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R-citalopram counteracts the effect of escitalopram in a rat conditioned fear stress model of anxiety

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Abstract

S-citalopram (escitalopram) mediates the serotonin reuptake inhibitory effect of the racemate, *R*,*S*-citalopram. The effect of escitalopram (0.5–3.9 mg/kg) was investigated in a rat conditioned fear stress model of anxiety and compared to the effects of *R*-citalopram (1.0–7.8 mg/kg), *R*,*S*-citalopram (4.0 and 8.0 mg/kg), and escitalopram (2.0 mg/kg) + *R*-citalopram (7.8 mg/kg). Diazepam (0.95 mg/kg) and buspirone (4.6 mg/kg) were included as positive controls. During an acquisition session, rats were allowed to freely explore a novel cage for 9 min. During that time, they received two inescapable footshocks through an electrifiable grid floor. Groups of nonshocked control rats were run in parallel. During an expression session on the next day, rats were treated with drug or vehicle 30 min before they were reintroduced into the test cage for a 9-min period this time without receiving footshocks and the total distance travelled was recorded. The distance travelled by vehicle-treated rats was markedly suppressed compared to a vehicle-treated group of nonshocked controls. Escitalopram produced a dose-dependent inhibition of the conditioned suppression of exploratory behaviour (minimal effective dose 1.0 mg/kg). Interestingly *R*,*S*-citalopram 4.0 and 8.0 mg/kg produced significantly smaller effect than escitalopram 2.0 and 4.0 mg/kg, receptively. *R*-citalopram, 7.8 mg/kg, produced a significant effect. However, in spite of this, *R*-citalopram (7.8 mg/kg) significantly inhibited the effect of escitalopram (2.0 mg/kg). The activity in drug-treated nonshocked groups was similar to the vehicle-treated group, except for the buspirone-treated group where a significant reduction was observed. The finding that *R*-citalopram inhibits the effect of escitalopram may be relevant to the improved clinical efficacy seen with escitalopram compared to *R*,*S*-citalopram in the treatment of anxiety and depression.

Keywords: 5-HT; Anxiolytic; *R*,*S*-citalopram; Escitalopram; *R*-citalopram; Conditioned fear stress

1. Introduction

The selective serotonin (5-HT) reuptake inhibitors (SSRIs) have gained extensive clinical use during the last 2 decades and are drugs of choice for the treatment of depression and also used frequently for treatment of anxiety disorders. The widely used SSRI, *R*,*S*-citalopram (normally referred to as citalopram), is a racemic mixture of *S*-(+)- and *R*-(−)-enantiomers (escitalopram and *R*-citalopram, respectively) in a 1:1 ratio. The 5-HT reuptake inhibitory activity of *R*,*S*-citalopram has previously been reported to reside in the *S*-enantiomer (Hyttel et al., 1992). During the last few years, escitalopram has been successfully used to treat major depressive and anxiety disorders (Montgomery et al., 2001; Burke et al., 2002; Wade et al., 2002).

The in vitro and in vivo 5-HT reuptake inhibitory activity and the effect of escitalopram in animal models predictive of antidepressant, anxiolytic, and antiaggressive activity have recently been characterised and compared with *R*,*S*-citalopram (Sánchez et al., 2003). In this study, escitalopram inhibited footshock-induced ultrasonic vocalisation in adult rats (a model suggested to be predictive of anxiolytic activity; e.g., review by Borsini et al., 2002; Sánchez, 2003) completely, while *R*,*S*-citalopram only produced a partial inhibition in the same dose range. *R*-citalopram also partially inhibited footshock-induced ultrasonic vocalisation but was much less potent than escitalopram and *R*,*S*-citalopram. The partial inhibition produced by *R*,*S*-citalopram and the biphasic nature of its dose–response curve could not be readily explained but suggests that *R*-citalopram interferes with the action of escitalopram. The present study was

