Altered response to the selective serotonin reuptake inhibitor escitalopram in mice heterozygous for the serotonin transporter: an electrophysiological and neurochemical study

Bruno P. Guiard1,2, Mostafa El Mansari1, Dennis L. Murphy3 and Pierre Blier1

1University of Ottawa Institute of Mental Health Research (IMHR), Ottawa, Ontario, Canada
2Laboratoire de Neuropharmacologie EA3544, Fac Pharmacie, Univ. Paris-sud XI, Chatenay-Malabry, France
3Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA

Abstract

A serotonin (5-HT) transporter (5-HTT; SERT) polymorphism has been associated with depressive states and poor responses to selective serotonin reuptake inhibitors (SSRIs). Given the similar attenuation of SERT activity in SERT+/− mice and in humans with short allele(s) of SERT in its promoter region, it is conceivable that SERT+/− mice offer an adequate model to mimic the human subpopulation with respect to their altered response to SSRIs. This study investigated the effects of the most selective SSRI escitalopram, in heterozygous SERT+/− mice using a combined electrophysiological and neurochemical approach. Results indicated that administration of escitalopram for 2 d resulted in a 72% and 63% decrease in dorsal raphe 5-HT neuronal firing rate in SERT+/+ and SERT+/− mice, respectively. In contrast, administration of escitalopram for 21 d produced a gradual recovery of 5-HT neuronal firing rate to basal level in SERT+/+, but not in SERT+/− mice. In the hippocampus, microdialysis revealed that sustained administration of escitalopram produced a greater increase in extracellular 5-HT ([5-HT]ext) outflow in SERT+/− than in the wild-types with or without a washout of the SSRI. Nevertheless, the ability of microiontophoretically applied 5-HT to inhibit the firing rate of CA3 pyramidal neurons was not different between SERT+/+ and SERT+/− mice given escitalopram for 21 d. The data indicate that the poor response to SSRIs of depressive patients with short allele(s) of SERT is not attributable to a lesser increase in 5-HT transmission in the hippocampus.

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Key words: Depression, electrophysiology, escitalopram, microdialysis, SERT.

Introduction

The serotonin transporter (SERT), a member of the super family of the 12 transmembrane domain sodium-dependent neurotransmitter transporters, regulates serotonin (5-HT) transmission (Blakely et al. 1994). Because 5-HT has been involved in the regulation of mood and anxiety, SERT became the principal molecular target for many drugs commonly used in the treatment of depression, in particular the selective serotonin reuptake inhibitors (SSRIs).

In humans, genetic searches have highlighted a polymorphism in the corresponding promoter region of the SERT gene (Lesch et al. 1996). This gene gives rise to a bi-allelic polymorphism designated long (L) and short (S). There is now strong evidence that S allele confers a greater susceptibility for the development of depression in the presence of stress, as first reported by Caspi et al. (2003) and recently confirmed in a meta-analysis that included all reported studies (Karg et al. 2011). Although post-mortem human tissue and imagery analysis initially failed to detect clear correlations between the distinct genotypes and SERT density (Mann et al. 2000; Parsey et al. 2006), more recent results using the [14C]DASB ligand have documented an increased binding potential of SERT in subjects homozygous for the L allele (Willeit &
Praschak-Rieder, 2010). Functional in-vitro and in-vivo studies of the activity of the SERT gene promoter in human lymphoblast cell lines or platelets showed that the L allele is associated with approximately 2-fold higher levels of transcriptional activity and rate of 5-HT uptake compared with the S variant (Greenberg et al. 1999; Lesch et al. 1996). These findings are consistent with data showing that messenger RNA levels and expression of SERT is higher in healthy humans or in post-mortem brains of LL homozygotes compared to S carriers (Heinz et al. 2000; Juhasz et al. 2003; Little et al. 1998). Taken together, these studies suggest that the L and S alleles of the SERT gene regulate the expression of SERT protein and appear to account for functional differences. Given that SSRIs act by blocking SERT, it was hypothesized that allelic variation of its gene expression could influence the individual response to these antidepressant drugs. To date, the results of 37 pharmacogenetic studies have been reported (Kato & Serretti, 2010) and 18 of them clearly demonstrate that patients with L/L genotype display a better response to SSRIs than S/L individuals either in term of latency to recover or proportions of responders (fluvoxamine: Kim et al. 2000; Pollock et al. 2000; Serretti et al. 2004; Smeraldi et al. 1998, 2006; Zanardi et al. 2000, 2001; paroxetine: Bozina et al. 2008; Ruhé et al. 2009; fluoxetine: Kim et al. 2000; Rausch et al. 2002; Yu et al. 2002; sertaline: Durham et al. 2004; Hong et al. 2006; Ng et al. 2006; citalopram: Arias et al. 2003; escitalopram: Huezo-Díaz et al. 2009; Margoob et al. 2008). This raised the possibility that an altered expression/function of SERT might limit the therapeutic activity of SSRIs. However, the cellular and molecular mechanisms underlying these pharmacogenetic observations remain unknown.

