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Serotonin Transporters: Implications for Antidepressant Drug Development

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Abstract

Due to the complexity of the disease, several hypotheses exist to explain the etiology of depression. The monoamine theory of depression suggests that disruptions in the serotonergic and noradrenergic systems result in depressive symptoms. Therefore, the serotonin transporter (SERT) has become a pharmacological target for treating these symptoms. This review will discuss what is known about the molecular interactions of antidepressants with SERT. The effects of antidepressants on SERT regulation and expression in addition to the receptors that may be involved in mediating these effects will be addressed. Specifically, how changes to SERT expression following chronic antidepressant treatment may contribute to the therapeutic benefits of antidepressants will be discussed. Furthermore, the effects of *SERT* gene polymorphisms on antidepressant efficacy will be examined. Finally, a brief overview of other hypotheses of depression will be addressed as well as factors that must be considered for future antidepressant development.

Keywords: SSRIs, antidepressant, serotonin transporter, depression, reuptake

Monoamine Theory of Depression

Functional deficiencies in serotonin (5-hydroytryptamine, 5-HT) and norepinephrine (NE) have been implicated in the pathophysiology of depressive syndromes, and restoring the normal function of 5-HT- and NE-associated signaling pathway has been the target of antidepressants. Restoration of monoamine deficiencies to normal levels as a therapeutic strategy is based on the monoamine hypothesis of depression.[1] The oldest antidepressants, the monoamine oxidase inhibitors (MAOIs), increase synaptic levels of 5-HT and NE by inhibiting the enzymatic degradation of these neurotransmitters. The tricyclic antidepressants (TCAs), as well as newer selective 5-HT reuptake inhibitors (SSRIs) and 5-HT/NE reuptake inhibitors (SNRIs), all increase synaptic levels of 5-HT or NE by inhibiting reuptake via the 5-HT transporter (SERT) or NE transporter (NET), respectively. Emerging evidence indicates that the monoamine hypothesis of 5-HT and NE modulation fails to explain the whole mechanism of antidepressants. Other hypotheses, including the cytokine hypothesis of depression, the hypothalamic-pituitary-thyroid hypothesis of depression, as well as the role of brain-derived neurotrophic factor and cyclic AMP response element binding protein will be considered later in this review.

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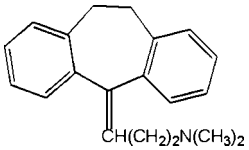
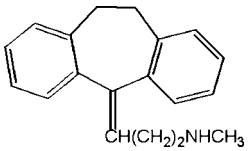
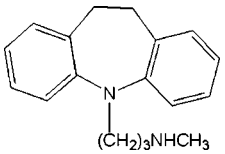
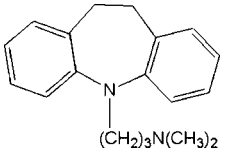
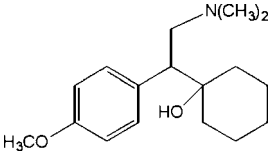
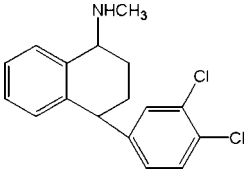
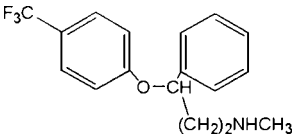
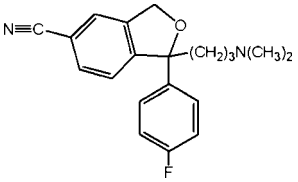
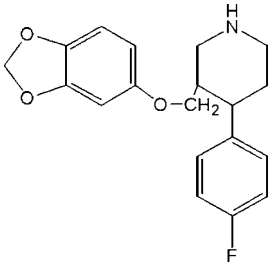
Antidepressant Development

The development of antidepressants began in the early 1950s with the fortuitous discovery that the antitubercular drug iproniazid, which functioned as an inhibitor of monoamine oxidase the enzyme responsible for breaking down 5-HT, had mood-elevating effects.[2] Subsequently, compounds with antihistamine activity such as imipramine and chlorpromazine were discovered to confer mood-elevating effects.[2] These discoveries led to the development of the first 2 classes of drugs used to treat depressed patients: the MAOIs and the TCAs. The first generation MAOIs (iproniazid, tranylcypromine, and phenelzine) were nonselective, irreversible inhibitors of MAO leading to increased levels of dopamine, 5-HT, and NE in patients. However, these drugs had dangerous and life-threatening side effects including hepatotoxicity and hypertensive crisis due to interactions with food high in tyramine. The risks of side effects and necessity for strict patient compliance led to the eventual disuse of these drugs. Nonetheless, MAOIs have seen a resurgence in recent years, particularly for the treatment of atypical and drug-resistant forms of depression.[3]

The first tricyclic antidepressant, imipramine, elicited antidepressant effects by inhibiting NE and 5-HT transport (Table 1). These compounds act as antagonists for SERT or NET and block neurotransmitter uptake, thus increasing synaptic concentrations of 5-HT or NE, respectively. However, imipramine was not without unfavorable side effects due to antihistaminic, antiadrenergic, and anticholinergic effects.[5,6] The development of second generation TCAs led to more selective inhibitors of NE uptake (desipramine, nortriptyline, maprotiline) and 5-HT uptake (clomipramine), but demonstrated no significant improvements in side effect profiles. More recently, the development of the SSRIs has consumed the mental health market (Table 1). The introduction of

Table 1. Structures of Commonly Prescribed Antidepressants and K_i Values for Inhibition at hSERT and hNET.*

Structure	Antidepressants	hSERT K_i (nM), [^{3}H]5-HT Uptake Inhibition	hNET K_i (nM), [^{3}H]NE Uptake Inhibition
	Amitriptyline	36 ± 1	102 ± 9
	Nortriptyline	279 ± 20	21 ± 0.77
	Desipramine	163 ± 5	3.5 ± 0.6
	Imipramine	20 ± 2	142 ± 8
	Venlafaxine	102 ± 9	1644 ± 84
	Sertraline	3.3 ± 0.4	1716 ± 151
	Fluoxetine	20 ± 2	2186 ± 142
	Citalopram	8.9 ± 0.7	30,285 ± 1600
	Paroxetine	0.83 ± 0.06	328 ± 25