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designed to investigate these observations in further detail in another rat model suggested to be predictive of anxiolytic activity, the conditioned fear stress model. This model is based on that reexposure to an environment where a rat previously has been exposed to an aversive stimulus, e.g., inescapable footshocks, induces a conditioned suppression of motor behaviour that is specifically related to this environment (e.g., Conti et al., 1990; Inoue et al., 1996). Treatment with benzodiazepines and serotonergic anxiolytics before the reexposure has consistently been found to counteract the behavioural suppression (e.g., review by Borsini et al., 2002). We used a test procedure that has previously been described and validated by Wedzony et al. (1992, 1996). We investigated the effect of escitalopram (0.5–3.9 mg/kg) and compared it to the effects of the presumed pharmacologically inactive *R*-citalopram (1.0–7.8 mg/kg) and the racemate *R*,*S*-citalopram (4.0 and 8.0 mg/kg, which equals 2.0 + 2.0 and 4.0 + 4.0 mg/kg, respectively, of *R*-citalopram + escitalopram). Because repeated administration of *R*,*S*-citalopram reveals a higher serum level of *R*-citalopram than escitalopram due to different metabolic rates (Foglia et al., 1997; Sidhu et al., 1997; Zheng et al., 2000), we did also include a group of rats treated with escitalopram (2.0 mg/kg) + *R*-citalopram (7.8 mg/kg). Diazepam (0.95 mg/kg) and buspirone (4.6 mg/kg) were included as positive controls.

2. Material and methods

2.1. Animals

The experiments used male Wistar rats (breeding stock at the Polish Academy of Science, Krakow) weighing approximately 200–230 g. The rats were habituated to the experimental room for 2 weeks prior to the experiments and were kept in groups of six, at a constant room temperature (22 °C ± 2), under a 12:12-h light/dark cycle (lights on at 7:00 am) with free access to tap water and laboratory chow. All experiments were performed between 9:00 a.m. and 5:00 p.m. The study was conducted in compliance with the Animal Protection Bill of August 21, 1997, and has been approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

2.2. Apparatus

The apparatus consisted of four Opto-Varimex cages (Columbus Instruments, OH). The cages (43/44 cm) were equipped with 15 infrared emitters, located on the *x*- and *y*-axes with a corresponding number of receivers on the opposite walls of the cage. The cages were equipped with an electrifiable metal grid floors attached to the footshock generator (Bialystok). Infrared emitters and receivers were located 2.5 cm above the level of metal grid floor. The cages were linked on line to an IBM PC-compatible computer and the movement of the rats was analysed by Auto-Track software (Columbus Instruments, OH). The position of the rats was registered 10 times per second and changes in their activity were expressed as distance travelled by animals in a selected time interval. In order to eliminate artefacts resulting from, for example, grooming behaviour, exploratory activity was calculated automatically only when rats had interrupted at least three consecutive lightbeams. Thus, all other movements resulting from repeated interruption of the same lightbeam were not included.

2.3. General procedure

2.3.1. Acquisition session

After 2 weeks of adaptation to laboratory conditions, rats were placed individually in the cages and were allowed to freely explore the novel environments for 9 min (a duration based on previous experience with the model; Wedzony et al., 1992, 1996). During that time, they received two inescapable footshocks through the electrifiable grid floor. The scrambled footshock had an intensity of 0.5 mA (200 ms/s) and a duration of 9 s. Footshocks were delivered in the 2nd and 5th min of the session. At the end of the acquisition session, rats were gently removed and placed in their home cages. Control animals were placed individually in the apparatus for the same period of time but footshocks were not delivered.

2.3.2. Expression session

On the next day, separate groups of rats received intraperitoneal injections of vehicle (1 ml/kg), escitalopram (0.50, 1.0, 2.0, and 3.9 mg/kg), *R*-citalopram (1.0, 2.0, 3.9, and 7.8 mg/kg), *R*,*S*-citalopram (4.0 and 8.0 mg/kg), escitalopram (2.0 mg/kg) + *R*-citalopram (7.8 mg/kg), diazepam (0.95 mg/kg), or buspirone (4.6 mg/kg) and 30 min later were again placed in the test cages and their activity was measured by Auto-Track software, as described above, for 9 min. The diazepam and buspirone doses were chosen based on previous experience with these drugs (Wedzony et al., 1996). During this session, the rats did not receive footshocks.

