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Revisiting Serotonin Reuptake Inhibitors and the Therapeutic Potential of "Uptake-2" in Psychiatric Disorders

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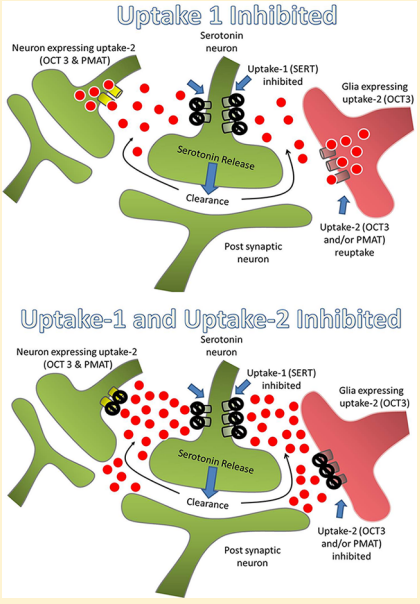
ABSTRACT: Depression is among the most common psychiatric disorders, and in many patients a disorder for which available medications provide suboptimal or no symptom relief. The most commonly prescribed class of antidepressants, the selective serotonin reuptake inhibitors (SSRIs), are thought to act by increasing extracellular serotonin in brain by blocking its uptake via the high-affinity serotonin transporter (SERT). However, the relative lack of therapeutic efficacy of SSRIs has brought into question the utility of increasing extracellular serotonin for the treatment of depression. In this Viewpoint, we discuss why increasing extracellular serotonin should not be written off as a therapeutic strategy. We describe how "uptake-2" transporters may explain the relative lack of therapeutic efficacy of SSRIs, as well as why "uptake-2" transporters might be useful therapeutic targets.



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Globally, major depressive disorder is among the most burdensome of psychiatric disorders, contributing to reduced worker productivity and unemployment.[1] In the United States, about 7% of the population suffers from major depression and it is the primary cause of disability for individuals 15−44 years of age.[2] These statistics are compounded by the relative lack of therapeutic benefit provided to patients by current antidepressant medications. Although reports vary, it is estimated that major depression is unsuccessfully treated in more than half the patient population. The most commonly prescribed antidepressant drugs act to inhibit uptake of serotonin (5-HT) from extracellular space. The prevalence of suboptimal therapeutic benefit in many patients has called into question the role of increasing extracellular 5-HT in triggering therapeutic effects. Here we revisit the utility of selective 5-HT reuptake inhibitors (SSRIs) for the treatment of depression. We present a model to help explain their relative lack of therapeutic efficacy and how "uptake-2" transporters can be targeted to improve therapeutic outcome.

REVISITING THE ROLE OF SEROTONIN IN DEPRESSION

Depression and related disorders are most commonly treated with SSRIs. This has propagated the belief that depression is etiologically linked, at least in part, to low brain extracellular 5-HT. However, among other evidence, the discovery of variants in the human *SERT* gene, which lead to reduced SERT protein expression and/or SERT function, has brought this long-standing belief into question.[3,4] The most studied of these *SERT* gene variants is a polymorphism that occurs in the promoter region of the *hSERT* gene, known as the serotonin transporter-linked polymorphic region (*5-HTTLPR*). The short or "*s*" allele confers lower transcriptional activity, leading to reductions in SERT mRNA, SERT binding, and 5-HT uptake into platelets and lymphoblasts when compared with individuals homozygous for the long "*l*" allele.[5−7] The *5-HTTLPR* is a relatively common polymorphism with carriers of one or two copies of the "*s*" allele representing approximately