*Data were Obtained in HEK cells Heterologously Expressing hSERT or hNET.[4]

fluoxetine in 1988 provided clinicians with a safer treatment alternative to the MAOIs and the TCAs since the side effect profile was considerably improved. A meta-analysis of 36 double-blind clinical trials of TCAs and SSRIs found that patients taking a TCA were more likely to drop out of a study due to an adverse side effect (22.4%) than those prescribed an SSRI (15.9%).[7] However, despite the improvement in side effects and drug interactions, the SSRIs are generally no more clinically efficacious at treating depression than any previous therapy.[8] Increasing response rates to the SSRIs are paralleled with increases in the placebo effect.[5] For example, response rates as measured by a 50% or greater decrease in the total depression score on the Hamilton Rating Scale for Depression were 79% and 60% for sertraline and fluoxetine, respectively. However, the corresponding placebo effect was 48% for sertraline, compared with only 33% for fluoxetine indicating a similar (~30%) response rate to these SSRIs.[5] Additionally, approximately 30% to 45% of patients fail to attain an adequate response to their initial pharmacotherapy.[9]

The failure of the SSRIs to treat some depressed patients led to the development of less selective agents. These SNRIs are inhibitors of both 5-HT and NE uptake. The SNRIs may have increased clinical utility over the SSRIs at treating refractory depression and anxiety indicating the need to target both the serotonergic and noradrenergic uptake systems in some patients.[10] Additionally, the SNRIs may have efficacy in reducing the physical symptoms associated with depression, including muscle tension, fatigue, appetite changes, and body aches, in addition to an alternative use as a treatment for fibromyalgia.[11] Drugs in this class include venlafaxine and duloxetine and although they are less selective than the SSRIs with regard to targeting specific transporters, they have limited affinity for the muscarinic, adrenergic, and histaminergic receptors, resulting in a favorable side-effect profile.[12] Finally, the shift from focusing completely on the 5-HT system to the NE system was complete with the development of 2 selective NE reuptake inhibitors, reboxetine and atomoxetine. Reboxetine is indicated for major depressive disorder, whereas atomoxetine is prescribed for attention-deficit/hyperactivity disorder.

The discussion of drugs used to treat affective disorders would not be complete without mentioning the atypical antidepressants. These drugs do not fall into a specific class and affect a wide range of physiological targets. Clinically used agents include the $5HT_{2A}$ receptor antagonists such as trazadone and its second generation compound nefazodone, the NE/dopamine reuptake inhibitor bupropion, and the $5\text{-}HT_{1A}$ receptor agonist and D2 dopamine receptor antagonist buspirone.

Molecular Interactions of Antidepressants with SERT

Antidepressants are represented by many structurally distinct classes of compounds each of which may interact with SERT in a unique manner (Table 1). Although the 3-dimensional structure of SERT and other monoamine transporters such as the dopamine transporter and NET has not been solved, experimental assays correlating functional potency data to structural changes provide some clues into the binding sites of these different drug classes as well as possible distinct binding. Species-scanning mutagenesis has also been employed to identify residues that contribute to inhibitor binding. This method exploits the differences in inhibitor potencies between variants of SERT (ie, human versus rat). Similarly, cross-species chimeras can be generated to determine the influences of specific transmembrane domain (TMD) regions on drug potencies. For example, Barker and colleagues[13] generated cross-species chimeras between the human and *Drosophila* SERT (hSERT and dSERT, respectively) homologs to determine the regions of the SERT responsible for the divergent pharmacology of SERT inhibitors at the *Drosophila* species as compared with hSERT. The inhibitor potency data from human/*Drosophila* SERT chimeras led to the observation that residues distal to the second TMD conferred potency specificities for most inhibitors including paroxetine and fluoxetine. Conversely, the region including TMDs I and II contained key sites for species discrimination of citalopram and mazindol. Further investigation revealed that mutation of tyrosine 95 in the hSERT to the corresponding dSERT phenylalanine residue altered mazindol and citalopram potency values nearly equivocal to those for dSERT.[12] Furthermore, computational modeling of the SERT-citalopram interaction suggests a dipole-dipole interaction of the nitril group of *S*-citalopram with Y95 providing further support for a specific molecular interaction with this amino acid.[14]

Additionally, an aspartate in the first TMD of the dopamine transporter has been implicated as a key residue for dopamine transport and cocaine analog recognition.[15] To determine the role of this residue in SERT, several mutations were generated in rat SERT (rSERT). The carboxylic acid of the D98E mutant was identified as an essential point of interaction for the amine in 5-HT, indicating this residue was important for substrate recognition. Furthermore, this mutation caused a significant decrease in citalopram potency for inhibiting 5-HT transport.[16] Therefore, this aspartate may be important for antidepressant recognition as well. In addition to the D98 residue in TMD I, key residues in the third TMD of hSERT appear critical for the recognition of cocaine, amphetamine, and antidepressants (C. Walline, unpublished data).

Other key residues have been implicated in antidepressant recognition in NET within the TMD V-IX region.[17-19] Although discussion of other transporters within the monoamine family is outside the scope of this review, 2 of these amino acids (P418 and S375) are sites of sequence divergence between hSERT and dSERT. Therefore, these residues

were ideal candidates for species-scanning mutagenesis in order to confirm their importance in antagonist recognition. Both P418 and S375 were independently mutated to the equivalent dSERT amino acid (serine and alanine, correspondingly).[20] Unfortunately, both mutants failed to show any statistically significant potency differences as compared with hSERT for a panel of TCA and SSRI antidepressants suggesting that these amino acids may not be critical sites for antidepressant recognition by SERT.[20]

Residues in SERT have also been discovered that confer high potency interaction with tricyclic antidepressants. Using species-scanning mutagenesis, F586 in hSERT was mutated to the corresponding valine in rSERT. The F586V hSERT mutant demonstrated a phenotype similar to the rSERT with regard to TCA potency.[21] The complementary rSERT mutation V586F showed hSERT-like potencies for the tricyclics imipramine, desipramine, and nortriptyline.[21] Taken together, these studies suggest that SERT TMD XII may contain distinct molecular determinants for binding of the TCAs. A more complete understanding of antidepressant recognition by SERT at the molecular level will be revealed by future efforts focused on elucidation of SERT structure.

