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Commentary

Consideration of allosterism and interacting proteins in the physiological functions of the serotonin transporter

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ABSTRACT

The serotonin transporter (SERT) functions to transport serotonin (5-HT) from the extracellular space into neurons to maintain homeostatic control of 5-HT. It is the molecular target for selective serotonin reuptake inhibitor (SSRI) antidepressants. Preclinical research has shown that some SERT inhibitors can bind to two distinct binding sites on the SERT, a primary high affinity binding site and a low affinity allosteric binding site. Mutational studies of the SERT and computational modeling methods with escitalopram resulted in the identification of key amino acid residues important for the function of the allosteric binding site. While this allosteric binding site appears to influence the clinical efficacy of escitalopram under physiological conditions, the molecular mechanism of this effect is still poorly understood and may involve a large network of protein–protein interactions with the SERT. Dynamic interfaces between the SERT and the SERT interacting proteins (SIPs) potentially influence not only the SERT on its uptake function, its regulation, and trafficking, but also on known as well as yet to be identified non-canonical signaling pathways through SIPs. In this commentary, we outline approaches in the areas of selective small-molecule allosteric compound discovery, biochemistry, *in vivo* genetic knock-in mouse models, as well as computational and structural biology. These studies of the intra-molecular allosteric modulation of the SERT in the context of the myriad of potential inter-molecular signaling interactions with SIPs may help uncover unknown physiological functions of the SERT.

1. Introduction

The serotonin transporter (SERT) is a member of the SLC6 family of neurotransmitter transporters that is structurally comprised of 12 transmembrane (TM) spanning domains and is responsible for the uptake of serotonin (5-HT) from the extrasynaptic space into presynaptic terminals. The SERT serve as a symporter, requiring ions including Na^+, K^+ and Cl^-, that are used as the driving force for transport. The SERT is a target for many of the currently used antidepressant drugs including the selective serotonin reuptake inhibitors (SSRIs).

SSRIs bind to the primary high affinity site, or orthosteric site, of the SERT to inhibit its uptake function, leading to increased extracellular levels of 5-HT. Although this has been regarded as the primary basis for the therapeutic actions of SSRIs, many questions related to the physiological and pathophysiological roles of the SERT as well as its exact role in antidepressant and analgesic treatment remain unanswered. The acute increase in 5-HT caused by SSRIs is not translated into immediate antidepressant effects. Traditional antidepressants, including SSRIs, require at least 2 or more weeks to demonstrate their therapeutic effects. The delayed onset of antidepressant therapeutic effect suggests that adaptive neuronal changes in the brain in addition to elevation of extracellular 5-HT levels are required for treating depression [1–3], but the precise mechanisms underlying this neuroadaptive process are far from well understood and are beyond the aim of this commentary. In this paper, we review the current findings suggesting that the overall machinery responsible for the 5-HT reuptake process by the SERT is more complex than previously anticipated. Firstly, we describe the intra-molecular evidence in the SERT indicating that allosteric ligand interactions may influence the reuptake mechanism and potentially account, at least in part, for some functional differences among SSRIs *in vivo*. Secondly, we discuss data from protein interaction studies at the inter-molecular level and demonstrate that the SERT carries out its main re-uptake function by concomitantly interacting with an extensive cadre of intracellular accessory signaling and scaffolding proteins. This property may not only affect the cellular processing of the SERT but also regulate its function through intracellular signaling cascades.

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2. Allosteric interactions in the SERT

Allosteric mechanisms are a common molecular theme for signal transduction by pharmacological ligands [4–6]. Allosteric binding properties of the SERT were initially reported in imipramine binding experiments [7] and allosteric activities were subsequently demonstrated for all three monoamine transporters [8,9]. Although several allosteric SERT modulators have been reported [10,11], most studies on the characterization of allosteric interactions within the SERT have been described using escitalopram.

Escitalopram is the S-enantiomer of racemic citalopram, which also contains an equal amount of the presumed non-therapeutic enantiomer, R-citalopram [12]. Escitalopram is more active than R-citalopram for its interactions with the SERT, the respective K_i values being 0.8 (S) and 62 nM (R) [13]. Based on preclinical *in vivo* and *in vitro* studies, R-citalopram counteracts the actions of escitalopram without pharmacokinetic interactions [12,14–16]. For example, in a microdialysis study using freely moving rats, extracellular 5-HT levels in the frontal cortex after escitalopram administration showed a greater maximal increase than with citalopram treatment [17]. R-citalopram alone did not affect the 5-HT levels, but counteracted the escitalopram-induced 5-HT increase when co-administered [17]. Similarly, R-citalopram blocked the effect of escitalopram in its potentiation of 5-hydroxytryptophan (5-HTP)-induced behavior in a dose-related manner, while it did not influence the effects of fluoxetine [18]. In a rat chronic mild stress model of depression, escitalopram showed a faster response than citalopram, and this effect was counteracted by R-citalopram [19].

