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Effects of Acute and Long-Term Administration of Escitalopram and Citalopram on Serotonin Neurotransmission: an *In Vivo* Electrophysiological Study in Rat Brain

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The present study was undertaken to compare the acute and long-term effects of escitalopram and citalopram on rat brain 5-HT neurotransmission, using electrophysiological techniques. In hippocampus, after 2 weeks of treatment with escitalopram (10 mg/kg/day, s.c.) or citalopram (20 mg/kg/day, s.c.), the administration of the selective 5-HT_{1A} receptor antagonist WAY-100,635 (20–100 μg/kg, i.v.) dose-dependently induced a similar increase in the firing activity of dorsal hippocampus CA_3 pyramidal neurons, thus revealing direct functional evidence of an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors. In dorsal raphe nucleus, escitalopram was four times more potent than citalopram in suppressing the firing activity of presumed 5-HT neurons (ED_{50} = 58 and 254 μg/kg, i.v., respectively). Interestingly, the suppressant effect of escitalopram (100 μg/kg, i.v.) was significantly prevented, but not reversed by R-citalopram (250 μg/kg, i.v.). Sustained administration of escitalopram and citalopram significantly decreased the spontaneous firing activity of presumed 5-HT neurons. This firing activity returned to control rate after 2 weeks in rats treated with escitalopram, but only after 3 weeks using citalopram, and was associated with a desensitization of somatodendritic 5-HT_{1A} autoreceptors. These results suggest that the time course of the gradual return of presumed 5-HT neuronal firing activity, which was reported to account for the delayed effect of SSRI on 5-HT transmission, is congruent with the earlier onset of action of escitalopram *vs* citalopram in validated animal models of depression and anxiety.

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INTRODUCTION

Although the physiopathology of major depression is not fully defined, a growing body of evidence implicates the serotonin (5-HT) system as a possible therapeutic target of antidepressant treatments (Heninger and Charney, 1987; Price *et al*, 1990; Van Praag *et al*, 1990; Maes and Meltzer, 1995; Blier and de Montigny, 1998). Indeed, clinical evidence indicates that reduction of brain 5-HT levels, induced by a dietary depletion of L-tryptophan, can reverse the antidepressant action of selective 5-HT reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOIs), and the α_2-adrenoceptor antagonist mirtazapine (Bremner *et al*, 1997; Nishizawa *et al*, 1997; Moreno *et al*, 2000). Preclinical studies show that antidepressant treatments increase 5-HT neurotransmission with a time course that is consistent with their delayed therapeutic effect. This enhancement would, however, be mediated via different mechanisms. Long-term administration of either MAOIs or SSRIs results in a desensitization of the somatodendritic 5-HT_{1A} autoreceptor of 5-HT neurons in the dorsal raphe nucleus (DRN), thereby allowing their firing rate to recover in the presence of the drugs (Blier *et al*, 1986; Chaput *et al*, 1986). In addition, long-term SSRI treatment also desensitizes terminal 5-$HT_{1B/1D}$ autoreceptors (Blier and Bouchard, 1994; Briley and Moret, 2000). Long-term treatment with the antidepressant mirtazapine increases 5-HT neurotransmission, as a result of a sustained increase in the firing activity

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of 5-HT neurons in the presence of decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals (Haddjeri *et al*, 1997). Finally, long-term treatment with 5-HT_{1A} receptor agonists, such as gepirone (Feiger *et al*, 2003), desensitizes the 5-HT_{1A} autoreceptors on soma but not the postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Blier and de Montigny, 1987; Schechter *et al*, 1990; Dong *et al*, 1998). Together, these indicate that adaptive changes in the 5-HT system may play a pivotal role in the therapeutic effect of antidepressant treatments.

Clinical and preclinical studies have shown that the citalopram effects on 5-HT reuptake inhibition and its antidepressant activity resides in the *S*-enantiomer (Hyttel *et al*, 1992; Montgomery *et al*, 2001; Burke *et al*, 2002; Wade *et al*, 2002; Lepola *et al*, 2004). In addition, using a variety of behavioral paradigms for depression/anxiety, it has been shown that escitalopram showed a more rapid and efficacious effect than citalopram, and that R-citalopram counteracted these effects *in vivo* and *in vitro* (Mørk *et al*, 2003; Sánchez *et al*, 2003a, b; Sánchez and Kreilgaard, 2004).

The present study was undertaken to determine and compare whether acute and sustained administration of citalopram and its enantiomer escitalopram in rats, modified the firing activity of dorsal raphe presumed 5-HT neurons, the responsiveness of auto- (pre-) and postsynaptic 5-HT_{1A} receptors, and overall 5-HT neurotransmission in the hippocampus. Parts of this work were previously published in abstract form (El Mansari *et al*, 2004).

MATERIALS AND METHODS

The experiments were carried out in male Sprague–Dawley rats (IFFA CREDO, L'Abresle, France) weighing 250–300 g at the day of the recording. They were kept under standard laboratory conditions (12:12 h light–dark cycle with free access to food and water). In the sustained administration experiments, groups of rats were treated with escitalopram (10 mg/kg/day) or citalopram (20 mg/kg/day) for 7, 14, and 21 days delivered subcutaneously (s.c.) with osmotic minipumps (Charles Rivers, France). Control rats received a minipump containing vehicle (NaCl 0.9%). The rats were tested with the minipumps in place. The animals were anesthetized with chloral hydrate (400 mg/kg, i.p.). Supplemental doses were given to maintain constant anesthesia and to prevent any nociceptive reaction to a tail pinch.