Acute Regulation of SERT Expression by Antidepressants

Alterations to the serotonergic system are thought to partially explain the therapeutic benefits of antidepressants. Since the discovery that antidepressants work in part by potentiating the actions of 5-HT within the serotonergic system (for review see Schloss and Williams[22] and Vetulain and Nalepa[23]), the effects these drugs elicit on the SERT protein have been an area of active research. Specifically, antidepressants are thought to initially function by acting as SERT antagonists resulting in an immediate increase in the synaptic 5-HT concentration.[22] Changes to the expression level of SERT in response to drugs could contribute to neuroadaptive changes associated with chronic antidepressant administration (see below). Surprisingly, the effects of antidepressants on regulation of SERT expression remain largely unevaluated.

Regulation of SERT activity by kinases seems plausible as SERT contains putative phosphorylation sites for protein kinase C (PKC), cAMP-dependent protein kinase (PKA), and cGMP-dependent protein kinase (PKG). Early studies in human embryonic kidney (HEK) cells heterologously expressing hSERT (HEK-hSERT) identified SERT as a phosphoprotein sensitive to rapid internalization following activation of PKC concurrent with a decrease in SERT activity.[24,25] Further studies in the HEK-hSERT cells showed that antidepressants alone could not affect the ability of PKC to phosphorylate SERT. The ligand 5-HT, however, caused a reduction in PKC-induced phosphorylation.[26] Therefore, in the presence of substrate, SERT would not become phosphorylated, the signal necessary for internalization. Interestingly, acute treatment of HEK-hSERT cells with 5-HT in the presence of the SERT-selective antidepressants, imipramine, paroxetine, and citalopram, but not the NET-selective nisoxetine, resulted in SERT phosphorylation by PKC.[26] The ability of antidepressants to counteract the action of 5-HT suggests a mechanism that would further increase the synaptic concentration of 5-HT by reducing the number of transporters at the cell surface available to eliminate 5-HT from the synapse.[26]

Although phosphorylation of SERT by acute PKC activation results in rapid changes in SERT activity and cellular distribution, regulation of SERT expression at the plasma membrane by PKA or PKG does not occur after acute kinase activation despite an increase in SERT phosphorylation.[25] One explanation for these results would be that although phosphorylation of SERT occurs through numerous kinase pathways, changes to SERT activsity and expression due to PKA or PKG phosphorylation occur over a longer period of time than PKC regulation. Another explanation is that HEK cells do not contain the proteins necessary to affect SERT expression following phosphorylation by PKA or PKG. Interestingly, chronic treatment with antidepressants increases the production of intracellular cAMP due to an increased coupling between the G protein Gs_{α} and adenylyl cyclase (for review see Donati and Rasenick[27]). Elevation of intracellular cAMP would result in increased translocation and activity of PKA suggesting a potential mechanism for PKA regulation of SERT after extended antidepressant administration. This effect could occur through changes to unidentified protein interactions facilitated by alterations in the phosphorylation state of SERT. Studies in human placental choriocarcinoma (JAR) cells, a cell line that endogenously expresses SERT, suggest that increases in cAMP should lead to an increase in SERT activity due to an increase in both SERT mRNA and protein levels.[28] This study is in conflict, however, with numerous studies that have reported a decrease in SERT levels following chronic antidepressant administration (see below) despite the increase in cAMP production explained above. Further investigation will be required to determine if this conflict is due to cell type-dependent variability in the role of PKA phosphorylation in SERT regulation under normal and antidepressant-treated conditions.

Not surprisingly, kinase pathways that increase the activity of SERT have also been reported. Studies have identified a mechanism for up-regulation of SERT expression through PKG-dependent pathways.[29] Previously, Miller and Hoffman[30] reported that the A_3 adenosine receptor regulated SERT in rat basophilic leukemia (RBL-2H3) cells through cGMP and nitric oxide, subsequently increasing the

V_{max} value for 5-HT transport. This regulation of SERT could occur under physiological conditions as RBL-2H3 cells endogenously express both the A_3 adenosine receptor and SERT. Interestingly, the increase in 5-HT transport was not associated with an increased expression of SERT at the cell surface.[30] One caveat of these experiments is that the cell-surface binding was performed using a membrane permeable ligand, thus, limiting the conclusions that can be drawn. Zhu et al,[29] however, reported that an up-regulation of SERT surface expression occurred when activating A_3 adenosine receptors in RBL-2H3 cells as measured by specific binding of the cocaine analog [^{125}I]RTI-55. Because SERT protein levels are low in RBL-2H3 cells, Chinese hamster ovary (CHO) cells were transiently transfected with A_3 adenosine receptor and SERT cDNAs to confirm these results. Similar increases in SERT protein surface expression levels following A_3 adenosine receptor activation occurred in the transfected CHO cells suggesting a common signaling pathway for SERT up-regulation within the 2 cell types.[29] Moreover, these studies extended those of Miller and Hoffman[30] by showing that activation of the PKG pathway required the upstream activity of phospholipase C, calcium, and guanylyl cyclase.[29] Additionally, investigation into other proteins involved in the PKG-signaling cascade led to the identification of p38 mitogen-activated protein kinase (MAPK) regulation of intrinsic SERT activity. Inhibition of p38 MAPK did not affect A_3 adenosine receptor-activated changes to SERT plasma membrane expression levels in RBL-2H3, thus suggesting that changes to SERT by the PKG activation of p38 MAPK occur independently of changes caused directly by PKG.[29] Interestingly, the studies of Samuvel et al[31] showed that application of the p38 MAPK-specific inhibitor PD169316 resulted in decreases in the activity, phosphorylation, and plasma membrane expression of SERT isolated in rat synaptosomes. Reduction of p38 MAPK by siRNA in HEK cells transiently expressing hSERT produced a reduction in SERT at the cell surface, and inhibition of p38 MAPK by PD169316 decreased delivery of SERT to the plasma membrane. Moreover, activation of either p38 MAPK or PKC also inhibited interaction of SERT with syntaxin 1.[31] Syntaxin 1 is a SNARE protein that has previously been found to interact with and regulate SERT's expression.[32-34] Therefore, p38 MAPK appears to influence SERT expression via regulation of plasma membrane insertion potentially by affecting SERT's interaction with syntaxin 1.[31] Additional investigations into the role of p38 MAPK regulation of SERT surface expression are necessary before the effects of antidepressants on these interactions can be investigated.

