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Pharmacology, Biochemistry and Behavior 71 (2002) 615–625

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Influence of the 5-HT2C receptor antagonist, SB-242084, in tests of anxiety

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Received 31 July 2001; received in revised form 14 September 2001; accepted 21 September 2001

Abstract

The 5-HT2C antagonist SB-242084 was examined in various anxiety tests at doses based on reversal of mCPP-induced hypoactivity (0.1–3 mg/kg ip). In the elevated plus-maze task, SB-242084 exhibited signs of anxiolysis (time spent, distance travelled, and entries into open arms), but this was potentially confounded by its general increase of locomotion; alprazolam selectively affected open-arm parameters. In a Geller–Seifter conflict test, SB-242084 produced a modest, nonsignificant increase in punished responding compared to the significant effect produced by diazepam. None of the treatments significantly affected unpunished responding. In the conditioned emotional response (CER) test, SB-242084 produced an increase in the suppression ratio (SR, smaller than diazepam). Since this 5-HT2C antagonist also increased lever pressing, an additional test was conducted with amphetamine that stimulated lever pressing but, nonetheless, failed to produce any change in SR. In the fear-potentiated startle task, SB-242084 was inactive in comparison to a significant effect of diazepam. The previously described reduction of schedule-induced polydipsia by fluoxetine and 5-HT2C receptor agonist Ro60-0175 was attenuated by SB-242084 pretreatment, however, the latter compound exhibited a potent increase in polydipsia when given alone. The present results demonstrate an anxiolytic potential of SB-242084, as well as an intrinsic response-enhancing property, however, both of these effects are task dependent.

Keywords: 5-HT2C receptor antagonist; SB-242084; Fluoxetine; Ro60-0175; Anxiety models; CER

1. Introduction

The broad medical use of drugs acting via modulation of serotonergic neurotransmission, particularly the selective serotonin reuptake inhibitors (SSRIs), for the treatment of a variety of CNS disorders, has emphasized the therapeutic importance of this neurotransmitter system (see Jones and Blackburn, in press). The diverse effects of serotonin are mediated by distinct 5-HT receptor subtypes (Boess and Martin, 1994; Barnes and Sharp, 1999), although the relative contributions of each to the development of CNS disorders and/or to the efficacy of available medicines are not fully understood. Recent studies showing that the discriminative stimulus effects of SSRIs may involve a 5-HT2C receptor component suggest that this subtype may have an important role in certain CNS effects of this drug class (Millan et al., 1999; see also Kennett, 1993; Jenck et al., 2000 for reviews). Drugs interacting with the 5-HT2C receptor may, therefore, offer an especially promising approach for the discovery of novel agents to use in the treatment of psychiatric disorders.

Martin et al. (1998) demonstrated that the 5-HT2C receptor agonist Ro60-0175 was effective in attenuating compulsive behaviors in diverse test situations and species: reducing schedule-induced polydipsia in rats, reversing whole-body scratching induced with 8-OH-DPAT in squirrel monkeys, reducing marble-burying in mice, and decreasing excessive feeding of palatable food in rats. Moreau et al. (1996) additionally demonstrated an attenuation of stress-induced anhedonia in rats following chronic treatment with Ro60-0175. However, despite showing activity in a model of panic, Ro60-0175 failed to exhibit anxiolytic-like effects in the elevated plus-maze task in rats up to doses that impaired motor responding (Martin et al., 1998; Jenck et al., 1998). Ro60-0175 is a potent agonist at both 5-HT2C and 5-HT2B receptors with approximately 10-fold select-

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0091-3057/02/$ – see front matter
PII: S0091-3057(01)00713-4

ivity over 5-HT2A receptors (Martin et al., 1998; Porter et al., 1999). However, in vivo Ro60-0175 appears to produce predominantly 5-HT2C receptor-mediated behaviors (Martin et al., 1998; Higgins et al., 2001). Furthermore, the discriminative stimulus produced by Ro60-0175 appears to be due to the activation of 5-HT2C receptors since it can be fully blocked by a selective 5-HT2C antagonist, but not by 5-HT2A or 5-HT2B antagonists (Dekeyne et al., 1999).

It has also been reported that the selective 5-HT2C receptor antagonist SB-242084 exhibited anxiolytic-like effects in rats at doses that failed to consistently affect motor function: increasing social interaction and increasing punished responding in Geller–Seifter and Vogel conflict tasks (Kennett et al., 1997). Similar effects have been reported with other drugs having 5-HT2C receptor antagonist properties although lacking the selectivity of SB-242084, e.g., mianserin, SB-200646, and SB-206553 (Kennett et al., 1994, 1996; Griebel et al., 1997; Dekeyne et al., 2000). These results indicate that 5-HT2C antagonists are active in certain animal tests of anxiety, whereas 5-HT2C agonists appear to exhibit effects in other animal tests of anxiety (in particular, those considered relevant to panic and obsessive–compulsive disorder).

The present investigation was designed to further investigate the effects of the selective 5-HT2C receptor antagonist SB-242084 in animal tests of motor function and models of anxiety and compulsive behavior. The focus of these investigations was to extend the characterization of SB-242084 to certain rodent anxiety tests additional to those used by Kennett et al. (1997). These included the elevated plus-maze task, conditioned emotional response (CER) task, schedule-induced polydipsia task, and fear-potentiated startle test.

2. Methods

2.1. Animals and maintenance conditions

Adult male rats (Sprague–Dawley strain; Biological Research Labs, Füllinsdorf, Switzerland) were used for all experiments, except for the fear-potentiated startle experiment in which adult male RORO rats were used (Ibm: RORO (SPF); Biological Research Labs). Animals were housed in Macrolon cages with sawdust bedding. The rats received food and tap water ad libitum in the home cage, except for the rats tested in the Geller–Seifter conflict task, the CER task, and the schedule-induced polydipsia task (the food restriction schedules are described in the respective method sections). The animal quarters were maintained on a 12:12 h light–dark cycle with light onset at 6:00 a.m. Room temperature (c. 22–24 °C) and humidity (c. 50–65%) were regulated. The rats were euthanized by means of CO_2 exposure upon completion of all testing. The experimental procedures used in the present investigation received prior approval from the City of Basel Cantonal Animal Protection Committee based on adherence to federal and local regulations.

