interaction (upper histogram) and concurrent locomotion (lower histogram) in a 15 min social interaction test under low light familiar conditions. All data cited as means ± SEM, $n = 12–16$. Significantly different from s.c. vehicle treated group $^{**}P < 0.01$, or from p.o. vehicle treated group $^{\#}P < 0.05$, by Newman-Keul's test and one-way ANOVA.

Table 1
Effect of RO 60 0175 on rat behaviour in a Vogel conflict test. All data cited as means ± SEM, $n = 3–13$. Significantly different from vehicle-treated group

Treatment (i.p. 30 min pre-test)	Number of shocks taken over 3 min (mean ± SEM)	Latency to drink (s)
Vehicle	6.1 ± 0.8	34.8 ± 13.2
RO 60 0175, 0.1 mg/kg	11.0 ± 2.4	36.3 ± 9.6
RO 60 0175, 0.3 mg/kg	9.0 ± 1.2	43.1 ± 7.3
RO 60 0175, 1.0 mg/kg	7.1 ± 0.6	64.3 ± 12.4
RO 60 0175, 3.0 mg/kg	4.0 ± 2.3	81.0 ± 27.9
Chlordiazepoxide 5 mg/kg	14.3 ± 1.8[a]	46.7 ± 8.2

[a] $P < 0.05$ by Dunnett's test and one-way ANOVA.

therefore received no punishment during the test, were excluded from the analysis.

3. Results

3.1. Effects of RO 60 0175 on locomotor activity

RO 60 0175 was administered s.c. 30 min pre-test at doses between 0.1 and 10 mg/kg. Doses above 0.5 mg/kg were found to markedly depress the number of cage transits over 10 min ($F(6, 54) = 34.9$, $P < 0.01$, Fig. 2). Following analysis by the iterative curve fitting program ''Allfit'', the ID_{50} dose was estimated as 0.75 ± 0.04 mg/kg s.c.

In experiments conducted to assess the possible contribution of 5-HT_{2C} receptor activation to the hypolocomotor activity of RO 60 0175, a box of slightly different dimensions (shorter and wider) was used. Nevertheless, vehicle treated rats completed a very similar amount of locomotor activity to that recorded in the first experiment. RO 60 0175 (3 mg/kg) also suppressed activity to a similar degree as previously. Prior administration of SB-242084 (5 mg/kg s.c.) significantly reversed the hypolocomotor effect of RO 60 0175 (3 mg/kg s.c.) and *m*CPP (4 mg/kg s.c.) ($F(2, 89) = 5.5$, $P < 0.01$, Fig. 3).

3.2. Effects of RO 60 0175 on rat behaviour in social interaction tests

Under high light unfamiliar conditions, RO 60 0175 did not increase the time spent in social interaction at any dose tested. However, a marked suppression of both time spent in social interaction [$F(4, 25) = 8.3$, $P < 0.01$] [$F(4, 25) = 119.5$, $P < 0.01$] and concurrent locomotion was seen at doses of 1 and 3 mg/kg (Fig. 4). The absence of any clear anxiolytic-like trend in this study argued against increasing the number of animals used from $n = 6$ per treatment group. RO 60 0175 had similar effects under low light familiar conditions also, suppressing both time spent in social interaction ($F(7, 116) = 14.1$, $P < 0.01$) and locomotion ($F(7, 116) = 24.4$, $P < 0.01$) at doses of 1 and 3 mg/kg (Fig. 5). This contrasts with the benzodiazepine inverse agonist, FG 7142, which at 30 mg/kg p.o. 1 h pre-test, selectively reduced social interaction without affecting locomotion in the test under low light familiar conditions (Fig. 5).

3.3. Effects of RO 60 0175 in the rat Vogel and Geller–Seifter conflict tests

In the rat Vogel conflict test, RO 60 0175 had no significant effect on either the number of shocks accepted or the latency to drink, unlike the benzodiazepine anxiolytic, chlordiazepoxide, which selectively increased the number of shocks accepted [$F(5, 62) = 4.9$, $P < 0.01$]. RO 60 0175 did tend to increase the latency to drink at 1 and 3 mg/kg s.c. (Table 1). This did not reach significance in the data included in the present study. However, 3/6 rats given 3 mg/kg of RO 60 0175 did not drink during the 3-min test period and were therefore excluded from the final analysis as non-responders. Inclusion of these might have resulted in a significant increase in latency to drink.