2.4. Statistical analysis

All data are presented as means ± S.E.M. Statistical significance was evaluated with a one-way analysis of variance (ANOVA) on ranks followed by post hoc comparison of means (Dunn's test). A significance level of .05 was applied.

2.5. Drugs

R,*S*-citalopram (HBr), escitalopram (oxalate), and *R*-citalopram (oxalate) were provided by H. Lundbeck, Denmark. Buspirone (HCl) was purchased from RBI (Natic,

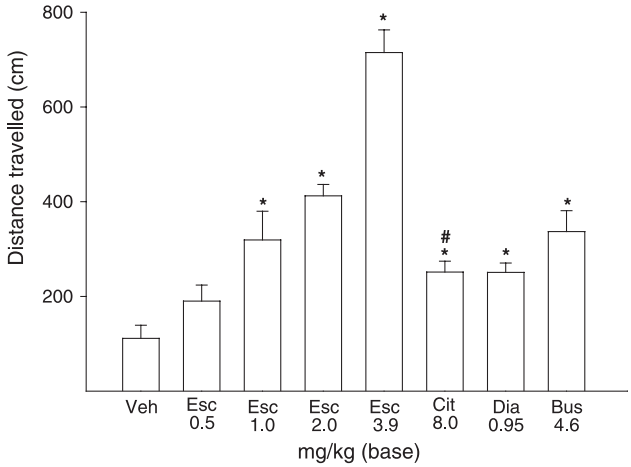

Fig. 1. Effects of escitalopram (Esc, 0.5–3.9 mg/kg ip), *R*,*S*-citalopram (Cit, 8.0 mg/kg ip), diazepam (Dia, 0.95 mg/kg ip), and buspirone (Bus, 4.6 mg/kg ip) on conditioned suppression of exploratory behaviour in rats. $N=9-18$. $^{*}P<.05$ compared to vehicle-treated controls (Veh) (one-way ANOVA on ranks followed by Dunn's test). $^{\#}P<.05$ between *R*,*S*-citalopram (8.0 mg/kg) and escitalopram (3.9 mg/kg). For further details, please refer to Material and methods section.

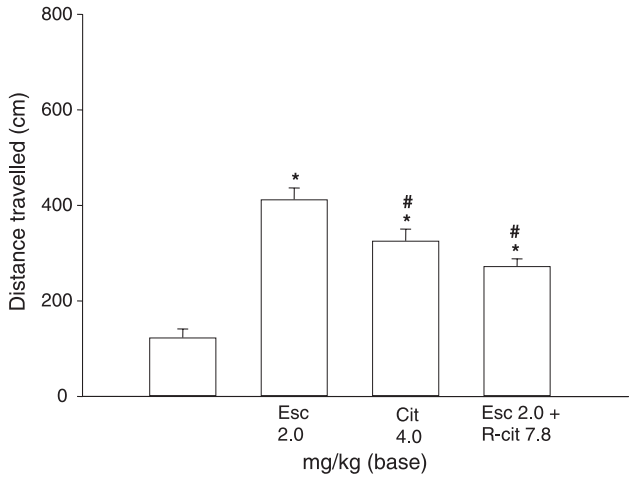

Fig. 3. Effects of escitalopram (2.0 mg/kg ip), *R*,*S*-citalopram (4.0 mg/kg ip) and combined administration of escitalopram (2.0 mg/kg ip), and *R*-citalopram (R-cit, 7.8 mg/kg ip) on conditioned suppression of exploratory behaviour in rats. $N=9-18$. $^{*}P<.05$ compared to vehicle-treated controls, $^{\#}P<.05$ compared to escitalopram, 2.0 mg/kg (one-way ANOVA on ranks followed by Dunn's test). For further details, please refer to Material and methods section.