2.2. Testing procedures

2.2.1. Spontaneous motor activity evaluation

Locomotor activity was measured in activity chambers ($36 \times 24 \times 19$cm; $L \times W \times H$; Benwick Electronics, UK) containing sawdust bedding. Three experiments were conducted to investigate SB-242084.

In the first experiment, the ability of SB-242084 to block mCPP (4 mg/kg ip)-induced hypoactivity was studied. An independent group design was used with naïve animals randomly assigned to 1 of 6 different treatment groups: (1) vehicle + vehicle; (2) vehicle + mCPP; (3) SB-242084 (0.01 mg/kg) + mCPP; (4) SB-242084 (0.03 mg/kg) + mCPP; (5) SB-242084 (0.1 mg/kg) + mCPP; and (6) SB-242084 (0.3 mg/kg) + mCPP ($n = 8$ per group). mCPP or its vehicle was injected 20 min before the test. SB-242084 or vehicle was injected 30 min before the test. All treatments were given intraperitoneally. Motor activity (consecutive photobeam interruptions) was measured for 20 min.

A second experiment examined the effect of SB-242084 (0.1–3 mg/kg ip) alone on locomotor activity in comparison to a vehicle control group. Again, an independent group design was used with all animals naïve to the activity chambers at the time of test ($n = 12$ per group). At 30 min postinjection, all animals were singly placed in the test chambers and activity was recorded for 2 h. A third experiment was conducted using a similar design to Experiment 2, except that the animals were habituated to the test chambers during two separate 2-h sessions on each of 2 days before testing began. A repeated-measures design ($n = 12$) was used with each animal receiving each dose of SB-242084 (0.1–3 mg/kg ip) or vehicle in a counterbalanced design with a 2–3-day interval between treatments. A dose of amphetamine (0.5 mg/kg) was also tested as a positive control.

Data were analyzed by one-way ANOVA for independent groups (Experiments 1 and 2) or within groups (Experiment 3). Following detection of a significant main effect, post hoc Newman–Keuls tests were conducted with an accepted level of significance of less than .05.

2.2.2. Elevated plus-maze task

The elevated plus-maze consisted of two open arms perpendicular to two closed arms (each arm was 10-cm wide × 50-cm long) extending from an open central area (10×10 cm). All parts of the apparatus were constructed of grey polyvinylchloride plastic. The plus-maze was located in a sound-attenuated observation room with controlled illumination (c. 200 lux on the central platform of the plus-maze). Testing was conducted during the light part of the light–dark cycle. At the time of testing, the rats weighed 110–140 g. Testing started by placing the animal onto the central platform facing an open arm. The duration of the test was 5 min. The maze was thoroughly cleaned with 70% ethanol after each successive test. The plus-maze was positioned in the middle of a closed, white environment

with the animal observed via a closed-circuit video camera mounted to the ceiling.

Behavioral analysis was conducted using a computerized system (Ethovision, Noldus Information Technology, The Netherlands). The key measures selected to represent anxiolytic-like behavior were the total time (s) spent in the open arms and the number of transitions from the open into the closed arms. The key measures used to quantify motor activity were the distance (cm) travelled within the open arms, within the closed arms, and the total distance travelled within open and closed arms of the plus-maze. Rats were randomly assigned to each treatment condition with 16 rats per treatment group. SB-242084 was tested at 0.1, 0.3, and 1 mg/kg ip following a pretreatment interval of 30 min. Alprazolam (0.3 mg/kg ip) was included as an active control condition. Differences between vehicle and drug treatments were evaluated with a single-factor ANOVA and when overall significance was obtained, post hoc Newman–Keuls tests were conducted. A P value less than .05 was accepted as statistically significant.

2.2.3. Operant tasks

2.2.3.1. Preliminary training. Rats were placed on a restricted food diet (12–15 g per day) and trained over a period of 1–2 weeks to press a lever for a food reward (45 mg Formula P Noyes pellet) on a continuous reinforcement (CRF) schedule (Operant boxes: Med Associates, USA; Kestrel Control System: Conclusive Marketing, UK). Operant schedule requirements were gradually increased from a variable interval 5-s schedule (VI5) to a final value of VI30 (conflict) or VI60 (CER). Subjects were trained for a period of 1–2 weeks until their rate of responding was stable over several consecutive days.

2.2.3.2. Geller–Seifter conflict test. This procedure was divided into three stages (one lever was extended throughout the whole session): (1) unpunished responding (5 min) during which the houselight was illuminated and animals received food reward following lever pressing on a schedule of VI30; (2) unrewarded responding (timeout period of 2 min) during which the houselight was extinguished and animals were able to press the lever, but did not receive a food reward; (3) punished responding (8 min) during which the houselight remained off and a cue light above the lever was illuminated. In the latter stage, a fixed ratio of 10 (FR10) was employed so that on every 10th lever press the rat received simultaneously a food reward and a footshock (0.6 mA, 0.5 s). These three stages were repeated in the same order so that the total daily session duration was 30 min.

Subjects were considered sufficiently trained when responding during the unpunished stage was high (approximately 40 lever presses per min) and stable across days and responding during the timeout and punished stages was low. Twelve trained rats were included in the experiment to evaluate SB-242084 and were tested, using a Latin-squares design, twice weekly with at least a 2-day interval between test sessions. Rats were trained between test days to maintain baseline performance. SB-242084 was tested at 0.1, 0.3, and 1 mg/kg ip following a pretreatment interval of 30 min. Diazepam (1 mg/kg ip) was included as an active control. At the end of the Latin-squares design testing, all rats were administered 3 mg/kg ip SB-242084 and tested on the same day. Data were analyzed using a repeated-measures ANOVA to compare all treatment groups and, in significant cases, this was followed by a paired t test with Bonferroni correction. A P value of less than .05 was accepted as statistically significant.

2.2.3.3. CER task. Rats were trained to a schedule of VI60 as previously described over a 1-h test session with the houselight illuminated. Once stable baseline responding had been attained, two 2-min conditioning periods were introduced into the session. A 2.9-kHz tone was delivered from a Sonalert system on the test chamber ceiling and a cue light above the lever was illuminated (CS). During the final 0.5 s of the CS presentation, animals received a footshock (0.8 mA, 0.5 s). The two CS periods were presented randomly at different time points from session to session, the first presentation of the CS approximately 20 min (range 15–25 min), and the second approximately 40 min (range 35–45 min) after the start of the session.