Table 2
Effect of RO 60 0175 on rat behaviour in a Geller–Seifter conflict test. All data cited as means ± SEM, $n = 6$ per group. Significantly different from vehicle treated group. Significant F values, VI RO 0.3 mg/kg $F(1, 5) = 61.3$, $P < 0.01$, RO 1 mg/kg $F(1, 5) = 283.1$, $P < 0.01$, FR, RO 0.3 mg/kg $F(1, 5) = 11.1$, $P < 0.01$, RO 1 mg/kg $F(1, 5) = 21.8$, $P < 0.01$, CDP $F(1, 15) = 54.2$, $P < 0.01$. Mean lever presses prior to treatment during test VI 608.5 ± 34.4, FR 20.7 ± 0.9

Treatment (s.c. 30 min pre-test)	% Change in no of lever presses from mean score on two preceding days after vehicle treatment	
	Unpunished (VI)	Punished (FR)
RO 60 0175 0.1 mg/kg s.c.	−4.4 ± 2.7	−8.5 ± 8.6
0.3 mg/kg s.c.	−23.1 ± 5.4[a]	−31.4 ± 13.0[b]
1.0 mg/kg s.c.	−35.8 ± 13.7[a]	−31.0 ± 15.6[b]
Chlordiazepoxide 5 mg/kg s.c.	+8.7 ± 5.8	+234.6 ± 97.0[a]

[a] $P < 0.01$ by 2-way ANOVA (treatments × subjects).
[b] $P < 0.05$ by 2-way ANOVA (treatments × subjects).

Of the animals given 10 mg/kg, RO 60 0175, 3/4 did not drink during the test (data not shown). In the Geller–Seifter procedure, the mean number of lever presses in vehicle treated rats prior to drug treatment on the test day was 608.5 ± 34.4 during the unpunished responding phases (VI) and 20.7 ± 0.9 during the punished phases (FR) of the test. RO 60 0175 significantly reduced both punished and unpunished responding at doses of 0.3 and 1 mg/kg, unlike chlordiazepoxide which selectively increased punished responding in the procedure (see Table 2 for both scores and F values).

4. Discussion

RO 60 0175 significantly reduced locomotor activity in a 10-min test as reported by Martin et al. (1998). Reversal of this sedative effect by the selective 5-HT_{2C} receptor antagonist, SB-242084 (Kennett et al., 1997), indicates that it is mediated via 5-HT_{2C} receptor activation, and demonstrates the potency of RO 60 0175 in stimulating this receptor.

In a rat social interaction test, under high light unfamiliar conditions optimal for the detection of anxiolytic-like responses (File and Hyde, 1978), RO 60 0175 failed to increase time spent in social interaction at any dose tested. Indeed, a significant reduction in both social interaction and concurrent locomotion was observed at higher doses, in keeping with a sedative-like profile. The profile of RO 60 0175 in this test therefore differs from that of the selective 5-HT reuptake inhibitor antidepressant and anxiolytic, paroxetine, which increases social interaction in this procedure, albeit after chronic administration (Lightowler et al., 1994).

There was also no evidence of anxiolytic-like activity in another test, the rat Vogel conflict procedure. Furthermore, in this test, no evidence of significant sedation was observed, although a doubling of the latency to drink in the test was associated with doses of the compound which elicited sedation in the locomotor and social interaction tests. The failure to detect a similar sedative effect in the Vogel test may be accounted for by the exclusion of 3/6 animals given 3 mg/kg and 3/4 animals given 10 mg/kg RO 60 0175 which failed to drink at all during the test. The absence of an anxiolytic-like response to RO 60 0175 in the present Vogel test, contrasts with the anticonflict action of the non-selective 5-HT_{2C} receptor agonist, *m*CPP, in a rat Vogel test reported by Chojnacka-Wojcik and Klodzinska, (1992). As no anticonflict effect of *m*CPP was observed under the conditions used in the present study (Kennett et al., 1998), the current test may be insensitive to any effect of RO 60 0175 as well.

Results from another conflict test of anxiety, the rat Geller–Seifter test, were similar, with the depression of both unpunished and punished responding by RO 60 0175 suggesting a sedative-like action (Geller et al., 1962). The onset of these effects at a somewhat lower dose than that required in the social interaction and Vogel conflict tests either suggests that this procedure is more sensitive to sedation, or that an additional action of RO 60 0175 may be exerted. One property associated with 5-HT_{2C} receptor activation (Kennett, 1993; Kennett et al., 1997; Curzon et al., 1997), and with RO 60 0175 in particular (Martin et al., 1998), is hypophagia. This may be uniquely relevant to the Geller–Seifter test, where rats are motivated to respond by a food reinforcement.