Experiments were performed in accordance with the European Communities Council Directives 86/609, OJ L 358,1, Dec 12, 1987, for the care and use of laboratory animals. All experiments were performed with the approval of the Regional Animal Care Committee (Faculty of Medicine, Claude Bernard University-Lyon 1), and complied with rules set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (publication 80-23).

Treatments

Two different lengths of treatment were used, one was short-term and the other was long-term treatment. On the one hand, in order to detect as early as possible changes in the sensitivity of 5-HT_{1A} autoreceptors and a return to baseline activity of DRN neurons, recordings were made after 7, 14, and 21 days of treatments. On the other, as the 5-HT_{1A} receptors in hippocampus adapt differently (see Blier and de Montigny, 1994 for a review), we aimed at recording the change in the sensitivity of these receptors following the putative return to normal discharge of DRN 5-HT neurons, that is, 14 days of treatment. In total, 10 mg/kg/day of escitalopram and 20 mg/kg/day of citalopram were used because they were shown to maximally increase extracellular 5-HT in the prefrontal cortex (see Ceglia *et al*, 2004).

Extracellular Unitary Recordings of Dorsal Raphe 5-HT Neurons

Extracellular recordings were performed with single-barreled glass micropipettes preloaded with fiberglass filaments in order to facilitate filling. The tip was broken back to 2–4 μm and filled with a 2 M NaCl solution saturated with Pontamine Sky Blue to stain the location of the last recording. The rats were placed in a stereotaxic frame and a burr hole was drilled on the midline, 1 mm anterior to lambda. Presumed DRN 5-HT neurons were encountered over a distance of 1 mm starting immediately below the ventral border of the Sylvius aqueduct. These neurons were identified using the criteria of Aghajanian (1978): a slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potentials; they were also verified to be located in DRN. For the acute studies, a dose–response curve of the suppressant effects on neuronal 5-HT firing of escitalopram and citalopram was constructed. To this end, in each rat only one neuron was studied with SSRIs and a dose of WAY 100635 (*N*-{2-[4(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; 50–100 μg/kg, i.v.) was used to reverse the suppressant effect of both escitalopram and citalopram on the firing activity of DRN 5-HT neurons. To determine a putative interaction between the enantiomers of citalopram on the firing activity of 5-HT neurons, a dose of 250 μg/kg (i.v.) of R-citalopram was injected prior to escitalopram (100 μg/kg, i.v. = ED_{100}). For the chronic studies, in order to determine the possible changes of the spontaneous firing activity of dorsal raphe 5-HT neurons, four to five electrode descents were carried out through this nucleus, in rats with minipumps in place. Finally, the sensitivity of 5-HT_{1A} receptors was evaluated by using the 5-HT_{1A} receptor agonist (±) 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin; ED_{100} = 5 μg/kg, i.v.).

Recordings from Dorsal Hippocampus CA_3 Pyramidal Neurons

Recording and microiontophoresis were performed with five-barreled glass micropipettes broken back to 8–12 μm under microscopic control. The central barrel was filled with a 2 M NaCl solution and used for extracellular unitary recordings. The pyramidal neurons were identified by their large amplitude (0.5–1.2 mV) and long-duration (0.8–1.2 ms) simple spikes alternating with complex spike discharges (Kandel and Spencer, 1961). The side barrels contained the following solutions: 5-HT creatinine sulfate

(2 mM in 200 mM NaCl, pH 4), quisqualate (1.5 mM in 200 mM NaCl, pH 8), and 2 M NaCl used for automatic current balancing. The rats were mounted in a stereotaxic apparatus and the microelectrodes were lowered at 4.2 mm lateral and 4.2 mm anterior to lambda into the CA_3 region of the dorsal hippocampus. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anesthesia, a leak or a small ejection current of quisqualate ($+2$ to -8 nA) was used to activate them within their physiological firing range (10–15 Hz; Ranck, 1975). A 10 or 20 nA ejection current of 5-HT was used, each ejection period lasting 50 s. To assess the effectiveness of the long-term treatment with citalopram and escitalopram, the recovery time 50 (RT_{50}) method was used. The RT_{50} value has been shown to be a reliable index of the *in vivo* activity of the 5-HT reuptake process in the rat hippocampus. This value is obtained by calculating the time in seconds required for the neuron to recover 50% of its initial firing rate at the end of the microiontophoretic application of 5-HT onto CA_3 pyramidal neuron. Thus, the blockade of the 5-HT transporter by an SSRI reveals a greater RT_{50} value than in controls (Piñeyro *et al*, 1994). The neuronal responsiveness to 5-HT was assessed using the IT_{50} method. It is the product of the current (in nA) used to eject 5-HT from the micropipette and the time (in seconds) required to obtain a 50% decrease from baseline of the firing rate of the recorded neuron. The more sensitive a neuron is to 5-HT, the smaller will be the IT_{50} value because the number of molecules ejected is proportional to the amount of coulombs (de Montigny and Aghajanian, 1978).