In addition to interactions with numerous kinases that control SERT availability at the plasma membrane, SERT forms a complex with protein phosphatase 2A (PP2A) in HEK-hSERT cells.[35] Previously, inhibitors targeting PP2A and protein phosphatase 1 were found to down-regulate SERT activity.[25] Furthermore, p38 MAPK appears to regulate the interaction between SERT and PP2A.[31] Additional research must be performed to elucidate the complex network of kinase and phosphatase activity involved in controlling SERT expression. Moreover, the extent of SERT-regulating phosphorylation that occurs in vivo remains unresolved. Further studies are necessary both in vivo and in vitro to establish the role of kinase phosphorylation on SERT expression under antidepressant-treated conditions both acutely and after chronic administration. Interestingly, an evaluation of kinase mRNA levels in rat brain following 21 days of fluoxetine or citalopram treatment showed a decrease in numerous kinases including PKC and PKA.[36] The effect this down-regulation confers on kinase protein levels and subsequent SERT regulation requires further analysis.

Effects of Chronic Antidepressant Exposure on SERT

Although antidepressants function to rapidly increase synaptic 5-HT concentrations, this initial effect does not instantly alleviate the symptoms of depression. In fact, a maximal clinical response to antidepressants will not become apparent until several weeks of continuous treatment (for review see Gelenberg and Chesen[37]). Thus, the acute blockade of SERTs to increase 5-HT signaling does not account for the typical delay in symptom improvement. Current thought proposes that the neuroadaptive changes necessary for the therapeutic benefits of some antidepressants results in part from down-regulation of postsynaptic β-adrenergic receptors.[38] Similar changes, however, are not characteristic for SSRIs and other antidepressants that act on SERT.[38] Therefore, neuroadaptive changes in SERT expression could be necessary for antidepressants to be effective. Reports of changes to SERT expression after treatment with antidepressants vary.

The effects of chronic antidepressant treatment on the expression level of SERT have been explored in few in vitro systems. Horschitz et al[39] treated HEK cells heterologously expressing rSERT with increasing concentrations of citalopram before determining the effects of treatment on [^{3}H]5-HT uptake and [^{3}H]-citalopram binding. Following a 3-day incubation with 500 nM citalopram, the maximal transport activity (V_{max} value) decreased to approximately 40% without a subsequent change in the K_m value for 5-HT. A similar reduction (~50%) in [^{3}H]-citalopram binding to rSERT at the plasma membrane occurred after this treatment; although a reduction in binding of approximately 25% was evident with as little as 20 nM citalopram. Moreover, a similar decrease in rSERT expression was not evident in cells chronically treated with the antidepressant, desipramine, a NET inhibitor.[39] Unfortunately, the precise mechanism for

citalopram-induced down-regulation of SERT in HEK cells remains unclear as well as how these results will translate to neuronal cell lines.

Although the above results were obtained in a heterologous expression system, similar patterns of SERT down-regulation have been documented after chronic treatment of rats and humans with antidepressants. Specifically, Benmansour and colleagues[40,41] reported that chronic treatment (15 or 21 days) with paroxetine or sertraline in rats reduced the number of SERT binding sites by as much as 80% in the CA3 region of the hippocampus without long-term changes to SERT mRNA levels. Furthermore, chronoamperometry measurements of 5-HT levels revealed an increase in the time necessary to clear 5-HT in chronic sertraline-treated rats compared with the time required following an acute fluvoxamine block of SERT in saline-treated rats.[41] These data show that an overall decrease in the expression level of SERT induced by chronic antidepressant exposure results in 5-HT remaining in the synapse longer than observed with acute blockade of SERT alone. Additionally, Gould et al[42] reported a 75% to 80% decrease in the lateral nucleus of the amygdala, dentate gyrus, and dorsal raphe SERT binding sites after 6 weeks of paroxetine administration to rats. Similar treatment with the selective NET inhibitor, reboxetine, did not result in a change to SERT binding.[42] A study of 17 healthy humans administered 40 mg/day of citalopram found a reduction in diencephalon and brainstem SERT binding following 8 days of treatment.[43] Surprisingly, no further reduction in SERT binding occurred after an additional 8 days of treatment.[43] Changes in platelet 5-HT and SERT have been suggested as possible biomarkers for psychiatric illness (for review see Plein and Berk[44]). Interestingly, Alvarez et al[45] reported a decrease in platelet SERT binding sites in depressed versus healthy individuals that was further reduced following 12-week administration of clomipramine or fluoxetine. Furthermore, a greater drop in platelet SERT binding sites was evident in antidepressant treatment responders versus nonresponders.[45] These data support a possible link between SERT expression and responsiveness to antidepressant treatment. Interestingly, the responders showed an initial increase in platelet inositol triphosphate levels that returned to normal following chronic antidepressant treatment. In nonresponders, the inositol triphosphate levels remained elevated. An increase in inositol triphosphate indicates an increase in the phosphoinositide signaling pathway that would also lead to an increase in 1,2-diacylglycerol (DAG). Activation of PKC by DAG could then regulate SERT surface expression.[45] A more complex system for regulation of SERT expression is suggested by these data, however, since a decrease in DAG, in addition to inositol triphosphate, should result in a decrease in PKC activity. Therefore, an increase in SERT expression would be expected if PKC alone were responsible for SERT regulation.