The number of lever presses during the 2-min conditioning period and the number of lever presses during the 2 min prior to this period were recorded and used to calculate suppression ratios (SR) using the following formula:

$$\mathrm{SR} = \frac{\mathrm{A}}{\mathrm{A} + \mathrm{B}}$$

A = number of lever presses prior to both CS periods

B = number of lever presses during both CS periods

An SR value of 0 indicates that animals have suppressed responding during the tone presentation due to a conditioned fear response. An SR value of 0.5 indicates that the animals are responding equivalently during the conditioning period and the 2 min immediately prior to this period. Animals were trained daily for a few weeks until their SR was 0.15 or lower. Sixteen rats were included in the experiment done to evaluate SB-242084 and were tested, using a Latin-squares design, twice weekly with at least a 2-day interval between test sessions. During a test session, the animals did not receive a footshock following the conditioning periods. However, animals always received footshock during intervening baseline days. SB-242084 was tested at 0.1, 0.3, and 1 mg/kg ip following a pretreatment interval of 30 min. At the end of the Latin-squares design testing, all rats were administered 3 mg/kg ip SB-242084 and tested on the same day. A separate group of 14 trained rats was used to test amphetamine at 0.1, 0.25, and 0.5 mg/kg ip following a pretreatment time of 10 min. Diazepam (3 mg/kg ip) was included as an active

control in both of these experiments. The SR data were analyzed using nonparametric statistics: Friedman's test was used to compare all treatment groups and, in significant cases, this was followed by a Wilcoxon Rank Sum test. A P value of less than .05 was accepted as statistically significant. To control for any motoric effects of drugs, the total number of lever presses made during the 1-h session was analyzed using a repeated-measures ANOVA, followed in significant cases by post hoc paired t tests with Bonferroni correction.

2.2.4. Palatability-induced feeding

Naïve rats with ad libitum access to food and water prior to testing were given access during daily 1-h sessions (5 days/week) to a wet mash diet (100 g Formula P diet per 200 ml water) in their home cage. After 3 weeks, food intake had stabilized with mean consumption approximately 8–10 g per rat, although marked differences were seen between individual animals. At this time, 10 rats were selected and the effect of SB-242084 (0.1–1 mg/kg ip) on food intake was evaluated with diazepam (3 mg/kg ip) included as a positive control. Rats received each treatment according to a balanced design with 2–3 days between successive cycles, and data were subsequently analyzed by repeated-measures ANOVA with a P value less than .05 accepted as statistically significant. Post hoc evaluation was done with the Newman–Keuls test.

2.2.5. Fear-potentiated startle

Testing was carried out in rats using eight SR-LAB startle response chambers with programmable animal shocker units (San Diego Instruments, San Diego, CA). Within each of these sound-attenuated test chambers there was a Plexiglas cylinder c. 8.8 cm in diameter and 20.5 cm in length mounted on a Plexiglas plate that contained the stabilimeter for measuring the magnitude of the startle response. Calibration was done at the start of each experiment. Acoustic tones were produced by a loudspeaker mounted in the ceiling of the chamber to induce the acoustic startle reaction. Background noise was 68 dB. The light (15 W) used as the conditioned stimulus was positioned at the rear of the chamber. Scrambled electric footshock could be delivered via the stainless-steel grid floor of the Plexiglas cylinder in which the rat was held. The stimuli were automatically delivered and the startle amplitudes (in voltage units) recorded via a computer.

Experimentally naïve rats (c. 260 g body weight) received training (pairing light and shock) on 2 consecutive days followed on the third day by fear-potentiated startle testing. Training and testing were done in a darkened chamber, except when the procedure required illumination. The training session was conducted on each of the initial 2 days and consisted of 5 min habituation in the test apparatus followed by 15 footshocks (0.25 mA) each delivered during the final 500 ms of a 3700-ms period of illumination (mean intertrial interval 30 s; range 20–40 s). On the test day, the 5-min habituation period was followed by 10 acoustic stimuli (95 dB; 50 ms) given in the dark (data not analyzed). Subsequently, five startle stimulus presentations of three intensity levels (90, 95, and 105 dB; 50 ms duration) were delivered during the 3700-ms period of illumination (occurring at the time point 3200–3250 ms). Five stimuli each of three intensities (90, 95, 105 dB; 50 ms) were delivered in the dark during a 3700-ms measurement period (occurring at the time point 3200–3250 ms). The mean intertrial interval was 30 s (range 20–40 s). Independent groups ($n = 12$ per group) received vehicle or SB-242084 (0.1, 0.3, 1 mg/kg ip). For comparison purposes, a parallel experiment was conducted with vehicle and diazepam (0.3, 1, 3 mg/kg ip). Treatment conditions were given 30 min prior to the test session. Data were averaged for each test condition and each rat was analyzed with a mixed-factor ANOVA for independent groups (drug treatment) and within groups (illumination condition). This was followed by Newman–Keuls tests with a P value less than .05 accepted as statistically significant.

2.2.6. Schedule-induced polydipsia task

The schedule-induced polydipsia paradigm induces excessive drinking through the administration of food to fasted rats at regular short intervals independent of the behavior of the rat. This test has been proposed as a model of obsessive–compulsive disorder based on pharmacological validation and symptomatic similarities (Woods et al., 1993). The excessive drinking was induced in these rats by use of a fixed-time operant schedule of reinforcement (FT-1 min delivery of 45 mg food pellets). The rats were food deprived overnight prior to each 60-min test session in a sound-attenuated operant test chamber with a water bottle attached. Once stable intake was obtained over several test sessions, evaluation of experimental compounds was initiated. At the start of the present experiments, the rats were already drug experienced and had undergone previous testing in this task. The rats weighted approximately 250–300 g. A repeated-measures design was used in each individual experiment with treatment presentation in a balanced order. Test days alternated with training days on which the session proceeded in the same manner as on test days. Total water intake (g) during the test session was measured. Group sizes of 8–16 per experiment were used. The statistical evaluation of the results was done using repeated-measures ANOVA followed by post hoc comparisons of the treatment groups with Newman–Keuls tests. A P value less than .05 was accepted as statistically significant.