In order to assess the degree of activation of the postsynaptic 5-HT_{1A} receptors exerting an inhibitory influence on the firing activity of CA_3 pyramidal neurons, WAY 100,635 was administered intravenously to disinhibit the hippocampal neurons resulting in an increase of their firing activity. The disinhibition would be best determined if the neurons were not firing at a high rate; therefore, their firing rate was decreased to about 10–20 Hz by reducing the ejection current of quisqualate and the selective 5-HT_{1A} receptor antagonist WAY 100,635 (20–100 μg/kg) was injected intravenously afterward (Haddjeri *et al*, 1998a; Besson *et al*, 2000). It is well established that the suppression of the firing activity of CA_3 pyramidal neurons by microiontophoretic applied 5-HT is mediated through the activation of 5-HT_{1A} receptors (de Montigny *et al*, 1984). If a tonic activation of postsynaptic 5-HT_{1A} receptors exits, the firing activity of hippocampus CA_3 pyramidal neurons will be lowered even though the responsiveness of these receptors to 5-HT is unchanged. Then the blockade of these receptors by WAY 100,635 will disinhibit the CA_3 pyramidal neurons resulting in an increase of their firing activity (for more details see Haddjeri *et al*, 1998a). An intravenous injection of saline preceded the first injection of WAY 100,635 to eliminate any effect due to the injection by itself. The saline injection corresponds to the 0 mg/kg dose. WAY 100,635 was administered in incremental doses of 20 μg/kg at time intervals of 2 min. To avoid residual drug effects, only one cell was studied in each rat. The change of firing activity was assessed by calculating the mean firing rate of neurons from about 1–2 min prior to and after (until a 'plateau') the i.v. administration of the drugs and the percent of change was calculated.

Statistical Analyses

Statistical analysis was performed with SigmaStat for Window version 4.0 software (Jandel Corporation). Average are shown as the mean $\pm$ SEM. Significance is considered for $^{*}P<0.05$ and $^{**}P<0.01$. A two-way analysis of variance (ANOVA) was performed on the firing rates on dorsal raphe 5-HT neurons in escitalopram and citalopram and control rats after 7-, 14-, or 21-days treatment. A Student's *t*-test or a one-way ANOVA was performed on the effects of 8-OH-DPAT, R-citalopram, and escitalopram on dorsal raphe 5-HT neuronal firing activity. One- and two-way repeated measures ANOVA were used to determine the effects of the different treatments on the firing rate of dorsal hippocampus CA_3 pyramidal neurons when repeated doses of WAY 100,635 were given. Correlation coefficients (*r*-values) for the dose–response relationship observed in the dorsal raphe were calculated using simple linear/curvilinear regression analysis. Differences between the two regressions were assessed by comparing the ED_{50} values using the 95% confidence interval method.

RESULTS

Effects of Acute and Sustained Administration of Escitalopram and Citalopram on the Firing Activity of Dorsal Raphe 5-HT Neurons

All dorsal raphe 5-HT neurons tested in the present study were inhibited, in a dose-dependent manner, by escitalopram and citalopram. As illustrated in Figure 1, escitalopram was more potent than citalopram in suppressing 5-HT neuronal firing activity in the DRN. In fact, the ED_{50} for citalopram and escitalopram was 254 and 58 μg/kg, respectively. This inhibitory effect was reversed by the intravenous administration of WAY 100,635, a selective 5-HT_{1A} receptors antagonist.

As illustrated in Figure 2, R-citalopram (250 μg/kg, i.v.) by itself did not modify the firing activity of dorsal raphe 5-HT neurons. In the absence of R-citalopram, the mean firing activity of 5-HT neurons was 1.6 ± 0.2 Hz before the administration of 100 μg/kg of escitalopram and 0 Hz after escitalopram injection ($n=6$). Interestingly, after R-citalopram administration, the firing rate was 1.7 ± 0.3 Hz before and $1.1+0.3$ Hz after escitalopram injection ($n=6$). R-citalopram prevented significantly, but did not reverse (see Figure 2a, $n=4$), the suppressant effect of escitalopram on the firing activity of 5-HT neurons ($P<0.01$ using unpaired Student's *t*-test, Figure 2).

In rats treated with escitalopram and citalopram for 7 days, there was a decrease of the spontaneous firing activity of dorsal raphe 5-HT neurons (by 61 and 70%, respectively, Figure 3). After 14 days of treatment with escitalopram, a significant decrease of the firing activity of 5-HT neurons was no longer present when compared to controls (11%) while there was still a significant decrease of 58% in rats treated with citalopram (Figure 3). This mean firing activity was back to control value after 21 days of treatment with citalopram (Figure 3).

The 5-HT_{1A} receptor agonist 8-OH-DPAT was used to assess the sensitivity of somatodendritic 5-HT_{1A} autoreceptors in rats treated with escitalopram or citalopram for 14 or