Not all studies of chronic antidepressant treatments report a decrease in SERT expression levels. Hébert et al[46] described no significant changes to brain SERT binding sites in rats treated 21 days with numerous antidepressants with the exception of decreased SERT levels in the entorhinal cortex induced by both fluoxetine and venlafaxine. Interestingly, treatment with the tricyclic antidepressants trimipramine or desipramine appeared to increase SERT binding in several cortical brain regions compared with the transporter inhibitors fluoxetine and venlafaxine.[46] Moreover, an increase in hippocampal SERT binding sites occurred after a 10-week treatment with amitriptyline in 24-month-old rats. The treatment did not change the SERT levels in 10-month-old rats.[47] An increase in SERT following antidepressant treatment could contribute to age-related differences in therapeutic effectiveness of antidepressants.[47] Finally, a study of depressed versus healthy individuals showed an initial decrease in lymphocyte SERT binding sites in depressed individuals compared with controls that increased after chronic fluoxetine treatment concurrent with a clinical improvement in depressed symptoms.[48] Further studies are necessary to confirm the link between clinical response and changes in SERT expression in areas outside of the brain as regulatory mechanisms could differ substantially between neuronal and non-neuronal cells.

Although the above-mentioned studies only represent a small portion of the studies examining the effects of chronic antidepressant treatment on SERT binding sites (for further review see Schloss and Silliams[22] and Schloss and Henn[49]), the disparities described could be reflective of the systems used to measure SERT levels. For instance, differences in the specificity and cell permeability of the radiolabels used for these studies could account for variability of the results. Moreover, different antidepressants may elicit unique effects on SERT availability due to differences in recognition of the antidepressants by SERT. Additionally, the dose of antidepressant administered as well as the route of antidepressant administration could affect the local concentration of drug in the brain. Benmansour et al[40,41] and Gould et al[42] used osmotic minipumps to continuously dispense the drugs to the rats, whereas Hébert et al[46] administered the drugs to the rats through a single daily ip injection. In addition, the region of the brain under examination could account for differences in expression levels.[46] Finally, the population size, ethnicity, and age of subjects in human studies must be considered before generalizing the results.

Receptor Regulation of SERT

As described earlier, SERT is subject to regulation through kinase and phosphatase activity. These signaling proteins themselves are regulated by the activity of neurotransmitter receptors. Thus, the effect of chronic antidepressant treatment on various receptors and the subsequent effect the

receptors confer on SERT is an area of active investigation. Unfortunately, specific receptor-mediated regulation of SERT remains poorly understood. One area of exploration involves the serotonin 5-HT_{1A} receptor. The 5-HT_{1A} receptor is believed to be important in the therapeutic effects observed upon chronic antidepressant treatment because this receptor is responsible for regulating firing of serotonergic neurons.[50] The increase in extracellular 5-HT caused by blockade of SERT by antidepressants results in a decrease in vesicular 5-HT release due to activation of the negative feedback mechanism facilitated by presynaptic 5-HT_{1A} receptors. Desensitization of the presynaptic 5-HT_{1A} receptor must then occur to return serotonergic neuronal firing to normal.[50,51] In the median and dorsal raphe, chronic treatment with fluoxetine leads to a decrease in presynaptic 5-HT_{1A} receptor G-protein coupling without a decrease in receptor binding sites. These findings suggest that receptor desensitization occurs at the level of effector coupling.[52] Currently, no data support a direct role for 5-HT_{1A} receptor regulation of SERT, although SERT expression is decreased in 5-HT_{1A} receptor knockout mice,[53] an effect that could be a result of disrupting serotonergic signaling.

Recent exploration of the 5-HT_{1B} receptor suggests a role for this receptor in regulating SERT activity in vivo.[54] Specifically, activating presynaptic 5-HT_{1B} receptors results in increased clearance of 5-HT by SERT independent of 5-HT_{1A} receptor activity.[55] In agreement with these findings, studies in 5-HT_{1B} receptor knockout mice report a larger increase in extracellular ventral hippocampus 5-HT concentration following a single ip injection of fluoxetine compared with wild-type mice, although an alteration in SERT levels in the knockout mice could explain these results.[56] Interestingly, no significant change to extracellular 5-HT concentrations was evident in the frontal cortex suggesting differences in SERT regulation between the 2 brain areas.[56] Additionally, treatment of rats for 21 days with fluoxetine decreased 5-HT_{1B} receptor mRNA levels in the dorsal raphe nucleus.[57] No concurrent measurement of changes to presynaptic 5-HT_{1B} receptor protein levels was assessed in this study. If a decrease in 5-HT_{1B} receptors were to occur, however, a decrease in SERT function would be considered likely since presynaptic 5-HT_{1B} receptors regulate 5-HT uptake.[55]

The therapeutic benefits of antidepressants may rely in part on desensitization of 5-HT_{1A} and 5-HT_{1B} receptors.[50] Interestingly, co-administration of the 5-HT_{1A} receptor antagonist pindolol with an SSRI increased the SSRI efficacy and decreased the lag between the start of drug treatment and a clinical response.[58] Studies by Shalom et al[59] showed that repeated application of a 5-HT_{1B} receptor antagonist in the rat frontal cortex prevents fluoxetine-induced 5-HT_{1B} receptor desensitization. Because of the importance of receptor desensitization in mediating the therapeutic benefits of chronic SSRI treatment, co-administration of an SSRI and a 5-HT_{1B} receptor antagonist may not be more effective over an extended period of time than an SSRI alone.[59] Further investigation into the mechanism behind 5-HT_{1B} receptor control of SERT activity could help with designing drug regimens for depressed individuals.