In preliminary experiments, dose–response evaluation was conducted separately for Ro60-0175 (vehicle, 0.1, 0.3, 1, and 3 mg/kg sc; 10 min pretreatment), fluoxetine (vehicle, 3, 10, and 10 mg/kg sc; 10 min pretreatment), and SB-242084 (vehicle, 0.03, 0.1, 0.3, and 1 mg/kg ip; 30 min pretreatment). Interaction experiments were also conducted using a 2×2 factorial design in which SB-242084 (0.3 mg/kg ip) was given in combination with Ro60-0175 (0.5 mg/kg sc) or fluoxetine (15 mg/kg sc) with appropriate vehicle

controls. In an additional set of control experiments, the same experimental procedure as above was used except that all 60 food pellets were given together at the start of the session with no subsequent food delivery during the 1-h test session ("low-stress condition"). Fluoxetine (7.5, 15, and 30 mg/kg sc) and Ro60-0175 (0.1, 0.3, and 1 mg/kg sc) were tested under this low-stress procedure.

2.3. Drugs

Ro60-0175 ([*S*]-2-(chloro-5-fluoro-indol-1-yl)-1-methyl-ethylamine 1:1 $C_4H_4O_4$), SB-242084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl] indoline, and D-amphetamine sulfate were synthesized at F. Hoffmann-La Roche (Basel, Switzerland). Fluoxetine was purchased from Sigma (St. Gallen, Switzerland). All compounds were prepared immediately prior to use in vehicle (either 0.3% Tween-80 in 0.9% NaCl or 8% hydoxypropyl-B-cyclodextrin and 25 nM citric acid in 0.9% NaCl) and ultrasonified (Model Digital S, TransSonic). The volume of administration was 1 ml/kg body weight for the CER and conflict tests and 5 ml/kg for all other tests. All doses were calculated as base.

3. Results

3.1. Spontaneous motor activity task

In Experiment 1, a statistically significant main effect of treatment group was found [$F(5,35) = 14.6$, $P < .01$]. Post hoc tests revealed a significant hypoactivity following mCPP treatment compared to vehicle-treated controls. This hypoactivity was significantly attenuated by SB-242084 (0.1–0.3 mg/kg) pretreatment with the doses 0.1 and 0.3 mg/kg producing significantly greater activity than the mCPP + vehicle treatment condition. The treatment group that received SB-242084 (0.3 mg/kg) + mCPP exhibited an activity level equivalent to the vehicle + vehicle treatment group (see Fig. 1A).

In animals naïve to the test environment, there was no statistically significant main effect of SB-242084 pretreatment on locomotor activity [$F(4,45) = 1.3$, NS] (Experiment 2; see Fig. 1B). However, there was a trend to suggest that SB-242084 might increase locomotor activity — particularly at the 1-mg/kg dose. Consequently, a further study was conducted using a repeated-measures design with animals habituated to the test apparatus. Both of these test modifications would be expected to increase the likelihood of detecting any potential hyperactivity effect of SB-242084. The results from this study are presented in Fig. 1C. A main effect of treatment was found on locomotor activity [$F(5,45) = 6.4$, $P < .01$], however, post hoc analysis only revealed a significant difference between vehicle control and low dose amphetamine (0.5 mg/kg). Despite a trend toward an increase in locomotion seen at the 0.3- and 1-mg/kg doses of SB-242084, again these effects failed to reach statistical significance.

3.2. Elevated plus-maze task

The effect of SB-242084 on the measures (1) time in open arm and (2) distance travelled in the open arms alone, (3) distance travelled in the closed arms alone, as well as (4) total distance travelled in both open and closed arms is illustrated in Fig. 2. Significant main effects of treatment were recorded on measures of total distance [$F(4,75) = 2.6$, $P < .05$], time in open arm [$F(4,75) = 4.7$, $P < .01$], transitions into open arm [$F(4,75) = 4.9$, $P < .01$], distance travelled in open arms [$F(4,75) = 4.5$, $P < 0.1$], transitions into closed arms [$F(4,75) = 3.6$, $P < .01$], and distance travelled in closed arms [$F(4,75) = 5.4$, $P < .01$]. SB-242084 in-

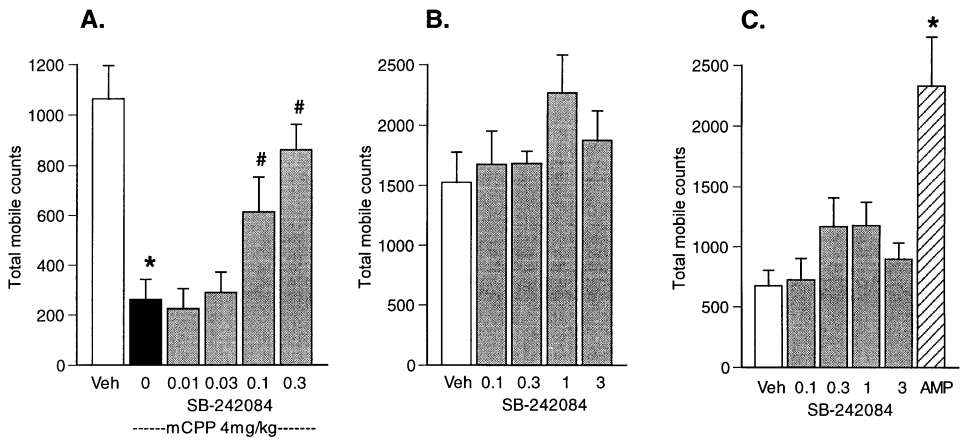

Fig. 1. (A) Effect of SB-242084 (0.01–0.3 mg/kg ip) against mCPP (4 mg/kg ip)-induced hypoactivity. * $P < .01$ vs. vehicle group, # $P < .01$ vs. mCPP + vehicle group. $n = 8$ rats per group. (B) Effect of SB-242084 (0.1–3 mg/kg ip) in a locomotor activity test of 2 h duration in rats unfamiliar with the test environment. $n = 12$ rats per group. (C) Effect of SB-242084 (0.1–3 mg/kg ip) and amphetamine (0.5 mg/kg ip; AMP) in a locomotor activity test of 2 h duration in rats familiarized to the test environment. $n = 12$ rats received each treatment in a randomized sequence in this experiment. * $P < .01$ vs. vehicle group.

