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The allosteric citalopram binding site differentially interferes with neuronal firing rate and SERT trafficking in serotonergic neurons

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Abstract
Citalopram is a clinically applied selective serotonin re-uptake inhibitor for antidepressant pharmacotherapy. It consists of two enantiomers, S-citalopram (escitalopram) and R-citalopram, of which escitalopram exerts the antidepressant therapeutic effect and has been shown to be one of the most efficient antidepressants, while R-citalopram antagonizes escitalopram via an unknown molecular mechanism that may depend on binding to a low-affinity allosteric binding site of the serotonin transporter. However, the precise mechanism of antidepressant regulation of the serotonin transporter by citalopram enantiomers still remains elusive. Here we investigate escitalopram's acute effect on (1) serotonergic neuronal firing in transgenic mice that express the human serotonin transporter without and with a mutation that disables the allosteric binding site, and (2) regulation of the serotonin transporter's cell surface localization in stem cell-derived serotonergic neurons. Our results demonstrate that escitalopram inhibited neuronal firing less potently in the mouse line featuring a mutation that abolishes the function of the allosteric binding site and induced serotonin transporter internalization independently of the allosteric binding site mechanism. Furthermore, citalopram enantiomers dose-dependently induced serotonin transporter internalization. In conclusion, this

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study provides new insight into antidepressant effects exerted by citalopram enantiomers in presence and absence of a functional allosteric binding site.

1. Introduction

According to the WHO, 350 million people of all ages suffer from major depressive disorders (MDD) and with annually 1 million patients committing suicide, MDD is the second leading cause of death among 15–29 year old people (WHO fact sheet No 369). Based on the projections of a steadily increasing numbers of patients, MDD will be the most common disease by the year 2030 (Mathers and Loncar, 2006). Currently, established antidepressant treatments display only a partial therapeutic effect and roughly a third of all patients suffer from chronic and refractory forms of MDD (Berton and Nestler, 2006; Gibbons et al., 2012). The main approach in antidepressant treatment is to enhance extracellular, bioactive concentrations of serotonin (5-hydroxytryptamine, 5-HT). One way to achieve this goal is the use of selective serotonin re-uptake inhibitors (SSRIs) that bind to cell surface-located serotonin transporter (SERT) and thereby inhibit 5-HT re-uptake and prolong serotonergic signaling (Haenisch and Bonisch, 2011; Morrissette and Stahl, 2014). SERT itself not only plays an essential role in serotonergic neurotransmission, as its activity determines the efficiency of serotonergic neurotransmission, but is also an easily accessible molecular target for clinically effective antidepressants as well as for drugs of abuse (Blakely and Baumann, 2000; Canli and Lesch, 2007; Murphy and Lesch, 2008; Schloss and Williams, 1998). Citalopram is one of several clinically applied SSRIs and consists of a racemic mixture of an S(+)-enantiomer (commonly also named escitalopram), which is clinically superior to most SSRIs including citalopram (Cipriani et al., 2009; Kennedy et al., 2006) and an R(−)-enantiomer (R-citalopram) (Sanchez, 2006). Of these two, escitalopram is implied to drive the antidepressant effect by inhibition of 5-HT uptake, whereas R-citalopram is thought to antagonize escitalopram's antidepressant effect. Indeed, of both enantiomers, escitalopram displays a 50 fold higher affinity to SERT as compared to R-citalopram (Chen et al., 2005; Sanchez, 2006; Zhong et al., 2009). On the molecular level, R-citalopram's antagonistic effect is assumed to depend on the binding to the low-affinity allosteric binding site which affects escitalopram binding to the high-affinity orthosteric binding site. In absence of R-citalopram, escitalopram binds to the allosteric binding site and thereby enhances its own binding to the orthosteric binding site (Plenge et al., 2007; Sanchez, 2006; Zhong et al., 2012a, 2012b). Then, this results in a stronger inhibition of 5-HT uptake. R-citalopram interferes with this process by interacting on the low-affinity allosteric binding site rather than competing for binding at the orthosteric site. Animal studies demonstrated that R-citalopram is able to antagonize escitalopram's antidepressant action in chronic mild stress paradigms (Sanchez et al., 2003), to abolish escitalopram-enhanced serotonergic signaling (Mansari et al., 2007; Mork et al., 2003), and to suppress acute escitalopram-mediated neuronal firing of dorsal raphé serotonergic neurons (Mansari et al., 2007; Mnie-Filali et al., 2007). One explanation for this phenomenon is that R-citalopram may interfere with escitalopram binding to SERT (Sanchez, 2006; Zhong et al., 2012a). However, respective findings for the human SERT are contradictory to the observations in animal studies. Various tomography studies showed that R-citalopram rather binds to human SERT (Klein et al., 2006; Lundberg et al., 2007) and that an antagonizing effect most likely is caused by chronic citalopram treatment (Klein et al., 2007).

The partial contradictory results may be caused by distinct regulation of rodent and human SERT (discussed in Jacobsen et al., 2014). Motivated by overcoming generalized pharmacology between species, Marc Caron and colleagues generated humanized mice expressing the human SERT gene. In doing so, they were able to pharmacologically characterize human SERT using methods restricted to animal models (Jacobsen et al., 2014). Their pharmacological analysis of wild type human SERT mice and mice carrying a mutation disabling the function of the allosteric binding site in human SERT revealed that the allosteric binding site may not be important for the inhibition of human SERT and that R-citalopram may not directly affect the escitalopram-mediated enhancement of serotonergic signaling (Jacobsen et al., 2014). However, the molecular mechanism how R-citalopram diminishes antidepressant effects induced by escitalopram still remain puzzling. Very recently, an elegant and important study on X-ray crystallographic structures of human SERT reveals that occupancy of the allosteric site sterically hinders ligand unbinding from the central site, providing a key explanation for the action of escitalopram as an allosteric ligand (Coleman et al., 2016).

To contribute to the understanding of the racemic regulation of SERT and thereby to the mechanisms triggered by citalopram enantiomers in antidepressant treatment, we have investigated for the first time the acute effect of SSRIs treatment on (1) neuronal firing activities of serotonergic neurons in transgenic mice that express the human SERT with and without a functional allosteric binding site (Jacobsen et al., 2014), and (2) the SSRI-mediated redistribution of human SERT after prolonged treatment on the cell surface of serotonergic neurons derived from mouse embryonic stem cells of the transgenic mouse lines. The present study provides new insights into the action of citalopram enantiomers and how they influence serotonergic neurotransmission via regulation at SERT *in vivo* and *in vitro*.

2. Experimental procedures

2.1. Drugs and reagents

During the experiments animals were intravenously (i.v.) injected and *in vitro*-generated serotonergic neurons and were incubated