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60% of the Caucasian population, although this varies with ethnicity. In individuals with lower SERT expression (e.g., carriers of the "*s*" allele), an obvious prediction is that basal extracellular 5-HT will be elevated compared to individuals who do not carry low functioning or expressing variants of the *SERT* gene. Studies in mice in which SERT expression is constitutively reduced support this prediction, and findings are consistent with a reduced ability of these mice to recapture released 5-HT.[8,9] It is interesting and somewhat paradoxical then that humans who carry the "*s*" allele of the *5-HTTLPR*, or other low expressing/functioning variants of *SERT*, and who presumably have higher extracellular levels of 5-HT, are not less prone to or even protected from depressive disorders. In fact, several groups have reported that when having encountered stressful life events, these individuals are more, not less likely to experience episodes of depression.[10−13] Clearly the role of 5-HT in depression is complex and cannot be explained simply in terms of basal 5-HT concentrations. One idea that we have advanced is that these data provide evidence that it is the *magnitude of the increase* of extracellular 5-HT that is important for initiating the cascade of events leading to antidepressant effects, regardless of basal 5-HT levels.[3]

Not surprisingly the *5-HTTLPR* has been among the most extensively studied human gene variant in terms of its predictive capabilities regarding therapeutic outcomes. Some studies report an association between the "*s*" allele and reduced treatment responses to antidepressants.[14−16] In contrast, other findings, including those arising from the "Sequenced Treatment Alternatives to Relieve Depression" (STAR*D) initiative, suggest that issues of tolerability might confound these reports and that treatment response is independent of SERT genotype.[17] It is important to note that STAR*D provides a detailed analysis of treatment response to one SSRI, citalopram, as initial treatment. Therefore, it might be premature to generalize lack of *SERT* genotype-dependency of treatment response to all SSRIs and all classes of antidepressants. Notwithstanding the controversy that remains regarding the relationship of *SERT* gene variants to antidepressant therapeutic outcomes, we do know that constitutive elevations in extracellular 5-HT, such as those occurring in SERT heterozygous (+/−) and homozygous (−/−) deficient mice, result in adaptive alterations in numerous receptors and other proteins, including transporters (see ref 18 for review). These adaptations likely serve to offset the effect of reduced SERT expression on serotonergic neurotransmission and could potentially contribute to poor therapeutic responses to antidepressant treatment.[3] It is also important to understand that low SERT expression is not only predicted by genetic makeup; for example, chronic treatment with SSRIs down-regulates SERT expression and function (for review, see ref 19). Therefore, regardless of SERT genotype, chronic pharmacological blockade of SERT is expected to lead to compensatory adaptations that might contribute to diminishing therapeutic efficacy of current antidepressants after long-term use.

TRANSPORTER PROMISCUITY AND COMPENSATION FOR REDUCED SERT FUNCTION

Regardless of their expression levels, transporters for biogenic amines are promiscuous. That is, they can transport more than one substrate. This is certainly not a new concept, with the idea of transporter promiscuity dating back to the 1960s. At this time, several groups provided evidence that 5-HT could be accumulated in catecholaminergic neurons by a low-affinity/high-capacity mechanism (for review, see ref 3). At that time, the concept of "uptake-2" was popularized; however, the precise identity of a second transporter for 5-HT remained elusive. Today, many "low-affinity, high-capacity", or "uptake-2" transporters for biogenic amines have been identified.

Other transporters for 5-HT have been largely overlooked because their expression in brain was discovered only a decade ago and the function they serve remains to be fully elucidated. These transporters, including the organic cation transporters (OCTs) and plasma membrane monoamine transporter (PMAT), are among the most efficient "low-affinity, high-capacity" uptake mechanisms for 5-HT and other biogenic amine neurotransmitters (see refs 20 and 21 for reviews). However, a role for these transporters in regulating central serotonergic neurotransmission remained unexplored until relatively recently. Feng and co-workers first investigated the effect of an OCT/PMAT blocker, decynium-22 (D-22), on 5-HT levels in brain.[22] They reported that perfusion of D-22 via a dialysis probe in the medial hypothalamus of rats produced robust and dose-dependent increases in dialysate 5-HT. The magnitude of this effect (∼200−650% increase, depending on dose) was remarkably similar to that reported for equivalent doses of the SSRI fluoxetine delivered by the same route to the hypothalamus (∼400% increase in extracellular 5-HT). These data suggest that even when 5-HT levels are "normal" (i.e., not elevated due to genetic or pharmacological inactivation of SERT), blockade of "uptake-2" transporters produces elevations of extracellular 5-HT that may be sufficient for antidepressant-like effects, and/or to augment the antidepressant effects of SSRIs.