MA, USA) and diazepam was purchased from Polfa (Warsaw, Poland). *R*,*S*-citalopram, escitalopram, *R*-citalopram, diazepam (commercially available solution diluted in distilled water), and buspirone were dissolved in distilled water. All drug injections were given in a volume of 2 ml/kg of body weight, 30 min before the test. All doses are shown as milligrams per kilograms base.

3. Results

Escitalopram (1.0–3.9 mg/kg), *R*,*S*-citalopram (8.0 mg/kg), diazepam (0.95 mg/kg), and buspirone (4.6 mg/kg) produced a significant increase in exploratory behaviour compared to vehicle-treated shocked controls [Fig. 1, $H(7)=78.7$; $P<.05$ Dunn's test]. Furthermore, escitalopram (3.9 mg/kg) produced a significantly larger increase than the same amount of escitalopram contained in *R*,*S*-citalopram ($P<.05$, Dunn's test). The effect size in the *R*,*S*-citalopram-treated group was comparable to that of the diazepam- and buspirone-treated groups.

R-citalopram (7.8 mg/kg) produced a significant [2; $H(4)=19.8$; $P<.05$ Dunn's test] effect, whereas lower doses were without effect (Fig. 2). Escitalopram (2.0 mg/kg) produced a significantly higher response than *R*,*S*-citalo-

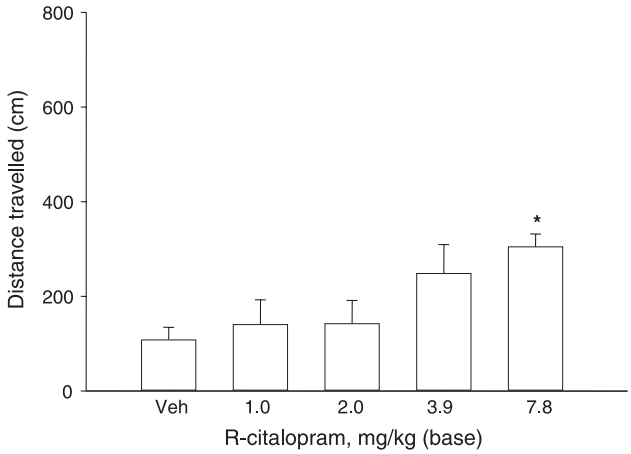

Fig. 2. Effects of *R*-citalopram (1.0–7.8 mg/kg ip) on conditioned suppression of exploratory behaviour in rats. $N=9-18$. $^{*}P<.05$ compared to vehicle-treated controls (one-way ANOVA on ranks followed by Dunn's test). For further details, please refer to Material and methods section.

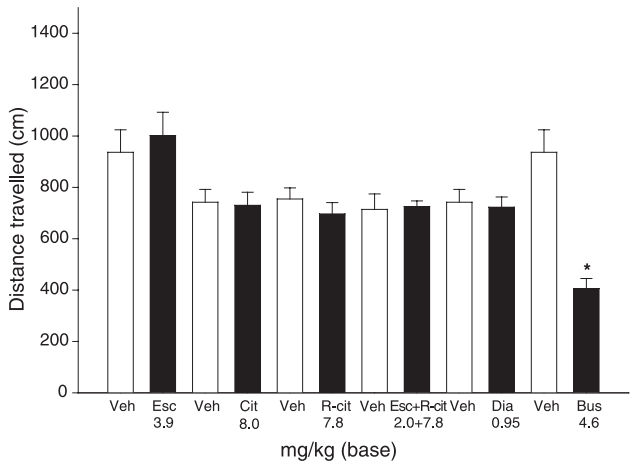

Fig. 4. Effects of vehicle (Veh, 2 ml/kg ip), escitalopram (Esc, 3.9 mg/kg ip), *R*,*S*-citalopram (Cit, 8.0 mg/kg ip), *R*-citalopram (R-cit, 7.8 mg/kg ip), escitalopram + *R*-citalopram (Esc 2.0 + R-cit 7.8 mg/kg ip), diazepam (Dia, 0.95 mg/kg ip), and buspirone (Bus, 4.6 mg/kg ip) on locomotor activity in nonshocked control rats. $N=9-18$. $^{*}P<.05$ compared to vehicle-treated controls (one-way ANOVA on ranks followed by Dunn's test). For further details, please refer to Material and methods section.

pram (4.0 mg/kg) or combined administration of escitalopram (2.0 mg/kg) + *R*-citalopram (7.8 mg/kg) [$H(2) = 12.0$; $P < .05$ Dunn's test] (Fig. 3).