Mice genetically engineered to express reduced amounts of SERT were created more than 10 yr ago (Bengel et al. 1998). In-vitro and in-vivo approaches have reported an altered 5-HT uptake in SERT+−/+ mice compared to their wild-type littermates (Bengel et al. 1998; Montanez et al. 2002; Perez et al. 2006). In particular, a 60% decrease in 5-HT uptake was detected in mice lacking only one copy of the SERT gene (Perez & Andrews, 2005). Consistent with these findings is the observation that the intensity of 5-HT uptake in striatal or hippocampal synaptosomes derived from SERT+−/+ mice is 50% lower than that found in wild-types (Montanez et al. 2003; Perez & Andrews, 2005). In this context, it is possible that heterozygous SERT+−/− mice offer a good model to study the interaction between SERT genotype and SSRI response.

Since the therapeutic effects of SSRIs may be dependent on 5-HT neurotransmission (Delgado et al. 1990), the present in-vivo studies were aimed at determining the firing activity of 5-HT neurons and 5-HT neurotransmission in SERT+−/− mice after long-term administration of the most selective SSRI escitalopram (K values for SERT, NET and DAT: 1.1, 7841 and 27 410 nM, respectively; Owens et al. 2001). Given this pharmacological profile, escitalopram appeared to be the most appropriate SSRI to investigate for the first time the effect of inhibiting 5-HT reuptake in these mutants.

Material and methods

Animals

Adult male SERT+−/+ and SERT+−/− mice with a CD1 x 129 sv background were generated by homologous recombination, as reported previously (Bengel et al. 1998). All of the mice used in the present study were 30-40 g. Mice were kept under standard laboratory conditions [12-h light/dark cycle (lights on 06:00 hours), with free access to food and water] and nesting material was provided. Animals were handled according to the guidelines of the Canadian Council on Animal Care (CCAC) and the local Animal Care Committee (Ottawa Health Research Institute, Canada) approved protocols.

Drugs

Chlortal hydrate, 5-HT creatinine sulfate, quisqualate, KCl and the 5-HT1A receptor antagonist WAY 100635 were purchased from Sigma-Aldrich (Canada). Escitalopram-oxalate was provided by Lundbeck (Denmark) and dissolved in 0.9% NaCl/dimethylsulfoxide (80/20). 5-HT creatinine sulfate and quisqualate were dissolved in NaCl (200 mM) while WAY 100635 was dissolved in distilled water for its subcutaneous administration.

Pharmacological treatments

SERT+−/+ and SERT+−/− mice were anaesthetized with a mixture of isoflurane and oxygen. Subcutaneously implanted osmotic minipumps (models: 1007D and 1002, Alzet, Durect Corporation, USA) were preloaded with escitalopram (10 mg/kg.d) and provided delivery for 0 (controls), 2, 7, 14 or 21 d. Distinct groups of mice were used for these treatment periods. Although all mice were tested without escitalopram washout, one group with minipumps removed 48 h prior to the microdialysis experiments was used to compare the basal extracellular levels of 5-HT ([5-HT]basal) with or without a washout of the SSRI. Control mice were

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submitted to the same surgical process with mini-pumps containing vehicle.

**In-vivo electrophysiological recordings**

Mice were anaesthetized with chloral hydrate (400 mg/kg i.p.) and placed in a stereotaxic frame (using the David Kopf mouse adaptor) with the skull positioned horizontally. The extracellular recordings were performed using single- or five-barrelled glass micropipettes (R&D Scientific Glass, USA) for recordings in the dorsal raphe (DR) and the dorsal hippocampus, respectively. Micropipettes were preloaded with fibreglass strands to promote capillary filling with a 2 M NaCl solution.

**Recording of DR 5-HT neurons**

Single glass micropipettes pulled on a pipette puller (Narishige, Japan) with impedances ranging from 2.5 to 5 mΩ, were positioned 0.2–0.5 mm posterior to the interaural line on the midline and lowered into the DR, usually attained at a depth of 2.5–3.5 mm from the brain surface. The DR 5-HT neurons were then identified according to the following criteria: a slow (0.5–2.5 Hz) and regular firing rate and a long-duration, positive action potential.