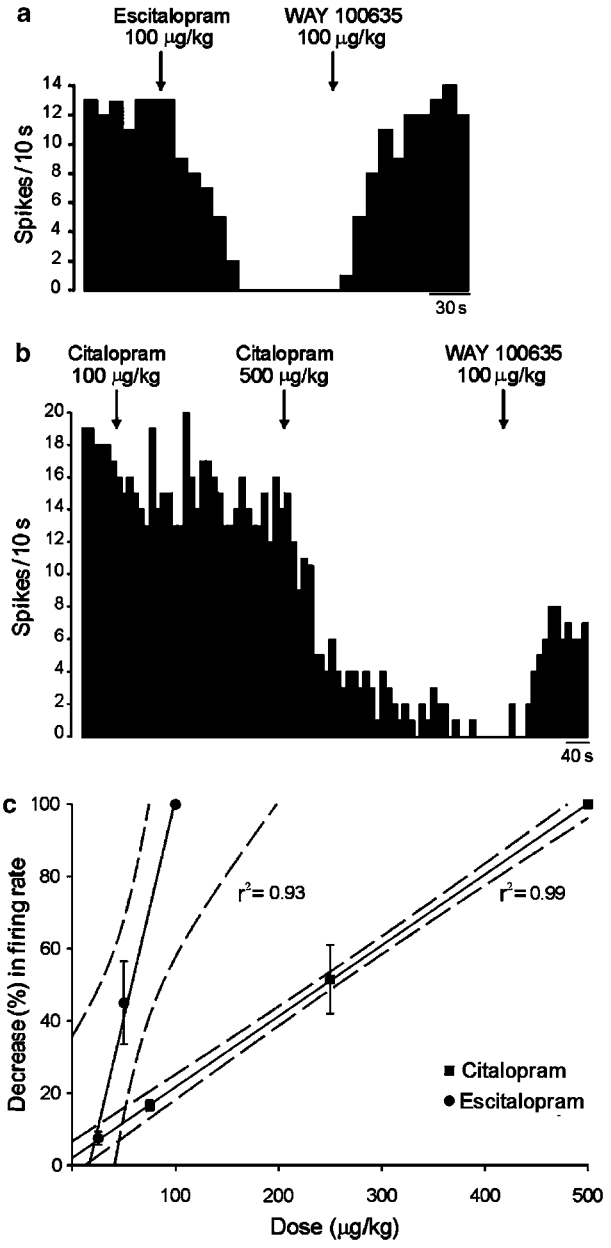

Figure 1 Integrated firing rate histograms of dorsal raphe 5-HT neurons in naive rats showing their response to escitalopram and WAY-100,635 administrations (a) and to citalopram and WAY-100,635 administrations (b). (c) Dose–response curves of the suppressant effect of escitalopram and citalopram (25–500 μg/kg, i.v.) on the firing activity of 5-HT neurons in naive rats. $P<0.05$ using confident limits of ED_{50} values. Two to three rats were used for each dose (in each rat only one neuron was tested).

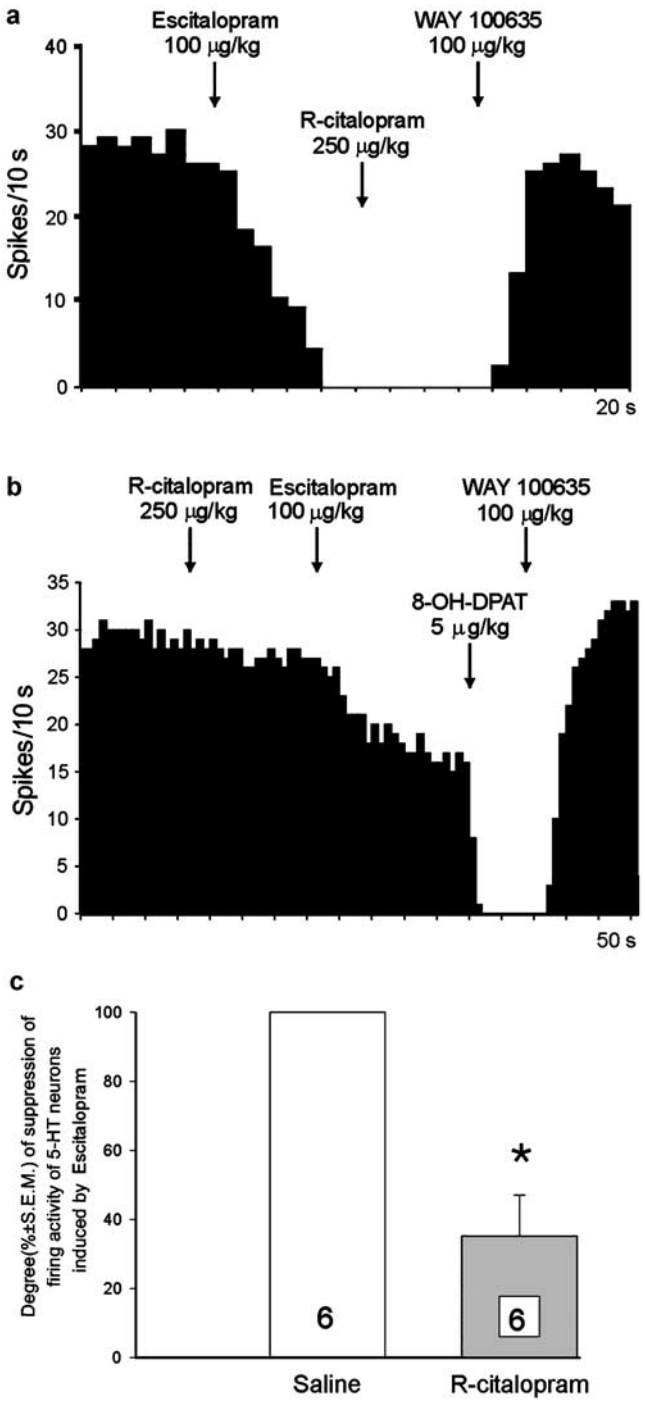

Figure 2 Integrated firing rate histograms of dorsal raphe 5-HT neurons in naive rats showing their response to escitalopram (a) and to R-citalopram, escitalopram and WAY-100,635 (b). (c) Mean results (±SEM) of the suppressant effect of escitalopram (100 μg/kg, i.v.) on the firing activity of 5-HT neurons in naive rats after saline or R-citalopram (250 μg/kg, i.v.) injections. The numbers at the bottom of the columns indicate the number of rats tested (in each rat only one neuron was tested). *$P<0.05$ vehicle *vs* R-citalopram using unpaired Student's *t*-test.