To date, evidence that "low affinity−high capacity" transporters of 5-HT can compensate for loss of SERT function has come from studies using SERT+/− or SERT−/− mice. The dopamine transporter (DAT), for example, is a compensatory alternative for transporting 5-HT in the ventral tegmental area, substantia nigra, and striatum of mice that lack SERT.[23,24] Conversely, there is no evidence so far for compensation for lost SERT activity by the norepinephrine transporter (NET), at least in the CA3 region of hippocampus where [^{3}H]nisoxetine binding to NET has been quantified using autoradiography.[25] Schmitt et al. reported that mRNA for OCT3 is increased in brains of SERT−/− mice.[26] This initial report, together with our findings of alcohol-induced inhibition of 5-HT clearance in hippocampus of SERT KO mice,[27] led us to a series of studies where we found that compared to wildtype mice, expression and function of OCT3 is markedly increased in SERT+/− and SERT−/− mice.[28] We also found that the OCT/PMAT blocker D-22 reduced immobility time in the tail suspension test, a measure of antidepressant-like activity. More recently, we discovered that D-22 can enhance antidepressant-like effects associated with pharmacological inactivation of SERT (Horton et al., in revision). Together, our findings in mice reveal a potentially important role for D-22 sensitive OCTs and/or PMAT in the regulation of 5-HT uptake and antidepressant-like responses when SERT function is compromised. Our findings are in keeping with those of others who have shown that D-22 (i) inhibits 5-HT uptake as well as augments the inhibitory effect of SSRIs on 5-HT uptake in mouse brain synaptosomes[29,30] and (ii) increases extracellular levels of 5-HT in rat hypothalamus.[22,31] In addition, knockdown of OCT3 using antisense oligonucleotides produces antidepressant-like effects in the forced swim test in mice[32] and studies using OCT2 knockout mice suggest a role for this transporter in

antidepressant responses.[33] However, can these findings help to explain poor therapeutic responses to antidepressants? We believe they can.

A MODEL TO EXPLAIN POOR THERAPEUTIC EFFICACY OF CURRENT ANTIDEPRESSANTS

Using SSRIs as an example, results from our group and others suggest that 5-HT uptake by a D-22-sensitive transporter(s) (putatively OCT3) may help to explain poor therapeutic responses to treatment with currently available antidepressant drugs. Based on our data,[28,34] together with literature reviewed in Daws,[3] we have developed a model (Figure 1) to illustrate

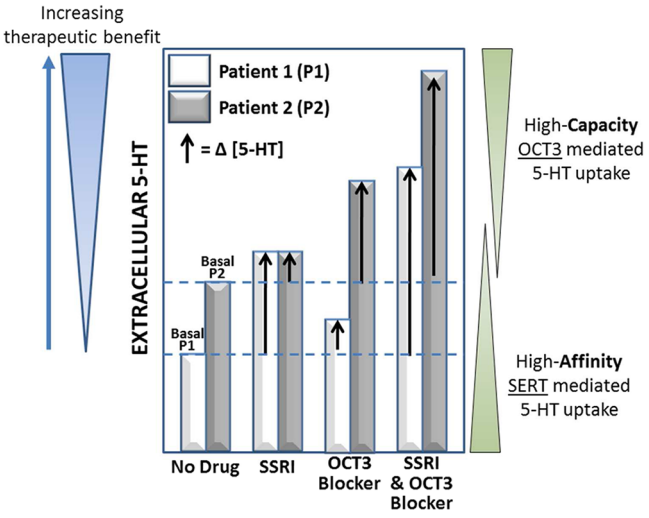

Figure 1. Model accounting for poor therapeutic responses to SSRIs and a hypothesized mechanism involving OCT3 blockade to improve therapeutic outcome. Arrows highlight the magnitude of increase in extracellular 5-HT following inhibition of SERT or OCT3, under two different scenarios: A patient (1) with "normal" [5-HT] and another (2) with higher basal [5-HT].

