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Behavioural and biochemical studies of citalopram and WAY 100635 in rat chronic mild stress model

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Abstract

Reversal of chronic mild stress (CMS)-induced decrease of sucrose consumption has been studied in rats after 2, 7, 14, and 35 days treatment with imipramine, citalopram (both 10 mg/kg per day, ip), WAY 100635 (0.2 mg/kg sc, b.i.d.), and citalopram plus WAY 100635. B_{max}, K_d, and functional status [cyclic AMP (cAMP) generation] of β_1-adrenoceptors were assessed in cortical tissue at the same time points. Citalopram reversed CMS-induced reduction of sucrose intake at an earlier time point than imipramine. WAY 100635 was not effective and did not potentiate the effect of citalopram. CMS produced increase of B_{max}. Imipramine decreased B_{max} in controls (Days 2, 7, 14, and 35) and normalised B_{max} in stressed animals (Day 35). Citalopram, WAY 100635, and the combination increased B_{max} in stressed animals and controls (Days 14 and 35). Inconsistent changes of K_d values and of cAMP responses to noradrenaline (NA) stimulation were observed. Thus stress- and drug-induced effects on β_1-adrenoceptors do not appear to be a common biochemical marker of antidepressant-like activity in the CMS model. © 2002 Published by Elsevier Science Inc.

Keywords: Depression; Chronic mild stress; 5-HT_{1A} receptor antagonist; SSRI; β_1-adrenoceptor

1. Introduction

The therapeutic effect of the selective serotonin (5-HT) reuptake inhibitors (SSRIs) is assumed to be related to enhanced serotonergic activity resulting from inhibition of reuptake of 5-HT in the terminal areas of the serotonergic neurones and subsequent stimulation of postsynaptic 5-HT receptors (reviewed by Blier et al., 1997). However, the increased 5-HT activity produced by acute antidepressant treatment is hypothesised to be too low to produce an immediate antidepressant effect due to the action of inhibitory serotonergic feedback mechanisms (e.g., reviewed by Blier and de Montigny, 1997; Artigas et al., 1996). Inhibition of 5-HT reuptake occurs in both the terminal and cell body regions and results in an elevation of the extracellular 5-HT levels (Bel and Artigas, 1992; Romero et al., 1997). Consequently, somatodendritic 5-HT_{1A} autoreceptors located in the cell body regions (i.e., dorsal and median raphe nuclei) are also stimulated. Somatodendritic 5-HT_{1A} receptors control firing of 5-HT neurones and stimulation of these receptors produces an inhibition of neuronal firing activity and subsequent reduction in 5-HT release (Romero et al., 1996a). It is hypothesised that prolonged stimulation of somatodendritic 5-HT_{1A} autoreceptors during chronic treatment with a 5-HT reuptake inhibitor produces a receptor desensitisation, and consequently, an attenuation of the inhibition of neuronal firing and 5-HT release in terminal areas and antidepressant effect (Blier and de Montigny, 1997).

Another and more rapid way of overcoming the inhibitory feedback loop is to block the somatodendritic 5-HT_{1A} autoreceptors with an antagonist. There is substantial experimental evidence in animals supporting this approach as applicable for achieving an enhanced serotonergic output. Electrophysiology studies have shown that the inhibitory effect of 5-HT or 5-HT_{1A} receptor agonists on raphe cell firing is antagonised by both (−)-pindolol and the more potent and selective 5-HT_{1A} receptor antagonist, WAY 100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-

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N-(2-pyridinyl) cyclo-hexanecarboxamide) (Romero et al., 1996a). Microdialysis studies have similarly shown that extracellular levels of 5-HT in terminal areas (e.g., cortex, hippocampus, and hypothalamus) are increased following acute administration of an SSRI. This increase is enhanced by coadministration of pindolol or WAY 100635 (Dreshfield et al., 1996; Gundlah et al., 1997; Hjorth et al., 1997; Invernizzi et al., 1997; Romero et al., 1996b,c). Similarly in a mouse forced swim test, it has been shown that the coadministration of pindolol enhances the effects of SSRIs (Jackson et al., 1996, 1997; Redrobe et al., 1996). There is also clinical evidence that the combined treatment with an SSRI and pindolol produces a faster onset of action and/or higher response rate than the SSRI alone (Artigas et al., 1994; Bordet et al., 1997; Tome et al., 1997; Isaac, 1999). However, other clinical studies have failed to show this (Berman et al., 1997; reviewed by Montgomery, 1999).

The chronic mild stress (CMS) procedure is suggested to be an appropriate model to study onset of antidepressant action in animals (reviewed by Willner, 1997). The pharmacological validation of the model is extensive compared to most of other animal models of depression, and prolonged antidepressant treatment over several weeks is required to reverse the stress-induced deficits. Antidepressant treatment including tricyclic antidepressants (TCAs), SSRIs, monoamine oxidase inhibitors, and electroconvulsive shock are effective and no falsely positive or falsely negative compounds have been detected so far (review by Willner, 1997).

Citalopram, the most selective 5-HT reuptake inhibitor on the market (e.g., Sánchez and Hyttel, 1999) has previously been studied in the CMS model and has consistently produced a faster normalisation of CMS-induced behavioural deficits than the TCA, imipramine, (Przegalinski et al., 1995; Sánchez and Papp, 2000).

The present study was designed to compare the time-course for reversal of the CMS-induced behavioural effects of citalopram alone and in combination with the 5-HT_{1A}-receptor antagonist, WAY 100635. Control groups treated with vehicle, imipramine, and WAY 100 635 alone were included in the study.