21 days. The mean spontaneous firing activity of representative 5-HT neurons tested was 1.3 ± 0.2 Hz in controls, 1.2 ± 0.5 Hz in rats treated with escitalopram for 2 weeks, 0.6 ± 0.1 Hz ($n=6$, $t=3.43$, df $=10$, $P<0.01$, using unpaired student's *t*-test) in rats treated with citalopram for 2 weeks, and 1.2 ± 0.3 Hz in rats treated with citalopram for 3 weeks. For all neurons tested in control rats, the i.v. administration of 8-OH-DPAT (5 μg/kg) produced a complete suppression of the firing activity of dorsal raphe 5-HT neurons (Figure 4a). The suppressant effect of 8-OH-DPAT on the firing activity of 5-HT neurons was unchanged in rats treated with citalopram for 2 weeks (20 mg/kg/day, s.c., $P>0.34$ using one-way ANOVA followed by PLSD Fisher's test). Interestingly, this suppressant effect of 8-OH-DPAT on the firing activity of 5-HT neurons was significantly attenuated by 46%, in rats treated with escitalopram for 2

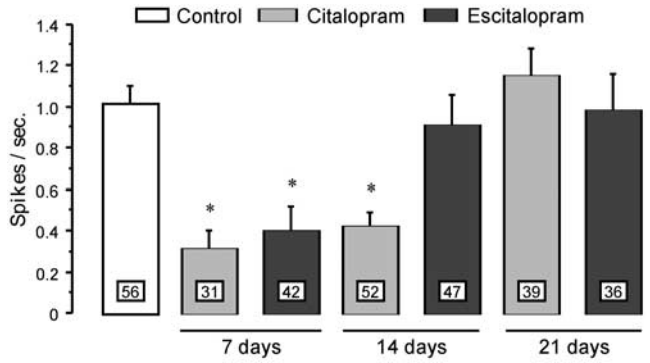

Figure 3 Mean (±SEM) of the firing rate of dorsal raphe 5-HT neurons in control rat and rats treated with escitalopram (10 mg/kg/day, s.c.) or citalopram (20 mg/kg/day, s.c.), for 7, 14, and 21 days. Note the recovery of firing of 5-HT neurons in rats treated with escitalopram for 14 days. *$P<0.05$, using two-way ANOVA. The numbers at the bottom indicate the total number of neurons recorded in each group of treated rats (six to seven rats were tested per group).

weeks (10 mg/kg/day, s.c., $P<0.03$ using one-way ANOVA followed by PLSD Fisher's test), and by 73% in rats treated with citalopram for 3 weeks (20 mg/kg/day, s.c., decrease in firing activity after 8-OH-DPAT injection $=27\pm19\%$, $n=5$, $P<0.01$). One-way ANOVA showed no significant difference between these two groups of treated rats.

Effects of Sustained Treatment with Escitalopram and Citalopram on the Dorsal Hippocampus CA_3 Pyramidal Neurons Responsiveness to WAY 100,635

For all CA_3 pyramidal neurons tested, 5-HT (10 or 20 nA) induced an inhibition of their firing activity (Figure 5). This suppressant effect occurred in the absence of any alteration of the shape of the action potentials. Short- or long-term treatment with escitalopram or citalopram did not modify the suppressant effect of microiontophoretically applied 5-HT on the firing activity of CA_3 pyramidal neurons. In fact, such treatments failed to modify the responsiveness of CA_3 pyramidal neurons to microiontophoretically applied 5-HT: the mean IT_{50} value for 5-HT was not significantly different in rats treated with escitalopram or citalopram for 14 days when compared to controls: IT_{50} value for 5-HT was 13 ± 2 ($n=9$) in control rats, 16 ± 4.5 ($n=6$) in rats treated citalopram for 2 weeks, and 15 ± 2 ($n=8$) in rats treated with escitalopram for 2 weeks ($P>0.05$, using one-way ANOVA). On the other hand, the mean RT_{50} value for 5-HT was significantly increased in rats treated with either escitalopram or citalopram. The mean RT_{50} value for 5-HT increased significantly following sustained administration of escitalopram and citalopram. The RT_{50} value for 5-HT was 13 ± 2 s in control rats ($n=9$), 40.5 ± 10 s in rats treated with escitalopram for 2 weeks ($P<0.05$, using one-way ANOVA followed by Dunnett test, $n=8$) and 40 ± 9 s in rats treated with citalopram for 2 weeks ($P<0.05$, using one-way ANOVA followed by Dunnett test, $n=6$).

The effect of WAY 100,635 on the quisqualate-activated firing activity of CA_3 pyramidal neurons was assessed in controls and in rats treated with escitalopram and citalopram for 14 days (Figure 5). WAY 100,635 increased slightly the quisqualate-activated firing activity of CA_3 pyramidal neurons within a dose range of 20–100 μg/kg (i.v.) in control rats (Figure 5). In rats treated with either escitalopram or citalopram for 14 days, WAY 100,635 induced a large and significant increase of the mean firing activity of CA_3 pyramidal neurons, when compared to