the potential therapeutic efficacy of D-22-like compounds in patients who respond poorly to treatment with SSRIs. In Figure 1, the white bars represent extracellular 5-HT in patient 1 who has not previously taken SSRIs and who does not carry low expressing/functioning genetic variants of SERT. The first white bar, labeled "no drug", shows "basal" extracellular 5-HT, which is predominantly determined by activity of the high-affinity SERT ("uptake-1"). When patient 1 takes an SSRI, it acts to block SERT-mediated 5-HT uptake and causes extracellular 5-HT to rise (second white bar). In some patients, an SSRI is therapeutically effective. In others, an SSRI is not able to produce a sufficiently large increase in extracellular 5-HT to initiate the cascade of events needed for therapeutic benefit. Our data suggest that extracellular 5-HT can be prevented from climbing higher after an SSRI because of the presence and activity of low-affinity, high-capacity (D-22 sensitive) "uptake-2" transporters for 5-HT. In other words, when extracellular 5-HT begins to climb, for example, following SSRI administration, the role of high-capacity transporters, such as OCT3, becomes increasingly prominent, limiting the extent to which extracellular 5-HT can increase following SSRI treatment (second white bar). The third white bar shows the predicted effect of an OCT3 blocker on extracellular 5-HT in patient 1, that is, a significant, but more modest increase in 5-HT limited by the activity of SERT. The fourth white bar shows what we predict will happen if an OCT3 blocker, such as D-22, is given in combination with an SSRI. With the low-affinity but high-capacity transporter(s) for 5-HT now blocked, in addition to the high-affinity, low capacity SERT, extracellular 5-HT is able to climb to a level sufficient to initiate downstream events required for therapeutic responses. This model is supported by data generated by us and others.[28,30]

The gray bars depict patient 2 who has higher basal levels of 5-HT to start (first gray bar), as might be the case in humans carrying the "*s*" allele of the *5-HTTLPR*, or who have been taking SSRIs for a prolonged period. In this scenario, the increase in extracellular 5-HT produced by an SSRI is modest (second gray bar). This is because the magnitude of increase in 5-HT that can occur before low-affinity, high-capacity transporters for 5-HT take over is much smaller when starting from higher basal 5-HT concentrations. According to our hypothesis, and supported by our data,[28,34] to help compensate for lower SERT activity in these individuals, OCT3 expression/activity increases. Thus, when patient 2 is given an OCT3 blocker, the prediction is that the resulting increase in extracellular 5-HT will be much greater (third gray bar). Likewise, when patient 2 is given an SSRI in combination with an OCT3 blocker, there may be a greater elevation in extracellular 5-HT (fourth gray bar) than in the patient with "normal" SERT function (fourth white bar). Of course, Figure 1 is an oversimplification of factors governing extracellular 5-HT concentrations in brain, but this model illustrates a testable hypothesis that provides a mechanistic framework to account for poor therapeutic responses to SSRIs, as well as to uncover novel targets for the development of improved therapeutics for depression.

This idea is shown another way in Figure 2, and it recapitulates the rationale that has led to the development of antidepressant drugs that block either SERT and NET (dual reuptake inhibitors) or SERT, NET, and DAT (triple reuptake inhibitors). Pertinent to this Viewpoint, evidence suggests that antidepressant drugs that inhibit uptake of 5-HT, NE, and DA have greater therapeutic utility than those that block uptake of only one of these biogenic amines.[35,36] Based on our views, this may be, in part, attributable to inhibition of uptake of one neurotransmitter, for example, 5-HT, by NET and DAT, as well as by its high-affinity transporter, SERT. Reductions in promiscuous uptake of norepinephrine (NE) and dopamine (DA) would similarly apply. We suggest that OCTs and/or PMAT may play a more prominent role as "uptake-2" transporters, such that their blockade, concurrently with an SSRI (or other antidepressant), would further improve the therapeutic efficacy of SSRIs, as well as dual and triple uptake inhibiting antidepressant drugs.