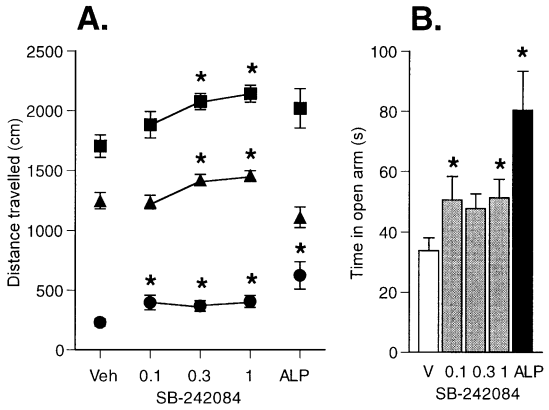

Fig. 2. Effect of SB-242084 (0.1–1 mg/kg ip) and alprazolam (0.3 mg/kg ip; ALP) on rat exploratory behavior in the elevated plus-maze. (A) Distance travelled (cm) in the open (●), closed (▲) arms and the total distance travelled (■). (B) Time spent on the open arms during the test. $n=16$ rats per group. * $P<.05$ vs. vehicle-pretreated controls following significant ANOVA.

creased time in open arm at the 0.1- and 1-mg/kg doses and transitions into the open arms also increased at 1 mg/kg (vehicle: 5.5 ± 0.7, SB-242084 1 mg/kg: 7.9 ± 0.7, $P<.05$) and with a tendency to increase at the lower doses. In addition to these changes, SB-242084 produced a dose-related increase in distance travelled within the open arms alone (0.1–1 mg/kg) and within the closed arms alone (0.3–1 mg;/kg)—consequently, total distance travelled in both open and closed arms was also increased (0.3–1 mg/kg). Closed-arm entries were also increased by SB-242084 (e.g., vehicle: 11.3 ± 1.3, SB-242084 1 mg/kg: 14.5 ± 0.9, $P<.05$). Alprazolam (0.3 mg/kg) increased time spent in open arm and open-arm entries (vehicle: 5.5 ± 0.7, alprazolam: 10.9 ± 1.4, $P<0.01$), both of these changes were significantly greater than that observed following SB-242084. For the measures of distance travelled, only open-arm activity was increased—thus alprazolam produced a selective increase in measures of open-arm exploration.

3.3. Geller–Seifter conflict task

Four animals were removed from the experiment because they exhibited a very high level of punished responding, consequently, a total of eight rats were included in the final analysis. A repeated-measures ANOVA revealed an overall statistically significant effect of treatment on punished responding [$F(5,35)=11.0$, $P<.01$]. SB-242084 produced a slight increase in punished responding (at 1 mg/kg), but this did not reach statistical significance. In contrast, diazepam significantly ($t=4.1$, $P<.03$) increased punished responding (Fig. 3). There was a significant effect of treatment on timeout responding [$F(5,35)=2.6$, $P<.05$] due to diazepam increasing this measure. The effect of treatment on unpunished responding failed to reach statistical significance [$F(5,35)=2.4$, $P=.056$].

3.4. CER task

In the SB-242084 experiment, analysis of the SR revealed an overall significant effect of treatment (Friedman test: $P<.01$) (Fig. 4). There was a robust increase in the SR following treatment with SB-242084 at 1 and 3 mg/kg compared to vehicle group. Diazepam (3 mg/kg), increased the SR to a magnitude significantly greater than SB-242084 ($P<.01$). There was a significant main effect of treatment [$F(5,75)=4.8$, $P<.01$] on the total number of lever presses made during the 1-h session (Fig. 4). SB-242084 significantly increased the number of lever presses at all doses. Diazepam did not significantly differ from the vehicle group on this measure.

In the amphetamine experiment, there was a significant main effect of treatment on the SR ($P<.01$). Diazepam significantly increased the SR, whereas amphetamine had no effect. There was also a significant effect of treatment on the total number of lever presses during the session [$F(4,52)=9.1$, $P<.01$]. Amphetamine significantly increased lever pressing at 0.25 and 0.5 mg/kg, whereas the effect produced by diazepam did not differ from that produced by vehicle treatment (Fig. 4).

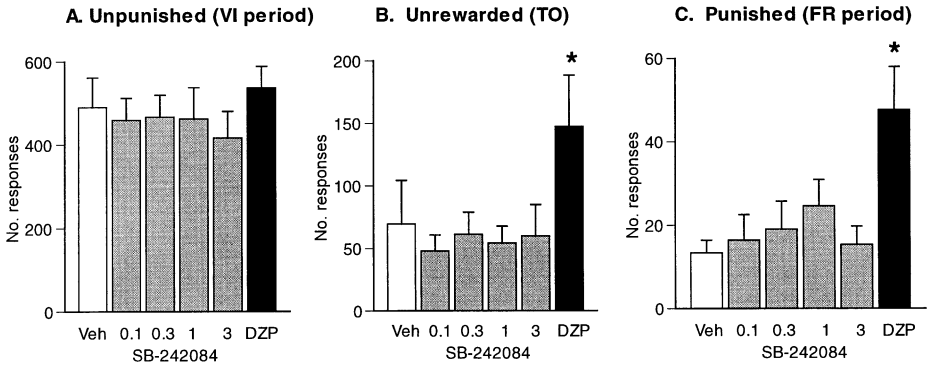

Fig. 3. Effect of SB-242084 (0.1–3 mg/kg ip) and diazepam (1 mg/kg ip; DZP) on rat behavior in the Geller–Seifter conflict test. The data are presented according to each component of the test schedule, (A) unpunished (VI30) period, (B) nonrewarded timeout (TO) period, (C) punished (FR10) period. $n=8$ rats were used in this study. * $P<.05$ vs. vehicle-pretreated controls following significant ANOVA.