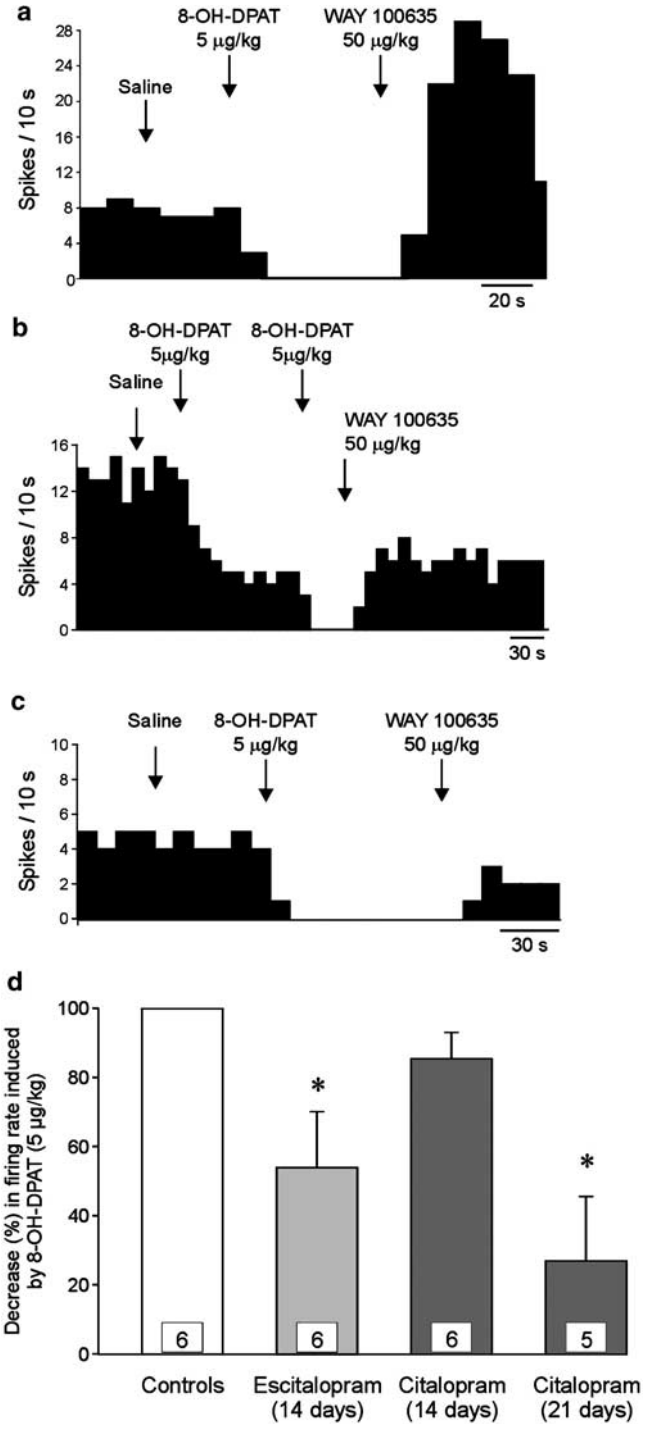

Figure 4 Integrated firing rate histograms of dorsal raphe 5-HT neurons showing their response to 8-OH-DPAT and WAY-100,635 injections in a control rat (a), in rat treated with escitalopram for 14 days (b) and in rat treated with citalopram for 14 days (c). Mean results±SEM is represented in (d). The numbers at the bottom of the columns indicate the number of rats tested (in each rat only one neuron was tested). *$P<0.05$ using one-way ANOVA followed by PLSD Fisher's test.