The role of the α_2-adrenergic receptor in the noradrenaline system, and the subsequent changes to the receptor's expression and function during depressed episodes are currently under intensive investigation. Specifically, changes to α_2-adrenergic receptors are known to occur when examining antidepressants that specifically inhibit NET with changes being dependent on the type of antidepressant.[49] Interestingly, Ansah and colleagues[60] recently reported that α_2-adrenergic receptor activation could inhibit SERT activity both in vitro and in vivo, and the presence of Ca^{2+} was essential for this inhibition. Application of an α_2-adrenergic receptor agonist at a concentration that resulted in maximal SERT inhibition in synaptosomal preparations did not decrease SERT activity in transfected cell lines suggesting activation of a pathway in vivo that was unavailable in transfected cells rather than a direct interaction of the receptor or drug with SERT. Furthermore, when analyzing the effects of the α_2-adrenergic receptor agonist UK14304 in vivo an increase in the amount of time necessary to clear 5-HT was evident. UK14304 did not change SERT phosphorylation or the V_{max} value, but did decrease the apparent K_m value for 5-HT.[60] Thus, regulation of SERT caused by the activation of α_2-adrenergic receptors does not appear to be changing the surface expression of SERT. Activation of α_2-adrenergic receptors, however, could influence the interaction of an ancillary protein with SERT that affects recognition of 5-HT. The influence that α_2-adrenergic receptors have on SERT expression and activity requires further investigation to determine what, if any, role these receptors play on changing SERT levels during antidepressant treatment.

SERT Polymorphisms and Antidepressant Response

Although the interplay between SERT, receptors, and signaling proteins must be considered for the current antidepressants, development of future drugs for the treatment of depression should not only consider these interactions, but also genetic polymorphisms of the *SERT* gene. Ramamoorthy and colleagues[61] isolated a single human *SERT* gene to chromosome *17q11.1-q12*. Later studies revealed a polymorphism in the promoter region of the human *SERT* gene that resulted in a deletion of a 44-base-pair repeat.[62] Further investigation into the functional implications of the promoter deletion showed a 3-fold decrease in activity of the short (*s*) versus the long (*l*) promoter in

JAR cells as measured by luciferase-promoter fusion constructs. Moreover, PKC- and cAMP-activated transcription was significantly greater at the *l SERT* gene promoter versus the *s* promoter.[62] Prevalence of the *s* versus the *l* alleles in 505 subjects revealed that 19% of the individuals were homozygous for the *s*/*s* genotype whereas 49% were *l*/*s* and the remaining subjects were *l*/*l*.[63] Basal transcriptional activity of the promoters were ascertained from lymphoblasts cultured from individuals with *l*/*l*, *l*/*s*, and *s*/*s* genotypes. Similar transcriptional activity to that shown in JAR cells was reported; a concurrent decrease in SERT protein expression was evident in lymphoblasts containing the *s*/*s* and *l*/*s* genotypes compared with the *l*/*l* genotype.[63]

Because these initial studies confirm that differences exist in the activities of the promoter as well as the distribution of the polymorphism in humans, the consequences of these allelic variations on antidepressant efficacy and response has become a subject of active inquiry. Rausch and colleagues[64] determined the SERT promoter polymorphism in 51 individuals diagnosed with major depression and examined the effects of fluoxetine on depression symptoms. Interestingly, patients with an *l* allele (either as *l*/*l* or *l*/*s*) exhibited an increase in the response to both fluoxetine and placebo as measured by a decrease in the Hamilton depression score compared with individuals with the *s*/*s* genotype.[64] In agreement with these findings, Chinese patients with the *l*/*l* genotype also showed a greater decrease in Hamilton scores after fluoxetine treatment compared with their *s*/*s* and *l*/s genotype counterparts.[65] Moreover, a more rapid onset of responsiveness for elderly people with the *l*/*l* genotype has been reported. Pollock et al[66] showed that after a week of treatment with paroxetine, *l*/*l* elderly patients had a decrease in the Hamilton depression score compared with both the *l*/*s* and s/*s* genotypes suggesting a faster rate of onset for the clinical effect. There was no change in onset of response in patients treated with nortriptyline suggesting a response unique to SERT-selective antidepressants.[66] Similar results indicating a faster rate of onset for response in *l*/*l* genotypes were obtained in a study of 176 elderly subjects treated with sertraline with no change in response to placebo-treated subjects.[67] Interestingly, neither study reported a further change in response subsequent to the initial response at the end of either a 12-week or 8-week study.[66,67] Thus, these data suggest a link between response to SSRIs and the age of the depressed subject.

Another polymorphism of the SERT gene results in a mutation in the eighth TMD of the SERT protein. This rare polymorphism was initially identified in 2 families with multiple neuropsychiatric illnesses.[68] The hSERT I425V mutation results in increased 5-HT transport in human cervical epitheloid carcinoma and COS-7 cells transfected with the I425V hSERT cDNA. The change in transport was accompanied with a decrease in the K_m value and an increase in the V_{max} value without a change in the surface expression suggesting a mutation-induced alteration to the transporter's activity.[69] Additionally, as previously shown with activation of SERT through the A_3 adenosine receptor,[30] a nitric oxide donor increased wild-type SERT activity. The increase in SERT activity following nitric oxide stimulation was equivalent to the activity of the I425V hSERT mutant in the absence of nitric oxide. hSERT I425V activity, however, was insensitive to nitric oxide. Therefore, hSERT I425V functions at a level equivalent to wild-type SERT after activating cGMP-signaling pathways.[69] Furthermore, the hSERT I425V polymorphism exists on the *l* allele.[68] Thus, individuals with this polymorphism would have increased expression of a catalytically enhanced transporter. Interestingly, individuals with the hSERT I425V polymorphism were resistant to antidepressant treatment.[68]

Although the present research strongly suggests genetic links to differences in response rates as well as overall response to antidepressants, specifically SSRIs, further investigation is necessary to determine how the changes in the *SERT* gene promoter alter the expression, regulation, and activity of the SERT protein. Rausch et al[64] reported a difference in the initial 5-HT K_m value by platelet SERT between subjects who responded to fluoxetine treatment versus nonresponders. This change in 5-HT interactions did not appear to be dependent on the allelic variant, although subjects with the *l* allele and an initial increase in the 5-HT K_m value did exhibit the most favorable response to treatment.[64] Furthermore, investigation into how the I425V polymorphism disrupts SERT regulation by PKG is necessary to further the understanding of how antidepressants affect SERT activity and expression. Such information could lead to the development of therapies for individuals who do not respond to common antidepressants.