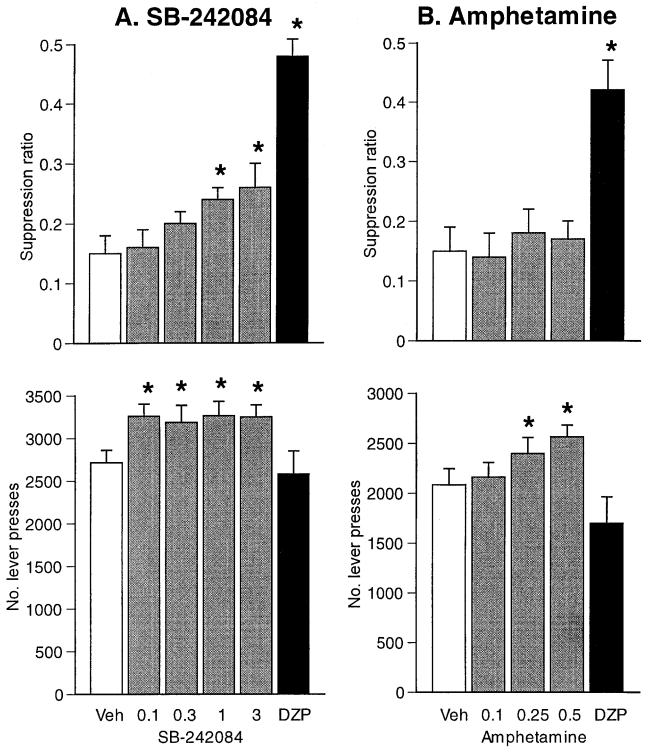

Fig. 4. Effect of (A) SB-242084 (0.1–3 mg/kg ip) and (B) amphetamine (0.1–0.5 mg/kg ip) on rat behavior in the CER test. Diazepam (3 mg/kg ip; DZP) was included as a positive control in each experiment. The upper histobars represent the effect of treatment on the SR, the lower histobars the total number of lever responses recorded over the test session. $n=16$ (SB-242084 study) or 14 (amphetamine study) rats were used in each experiment. * $P<.05$ vs. vehicle-pretreated controls following significant ANOVA.

3.5. Palatability induced feeding

Nonfasted rats given a 1-h access to a wet mash diet consumed a mean of 8.2 ± 1.1 g. A significant main effect of treatment was recorded in the SB-242084 study [$F(4,36)=2.7$, $P<.05$], however, post hoc analysis revealed that only food intake of the diazepam-treated (3 mg/kg) group significantly differed from that of the vehicle group. Thus, at no dose did SB-242084 (0.1–1 mg/kg) affect feeding in this paradigm (see Fig. 5).

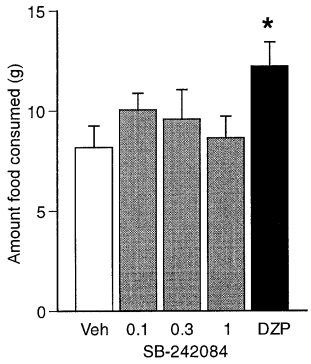

Fig. 5. Effect of SB-242084 (0.1–1 mg/kg ip) and diazepam (3 mg/kg ip; DZP) on the consumption of a wet mash diet in a 1-h access period. $n=10$ rats. * $P<.05$ vs. vehicle-pretreated controls following significant ANOVA.

3.6. Fear-potentiated startle

Following SB-242084 (0.1–1 mg/kg ip) pretreatment, there was a highly significant effect of test condition [$F(1,11)=50.9$, $P<.01$] reflecting potentiated startle, but not treatment [$F(3,33)=0.3$, NS] or Treatment × Condition interaction [$F(3,33)=1.1$, NS]. Therefore, SB-242084 failed to influence startle responding under either the light or dark test condition. Despite a slight trend for SB-242084 (0.3 mg/kg) to increase startle under the dark condition and reduce startle in light, analysis of the difference scores again failed to reveal a treatment effect [$F(3,33)=1.1$, NS] (Fig. 6).

A main effect of condition [$F(1,11)=81.6$, $P<.01$], treatment [$F(3,33)=8.0$, $P<.01$] and Treatment × Condition [$F(3,33)=6.2$, $P<.01$] was recorded in the diazepam experiment. Thus, diazepam reduced startle under both the light and dark conditions, yet the potentiated startle occurring under the former condition was more sensitive to suppression. Analysis of difference scores revealed a main effect of treatment [$F(3,33)=6.2$, $P<.01$], with all doses of diazepam (0.3–3 mg/kg) affecting this measure (Fig. 6).

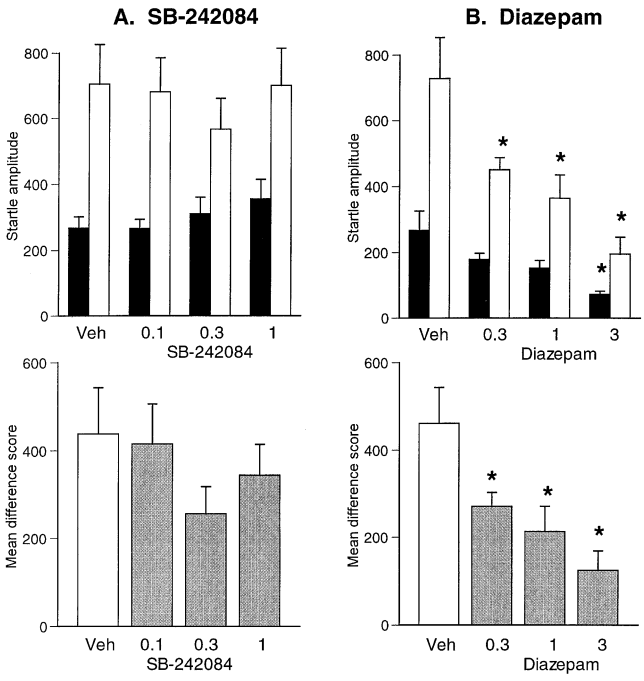

Fig. 6. Effect of (A) SB-242084 (0.1–1 mg/kg ip) and (B) diazepam (0.3–3 mg/kg ip) in the fear-potentiated startle test. The upper set of histobars represent the effect of treatment on startle according to condition: ■ = dark condition, □ = light condition. The lower set of histobars represent the mean difference score for each treatment (i.e., light–dark startle score). $n=12$ rats per group. * $P<.05$ vs. the startle value for vehicle-pretreated controls at the respective startle condition following significant ANOVA.