**Recording of CA3 dorsal hippocampus pyramidal neurons**

Three-barrelled glass micropipettes were lowered at 2–3 mm lateral and 2.3–2.5 mm posterior to bregma into the CA3 region of dorsal hippocampus (Paxinos & Franklin, 2001). The impedance of the central barrel and of the side-barrels typically ranged from 2 to 5 and 30 to 80 MΩ, respectively. The side-barrels contained either solutions of 5-HT creatinine sulfate (50 mM in NaCl 200 mM; pH 4) or quisqualate (1.5 mM in 400 mM; pH 8). Another barrel used for automatic current balancing was filled with a 2 M NaCl solution. 5-HT was ejected as cations while quisqualate was ejected as anions. To assess the effectiveness of the long-term escitalopram treatment in SERT+/+ and SERT+/- mice, the recovery time 50 (RT50) method was used. The RT50 value has been shown to be a reliable index of *in-vivo* activity of the 5-HT reuptake process in the 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 CA3 pyramidal neurons. Initial studies have shown that RT50 values were higher in SSRI-treated animals than in controls (Pineyro *et al.* 1994). The neuronal responsiveness to 5-HT was assessed using the IT50 method. This parameter 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 IT50 (de Montigny & Aghajanian, 1978). Finally, in order to assess the degree of activation of the post-synaptic 5-HT1A receptors exerting an inhibitory influence on the firing activity of CA3 pyramidal neurons, the selective 5-HT1A receptor antagonist WAY 100635 (50–300 µg/kg) was administered subcutaneously to disinhibit the hippocampal neurons resulting in an increase of their firing activity.

**In-vivo intracerebral microdialysis**

Concentric dialysis probes (0.30 mm outer diameter) were constructed of cuprophane and set up as described previously (Malagie *et al.* 2001). The size of the dialysis membranes was 4 mm long in the hippocampus with an active length of 2 mm. Mice anaesthetized with chloral hydrate (400 mg/kg i.p.), were placed in a stereotaxic frame and implanted with two probes in the hippocampus using the following coordinates (in mm from bregma and top of the skull according to the atlas of Paxinos & Franklin, 2001): AP −2.8, L ± 3.0, V 4. The probes were cemented in place and mice were allowed to recover from the surgery overnight. The next day, 24 h after the surgery, the probes were continuously perfused with an artificial cerebrospinal fluid at a flow rate of 1.5 µl/min, using a CMA/100 pump (Carnegie Medicin, Sweden). After a 2-h equilibration period, dialysate samples were collected every 15 min in Eppendorf tubes and were analysed by high performance liquid chromatography (Hewlett-Packard 1049A amperometric detector, limit of detection for standard 5-HT: 0.5–1 fmol/sample; signal-to-noise ratio = 2). Six 15-min baseline dialysate samples were collected (from t0 to t75). A KCl pulse (120 mM for 15 min) was perfused locally through the dialysis probe. Six subsequent 15-min samples were collected (from 90 to 165 min).

**Statistical analyses**

Electrophysiological data obtained from recordings in the DR were expressed as means ± S.E.M. of the firing rate of 5-HT neurons or number of 5-HT neurons recorded per tract. Electrophysiological data obtained from recordings in the CA3 region of the dorsal hippocampus were expressed as means ± S.E.M. of RT50 or IT50 values. A two-way analysis of variance (ANOVA) with duration of escitalopram treatment (0, 2, 7, 14, 21 d) and genotype (SERT+/+ vs. SERT+/-) as main
factors was performed to compare the firing rate of 5-HT neurons and number of 5-HT neurons recorded per tract. A two-way ANOVA with treatment (vehicle vs. escitalopram) and genotype (SERT+/+ vs. SERT+/−) as main factors was performed to compare RT_{SS} or IT_{SS} values. A two-way ANOVA (genotype × treatment) with repeated measures was applied to analyse the effect of cumulative doses of WAY 100635 on the firing activity of CA3 pyramidal neurons. For microdialysis, the 5-HT contents of dialysate samples were expressed as femtomoles per 20 μl sample. The effects of KCl were measured from area under the curve (AUC) values calculated for the 90- to 165-min period. Statistical analyses were performed using a two-way ANOVA with treatment and genotype as main factors. In all cases, when appropriate, pairwise comparisons were performed using the protected least significance (PLSD) post-hoc test in all experiments using the computer software StatView 4.02. Accepted level of significance was set at p ≤ 0.05.