None of the treatments except buspirone changed the exploratory activity in nonshocked controls [Fig. 4; $H(11) = 44,4$; $P < .05$, Dunn's test for buspirone compared to corresponding vehicle control group].

4. Discussion

In the present study, escitalopram produces a significant and marked reversal of conditioned suppression of exploratory behaviour produced by previous exposure to footshocks in the same environment. The behaviour measured in this model can be considered to be truly conditioned as the activity measured in another environment remained unaffected (Wedzony et al., 1992). Furthermore, escitalopram did not affect the motor activity in nonshocked control rats. This is consistent with the anxiolytic-like effects observed in other models predictive of anxiolytic activity (i.e., the footshock-induced ultrasonic vocalisation in adult rats and the mouse black and white box; Sánchez et al., 2003). The minimal effective dose of escitalopram was 1.0 mg/kg. Interestingly, the effect of escitalopram, 3.9 mg/kg, was significantly greater than that of *R*,*S*-citalopram, 8.0 mg/kg, which is equivalent to escitalopram 4.0 + *R*-citalopram 4.0 mg/kg. *R*-citalopram, when given alone, reversed the conditioned suppression of exploratory behaviour, with a minimal effective dose of 7.8 mg/kg. However, in spite of producing an effect by itself, *R*-citalopram (7.8 mg/kg) inhibited the effect of escitalopram (2.0 mg/kg) significantly. These observations suggest that *R*-citalopram interferes with the effect of escitalopram and is consistent with the results of the footshock-induced ultrasonic vocalisation model in adults rats (Sánchez et al., 2003).

The mechanism by which *R*-citalopram inhibits escitalopram-induced reversal of conditioned suppression of exploratory behaviour is not known at present. Facilitation of brain 5-HT neurotransmission has been reported to decrease conditioned fear-induced suppression of motor behaviour by Hashimoto et al. (1999) in a study where extracellular 5-HT levels in medial prefrontal cortex were measured concomitantly with assessment of conditioned freezing behaviour in vehicle- and *R*,*S*-citalopram-treated rats. Coadministration of *R*,*S*-citalopram and a low dose of a 5-HT_{1A} receptor antagonist produced a further enhancement of the effect of *R*,*S*-citalopram (Hashimoto et al., 1997). These results suggest that the stimulation of postsynaptic 5-HT receptors in the terminal areas is involved in mediating the anxiolytic-like responses. However, the role of 5-HT in mediation of anxiety responses is complex and a hypothesis of a dual role-enhancing learned responses to potential or distal threat through actions in the forebrain while inhibiting unconditioned responses to proximal threat by acting on the periaquaductal gray area has been put forward (reviewed by Graeff, 2002). Various studies suggest that conditioned fear responses in rats involve 5-HT_{1A} receptors. For example, the 5-HT_{1A} receptor agonists, buspirone and flesinoxan, have been found to inhibit conditioned fear behaviour (Wedzony et al., 1996; Li et al., 2001; and the present study). The 5-HT depleting agent, *p*-chlorophenylalanine, is inactive while ipsapirone inhibits conditioned fear behaviour in these depleted animals suggesting that postsynaptic 5-HT_{1A} receptors are mediating the effect (Inoue et al., 1996). However, *R*,*S*-citalopram and its enantiomers do not have any significant affinity for 5-HT_{1A} receptors, as shown in the receptor-binding studies performed with rat brain homogenate (Sánchez et al., 2003). It is therefore highly unlikely that *R*-citalopram modulates 5-HT_{1A} receptor-mediated activities by a direct effect on these receptors.