The study also included assessments of β_1-adrenoceptor binding properties (B_{max} and K_d values) and functional status [cyclic AMP (cAMP) generation] at different time points, i.e., after 2, 7, 14, and 35 days of treatment. The observations that chronic antidepressant treatment may down-regulate the β_1-adrenoceptor-mediated cAMP formation (Vetulani and Sulser, 1975) and the β_1-adrenoceptor density (Banerjee et al., 1977) have been confirmed in many studies over the years (reviewed by Sulser and Mishra, 1983; Leonard and Spencer, 1990; Vetulani, 1991). However, most of these studies have been performed in normal animals and the outcome with SSRIs has been variable (Hyttel et al., 1994; Nalepa and Vetulani, 1993; reviewed by Vetulani and Nalepa, 2000). A previously published study in the CMS model of imipramine and β_1-adrenoceptors function revealed that CMS increased the density and responsiveness of the β-receptors and that imipramine reversed this effect (Papp et al., 1994a).

2. Method

2.1. Subjects

A total of 320 male Wistar rats (Gorzkowska, Warsaw) were brought into the laboratory 2 months before the start of the experiment. The animals were singly housed with food and water freely available, and maintained on a 12-h light/dark cycle at a temperature of 22 ± 2 °C, except as described below.

The study was conducted in compliance with the Animal Protection Bill of August, 21, 1997, and was approved by the Bioethical Committee at the Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland.

2.2. CMS procedure

The animals were first trained to consume a 1% sucrose solution; training consisted of eight 1-h baseline tests in which sucrose was presented, in the home cage, following 14 h food and water deprivation; intake was measured by weighing preweighed bottles containing the sucrose solution, at the end of the test. Subsequently, sucrose consumption was monitored, under similar conditions, throughout the whole experiment.

On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group of animals was subjected to a CMS procedure for a period of eight consecutive weeks. The weekly stress regime consisted of two periods of food or water deprivation, 45° cage tilt, intermittent illumination (lights on and off every 2 h), soiled cage (250 ml water in sawdust bedding), paired housing, and low intensity stroboscopic illumination (150 flashes/min), and two periods of no stress. All stressors were 10–14 h of duration and were applied individually and continuously, day and night. Control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for the 14 h preceding each sucrose test, but otherwise food and water were freely available in the home cage.

On the basis of their sucrose intake scores following 3 weeks of stress, both stressed and control animals were divided into matched subgroups ($n = 8$). These groups were, for the subsequent 2–35 days, injected with vehicle (1 ml/kg ip), imipramine (10 mg/kg ip), citalopram (10 mg/kg ip), WAY 100635, (0.2 mg/kg sc, b.i.d.), or WAY 100635 (0.2 mg/kg sc, b.i.d.) plus citalopram (10 mg/kg ip). Drugs were administered at 10:00 a.m., with a second injection in the case of b.i.d. regimes at 5:00 p.m. WAY 100635 was injected approximately 30 min before citalopram. Sucrose tests were carried out once weekly 24 h following the last

drug treatment; in the case of b.i.d. regimes, the second injection (5:00 p.m.) preceding the sucrose test was omitted. Stress was continued throughout the entire treatment period.

On Days 2, 7, 14, and 35 of treatment (24 h after the final injection), all groups of control and stressed animals receiving vehicle, imipramine, citalopram, WAY 100635, or WAY 100635 plus citalopram were decapitated. Immediately after decapitation the brains were excised, dissected on an ice-cold porcelain plate and used for further biochemical analysis.

2.3. Binding studies

The β_1-adrenoceptor binding parameters were investigated in the crude synaptosomal fraction obtained from cortical tissue. One half of cortex from each animal was homogenised separately at 0 °C in 20 vol. of 50 mmol/l Tris–HCl buffer, pH 7.6. The homogenate was centrifuged at $1000 \times g$ for 10 min. The supernatant was decanted and recentrifuged at $25{,}000 \times g$ for 30 min, and the resulting pellet was resuspended in the buffer and recentrifuged under the same conditions. The final pellet was stored in −20 °C for 24 h. For binding studies the pellet was reconstituted in 50 mmol/l Tris–HCl buffer pH 7.6 to obtain a final protein concentration (measured according to Lowry et al., 1951) of approximately 0.8 mg/ml. The radioligand [^{3}H]CGP12177 (48 Ci/mmol) for β_1-adrenoceptors was prepared in six concentrations from 0.1–3 nM in Tris–HCl buffer. The incubation mixture (final volume 550 μl) consisted of 450 μl of membrane suspension, 50 μl of [^{3}H]CGP12177 solution, and 50 μl of the buffer without (total binding) or with (nonspecific binding) propranolol (final concentration 10 μM). The incubation was carried out in duplicate in a shaking water bath at 25 °C for 30 min, and terminated by filtration through GF/C Whatman fibreglass filters. The filters were then rinsed twice with 5 ml of ice-cold incubation buffer and placed in plastic scintillation minivials. The samples were counted for radioactivity in a Beckman scintillation counter. Specific binding was defined as the difference between total and nonspecific binding, and is expressed in fmol/mg protein. B_{max} (density) and K_d (affinity) values were achieved by means of Scatchard analyses.