Results

Effects of short- and long-term administration of escitalopram on the firing rate of 5-HT neurons in SERT+/+ and SERT+/− mice

SERT+/+ and SERT+/− mice were treated for 0, 2, 7, 14 and 21 d with escitalopram (10 mg/kg.d s.c.). Experiments were performed with osmotic minipumps in place. A two-way ANOVA for firing rate of DR 5-HT neurons indicated an overall significant effect of duration of escitalopram treatment [F(4,350) = 20.4; p < 0.001] and genotype [F(1,350) = 22.1, p < 0.001]. A significant interaction between those two independent variables was also detected [F(4,350) = 3.6, p = 0.006].

As described previously (Gobbi et al. 2001), the mean spontaneous firing rate of DR 5-HT neurons was significantly higher in SERT+/+ mice than in SERT+/− littermates (1.6 ± 0.1 vs. 1.1 ± 0.1 Hz, respectively; p < 0.01, Fig. 1). Two days’ treatment with escitalopram resulted in a 72% and 63% decrease in DR 5-HT neuronal firing rate in SERT+/+ (0.47 ± 0.08 Hz, n = 4) and SERT+/− mice (0.41 ± 0.08 Hz, n = 4), respectively (Fig. 1), but no significant differences were noted between both genotypes. In contrast, although sustained treatment with escitalopram (7, 14, 21 d) produced a gradual recovery of DR 5-HT neuronal firing rate to basal level in SERT+/+ mice (0.7 ± 0.1 Hz, n = 4; 0.9 ± 0.05 Hz; n = 3; 1.4 ± 0.1 Hz, n = 4, respectively), it failed to do so in SERT+/− mice (0.7 ± 0.1 Hz, n = 5; 0.6 ± 0.1 Hz, n = 4; 0.7 ± 0.07 Hz, n = 5, respectively). Interestingly, after the 21-d regimen of escitalopram, the firing rate of DR 5-HT neurons in SERT+/− was half of that in SERT+/+ mice (0.7 ± 0.07 vs. 1.4 ± 0.1 Hz, respectively; p < 0.001; Fig. 1). The number of neurons recorded per track in each experimental condition is given in Table 1. This parameter is known as a valid, indirect index of the percentage of neurons that are spontaneously discharging (active) during in-vivo electrophysiological recordings (Bambico et al. 2007).

![Graph](http://ijnp.oxfordjournals.org/)

**Fig. 1.** Comparison of the effects of short- and long-term treatment with escitalopram on the electrophysiological activity of dorsal raphe (DR) 5-HT neurons in SERT+/+ and SERT+/− mice. Data are means (± S.E.M.) of frequency (Hz) of 5-HT neurons recorded in the DR of SERT+/+ and SERT+/− mice administered escitalopram for 0 (controls), 2, 7, 14 and 21 d (3–5 mice per group). * p < 0.05 and *** p < 0.001 indicate significantly different from the corresponding genotype of control mice. ### p < 0.01 and #### p < 0.001 indicate significant differences between both genotypes of mice (n.s., not significant). The numbers within the histograms indicate the number of neurons recorded.
Table 1. Comparison of the effects of short- and long-term treatment with escitalopram on the number of dorsal raphe (DR) 5-HT neurons recorded per tract in SERT^{+/+} and SERT^{+-} mice

<table>
<thead>
<tr>
<th>Number of DR 5-HT neurons recorded per tract</th>
<th>Escitalopram (10 mg/kg, d.s.c.)</th>
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<tr>
<td></td>
<td>Vehicle</td>
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<tr>
<td>SERT^{+/+}</td>
<td>2.5 ± 0.2 (n = 5)</td>
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<tr>
<td>SERT^{+-}</td>
<td>2.4 ± 0.4 (n = 5)</td>
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</table>

n = Number of mice per group.

Although a two-way ANOVA for the number of neurons recorded per tract indicated significant effect of genotype factor [F(1, 33) = 4.4, p < 0.05], pairwise comparison revealed no difference between SERT^{+/+} and SERT^{+-} mice treated with vehicle or escitalopram for 2, 7, 14 or 21 d.

**Effects of WAY 100635 on firing rate of 5-HT neurons in SERT^{+/+} and SERT^{+-} mice treated with escitalopram for 21 d**

To determine whether sustained administration of escitalopram resulted in increased activation of 5-HT_{1A} autoreceptors by endogenous 5-HT, the firing rate of DR 5-HT neurons in SERT^{+/+} and SERT^{+-} mice was determined before and after injection of WAY 100635. A two-way ANOVA for firing rate of DR 5-HT neurons indicated an overall significant effect of treatment [F(1, 61) = 20.2, p < 0.001] but not of genotype [F(1, 61) = 0.41, p = 0.52]. However, a significant interaction between those two independent variables was detected [F(1, 61) = 5.35, p < 0.05]. In these experiments, the firing rates of DR 5-HT neurons in SERT^{+-} mice returned to the level observed in SERT^{+/+} mice after injection of WAY 100635 in both groups (Fig. 2).