A postulated hallmark for the need of a 2–3 week treatment before the onset of antidepressant action is 5-HT_{1A} receptor desensitization, which can be shown as a recovery of neuronal firing in the dorsal raphe [1,15]. This firing rate recovery is evident after 2 weeks of treatment with escitalopram, but requires a 3-week period when racemic citalopram is used [15]. Subsequently, Mnie-Filali et al. showed that the faster recovery of 5-HT neuronal firing and increased hippocampal neurogenesis elicited by escitalopram were prevented by a co-treatment with R-citalopram [16]. The faster neuroadaptive changes caused by escitalopram compared to other SSRIs may be due to the higher 5-HT levels elicited by escitalopram [14,20]. The enhanced efficacy of escitalopram and the antagonistic effect of R-citalopram on escitalopram are thought to be mediated via interactions at an allosteric binding site on the SERT.

The allosteric binding activity of escitalopram was initially demonstrated by its ability to delay the dissociation of labeled escitalopram from the primary binding site, thus increasing the affinity of the latter [21,22]. Escitalopram and R-citalopram do not appear to show as greater degree of stereoselectivity in this allosteric effect as in the primary site binding, since the EC_{50} values of the two compounds in slowing the dissociation rate of escitalopram are approximately 5 and 25 μM, respectively [13,21]. Given the micromolar concentrations required to demonstrate the allosteric effect as compared to the high affinity primary site binding, its physiological role remains to be understood. It is possible that under *in vivo* conditions, escitalopram may be able to induce SERT conformational changes through its allosteric site with greater sensitivity than under *in vitro* situations. Nonetheless, based on this characterization, escitalopram, paroxetine and R-citalopram have been shown to have allosteric activities, while many other SERT inhibitors including fluoxetine are devoid of allosteric effects [22]. Yet additional evidence corroborates the presence of an allosteric interaction at the SERT. R-citalopram at concentrations (below 100 nM) that are similar to those obtained after therapeutic doses of citalopram [23], can attenuate the association rate of [^{3}H] escitalopram binding to the SERT through an allosteric mechanism [20]. Therefore, escitalopram may elicit a more complete inhibition of 5-HT reuptake due to its dual activity at the primary and allosteric binding sites, leading to higher extracellular 5-HT levels *in vivo* and more rapid 5-HT_{1A} autoreceptor desensitization, and hence greater efficacy and faster onset of action than citalopram [1,14,15].

The existence of an allosteric site has also been corroborated by structural studies showing that key amino acid residues influence primary site versus allosteric binding. These structural studies including mutational and computational methods have been further aided by the crystal structures of the bacterial homolog of the SERT, the leucine transporter [24,25]. The schematic Fig. 1 summarizes these residues with their differential roles. For SSRIs, the primary binding site is likely to be within the deep substrate binding pocket in the SERT, which involves S545, Y95, and I172 residues [26,27]. Escitalopram fits well in this pocket in a reversed

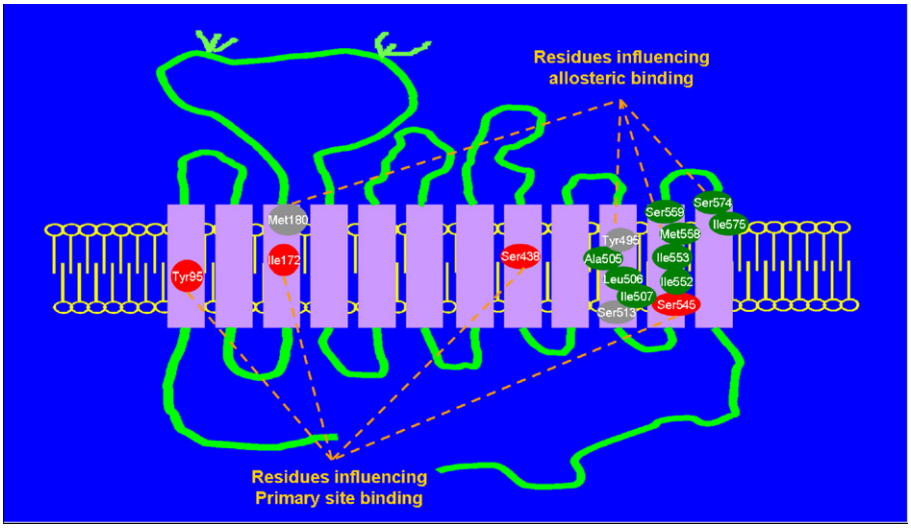

Fig. 1. Schematic representation of the amino acid residues influencing allosteric and primary site binding of the SERT. Allosteric binding can be modulated by the following amino acid residues within TMs 10, 11 and 12 of the SERT: Ala505, Leu 506, Ile507, Ile552, Ile553, Met558, Ser559, Ser574 and Ile575 (labeled in green) [31]. Other SERT residues within TMs 3 and 10, Met180, Tyr495 and Ser513 (labeled in grey), can also partially influence allosteric binding [21]. In comparison, the primary binding site is influenced by a different set of residues, represented by Tyr95, Ile172, Ser438, Ser545 on TMs 1, 3, 8, and 11 (labeled in red) [26,27,30]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)