2.4. cAMP studies

The second half of the cerebral cortex was used for an assessment of cAMP generation as response to noradrenergic stimulation. The tissue was sliced with an McIlwain tissue chopper (0.35 mm prisms), and the slices were suspended in O_2/CO_2 (95:5) gassed, glucose-containing modified Krebs–Henseleit medium (NaCl 118 mM; KCl 5 mM; $CaCl_2$ 1.3 mM; $MgSO_4$ 1.2 mM; KH_2PO_4 1.2 mM; $NaHCO_3$ 25 mM; glucose 11.7 mM; pH 7.4) at 37 °C, which was used throughout all incubations. cAMP was assayed by the method of Shimizu et al. (1969) with modifications described earlier (Nalepa and Vetulani, 1991). Briefly, after a 15-min adaptation period the buffer was changed and [^{3}H]adenine (S.A. 26.8 Ci/mmol, NEN) was added to the incubation mixture in final concentration ~100 nM. After 45 min of incubation, the slices were washed, gravity-packed, and distributed in 50-μl portions into vials containing 440 μl of the buffer. After 5 min of preincubation, noradrenaline (NA) was added in a volume of 10 μl. The final concentration of NA was 100 μM and the incubation proceeded for 10 min, after which it was stopped with 550 μl of 10% trichloroacetic acid. The mixture was homogenised and centrifuged, and the supernatant was decanted into test tubes. [^{3}H]cAMP was purified by chromatography with a tracer [^{14}C]cAMP (S.A. 51.2 mCi/mmol, NEN) using a two-column system (Dowex 50W × 4 and alumina) according to Salomon et al. (1974). The system was eluted with water and then the alumina column was eluted with 0.1 M imidazole solution. The final elutes were tested for radioactivity in a Beckman LS 3801 liquid scintillation counter ([^{14}C]/[^{3}H] channel). All experiments were carried out in triplicates.

2.5. Drugs

The following agents were used: imipramine HCl (RBI, USA), citalopram HBr and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl] ethyl]-*N*-(2-pyridinyl) cyclo-hexanecarboxamide oxalate (WAY 100635) (both synthesised in the Department of Medicinal Chemistry, H. Lundbeck, Denmark). All drugs were dissolved in distilled water, which was used for vehicle injections.

2.6. Statistics

Sucrose intake data were analysed by multiple analyses of variance with drug treatments in stress and control groups as between-subjects factors and sucrose test day as within-subject factor. Receptor binding parameters (B_{max} and K_d) and cAMP generation were subjected to analysis of variance with between-subjects factors test day, drug treatments in stress and control groups. In all analysis the Fisher's LSD test was used for post hoc comparisons of means. P values lower than 0.05 were considered statistically significant.

3. Results

CMS caused a gradual decrease in the consumption of 1% sucrose solution. In the final baseline test, sucrose intakes were approximately 13 g and the animals were allocated to control and stress groups in a balanced way. Following 3 weeks of stress the intakes remained at approximately 14 g in controls but fell to approximately 9 g in stressed animals (Fig. 1). Similar differences were maintained between vehicle-treated control and stressed groups throughout the studies. Significance levels between stress and control groups treated with vehicle for 2, 7, 14, and

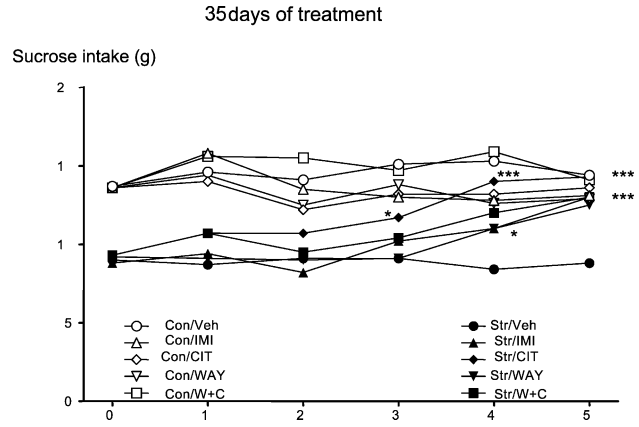

Fig. 1. Sucrose consumption in control (Con) and stressed (Str) animals treated daily with imipramine (10 mg/kg), citalopram (10 mg/kg), WAY 100635 (0.2 mg/kg), and citalopram + WAY 100635. Values are presented as means. Standard errors have been omitted in the figure for the sake of clarity. $N = 8$ per treatment group. Statistically significant differences indicated by $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ are calculated for drug-treated stressed groups relative to vehicle-treated stressed animals at the indicated time points.

35 days were $F(1,14) = 5{,}609$ ($P = .033$), $F(1,14) = 19{,}774$ ($P < .001$), $F(1,14) = 10.788$ ($P = .005$), and $F(1,14) = 48.595$ ($P < .001$), respectively.

After the initial 3 weeks of stress the control animals were slightly but significantly heavier than the stressed animals (i.e., 397 ± 6.8 vs. 377 ± 6.6, $P = .039$).

3.1. Drug-induced effects on sucrose intake

Sucrose intake was unaffected by drug treatment in unstressed and stressed groups that were sacrificed after 2 (unstressed $F(4,35) = 0.000924$ and stressed $F(4,35) = 0.0207$), 7 (unstressed $F(4,35) = 0.00663$ and stressed $F(4,35) = 0.419$), and 14 days (unstressed $F(4,35) = 0.479$ and stressed $F(4,35) = 0.346$) of drug treatment (data not shown).

There was no effect of drug treatment in the unstressed groups treated for 35 days [$F(4,35) = 0.848$], whereas there was a significant effect of drug treatment in the stressed groups [$F(4,35) = 2.633$, $P = .05$; Fig. 1]. Compared to the vehicle-treated stressed group there were no significant overall effect of imipramine [$F(1,14) = 2{,}832$, $P = .115$] but a significant interaction between treatment and test day [$F(5,70) = 2.426$, $P = .044$]. Subsequent comparisons of means at individual time points revealed significant differences between imipramine and vehicle at Weeks 4 and 5 ($P = .05$ and $P = .002$, respectively). There was a significant overall effect of citalopram compared to vehicle [$F(1,14) = 14.167$, $P = .002$] and a significant interaction between treatment and test day [$F(5,70) = 3.146$, $P = .013$]. Comparison of means revealed a significant difference between citalopram and vehicle at Weeks 3, 4, and 5 ($P = .05$, $P < .001$, and $P < .001$, respectively). Overall, the WAY 100635 + citalopram treatment was significantly different from vehicle [$F(1,14) = 8.366$, $P = .012$]. However, no significant differences between vehicle and drug treatment were achieved at the specific time points. The group treated with WAY 1000635 did not reached the 5% level of significance [$F(1,14) = 1.110$, n.s.) when compared to the vehicle group.