Cytokines and Depression

In addition to antidepressants acting on the biogenic amine neurotransmitters, other signaling pathways may be involved with antidepressant action as well as the pathophysiology of affective disorders. One alternative to the monoamine hypothesis of depression is the macrophage hypothesis of depression developed by RS Smith in 1991.[70] Smith hypothesized that gross secretion of macrophage cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-α (IFN-α) are responsible for some cases of major depression. Increases in these substances may elicit a hypersecretion of corticotrophin-releasing factor, resulting in hypercortisolemia, which may play a role in depressive diseases as a result of defects in negative feedback mechanisms controlling these substances.[71] In fact, a recent study has indicated that patients suffering from depressive syndromes with a history of suicidal attempts were associated with lowered adrenocorticotropin and cortisol levels, particularly after a recent suicide attempt.[72]

Additionally, cancer patients receiving IFN-α or interleukin-2 therapy had significant decreases in serum tryptophan, the amino acid precursor to 5-HT.[73] Decreased serum tryptophan upon cytokine treatment may be due to induced tryptophan catabolism leading to increases in kynurenine and quinolinic acid. Patients who received IFN-α treatment for malignant melanoma showed significant increases in kynurenine as well as decreases in tryptophan and approximately half of these patients developed major depression during treatment.[74] However, patients who received paroxetine 2 weeks prior to IFN-α treatment did not develop major depression despite increases in kynurenine and quinolinic acid.[74] Although major depression frequently occurs in patients receiving cytokine therapy, these depressive symptoms can be attenuated with antidepressant treatment. Although a detailed discussion of cytokines and their purported link to depression via the hypothalamic-pituitary-adrenal axis is outside the scope of this review, it has been reviewed in detail elsewhere.[71,75]

HYPOTHALAMIC-PITUITARY-THYROID AXIS

The hypothalamus is directly responsible for regulating the pituitary, which in turn secretes thyroid-stimulating hormone (TSH). Dysregulation of this tightly controlled system can result in thyroid dysfunction. Interestingly, hypothyroidism and depression share many of the same psychological and physical symptoms including anhedonia, fatigue, depressed mood, and cognitive disturbances, as well as sleep disturbances and weight change. Depression, in addition to hypothyroidism, may be a result of an imbalance of the regulatory functions of the hypothalamic-pituitary-thyroid (HPT) axis. Recently, Bschor and colleagues[76] reported a relationship between thyroid function and lithium pharmacotherapy. Although lithium therapy is one of the oldest treatments for mood disorders and remains a useful strategy for the treatment of refractory depression, the mode of lithium action has been poorly understood. Following lithium treatment, Bschor et al[76] reported significantly higher serum TSH levels, as well as significantly decreased triiodothyronine (T_3) and thyroxine (T_4) levels, resulting in a significant reduction of HPT system activity. Although the study did not provide evidence that thyroid status would predict responses to lithium therapy, it does provide support for a relationship between the HPT and depression.

Direct effects of psychotropic therapy on the HPT axis have been difficult to assess. Many studies are inconclusive and have considerable methodological differences making the results difficult to interpret. At the molecular level, in vitro studies have shown that incubation of thyroid cells with chlorpromazine causes a decrease of ^{131}I uptake, an important step in thyroid hormone synthesis.[77] Additionally, many antipsychotics and antidepressants with an alkylamino side chain (alimemazine, chlorpromazine, clomipramine, imipramine, and desipramine) can have a strong donor/acceptor interaction with iodine that could lead to iatrogenic hypothyroidism or thyroiditis.[77]

The possible interactions between antidepressants and thyroid metabolites are less clearly interpreted in clinical studies. Shelton and colleagues[78] reported that T_3 levels decreased transiently for both desipramine and fluoxetine treatment groups whereas desipramine treatment caused a significant increase in total T_4. Also, no significant treatment effects on free T_4, TSH, or TSH response to a thryrotropin-releasing hormone test were observed.[78] A similar study determined the antidepressant effects on thyroid function while controlling for the circadian activity of the thyrotrophs. Depressed patients had a significantly lower TSH response to protirelin, free T_4, and free T_3 than control patients.[79] The authors concluded that significant changes in thyroid function tests were associated with clinical recovery and not due to a direct effect of the antidepressant drug treatment.

Conversely, thyroid hormone levels could exert a positive influence on antidepressant treatment of depressive disorders. Sauvage et al[77] postulated that T_3 may enhance the effects of antidepressants through a potentiation of the β-adrenergic receptor system. Co-administration of T_3 during an antidepressant regimen may accelerate a response to antidepressant treatment to improve the overall treatment outcome.[77] Overall, depression and the HPT axis seem to be intimately linked, although this link remains to be clearly defined.