3.7. Schedule-induced polydipsia

Both Ro60-0175 [$F(4,60) = 23.1$, $P < .01$] and fluoxetine [$F(3,45) = 27.6$, $P < .01$] produced a marked dose-related decrease in water consumption in the schedule-induced polydipsia paradigm (Fig. 7). Subsequent interaction studies with SB-242084 (0.3 mg/kg) revealed a significant antagonism of both Ro60-0175 (0.5 mg/kg)- and fluoxetine (15 mg/kg)-induced suppression of water intake. However, in each of these interaction studies, SB-242084 itself produced a statistically significant increase in water consumption. Consequently, a dose–response experiment with SB-242084 (0.03–1 mg/kg) in the schedule-induced polydipsia paradigm was conducted. A main effect of treatment was recorded [$F(4,60) = 10.5$, $P < .01$], with SB-242084 doses of 0.1, 0.3, and 1 mg/kg each significantly increasing fluid intake (Fig. 7). Under the low-stress condition with all pellets given together at the start of the session (mean control intake of 4.6–5.4 g), neither fluoxetine (7.5–30 mg/kg) nor Ro60-0175 (0.1–1 mg/kg) significantly reduced water consumption (data not shown).

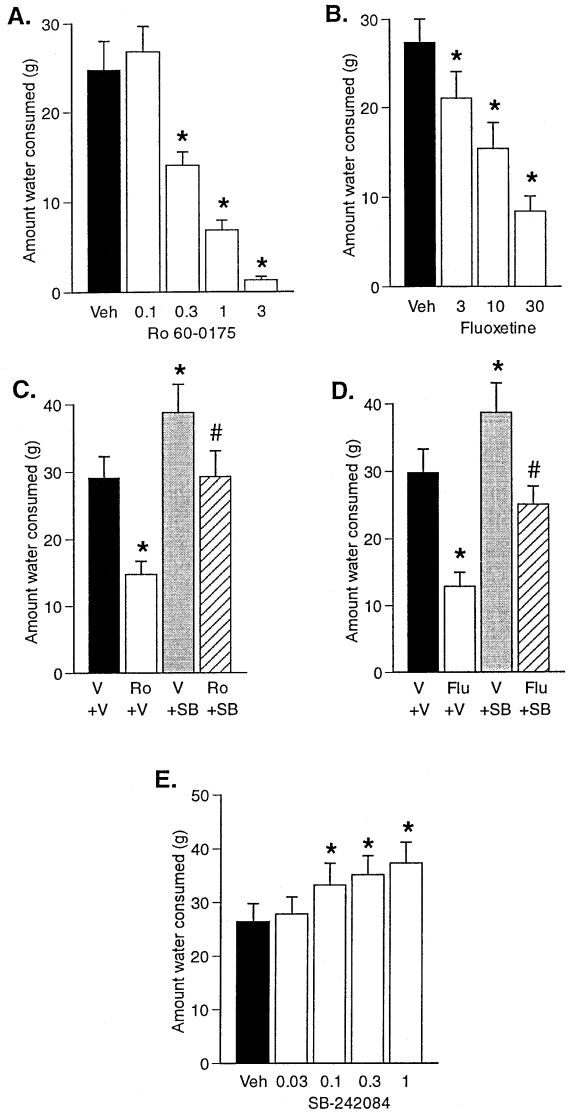

Fig. 7. Effect of Ro60-0175, fluoxetine, and SB-242084 on a schedule-induced polydipsia test with food reward available under a VI60 operant schedule. Dose–response for (A) Ro60-0175 (0.1–3 mg/kg sc) and (B) fluoxetine (3–30 mg/kg sc) on the amount of water consumed in the 1-h test. * $P < .01$ vs. vehicle pretreatment. The effect of SB-242084 (0.3 mg/kg ip) against the reduction of water consumed by (C) Ro60-0175 (0.5 mg/kg sc) and (D) fluoxetine (15 mg/kg sc) is also presented. * $P < .01$ vs. vehicle pretreatment, $^{\#}P < .01$ vs. Ro60-0175 or fluoxetine only treatment. (E) Effect of SB-242084 (0.03–1 mg/kg ip) on the amount of water consumed in the 1-h test. * $P < .01$ vs. vehicle pretreatment. For each experiment, groups of 8–16 rats were used.

4. Discussion

In the first part to this investigation, we examined the effect of SB-242084 against the hypoactivity response produced by mCPP. Since this effect of mCPP is generally accepted to be mediated through activation of central 5-HT2C receptors (Kennett and Curzon, 1988; Heisler and Tecott, 2000), this test has value in estimating doses of SB-242084 necessary to occupy 5-HT2C receptors in vivo. In accordance with the studies of Kennett et al. (1997), SB-242084 had an approximate ED_{50} of 0.1–0.2 mg/kg ip, consequently, we focused within a dose range of 0.1–3 mg/kg for subsequent experiments. Based on the published pharmacology of SB-242084 (Kennett et al., 1997; Bromidge et al., 1997), we reasoned that larger doses would presumably be nonselective for the 5-HT2C receptor.

In separate groups of rats, either naïve or habituated to the test chamber, SB-242084 tended to produce a small increase in locomotor activity that only reached borderline statistical significance. This is consistent with previous studies we have conducted with this drug (Higgins et al., 2001; Fletcher et al., in press) although Hutson et al. (2000) and Kennett et al. (1997) failed to observe any changes in locomotor activity at a 1-mg/kg dose of SB-242084. It was interesting to compare this with the robust increase in locomotor activity recorded in the elevated plus-maze. Here, SB-242084 increased transitions into open arms, distance travelled in open arms, and time in open arm consistent with an anxiolytic effect, although the equivalent closed-arm measures were also increased reflecting a more generalized motor effect. However, there was some dissociation between these effects, since at the 0.1-mg/kg dose SB-242084 selectively increased the former measure. Nonetheless, the generalized increase in exploratory behavior questions the interpretation of the increased open-arm exploration as simply an anxiolytic effect (Dawson et al., 1995), and emphasizes a need for further anxiety tests to complement observations from the plus-maze. By way of contrast, the benzodiazepine anxiolytic alprazolam tended to increase open-arm exploration at the expense of closed-arm activity, reflecting a shift in exploratory pattern rather than a generalized increase. Intuitively, this seems more consistent with an anxiolytic response (Pellow et al., 1985).