Body weights (data not shown) remained unaffected by drug treatment throughout the study both in unstressed (Day 2: $F(4,35) = 0.312$, Day 7: $F(4,35) = 0.569$, Day 14: $F(4,35) = 2.415$, Day 35: $F(4,35) = 1.995$, n.s.) and stressed groups (Day 2: $F(4,35) = 0.527$, Day 7: $F(4,35) = 2.203$, Day 14: $F(4,35) = 0.435$, Day 35: $F(4,35) = 1.021$, n.s., data not shown).

3.2. Effects on β-adrenoceptor binding and function

3.2.1. Effects on B_{max}

A three-way analysis of variance revealed overall significant effects of sucrose test day [$F(3,280) = 4.673$, $P = .003$], stress level [$F(1,280) = 15{,}375$, $P < .001$], and drug treatment [$F(4,280) = 32{,}509$, $P < .001$] and significant interaction between test day and drug treatment [$F(12,280) = 2.127$, $P = .016$] and stress level and drug treatment [$F(4,280) = 5{,}242$, $P < .001$].

On treatment day 2 the number of β_1-adrenoceptors, B_{max}, was significantly affected by treatment in unstressed (overall treatment effect: $F(4,35) = 3.692$, $P < .05$) as well as stressed groups (overall treatment effect: $F(4,35) = 6.145$, $P < .001$). B_{max} was significantly increased by CMS in the vehicle group (Fig. 2). Imipramine decreased B_{max} significantly both in the unstressed and the stressed group compared to the corresponding vehicle groups. Unstressed animals treated with citalopram, WAY 100635, or citalopram + WAY 100635 did not differ significantly from the unstressed vehicle controls. Stressed animals treated with citalopram, WAY 100635, or citalopram + WAY 100635 showed significantly lower B_{max} values than the stressed vehicle group.

On treatment day 7 B_{max} values for β_1-adrenoceptor binding were significantly affected in both unstressed groups [overall treatment effect: $F(4,35) = 2.951$, $P < .05$] and in stressed groups [overall treatment effect: $F(4,35) = 2.937$, $P < .05$]. Groupwise comparisons of drug-treated and vehicle-treated animals revealed significantly lower B_{max} values in the imipramine treated groups (Fig. 2).

On treatment day 14 B_{max} values for β_1-adrenoceptor binding were significantly affected in both unstressed groups [overall treatment effect $F(4,35) = 10.063$, $P < .001$] and stressed groups [$F(4,35) = 25.881$, $P < .001$]. B_{max} was significantly increased by CMS (Fig. 2) and B_{max} was significantly decreased in imipramine-treated groups, unstressed and stressed animals. The B_{max} values were significantly increased in citalopram- and WAY 100635-treated unstressed groups. Among the stressed groups WAY 100635 and combined treatment with WAY 100635 and citalopram produced a significant decrease of β_1-adrenoceptors density compared to vehicle-treated stressed animals.