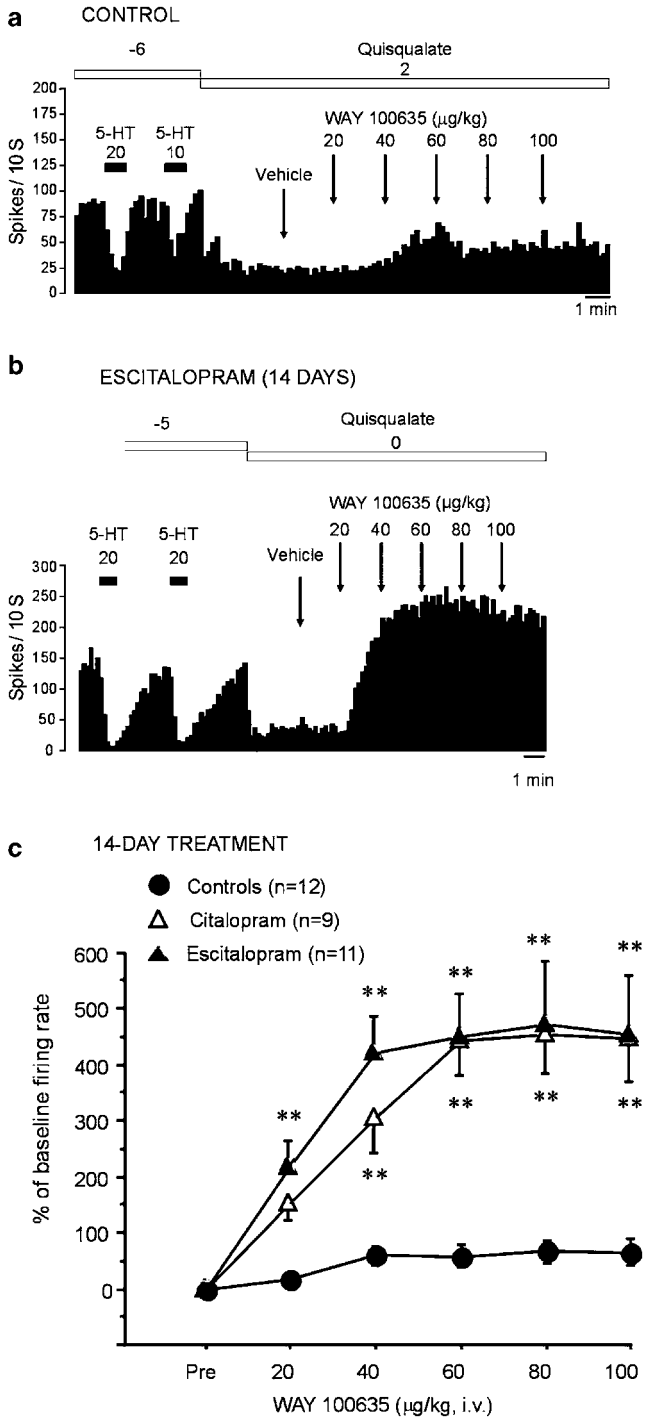

Figure 5 Integrated firing rate histograms of dorsal hippocampus CA_3 pyramidal neurons showing their responsiveness to the microiontophoretic application of 5-HT and i.v. injection of WAY-100,635 in a control rat (a) and a rat treated for 2 weeks with escitalopram (10 mg/kg/day, s.c. (b)). These neurons were activated with quisqualate and horizontal bars indicate the duration of the applications for which the current is given in nanoamperes. Note the increase in firing activity of CA_3 pyramidal neurons after the injection of WAY-100,635. (c) Degree (%±SEM) of increase of the firing activity of CA3 pyramidal neurons in control rats and treated rats with escitalopram and citalopram for 14 days (c) prior to and after the administration of WAY-100,635 (20–100 μg/kg, i.v.). *n* values indicate the number of neurons tested (in each rat only one neuron was tested). $^{**}P<0.01$ using one-way ANOVA followed by PLSD test of Fisher.

control (Figure 5). This significant increase was dose-dependent at 20 and 40 μg/kg and reaching a plateau starting at dose of 60–100 μg/kg, with an overall significantly greater increase after 14-day treatment of escitalopram and citalopram administration than control ($P<0.001$, using one-way ANOVA followed by PLSD test of Fisher). The increase firing activity of these neurons induced by WAY 100,635 was, however, similar in rats treated with escitalopram and citalopram for 14 days (increase of 400–600%, Figure 5).

DISCUSSION

In DRN, the marked decrease of the firing activity of 5-HT neurons followed by recovery after long-term administration of escitalopram and citalopram is in agreement with previous studies using citalopram and other SSRIs (Chaput *et al*, 1986; Blier and de Montigny, 1994). This decrease has been previously reported to result from a greater activation of somatodendritic 5-HT_{1A} autoreceptors (Blier and de Montigny, 1983; Blier *et al*, 1984; Chaput *et al*, 1986; Le Poul *et al*, 1995) due to the increased levels of extracellular 5-HT in the DRN produced by SSRIs (Bel and Artigas, 1992; Hjorth *et al*, 1995; Romero *et al*, 1996). As with other SSRIs, the inhibitory effect 8-OH-DPAT on 5-HT neurons activity was attenuated in treated rats with escitalopram for 2 weeks and citalopram for 3 weeks in the present study, confirming the desensitization of 5-HT_{1A} receptors in DRN (see Blier and de Montigny, 1994). With autoreceptor regulation decreased, there can be a return to normal firing activity and a greater release of 5-HT per impulse, thus leading to an increased serotonergic transmission at 5-HT_{1A} receptors. In the present study, a complete recovery of 5-HT discharge was observed after 14-day treatment with escitalopram but not with citalopram (Figure 3). If the degree of 5-HT_{1A} receptor desensitization is proportional to recovery of the activity of 5-HT neurons, one would expect levels of 5-HT_{1A} receptor desensitization to translate into proportional change in firing activity of 5-HT neurons. Indeed, following 2 weeks of treatment with citalopram, while around 20% of neurons discharged at control rate and had attenuated response to 8-OH-DPAT (data not shown), the majority of 5-HT neurons fired at lower rate than control and presented no desensitized 5-HT_{1A} autoreceptor, most likely explaining the lack of firing recovery of these neurons. In this regard, using *in vitro* recordings in DRN, a progressive increase in the proportion of 5-HT cells with desensitized 5-HT_{1A} autoreceptors was observed with prolonged SSRI administration (Le Poul *et al*, 1995). The authors suggested that rather than the degree of 5-HT_{1A} autoreceptor desensitization *per se* as being the critical parameter with regard to the mean recovery of 5-HT neuronal activity, it may be the number of 5-HT cells with desensitized 5-HT_{1A} autoreceptors that is more important.

The reason for which there was a complete recovery of firing activity of 5-HT neurons following 2-week escitalopram treatment but not with citalopram is still unknown, but may involve additional effects of these SSRIs. It is conceivable that the difference in the time constant of 5-HT firing recovery with escitalopram *vs* citalopram may be due to the presence of R-citalopram attenuating the effect of

escitalopram on the recovery of activity of 5-HT neurons. It was reported that escitalopram mediates citalopram's *in vivo* 5-HT uptake inhibitory activity (Hyttel *et al*, 1992; Owens *et al*, 2001). The present study shows that the inhibitory effect of escitalopram on DRN 5-HT neurons was prevented but not reversed by the administration of R-citalopram, suggesting that for racemate citalopram, the R-citalopram could counteract the effect of escitalopram. Indeed, microdialysis studies have shown that R-citalopram attenuated the augmenting effect of escitalopram on 5-HT release in frontal and prefrontal cortex (Mørk *et al*, 2003; Ceglia *et al*, 2004). In addition, it has been shown that R-citalopram attenuates antidepressant effect of escitalopram in rat chronic mild stress model (Sánchez *et al*, 2003a) and prevents anxiolytic effect of escitalopram in a rat ultrasonic vocalization model (Sánchez, 2003). The mechanism by which R-citalopram prevents the effect of escitalopram on 5-HT transmission is not yet fully elucidated. A putative competitive action between R-citalopram and escitalopram on the 5-HT transporter can be envisaged. Recently, a kinetic interaction of R-citalopram and escitalopram has been suggested to be a critical factor in the antagonistic effects of R-citalopram (Stórustovu *et al*, 2004; for a review see Sánchez *et al*, 2004). Furthermore, it was shown that S- and R-enantiomers stereoselectivity differs for the primary and allosteric sites and that allosteric ligands increase the binding affinity at the primary site by inducing conformational changes (Chen *et al*, 2004).