Of the 144 targets tested in in vitro binding affinity studies (Sánchez et al., 2003), the only target for which *R*,*S*-citalopram and *R*-citalopram, but not escitalopram, showed appreciable affinity was that of the histamine H_1 receptor. In vitro studies in isolated guinea pig ileum show that *R*-citalopram is a weak histamine H_1 receptor antagonist (unpublished observation). Histamine H_1 receptors have weak or no effect in animal models predictive of anxiolytic activity (Hasenohrl et al., 1999; Privou et al., 1998; Kennett et al., 1994). Thus, it would be expected that *R*-citalopram's histamine H_1 receptor antagonistic activity would enhance rather than attenuate the effect of escitalopram on footshock-induced suppression of exploratory behaviour. This is consistent with the anxiolytic-like effect of *R*-citalopram in the present study.

There may be a possibility that *R*-citalopram counteracts the effect of escitalopram by interfering with escitalopram's binding to the 5-HT transporter protein and thereby affects the 5-HT levels at the synapse. Recently, accomplished microdialysis studies measuring the 5-HT level in prefrontal cortex of freely moving rats support this hypothesis (Mørk et al., 2003). In these studies, *R*-citalopram was found to reduce escitalopram-induced increases of the 5-HT level. This effect could not be ascribed to a pharmacokinetic interaction between the two enantiomers as the extracellular concentration of escitalopram (2 mg/kg) in the brain was unaffected by the presence of *R*-citalopram (7.8 mg/kg). Furthermore, the inhibitory effect of *R*-citalopram on escitalopram-induced increases of the 5-HT level was confirmed in studies where the enantiomers were applied locally via the dialysis probe (Mørk et al., 2003). *R*-citalopram is 30- to 100-fold less potent than escitalopram in its ability to inhibit 5-HT reuptake in vitro, and *R*-citalopram is practically devoid of in vivo 5-HT reuptake inhibitory activity, as measured by the potentiation of 5-HTP-induced behavioural changes (Owens et al., 2001; Sánchez et al., 2003). However, previously published studies of in vitro binding kinetics of ^{3}H-*R*,*S*-citalopram in rat brain homogenates and human platelets have shown that higher concentrations of escitalopram or *R*-citalopram stabilise ^{3}H-*R*,*S*-citalopram binding, resulting in a low dissociation rate (Plenge and Mellerup, 1985). This is

suggested to be due to an allosteric effect on the 5-HT transporter protein via binding to a low-affinity site and is a unique property of *R*,*S*-citalopram (Plenge et al., 1995). Recent studies with membrane preparations of COS-1 cells expressing the human 5-HT transporter protein have compared effects of escitalopram and *R*-citalopram on dissociation rates of ^{3}H-escitalopram, ^{3}H-MADAM [N,*N*-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine; Chalon et al., 2002], and ^{125}I-RTI [β-carbomethoxy-3β-(4-iodophenyl)tropane or β-CIT] and have demonstrated differences between escitalopram and *R*-citalopram (Wiborg and Sánchez, 2002). The selectivity ratio between the enantiomers was much lower than for 5-HT uptake inhibition, indicating that the *R*-enantiomer may have a significant allosteric effect. Furthermore, the potency ratio depended on the radioligand studied, indicating that the enantiomers interact with the 5-HT transporter protein in different ways. A possible interference of *R*-citalopram on the binding kinetics of escitalopram and the relevance of this phenomenon in vivo remains to be established.

In conclusion, the results of the present study indicate that *R*-citalopram inhibits the anxiolytic-like effect of escitalopram in a rat conditioned fear model, as *R*,*S*-citalopram (4.0 or 8.0 mg/kg) and concomitant administration of *R*-citalopram (7.8 mg/kg) and escitalopram (2.0 mg/kg) produced significantly smaller effects than the same escitalopram doses (2.0 or 3.9 mg/kg) administered in the absence of *R*-citalopram. The mechanism involved is unknown but may be relevant to the improved clinical activity on anxiety and depression symptoms seen with escitalopram in comparison with the racemix mixture, *R*,*S*-citalopram (Montgomery et al., 2001; Gorman et al., 2002; Reines et al., 2002).

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