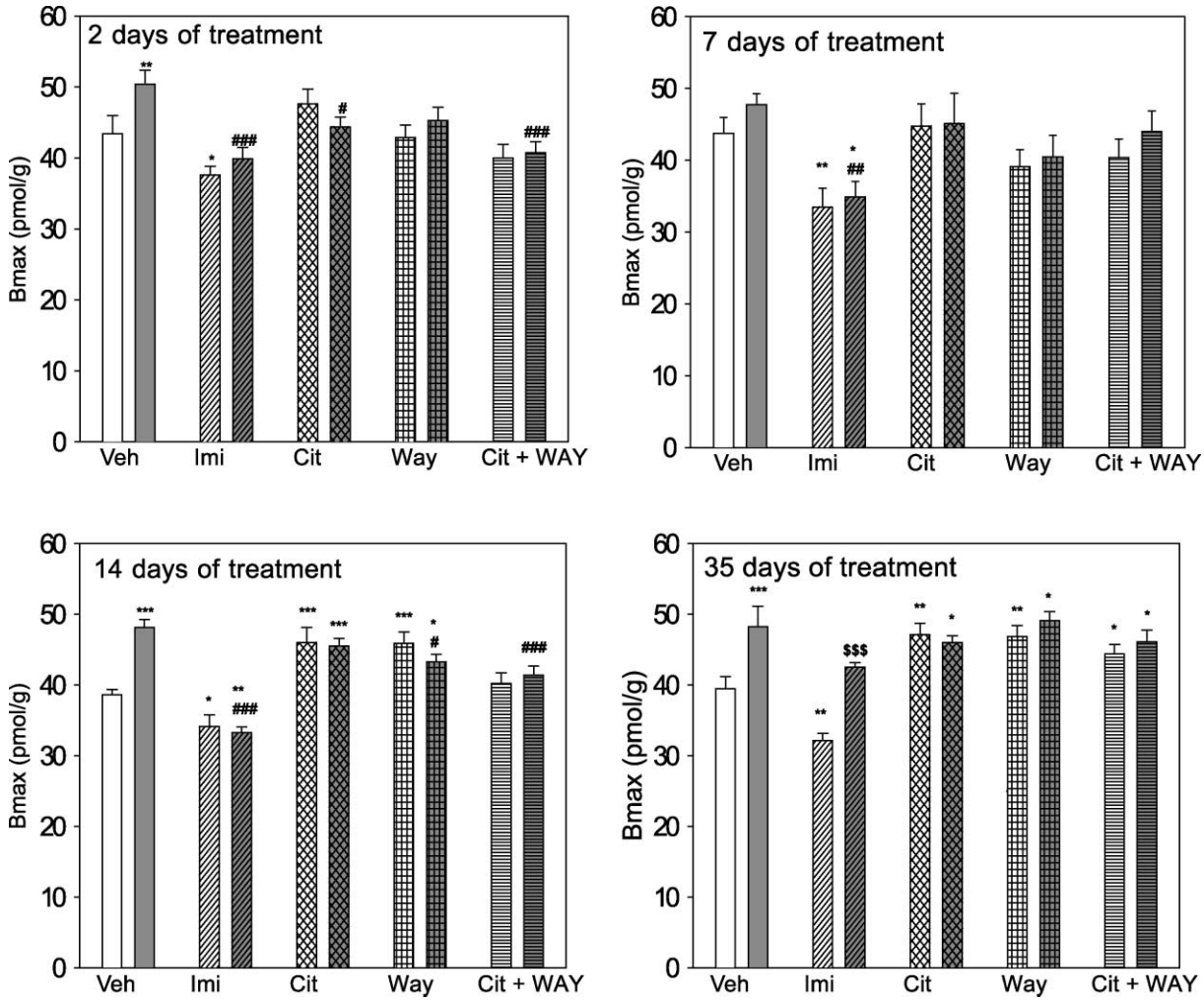

Fig. 2. Density (B_{max}) of cortical β_1-adrenergic receptors in control (Con, white bars) and stressed (Str, grey bars) animals treated daily with imipramine (10 mg/kg), citalopram (10 mg/kg), WAY 100635 (0.2 mg/kg), and citalopram + WAY 100635. Values are means ± S.E.M. of eight assays. $^{*}P<.05$, $^{**}P<.01$, and $^{***}P<.001$; relative to vehicle-treated control animals. $^{\#}P<.05$ and $^{\#\#\#}P<.001$; relative to vehicle-treated stressed animals; $^{\$\$\$}P<.001$ relative to appropriate drug-treated control.

On treatment day 35 B_{max} values for β_1-adrenoceptor binding were significantly affected by drug treatment in unstressed groups [overall treatment effect $F(4,35)=18.341$, $P<.001$] and stressed groups [overall treatment effect $F(4,35)=3.332$, $P<.05$]. B_{max} was significantly increased in stressed vehicle or imipramine-treated animals compared to their corresponding unstressed groups (Fig. 2). Citalopram, WAY 100635, and the combination of the two drugs produced a significant increase of the density of β_1-adrenoceptors in unstressed rats whereas imipramine produced a significant decrease compared to vehicle-treated animals. Five weeks treatment with citalopram, WAY 100635, and WAY 100635 + citalopram did not antagonise the CMS-induced up-regulation of β_1-adrenoceptors (Fig. 2).

3.2.2. Effects on K_d

A three-way analysis of variance revealed overall significant effects of test day [$F(1,280)=22.365$, $P<.001$], stress level [$F(1,280)=5.364$, $P=.021$], and drug treatment [$F(4,280)=2.840$, $P=.025$] and significant interaction between stress level and drug treatment [$F(4,280)=2.612$, $P=.036$].

Subsequent analysis of drug effects in unstressed and stressed groups revealed a significant drug effect in unstressed groups [$F(4,140)=4.654$, $P<.001$]. Citalopram produced a significant increase of K_d values compared to vehicle-treated animals ($P=.003$). There was no significant effect of drug treatment in the stressed groups [$F(4,140)=0.733$, n.s.] (Table 1).

3.2.3. Effects on cAMP response to noradrenergic stimulation

A three-way analysis of variance revealed overall significant effects of drug treatment [$F(4,280)=12.781$, $P<.001$] and significant interactions between test day and stress level [$F(3,280)=3.224$, $P=.023$], test day and drug

Table 1
Affinities (K_d) for β_1-adrenoceptors in control (Con) and stressed (Str) animals treated daily with imipramine (IMI, 10 mg/kg), citalopram (CIT, 10 mg/kg), WAY 100635 (WAY, 0.2 mg/kg b.i.d.), and citalopram + WAY 100635. Displacement studies of the radioligand [^{3}H]CGP12177 were performed in the crude synaptosomal fraction obtained from cortical rat brain tissue. Values are means (nM) ± S.E.M. See text for further details. $N = 8$ per treatment group

	Day 2		Day 7		Day 14		Day 35	
	Con	Str	Con	Str	Con	Str	Con	Str
Vehicle	0.32 (0.02)	0.36 (0.03)	0.35 (0.02)	0.38 (0.02)	0.29 (0.01)	0.32 (0.02)	0.30 (0.03)	0.33 (0.03)
IMI	0.31 (0.02)	0.31 (0.02)	0.37 (0.02)	0.37 (0.03)	0.27 (0.02)	0.27 (0.02)	0.33 (0.03)	0.39 (0.03)
CIT	0.36 (0.02)	0.35 (0.01)	0.41 (0.03)	0.37 (0.03)	0.30 (0.02)	0.30 (0.02)	0.35 (0.04)	0.31 (0.03)
WAY	0.33 (0.03)	0.36 (0.02)	0.34 (0.03)	0.37 (0.04)	0.31 (0.04)	0.28 (0.02)	0.32 (0.03)	0.35 (0.02)
WAY + CIT	0.29 (0.02)	0.36 (0.05)	0.32 (0.02)	0.41 (0.02)	0.26 (0.01)	0.26 (0.02)	0.30 (0.02)	0.31 (0.02)

treatment [$F(12,280) = 2.563$, $P = .003$], stress level and drug treatment [$F(4,280) = 4.152$, $P = .003$], and between test day, stress level, and drug treatment [$F(12,280) = 2.210$, $P = .012$].