**Assessment of SERT and 5-HT_{1A} receptor functions in the hippocampus of SERT^{+/+} and SERT^{+-} mice treated with escitalopram for 21 d**

Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, a variable ejection of quisqualate current (from -1 to -5 nA) was used to activate them within their physiological firing rate (8–15 Hz). To test the activity of the 5-HT reuptake process in the hippocampus, the recovery time, from the suppression of pyramidal neuron firing activity after microiontophoretic application of 5-HT was assessed by determining RT_{50} values in both genotypes after the sustained administration of escitalopram (without a washout period) or vehicle (Fig. 3). A two-way ANOVA for RT_{50} values indicated an overall significant effect of treatment [F(1, 25) = 85.6, p < 0.001] and genotype [F(1, 25) = 24.2, p < 0.001]. A significant interaction between those two independent variables was also detected [F(1, 25) = 16.8, p < 0.001]. In agreement with previous data (Gobbi et al. 2001), the RT_{50} values were not different.
between vehicle-treated SERT+/+ and SERT+/− mice (23 ± 2, ns. 28 ± 6 s, p = 0.45; Fig. 3e). Sustained administration of escitalopram significantly prolonged the mean RT50 value for 5-HT in SERT+/+ (52 ± 5 s, p < 0.01, Fig. 3e) and SERT+/− (102 ± 6 s, p < 0.001, Fig. 3e) mice compared to the corresponding control.
group of mice treated with vehicle. Interestingly, the $RT_{2s}$ values were significantly higher in SERT $^{+/−}$ than in SERT $^{+/+}$ ($p<0.001$) mice after sustained administration of escitalopram (Fig. 3a–e).

To test the sensitivity of post-synaptic 5-HT$_{1A}$ receptors, the $RT_{2s}$ values were determined. A two-way ANOVA for $RT_{2s}$ value indicated no significant effect of treatment [$F(1,25) = 1.9$, $p = 0.1$] and genotype [$F(1,25) = 0.6$, $p = 0.4$] factor. No significant interaction between those two independent variables was detected [$F(1,25) = 0.009$, $p = 0.9$]. Thus, escitalopram did not modify the suppressant effect of microiontophoretically applied 5-HT on the firing activity of CA3 pyramidal neurons in both SERT $^{+/+}$ and SERT $^{+/−}$ mice compared to the corresponding control group of mice treated with vehicle. No statistical difference was detected between SERT $^{+/+}$ and SERT $^{+/−}$ mice after administration of vehicle or escitalopram for 21 d (Fig. 3a–d, f) indicating that post-synaptic 5-HT$_{1A}$ receptors are normosensitive in mutants. Interestingly, at the end of each recording, the effect of WAY 100635 on the responsiveness of CA3 dorsal hippocampus pyramidal neurons to microiontophoretically applied 5-HT was examined in both genotypes. A two-way ANOVA on the number of spikes suppressed/nA indicated an overall significant effect of treatment [$F(1,20) = 11.9$, $p<0.001$] but not of genotype [$F(1,20) = 0.6$, $p = 0.5$]. No interaction between those two independent variables was detected [$F(1,20) = 0.02$, $p = 0.95$]. Although WAY 100635 significantly decreased the inhibitory response to 5-HT in SERT $^{+/+}$ ($p<0.05$) and SERT $^{+/−}$ ($p<0.05$) mice, no difference between genotypes was observed ($p>0.05$, Table 2) confirming the lack of change in post-synaptic 5-HT$_{1A}$ receptor sensitivity.