Clearly, despite evidence that RO 60 0175 has antidepressant-like properties as indexed by the reversal of chronic mild stress-induced deficits in rates of self stimulation through electrodes implanted in the nucleus accumbens (Moreau et al., 1996), the compound did not elicit anxiolytic-like responses in the three tests described. This is consistent with the lack of activity of RO 60 0175 in a fourth rat model of anxiety, the elevated plus maze (Martin et al., 1998). Indeed, only a marked sedative-like action was observed which becomes apparent at doses similar to those reported antidepressant-like in another rodent model (Moreau et al., 1996). It is conceivable that this sedative-like effect actually reflects anxiogenic-like activity, as non-selective 5-HT_{2C} receptor agonists have anxiogenic-like consequences in both rats and man (see Kennett, 1993) while selective 5-HT_{2C} receptor antagonists such as SB-242084 have anxiolytic-like actions in both the rat social interaction and Geller–Seifter models (Kennett et al., 1997). Furthermore, mutant mice lacking the 5-HT_{2C} receptor also exhibit reduced anxiety in tests such as the open field procedure and the elevated 0- and X-mazes (Tecott, 1996; Das and Tecott, 1996). As the social interaction test under high light unfamiliar conditions and the rat Vogel or Geller–Seifter conflict tests used are suboptimal for the detection of anxiolytic responses, RO 60 0175 was tested in a differently configured social interaction test that was. Under the low light familiar conditions used, RO 60 0175 simultaneously reduced both social interaction and locomotion. Thus, RO 60 0175 did not exhibit the selective reduction of social interaction expected of an anxiogenic agent (File and Hyde, 1978) which was exemplified in this study by the benzodiazepine inverse agonist and anxiogenic agent, FG 7142 (Dorow et al., 1983). The failure to observe clear evidence of anxiogenesis may be due to the use of inappropriately large dose increments of RO 60 0175. This is supported by previous studies with the anxiogenic 5-HT_{2C} receptor agonist, *m*CPP, which suggest that there is little separation between doses that elicit anxiogenic-like and sedative-like responses in the social interaction test (Kennett et al., 1989). Further studies to investigate the effects of RO 60 0175 over smaller dose increments are thus warranted.

The present studies do not suggest that acute administration of RO 60 0175 has anxiolytic-like properties, in contrast to the actions of chronic administration of 5-HT

reuptake inhibitors (Lane et al., 1995) which were reported to increase the level of terminal 5-HT in the brain (Bel and Artigas, 1993; Blier and deMontigny, 1994). It is also of note that, in contrast to previous studies with *m*CPP, a 5-HT_{2C} receptor agonist and a known anxiogenic agent, in both the social interaction and elevated 0-maze tests (Kennett et al., 1989; Weiss et al., 1998), no clear evidence that RO 60 0175 induced anxiogenesis was recorded either. This may be accounted for by methodological differences, particularly as selective 5-HT_{2C} receptor antagonists (Kennett et al., 1997) or 5-HT_{2C} receptor knock out mice (Tecott, 1996) are associated with anxiolytic-like profiles. It is possible, however, that the anxiogenic actions of *m*CPP which can be reversed by the 5-$HT_{2C/2B}$ receptor antagonist, SB-200646-A (Kennett et al., 1994) are the result of coactivation of the 5-HT_{2C} and other receptors which are not stimulated by the more selective RO 60 0175.

Finally, since RO 60 0175 was associated only with marked sedation in the present study, the possibility that this property accounts for the acute drug's anti-obsessive–compulsive disorder-like and antipanic-like actions in the polydipsia (Martin et al., 1998) and periaqueductal gray (Jenck et al., 1996) models must be considered. In the polydipsia model, hungry rats given access only to water, indulge in bouts of prolonged drinking. In the periaqueductal gray model, electrical stimulation of the periaqueductal gray results in profound motor activation. It is therefore conceivable that the effects of RO 60 0175 in these models might be secondary to sedation.

In conclusion, RO 60 0175 was markedly sedative via 5-HT_{2C} receptor activation, but was neither anxiolytic nor anxiogenic when acutely administered at the doses tested in this study. This sedative profile differs from the anxiogenic-like actions of the 5-HT_{2C} receptor agonist, *m*CPP, for reasons that are currently unclear. However, RO 60 0175-induced sedation may at least contribute to the compound's reported antipanic-like and anti-obsessive compulsive disorder-like actions in the rat periaqueductal gray stimulation and polydipsia models, respectively.

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