On Day 2 no changes were observed with respect to cAMP generation in the response to noradrenergic stimulation in unstressed [$F(4,35) = 0.458$, n.s.] and in stressed animals [$F(4,35) = 0.826$, n.s.; Fig. 3]. On Day 7 significant effects were observed in unstressed [$F(4,35) = 5.897$, $P < .001$] and stressed groups [$F(4,35) = 4.435$, $P < .01$]. The cAMP response was significantly lower than the corresponding vehicle groups for imipramine-treated animals, unstressed as well as stressed, and in stressed animals treated with WAY 100635. On Day 14 the NA-stimulated cAMP generation was significantly affected by drug treatment in unstressed [$F(4,35) = 10.433$, $P < .001$] as well as

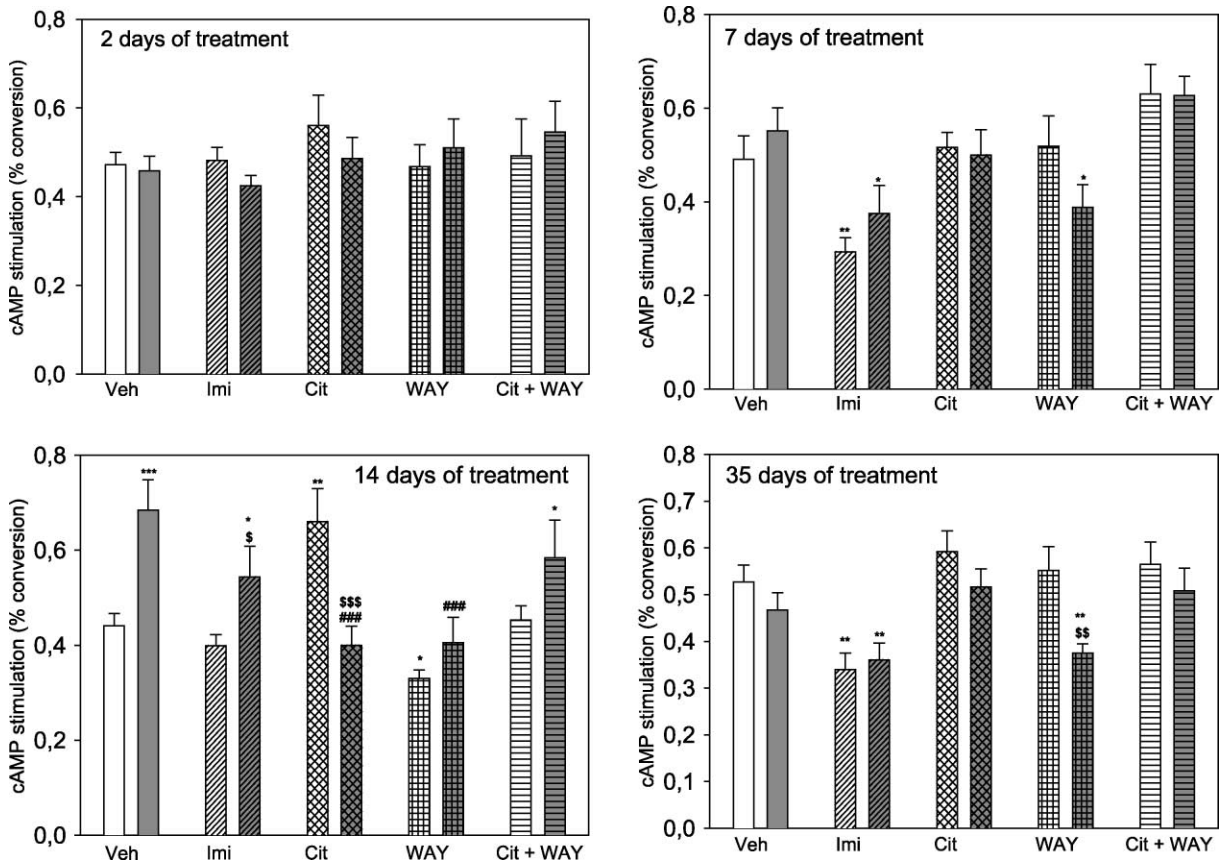

Fig. 3. NA-induced cAMP generation measured in cortical slices of control (Con, white bars) and stressed (Str, grey bars) animals treated daily with imipramine (10 mg/kg), citalopram (10 mg/kg), WAY 100635 (0.2 mg/kg), and citalopram + WAY 100635. The cAMP response was induced by 100 μM NA. Values are means ± S.E.M. of seven to eight assays and represent the percentage of conversion of [^{3}H]adenine to [^{3}H]cAMP presented as net stimulation over basal conversion level. Ranges: 0.16–0.24 (vehicle Con); 0.18–0.21 (vehicle Str); 0.16–0.20 (imipramine Con); 0.16–0.24 (imipramine Str); 0.18–0.21 (citalopram Con); 0.18–0.23 (citalopram Str); 0.17–0.22 (WAY Con); 0.18–0.24 (WAY Stress); 0.19–0.24 (WAY + citalopram Con); 0.16–0.19 (WAY + citalopram Str). * $P < .05$, ** $P < .01$, and *** $P < .001$; relative to vehicle-treated control animals. $^{###}P < .001$; relative to vehicle-treated stressed animals; $^{\$\$}P < .01$ and $^{\$\$\$}P < .001$ relative to appropriate drug-treated control animals.

stressed groups [$F(4,35) = 3.888$, $P < .01$]. The cAMP response was significantly higher in stressed vehicle controls and imipramine-treated animals than the corresponding unstressed groups whereas the citalopram-treated stressed animals showed significantly lower cAMP response than the corresponding unstressed group. Finally, on Day 35 the cAMP response to noradrenergic stimulation was significantly affected by drug treatment in the unstressed [$F(4,35) = 5.429$, $P < .01$] as well as stressed groups [$F(4,35) = 3.945$, $P < .01$]. Imipramine produced a significant decrease of cAMP generation both in the stressed and unstressed group, and WAY 100635 produced a significant decrease in stressed animals compared to corresponding unstressed controls.