Finally, the ability of WAY 100635 to disinhibit neuronal firing activity of CA3 pyramidal neurons in both SERT $^{+/+}$ and SERT $^{+/−}$ mice treated with vehicle or escitalopram for 21 d was determined. A two-way ANOVA with repeated measures on the changes of baseline firing rates indicated an overall significant effect of genotype [$F(1,25) = 9.37$, $p<0.01$] and treatment [$F(1,25) = 27.02$, $p<0.001$]. No significant interaction between those three independent variables was detected [$F(1,25) = 2.01$, $p = 0.16$]. WAY 100635 similarly increased the mean firing rate of CA3 pyramidal neurons in vehicle-injected SERT $^{+/+}$ and SERT $^{+/−}$ mice (197 ± 17 vs. 255 ± 39% at the highest dose tested, $p = 0.16$; Fig. 3g). Sustained administration of escitalopram significantly enhanced the ability of WAY 100635 (150–300 µg/kg) to increase the mean spontaneous firing rate of CA3 pyramidal neurons of SERT $^{+/+}$ and SERT $^{+/−}$ mice compared to the corresponding control group of mice treated with vehicle (332 ± 26 vs. 197 ± 17%, $p<0.01$, and 485 ± 47 vs. 255 ± 39%, $p<0.01$, at the highest dose tested respectively). It is noteworthy that this increase in firing activity was significantly greater in SERT $^{+/−}$ and SERT $^{+/+}$ mice treated with escitalopram for 21 d ($p<0.01$, Fig. 3a–d, g).

**Neurochemical assessment of basal levels and KCl-evoked release of dialysate 5-HT in SERT $^{+/+}$ and SERT $^{+/−}$ mice treated with escitalopram for 21 d**

A two-way ANOVA on basal [5-HT]$_{ext}$ in the hippocampus with and without a SSRI washout indicated an overall significant effect of genotype [$F(1,41) = 27.9$, $p<0.001$] and treatment [$F(2,41) = 33.5$, $p<0.001$]. No significant interaction between those two independent variables was detected [$F(2,41) = 1.3$, $p = 0.05$]. The mean basal [5-HT]$_{ext}$ in the hippocampus, measured either 48 h after removal (washout) or in the presence (no washout) of osmotic minipumps, was significantly higher in SERT $^{+/−}$ than in SERT $^{+/+}$ (treated with escitalopram for 21 d; $p = 0.008$ and $p = 0.002$, respectively; Fig. 4).

The effects of constitutive reductions in SERT expression on evoked 5-HT release were then investigated. An immediate increase in hippocampal [5-HT]$_{ext}$ was observed in each group of mice after the local perfusion of KCl through the microdialysis probe (120 mm for 15 min, Fig. 5a, b). In SERT $^{+/+}$ mice that had received vehicle or escitalopram for 21 d or in SERT $^{+/−}$ mice administered with vehicle, KCl produced an increase in [5-HT]$_{ext}$ that returned to

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**Table 2. Effects of the 5-HT$_{1A}$ receptor antagonist WAY 100635 on the responsiveness of CA3 dorsal hippocampus pyramidal neurons to 5-HT in SERT $^{+/+}$ and SERT $^{+/−}$ mice treated with escitalopram for 21 d**

<table>
<thead>
<tr>
<th></th>
<th>Before WAY 100635</th>
<th>After WAY 100635</th>
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<tbody>
<tr>
<td>SERT $^{+/+}$</td>
<td>4.1 ± 0.6</td>
<td>2.3 ± 0.4 (n = 7)*</td>
</tr>
<tr>
<td>SERT $^{+/−}$</td>
<td>3.6 ± 0.3</td>
<td>1.7 ± 0.4 (n = 5)*</td>
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</table>

$n =$ Number of mice per group.

Data are means (±S.E.M.) of number of spikes suppressed/nA induced by microiontophoretically applied 5-HT on hippocampus pyramidal neurons of SERT $^{+/+}$ and SERT $^{+/−}$ mice before and after WAY100635 (300 µg/kg s.c.) administration. *$p<0.05$: significantly different compared with the number of spikes suppressed/nA before the administration of WAY 100635.

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**Altered response to escitalopram in SERT mice**

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The lower spontaneous firing rate of 5-HT neurons (percentage of baseline) are calculated for a 120-min period. Basal extracellular levels of 5-HT in the hippocampus (Fig. 5) were determined with and without escitalopram washout. Data are means (± S.E.M.) of extracellular 5-HT concentrations, expressed as femtomoles per 20 μl. Areas under the curve (percentage of baseline) are calculated for a 120-min period. *** p < 0.001 indicates significantly different from the corresponding genotype of mice treated with vehicle. * p < 0.05 and ** p < 0.01 indicate significant differences between both genotypes of mice. Data are not corrected for the in-vitro probe recovery. The numbers within the histograms indicate the number of mice.