In hippocampus, the assertion that the inhibitory effect of 5-HT on the firing activity of CA_3 pyramidal neurons is mediated by 5-HT_{1A} receptor subtype, is supported by the observation that the selective 5-HT_{1A} receptor antagonist WAY 100,635 produced marked reduction of the effect of microiontophoretically applied 5-HT (Haddjeri *et al*, 1998a; Besson *et al*, 2000). The sensitivity of postsynaptic 5-HT_{1A} receptors was not changed after 14-day treatment with escitalopram and citalopram, as demonstrated by the ability of 5-HT, when applied microiontophoretically, to inhibit the CA_3 pyramidal neurons to same extent as in control rats (see IT_{50} values). This result is in agreement with previous studies showing that 5-HT_{1A} receptors in the hippocampus are normosensitive in rat treated with SSRIs (Piñeyro *et al*, 1994; Haddjeri *et al*, 1998a) or in 5-HT transporter knockout mice (Mannoury la Cour *et al*, 2001).

Should there be a greater tonic activation of these postsynaptic 5-HT_{1A} receptors, one would expect their blockade by WAY 100,635 to result in a greater enhancement of the baseline firing activity of CA_3 pyramidal neurons (Haddjeri *et al*, 1998a). In the present study, systemic injection of WAY 100,635 increased the firing activity of one-third of hippocampal cells in control rats. Although this disinhibition has not been reported in previous studies (Haddjeri *et al*, 1998a, b; Rueter *et al*, 1998; Besson *et al*, 2000), Kasamo *et al* (2001) have shown that WAY 100,635 administration significantly increased the firing activity of CA_1 pyramidal neurons of the dorsal hippocampus in quiet awake rats. This indicates that in nontreated rats endogenous 5-HT would tonically inhibit the spontaneous firing activity of CA_1 pyramidal neurons through the activation of 5-HT_{1A} receptors. As previously shown with several classes of antidepressants, the selective monoamine oxidase-A inhibitor befloxatone (Haddjeri *et al*, 1998b), the dual 5-HT/noradrenaline reuptake inhibitor, duloxetine (Rueter *et al*, 1998), or the α2-adrenoceptor antagonist mirtazapine and the SSRI paroxetine (Besson *et al*, 2000), the present study shows that sustained treatment with escitalopram and citalopram induced a strong but similar enhancement of the tonic activation of the postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus. One would expect a putatively greater tonic inhibitory activation of 5-HT_{1A} receptors on hippocampal neurons in the presence of greater amount of 5-HT in the extracellular space. In frontal cortex, a greater increase in extracellular 5-HT was observed with escitalopram than citalopram, using microdialysis method (Mørk *et al*, 2003). More importantly, the increase of extracellular 5-HT in prefrontal cortex was greater following 13-day compared to 2-day treatment with escitalopram, but not after citalopram treatment (Ceglia *et al*, 2004). However, the increase of 5-HT level was similar in rats treated for 13 days with escitalopram (10 mg/kg/day) and citalopram (20 mg/kg/day) (Ceglia *et al*, 2004). Although no microdialysis studies were undertaken in hippocampus following long-term administration of these two SSRIs, several other studies have shown that the net impact of different SSRIs on extracellular 5-HT could vary with brain regions, such as between the frontal cortex and hippocampus (Malagié *et al*, 1996; Hervas and Artigas, 1998; see for a review Hjorth *et al*, 2000) depending on their innervation by DRN *vs* median raphe nucleus, respectively. The greater efficacy of escitalopram compared to citalopram in increasing extracellular 5-HT in hippocampus, if any, does not appear to result in differential increase in tonic activation of postsynaptic 5-HT_{1A} receptors, as was shown with other antidepressant, thus suggesting that other mechanisms are involved. On the one hand, this discrepancy could stem from the fact that citalopram may be unique in that it exerts their antidepressant action differently from other SSRIs. In norepinephrine-deficient mice, unlike paroxetine, fluoxetine, and sertraline, citalopram has been shown to partially increase 5-HT levels in the hippocampus and reduce immobility, in tail suspension test in mice, independently of norepinephrine system integrity (Cryan *et al*, 2004). On the other, the regulation of postsynaptic 5-HT_{1A} receptor function in hippocampus may occur at a level distal to the receptor, such as regulation of G protein expression. Indeed, although no change or increase in 5-HT_{1A} receptor-stimulated [^{35}S]GTPγS binding was observed in hippocampus following treatment with fluoxetine (see Hensler, 2003 for a review), changes in hippocampal 5-HT_{1A} receptor-mediated inhibition of adenylyl cyclase activity have been reported following a such treatment (Newman *et al*, 1992).

The precise regulatory mechanism by which SSRIs increase 5-HT neurotransmission is still to be determined. Although complex, the combination of several adaptive changes such as desensitization of the 5-HT_{1A} receptors in the raphe nucleus, amygdala and hypothalamus, normosensitive 5-HT_{1A} receptors in hippocampus and cortex, and the interplay between 5-HT soma and nerve terminal might contribute, at least in part, to the antidepressant responses of SSRIs (for a review see Hjorth *et al*, 2000; Hensler, 2003). Nevertheless, the results obtained from raphe dorsalis suggest that the gradual recovery of normal firing activity of DRN neurons, which was reported to underlie the

delayed effect of SSRI on 5-HT neurotransmission (Blier and de Montigny, 1994), is in accordance with the earlier onset of action of escitalopram *vs* citalopram in animal models of depression and anxiety (Sánchez *et al*, 2003a, b; Fish *et al*, 2004).

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