Given these observations from the plus-maze experiment, we focused further experiments with SB-242084 on non-exploratory-based anxiety tests. In the CER test protocol used, rats are trained to associate a light/tone cue with shock, although during drug testing no shock is actually delivered (Costello et al., 1991; Stanhope and Dourish, 1996). This test variant has an advantage that any potential drug effect on nociception is avoided as a confounding factor. SB-242084 produced a robust, dose-related increase in suppressed responding at the 1- and 3-mg/kg doses. A further feature of SB-242084 pretreatment was a significant increase in lever pressing recorded throughout the test session. This observation prompted the inclusion of amphetamine in a subsequent study to see if such an activity-enhancing effect of SB-242084 per se might contribute to its apparent anxiolytic effect in this test. Our findings suggest that this is not the case since amphetamine doses that increased overall response rate nonetheless failed to affect the SR. Of further note, the peak change in SR seen with SB-242084 was lower than that obtained with diazepam (vehicle: 0.15 ± 0.03, SB-242084 3 mg/kg: 0.26 ± 0.04, diazepam 3 mg/kg: 0.48 ± 0.03). Since response rates were relatively unaffected by diazepam pretreatment, this difference suggests a greater maximal release of suppressed responding by a benzodiazepine compared to a 5-HT2C receptor antagonist.

In the Geller–Seifter conflict task, SB-242084 tended to increase punished responding, although this did not reach statistical significance, whereas diazepam significantly increased punished responding. These observations contrast with those from Kennett et al. (1997), where it was reported that SB-242084 significantly increased punished responding. However, differences in experimental protocol, e.g., FR5 vs. FR10 punishment schedule or titrating the shock level for individual rats to ensure more uniform response rates during punishment, may explain the different findings. Other research groups have described anticonflict effects of drugs having 5-HT2C antagonist properties, e.g., mianserin, SB-206443 (Kennett et al., 1996; Griebel et al., 1997; Dekeyne et al., 2000). Therefore, taken together, it would seem that 5-HT2C receptor antagonists are active in conflict-based tests, although their activity may be dependent on certain procedural variables and, consequently, they may lack the robustness of benzodiazepine anxiolytics. It must also be recognized, however, that other behavioral effects of benzodiazepines possibly unrelated to anxiety per se, e.g., hyperphagia, decision-making, and response inhibition (Cooper, 1985; Ljungberg et al., 1987; Thiebot et al., 1985), are also likely to contribute to their robust anticonflict effects (Martin et al., 1993; Sepinwall and Cook, 1980). Given the lack of effect of SB-242084 in a test of palatability induced feeding and timeout responding in the Geller–Seifter task, 5-HT2C receptor antagonists appear to lack such additional behavioral effects.

The positive effects of SB-242084 in a test of conditioned fear prompted the evaluation of this drug in the fear-potentiated startle paradigm, which shares certain features common to the CER task, yet response output is measured by startle rather than lever pressing for food (Davis, 1990, 1992). In contrast to significant effects in the CER paradigm, SB-242084 was ineffective in attenuating a fear-potentiated startle response. We have recently reported effects of SB-242084 on baseline startle (Ouagazzal et al., in press), which might have influenced the outcome from the present study. However, assessment of difference scores between either test condition again failed to reveal a statistically significant main effect of drug. Although apparent baseline startle (i.e., observed under the dark condition) was not significantly increased by SB-242084, there is evidence to suggest that some contextual fear conditioning may be evident under this test situation leading to potentiated startle (Guscott et al., 2000), which conceivably might mask a drug effect on baseline startle. Thus, further experiments should evaluate SB-242084 on fear-potentiated startle, using distinct environments for conditioning and testing. This should allow a more effective means of distinguishing drug effects on anxiety from startle in this test. Diazepam was effective in reducing fear-potentiated startle under both 'light' and 'dark' test conditions. This apparent effect on baseline startle may, therefore, reflect the anxiolytic and/or myorelaxant effect of benzodiazepine anxiolytics.

It has previously been shown that both Ro60-0175 and fluoxetine reduced the amount of water consumed in the schedule-induced polydipsia task (Martin et al., 1998), an effect replicated in the present studies. In subsequent interaction experiments, we have demonstrated that pretreatment with SB-242084 significantly attenuates the effect of both fluoxetine and Ro60-0175 in this task. However, during the course of these experiments, it emerged that SB-242084 itself actually increased schedule-induced water consumption. Consequently, it is unclear whether this antagonism is strictly a pharmacological or, alternatively, a functional interaction. The demonstration of increased water consumption in the schedule-induced polydipsia paradigm is of interest and consistent with other test situations, i.e., CER and plus-maze tasks (present study), ethanol consumption (Tomkins et al., in press), which have revealed a robust and significantly increased behavioral output following SB-242084 pretreatment. Considering the lack of a significant locomotor effect of SB-242084 (see also Kennett et al., 1997; Hutson et al., 2000), these data may suggest an interaction between the behavioral effects of a 5-HT2C receptor antagonist and the arousal state. Thus, in states of high arousal, the level of serotonergic tone at the 5-HT2C receptor may increase and this may serve to reduce behavioral output. A potential locus for this interaction is the ventral tegmental area (VTA), where activation of 5-HT2C receptors have been shown to reduce dopaminergic cell firing and consequently mesolimbic DA function via enhancement of inhibitory GABAergic interneuron activity (see Di Matteo et al., 2001, in press). Serotonergic inputs to the VTA are derived from the midbrain raphe nuclei (DRN,

MRN), and raphe cell firing rates are known to be influenced by changes in arousal (see Jacobs and Fornal, 1999).

It has previously been reported that 5-HT2C receptor agonists, including Ro60-0175 ameliorate the panic reaction in rats induced by electrical stimulation of the periaqueductal grey (Jenck et al., 1998) and attenuate schedule-induced polydipsia in rats (Martin et al., 1998), thus providing evidence that 5-HT2C agonists may be effective in certain animal tests of anxiety. However, Kennett et al. (2000) recently suggested that some of these effects may be secondary to the locomotor changes produced by Ro60-0175. The present series of experiments suggest that in certain tests, notably the CER task, SB-242084 exhibits anxiolytic properties. Together with related reports (Kennett et al., 1997; Griebel et al., 1997; Dekeyne et al., 2000), this suggests that 5-HT2C receptor antagonists have anxiolytic potential. It remains to be established whether these findings translate into clinical benefit, although the present studies do emphasize the care necessary in interpreting findings from preclinical anxiety tests.

Acknowledgments

We would like to thank Brigitte Algeyer, Michel Enderlin, Marie Haman, Françoise Kahn, Martine Maco, and Yvan Peterschmidt for their excellent technical assistance and Dr. Stephan Roever for the synthesis of SB-242084.

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