In basal conditions, SERT+/− mice were previously shown to display a reduced spontaneous neuronal firing activity of DR 5-HT neurons (Gobbi et al. 2001). Such an alteration was confirmed in the present study, as evidenced by the 30% decrease in DR 5-HT neuronal activity in SERT+/− mice compared to wild-types. These electrophysiological data in SERT+/− mice have been hypothesized to result from an increase in [5-HT]ext, particularly in the vicinity of the soma of 5-HT neuronal cell bodies. This, however, has never been reported in the DR. The present data showed no effect of the genotype on basal hippocampal [5-HT]ext as previously reported in the striatum and frontal cortex (Mathews et al. 2004), thus suggesting that the partial loss of SERT was not sufficient to alter the homeostasis of the serotonergic system at nerve terminals. In keeping with this hypothesis, the time for CA3 pyramidal neurons to recover 50% of their initial firing frequency in response to local application of 5-HT, a parameter reflecting the degree of SERT inhibition (Pineyro et al. 1994), were similar in SERT+/+ and SERT+/− mice. Nevertheless, in an attempt to determine whether subtle, but biologically significant, alterations in [5-HT]ext occur in SERT+/− mice, the method of zero net flux (ZNF) has been employed. In these experimental conditions, increases in basal striatal and cortical [5-HT]ext have been detected in SERT+/− mice (Mathews et al. 2004). Consequently, in the present study the fact that basal hippocampal [5-HT]ext in SERT+/+ and SERT+/− mice were similar, does not necessarily mean an absence of differences between these groups. Higher [5-HT]ext in SERT+/− mice was expected given the primary function of SERT to clear 5-HT from the extracellular space but, also taking into consideration the desensitization of somatodendritic 5-HT1A receptors reported in SERT+/− mice (Fabre et al. 2000; Gobbi et al. 2001).
It can appear surprising that despite an approximately 50% reduction of the sensitivity of 5-HT$_1$A autoreceptors in SERT$^+/x$ mice (Gobbi et al. 2001), the mean basal firing rates of 5-HT neurons was still attenuated. One explanation could be that such a degree of 5-HT$_1$A autoreceptor desensitization is not sufficient to prevent the attenuation of DR 5-HT neuronal firing induced by a putative local excess of endogenous 5-HT. It is important to note that the desensitization of somatodendritic 5-HT$_1$A autoreceptors previously reported in SERT$^+/x$ mice, is consistent with recent clinical data showing a lower 5-HT$_1$A receptor binding in the raphe in S/S or S/L genotypes compared to individuals with L/L genotypes (David et al. 2005).

As previously demonstrated in rats with a normal expression of SERT, the subchronic administration of SSRIs in SERT$^{+/+}$ mice decreased the firing rate of DR 5-HT neurons while prolonged administration is associated with a progressive recovery to normal activity (Blier & de Montigny, 1983). The most marked result obtained herein was the observation that 5-HT neurons did not even regain their baseline firing activity in SERT$^{+/−}$ mice after administration of escitalopram for 21 d, suggesting that both copies of the SERT gene are necessary for antidepressants to produce their optimal effects on firing rate recovery. Since the time-course for this recovery in rodents is congruent with the onset of therapeutic activity of SSRIs in depressed patients (see Blier & de Montigny, 1994), it is possible that such electrophysiological alteration in SERT$^{+/−}$ mice may be relevant to the lesser or delayed SSRI response observed in S/L or S/S patients (Smeraldi et al. 1998; Zanardi et al. 2000).

Another interesting parallel between the present preclinical findings in SERT$^{+/−}$ mice and clinical data in S/L and S/S patients is the fact that WAY 100635 reversed to normal levels the firing rates of 5-HT neurons in SERT$^{+/−}$ mice chronically treated with escitalopram. Similar conclusions have been reported in clinical data showing that S/L depressive patients exhibited a poor response to the SSRI fluvoxamine while the addition of the non-selective 5-HT$_1$A antagonist pindolol ameliorated the rate of response in these individuals (Smeraldi et al. 1998; Zanardi et al. 2001).

The functional consequences of these electrophysiological differences in the DR between SERT$^{+/−}$