4. Discussion

In the present investigation we studied the time-course of the antidepressant-like effect of imipramine and citalopram, the selective 5-HT_{1A} receptor antagonist, WAY 100635, and the combination of citalopram and WAY 100635 in the rat CMS model of depression. All treatments produced a reversal of stress-induced suppression of sucrose intake. Interestingly, citalopram produced a faster reversal of stress-induced suppression of sucrose intake than imipramine. This confirms the results of two previously published studies where citalopram and imipramine have been studied in parallel in the CMS model of depression (Przegalinski et al., 1995; Sánchez and Papp, 2000). It may be argued that the differences in onset of action could be ascribed to different potencies of the two drugs. However, a lower citalopram dose (5 mg/kg) was inactive in the CMS model (unpublished observation). Furthermore, citalopram and imipramine are found to be equipotent in the forced swim test, a frequently used model of antidepressant activity (Sánchez and Meier, 1997). The clinical significance of this finding remains to be established as no clinical studies designed specifically to address differences in time to onset of effect between citalopram and TCAs have been conducted.

Combined treatment with WAY 100635 and citalopram failed to produce a faster onset of action than the SSRI alone and actually attenuated the effect of citalopram in the present study. There are several possible explanations for this finding. It may be that the dose of WAY 100635 was not optimal. Results from combination studies with SSRIs and 5-HT_{1A} receptor antagonists in the forced swim test suggest that there may be an optimal ratio between 5-HT uptake inhibitory and 5-HT_{1A} receptor antagonistic potency (unpublished observation). In the present study we were only able to study one dose of WAY 100635, as it was not possible to include more animal groups at a time. Another possible explanation could be that a partial 5-HT_{1A} receptor agonist rather than an antagonist should be used in combination with the SSRI. The clinical studies used a combination of SSRI and pindolol. The latter drug has in many in vivo and in vitro studies proven to be a partial 5-HT_{1A} receptor agonist rather than an antagonist (e.g., Newman-Tancredi et al., 1998; Clifford et al., 1998; Gartside et al., 1999; Sprouse et al., 2000; Hirani et al., 2000; Arborelius et al., 2000). Some preclinical and clinical studies of combinations of the partial 5-HT_{1A} receptor agonist, buspirone and an SSRI report on enhanced efficacy (Redrobe and Bourin, 1998; Van-Amerigen et al., 1996; Bouwer and Stein, 1997; Appelberg et al., 2001), but this is not a consistent finding (Fischer et al., 1998; Landen et al., 1998). There is substantial evidence from microdialysis studies that combinations of SSRIs and pindolol or WAY 100635 produce significantly increased 5-HT output compared to SSRIs alone and thereby an enhanced serotonergic neurotransmission. This is ascribed to the blockade of somatodendritic 5-HT_{1A} receptors and subsequent attenuation of the inhibitory effect on 5-HT output mediated by these receptors. However, these studies were conducted in nonstressed rats and it cannot be excluded that the discrepancy between these data and our findings arise because we are using stressed rats. Chronic stress conditions have been reported to have a major impact on serotonergic neurotransmission, e.g., social isolation produced a significant decrease of the basal serotonin turnover (Heidbreder et al., 2000) and 5-HT release as response to acute tail-shock stress was increased in rats subjected to the learned helplessness paradigm (Petty et al., 1994).

Stimulation of postsynaptic 5-HT_{1A} receptors is hypothesised to play an important role for mediating the antidepressant activity in patients. If this hypothesis can be extrapolated to the rat CMS model of depression, it may be that WAY 100635 counteracts the presynaptically produced enhancements of 5-HT by inhibition of the postsynaptic 5-HT_{1A} receptor-mediated neurotransmission and thereby produce a delay in the onset of effect in the CMS model. The role of 5-HT_{1A} receptors in mediating the CMS response has not been studied in full detail. It has previously been found that CMS increases the number of 5-HT_{1A} receptors in terminal areas, e.g., in hippocampus (Papp et al., 1994b). However, chronic treatment with imipramine increased the number of 5-HT_{1A} receptors in unstressed controls and did not normalise the number of receptors in stressed animals (Papp et al., 1994b). Furthermore, the 5-HT_1 receptor antagonist, metergoline, failed to reverse the effect of imipramine in the CMS model of depression (Muscat et al., 1990).

A study comparing time-effect profiles of paroxetine and paroxetine plus pindolol on olfactory bulbectomy-induced hyperactivity in rats has also failed to show a faster onset of action of the combined treatment (Cryan et al., 1998). As expected, paroxetine reversed the hyperactivity after 14 days of treatment but the combined pindolol and paroxetine treatment actually did not reverse the behaviour at that time point, suggesting that pindolol counteracts the effect of paroxetine. It would have been interesting to see whether a longer lasting treatment with pindolol plus paroxetine would have been effective. In the same study pindolol plus parox-

etine attenuated 8-OH-DPAT-induced hypothermia already after 3 days of treatment whereas paroxetine alone was active after 14 days. Thus the combined treatment produced a faster adaptive change of 5-HT_{1A} receptor function than paroxetine alone. It remains to be studied whether CMS produces changes in the function of 5-HT_{1A} receptors and whether antidepressants can reverse this.

The most consistent effects on β_1-adrenoceptors were observed with respect to changes in their densities (B_{max}). The overall picture is that CMS produces an increase of number of β_1-adrenoceptors throughout the study period in the vehicle-treated group exposed to CMS, although this increase failed to reach statistical significance on Day 7. This agrees with previously published data on β-adrenoceptor binding properties during the CMS procedure where [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol were used as ligands (Papp et al., 1994a,b).