THE FUTURE FOR "UPTAKE-2" BLOCKADE IN THERAPEUTICS

OCTs and PMAT, like SERT, NET and DAT, are expressed in many peripheral organs including heart, kidney, and liver, where they function to translocate, bidirectionally, a variety of organic cations with widely differing molecular structures. Disruption of OCTs or PMAT function could potentially lead to a buildup of cationic drugs that might lead to toxicity. Clearly careful assessment of the potential toxic effects of drugs that block these transporters is needed before they could be considered for clinical trials. That said, the fact that OCT1, OCT2, and OCT3 knockout mice are phenotypically normal across a wide range of physiological and behavioral measures suggests that there is sufficient redundancy among these

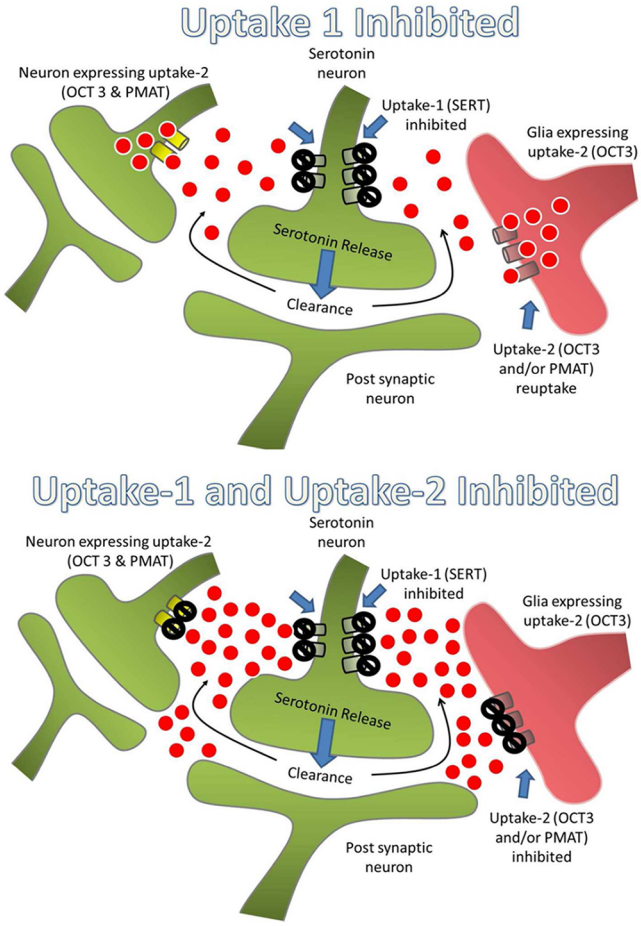

Figure 2. Serotonin clearance from extracellular space by uptake-2 may undermine the ability of SSRIs to increase extracellular serotonin by inhibiting SERT (uptake-1) (top panel). Concurrent blockade of SERT and uptake-2 may bolster extracellular serotonin levels to those necessary for improved therapeutic outcome (bottom panel).

transporters that drugs selective for a particular subtype might be a promising path for future investigations.[20,21,37,38] For example, biogenic amine uptake by OCT1 is very limited, or nonexistent. OCT2 can transport 5-HT and other biogenic amines, with an equivalent or greater affinity than OCT3 and PMAT. However, unlike OCT3 and PMAT, OCT2 expression levels in brain are low and confined primarily to the subventricular region.[39,40] On the other hand, OCT3 and PMAT expression in brain are largely overlapping and prominent in 5-HT rich regions that are implicated in mediating mood and antidepressant actions (e.g., dorsal raphe, cortex, hippocampus, amygdala, and hypothalamic nuclei).[41−45] Therefore, selective targeting of OCT3 and/or PMAT may be a therapeutically beneficial strategy for treating depression, while leaving OCT1 and OCT2 function intact to maintain transport of cations in the periphery.

CLOSING REMARKS

We would be remiss to close without acknowledging those who pioneered this field, including Bertler et al., Burgen and Iversen, Lichtensteiger et al., Fuxe and Ungerstedt, Butler et al., Shaskan and Snyder, Schildkraut and Mooney,[46−52] and others, who identified "uptake-2" decades ago and put forth similar hypotheses regarding the potential for "uptake-2" to play an important role in the regulation of biogenic amine neurotransmission. It is now left for future investigations to decipher which "uptake-2" transporter(s) might be the best to target for the development of improved antidepressant medications.

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