![Graph](image-url)
and SERT+/+ mice chronically treated with escitalopram were then examined at post-synaptic levels in the hippocampus. As mentioned previously, although conventional microdialysis is not the best method to unveil subtle changes in basal [5-HT]ext, a significant higher hippocampal 5-HT outflow was observed in SERT+/− mice that had received escitalopram for 21 d compared to the corresponding group of wild-type-treated mice. Such neurochemical differences at nerve terminals may be explained by a more pronounced desensitization of 5-HT1A autoreceptors in SERT+/− escitalopram-treated mice than that observed in untreated SERT+/− mice (Gobbi et al. 2001), thus effectively reducing their inhibitory feedback on 5-HT neurotransmission. However, this hypothesis does not explain the observation that the firing rate of DR 5-HT neurons is lower in SERT+/− mice than in wild-types after 21-d escitalopram treatment. In microdialysis experiments, changes in [5-HT]ext reflect changes not only in the release of the neurotransmitter from nerve endings, but also in its reuptake and metabolic degradation (Guiard et al. 2006). The present study assessed whether the effect of a direct perfusion of KCl into the hippocampus, causing neuronal depolarization and exocytotic release of 5-HT, was modified in escitalopram-treated SERT+/+ and SERT+/− mice. As previously demonstrated in the striatum (Mathews et al. 2004), KCl enhanced 5-HT release at the same extent in the hippocampus of control SERT+/+ and SERT+/− mice. However, although sustained administration of escitalopram failed to potentiate KCl-evoked 5-HT release in the hippocampus of SERT+/+ mice, an increased effect was detected in escitalopram-treated SERT+/− mice. As expected, these data suggest that the combination of partial genetic and pharmacological inactivation of SERT produced additive effects. Considering the fact that SERT+/− mice treated with escitalopram for 21 d displayed a reduced 5-HT reuptake and a probable desensitization of 5-HT1A autoreceptors, central serotonergic transmission would likely be enhanced in these mice more than in their SERT+/+ littermates as reported herein.

In a final series of experiments, the present study compared the tonic activation of post-synaptic 5-HT1A receptors in SERT+/+ and SERT+/− mice chronically treated with escitalopram. It is well established that the suppression of the firing activity of CA3 pyramidal neurons by microiontophoretically applied 5-HT is mediated through the activation of 5-HT1A receptors. If a tonic activation of post-synaptic 5-HT1A receptors exits, the firing activity of hippocampus CA3 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 100635 will disinhibit the CA3 pyramidal neurons resulting in an increase of their firing activity. In SERT+/− mice, the sustained administration of escitalopram increased the tonic activation of hippocampal post-synaptic 5-HT1A receptors, as evidenced by the enhancing effect of WAY 100635 on the firing rate of CA3 pyramidal neurons. This effect, previously reported in rats chronically treated with various antidepressants (Haddjeri et al. 1998) including SSRIs (El Mansari et al. 2005), is believed to reflect an increased synaptic 5-HT concentration in the dorsal hippocampus. In agreement with microdialysis findings reported in the present work (i.e. a higher [5-HT]ext in the hippocampus of SERT+/− mice treated with escitalopram), a higher tonic activation of post-synaptic 5-HT1A receptors was also detected in SERT+/− mice treated with escitalopram. Thus, a higher accumulation of 5-HT in the hippocampus and the subsequent higher activation of post-synaptic 5-HT1A receptors were achievable despite the decreased firing rate of DR 5-HT neurons in these mice. Since post-synaptic 5-HT1A receptors have been involved in the antidepressant/anxiolytic-like effect of SSRIs (Berrocoso & Mico, 2009; Gross et al. 2002; Redrobe et al. 1996), the possibility that their functional desensitization occurred specifically in SERT+/− mice treated with escitalopram was investigated. Indeed, it is conceivable that the poor response to SSRIs of depressive patients with S/L or S/S genotypes results from a desensitization of this receptor subtype at the post-synaptic level. However, in the present study, the observation that the local application of 5-HT similarly reduced the firing rate of CA3 pyramidal neurons in SERT+/+ and SERT+/− mice treated with escitalopram for 21 d does not support this hypothesis. It thus appears that sustained administration of escitalopram desensitized presynaptic, but not post-synaptic 5-HT1A receptors as previously reported (Riad et al. 2004). Such adaptive regulations may be attributable to regional differences in the coupling of these receptors to G proteins (Mannoury la Cour et al. 2001). Consequently, the possibility that other(s) post-synaptic 5-HT receptor(s), in other brain regions in SERT+/− mice, are involved in the antidepressant response to SSRIs should be the subject of future investigations. Among these post-synaptic 5-HT receptor(s), 5-HT1A/C subtypes may participate in attenuating the effects of escitalopram. Indeed, evidence suggest that their activation can lead to a greater inhibition of the firing of norepinephrine and dopamine neurons (Boothman & Sharp, 2005; Di Matteo et al. 2000; Dremencov et al. 2009; Szabo & Blier, 2002).
Since enhanced norepinephrine and dopamine transmission can contribute to antidepressant responses (see El Mansari et al. 2010), their dampening in ‘S’ carriers treated with SSRIs, may diminish their therapeutic benefit.

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References