G-PROTEINS, CAMP RESPONSE ELEMENT BINDING PROTEIN

Several hypotheses regarding the mechanism of action of antidepressants have been developed, including the monoamine, cytokine, and HPT hypotheses. However, all of these hypotheses fail to address the significant lag time (approximately 2 to 4 weeks) between initiating a treatment regimen and detecting a clinical response. Molecular interactions of antidepressants at targets such as transporters and receptors are well studied and these interactions are known to take place following the first dose of drug treatment. Additional mechanisms must account for the lengthy response time. One such mechanism proposed is an increase in G-protein coupled signaling.[27] An increase in the coupling of the heterotrimeric G-protein subunit Gs_α to adenylyl cyclase results in increased cAMP, cAMP-dependent activation of protein kinase A, and subsequent phosphorylation and activation of a multitude of downstream targets. The up- or down-regulation of these targets is dependent on transcriptional and translational events that require days or even weeks for full molecular and cellular effects to be achieved and may contribute to the lag time between initiation of pharmacotherapy and evidence of a clinical response. These

targets may include the cAMP response element binding protein (CREB), a nuclear transcription factor that up-regulates gene expression. Chronic antidepressant treatment has been linked to the up-regulation of CREB in the hippocampus, cortex, and amygdala.[80,81] Wallace and coworkers[81] demonstrated that overexpression of CREB in the basolateral amygdala after training in the learned helpless model of depression caused a decrease in escape failures, an antidepressant response. In contrast, induction of CREB before training caused an increase in escape failures, a converse effect, as well as increased immobility in the forced swim test, indicating a prodepressive effect. Although the temporal expression of CREB was critical in determining antidepressant outcomes, it nonetheless implicates CREB as a key player in depression, learning, and memory, and signifies the need for controlled regulation.

Brain-derived Neurotrophic Factor

Brain-derived neurotrophic factor (BDNF) is a neurotrophin (ie, a peptide that regulates growth and survival of neurons) that functions as a neurotransmitter modulator and a regulator of plasticity mechanisms.[82] Human studies have shown that serum BDNF levels in antidepressant-naïve depressed patients are lower than those in antidepressant-treated patients or healthy controls. These findings indicate that decreased BDNF may be important in the pathophysiology of depression.[82] Shimizu et al[82] hypothesized that a decrease in BDNF may reduce the neuroprotective effect and cause stress-induced neuronal damage, leading to biological vulnerability and susceptibility to depressive diseases.[82] Several studies have reported robust increases in BDNF mRNA levels in several parts of the brain including the cortex and hippocampus after various (SSRI, TCA, MAOI) antidepressant treatments.[83] Coppell and coworkers[84] replicated this finding in rats with repeated administration of tranylcypromine, fluoxetine, paroxetine, or sertraline. Their data show BDNF mRNA levels were up-regulated in the hippocampus after long-term drug treatment. However, 4 hours after the last injection BDNF mRNA levels were down-regulated indicating that BDNF expression is differentially regulated in acute and chronic drug treatments. Interestingly, desipramine, maprotiline, and mianserin did not increase BDNF levels 24 hours post-injection.[84] Therefore, the regulation of BDNF may be drug specific as well as temporally regulated. Finally, CREB-deficient mice, unlike wild-type controls, do not exhibit increases in BDNF mRNA levels upon chronic desipramine administration.[85] Conti and colleagues[85] were unable to detect increased levels of BDNF mRNA after chronic fluoxetine administration in either control or CREB-deficient mice providing further evidence that this regulatory effect may be antidepressant specific. In summary, the data demonstrate that CREB may act upstream of BDNF and is necessary for antidepressant-induced alterations in BDNF mRNA expression.

Future Horizons of Antidepressant Development

Future treatments for depression will likely diverge further from the single target specificity of the SSRIs or the tricyclic antidepressants. Currently, novel 5-HT_{1D} and 5-HT_{1B} receptor antagonists that block presynaptic autoreceptors are under development. These agents would reverse the inhibitory role of the autoreceptors resulting in the subsequent release of 5-HT.[86] In addition, selective, reversible inhibitors of MAOs (RIMAs) such as befloxatone, teloxantrone, moclobemide, and brofaromine are being redeveloped.[11] If taken at low to moderate doses, RIMAs can be displaced from the enzyme and do not require the dietary restrictions of conventional MAOIs.[11] The emergency contraceptive mifepristone (RU-486) in addition to antagonizing progesterone receptors also antagonizes glucocorticoid receptors. Blocking the toxic effects of cortisol on glucocorticoid receptors may have therapeutic benefits in treating psychotic depression and bipolar disorder by enhancing neurocognitive function.[11,87] Furthermore, novel peptides that act as antagonists for the corticotropin-releasing factor are under development.[11] In fact, a recent study found that patients with mood disorders had a statistically significant increased number of corticotrophin-releasing hormone immunoreactive (CRH-IR) neurons in the paraventricular nucleus of the hypothalamus as well as a significantly higher number of CHR-IR neurons expressing the estrogen receptor alpha.[88] Finally, antagonists for the neurokinin receptors are being developed as inhibitors of substance P and other neurokinins. Antagonizing the effects of these peptides may have efficacy in treating mood disorders, psychosis, and anxiety.[11] Indeed, several neurokinin receptor antagonists have demonstrated the same antidepressant effect as amitriptyline and desipramine in a rodent forced-swim test model.[89]

Conclusion

Clinical depression is thought to result in part from disruptions in the serotonergic system. Numerous studies have identified SERT as a pharmacological target for treating these symptoms. Not surprisingly, an interaction of antidepressants with SERT alone is not sufficient for immediate therapeutic benefit. Although a preponderance of research has focused on determining the regulation of SERT expression, the exact mechanisms responsible remain undefined. Thus, until the normal processes of SERT regulation are delineated, the implications of discrepancies in SERT levels and function due to chronic antidepressant treatment cannot be ascertained. Furthermore, development of new antidepressants with greater therapeutic benefits will be hindered

without knowing the contribution of the various receptors and signaling proteins to this regulation as well as the contribution of non-amine systems to the etiology of depression. Finally, recent evidence shows that allelic differences exist in the responsiveness to current antidepressants. Due to the prevalence of depression in society, further investigation into why the *l*/*l* genotype of SERT allows for faster onset of therapeutic response to antidepressants and how drugs can be designed to increase the responsiveness of the *s*/*s* SERT genotype must be addressed.

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