Down-regulation of cortical β-adrenoceptors has been suggested as a common effect of long-term antidepressant treatment (see, for review, Vetulani and Nalepa, 2000). In unstressed animals, imipramine produced a decrease of B_{max} of β_1-adrenoceptor as soon as after 2 days treatment and this effect persisted throughout the study. This early effect of imipramine is in line with that observed by Hosoda and Duman (1993), who reported a decreased level of [^{3}H]CGP-12177 binding after 3 days of drug administration. Duncan et al. (1994) showed a similar rapid down-regulation of β-adrenoceptors after 2 days treatment with imipramine and reported that cerebral cortical regions were the most rapidly affected regions.

We found a similar decrease in β_1-adrenoceptor density in stressed animals at Days 2, 7, and 14 but not at 35 days of treatment (Fig. 2). The latter is in agreement with our previously reported results concerning cAMP generation and indicating that 5 weeks of imipramine administration abolished the effect of stress and at the end of the treatment period no down-regulation was observed in stressed animals (Papp et al., 1994a). Thus the onset of imipramine-induced β-adrenoceptors down-regulation in stressed rats was observed much earlier than the reversal of CMS-induced suppression of sucrose intake.

In unstressed rats both citalopram and WAY 100635 produced similar effects, i.e., increased density of β_1-adrenoceptors in the cortex of unstressed as well as stressed rats after 14 and 35 days treatment. This increase was comparable to the increase produced by CMS in vehicle-treated animals. This agrees with the results of previous citalopram studies where up-regulation of β_1-adrenoceptors and enhanced responsiveness (i.e., cAMP generation) of cortical adrenoceptors were reported after chronic treatment of normal rats (Palvimaki et al., 1994; Nalepa and Vetulani, 1993). In light of these results it may be concluded that β_1-adrenergic receptor down-regulation is not the biochemical marker of antidepressant-like activity of the investigated drugs.

Changes in receptor numbers were only partially correlated with the response of cAMP to noradrenergic stimulation. In the present study an increased cAMP generation appeared only at the 5th week of stress (drug treatment day 14), while in a previous study this effect occurred after 8 weeks of stress (Papp et al., 1994a). Thus, it may be questioned whether the increase in cAMP generation found in the CMS model is a consistent finding. On the other hand, it could be speculated that an enhancement of cAMP generation occurring in the course of the stress procedure is transient. This may reflect an early compensatory mechanism of the adenylyl cyclase-coupled signal transduction cascade that precedes other changes resulting in the development of behavioural deficits.

Unlike imipramine, citalopram (10 mg/kg ip, b.i.d. for 14 days) was reported to produce an up-regulation of the NA-stimulated cAMP formation in normal rats (Nalepa and Vetulani, 1993). A similar effect of citalopram was seen in unstressed animals in the present study, but only at 14 days. The lack of effect on cAMP formation at Day 35 may be related to the different dose regimen used in the present study. Citalopram abolished the CMS-induced increase of cAMP response in stressed rats at Day 14 without reducing the stress-induced increase in β_1-adrenoceptor density, and B_{max} values in stressed and unstressed citalopram-treated animals were significantly increased compared to unstressed vehicle controls throughout the study. Since citalopram does not interact directly with any of the adrenergic receptors (Nalepa and Vetulani, 1993), changes in the beta-adrenoceptor density and cAMP response induced by this drug may reflect an adaptation of the noradrenergic system to the increased serotonin availability. Results from a number of studies suggest that citalopram-induced adaptive changes in the β_1-adrenergic system is produced by a modulating action of serotonin on NA release involving various serotonin receptor subtypes. Data from microdialysis studies indicate that an inhibitory tone mediated via 5-HT_2 receptor stimulation reduce NA release while stimulation of 5-HT_{1A} receptors in the hippocampus (Done and Sharp, 1994; Hajos-Korcsok et al., 1999) and in the frontal cortex of rats (Gobert et al., 1999) enhance NA release. Acute treatment with citalopram or fluoxetine enhanced NA release (Hughes and Stanford, 1998). Conflicting results are reported for long-term treatment with SSRIs. Repeated citalopram treatment resulted in a reduced NA-stimulated cAMP response (Petersen and Mørk, 1996), whereas repeated treatment with fluoxetine produced enhanced electrically evoked release of NA (Mongeau et al., 1994) and chronic paroxetine lead to further increase in NA release (Hajos-Korcsok et al., 2000).

Fourteen days of treatment with WAY 100635 produced a reduced cAMP response in unstressed animals. To our knowledge, this is the first report showing that chronic treatment with a 5-HT_{1A} antagonist affects the β_1-adrenoceptor function. Moreover, the CMS-produced increase of cAMP formation was reversed at Day 14. The latter effect was accompanied by a decrease in the β_1-adrenoceptor density. A combined treatment with WAY 100635 and citalopram abolished the changes that in stressed animals were

induced by each compound administered alone. Recently, Cremers et al. (2000) reported that WAY 100635-induced augmentation of citalopram-induced increase of serotonin release critically depends on the citalopram dose. If adaptive changes of β_1-adrenoceptors function depend on the level of serotonin and the latter effect is under control of 5-HT_{1A} receptors, it cannot be excluded that the citalopram dose used in present experiment was too low to induce WAY 100635 augmentation of behavioural changes. Nevertheless, it is worth noting that imipramine-, citalopram-, and WAY 100635-induced changes in β_1-adrenoceptor function in stressed animals preceded antidepressant-like effects in the rat CMS model of depression. Our experiment indicates that early changes of behavioural response have appeared a week after those seen in cAMP response.

In conclusion, the stress- and drug-induced effects on β-adrenoceptor number, affinity, and function are complex and do not appear to be a common biochemical marker of antidepressant-like activity in the rat CMS model of depression. Citalopram produced a faster onset of the antidepressant-like effect than imipramine in the rat CMS model. The 5-HT_{1A} receptor antagonist, WAY 100635, did not potentiate the effect of citalopram.

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