Beyond Monoamine-Based Therapies: Clues to New Approaches

Phil Skolnick, Ph.D.

Advances in antidepressant therapy have resulted in agents with fewer serious side effects than, for example, nonselective monoamine oxidase inhibitors and tricyclic antidepressants. Nonetheless, these newer agents are far from the ideal. Many of the drawbacks associated with these newer agents—slow onset, low rate of response, and low rate of remission—are likely to be mechanism related. In order to overcome these problems, researchers must either improve upon these traditional, biogenic amine–based mechanisms or explore nontraditional mechanisms. Strategies for improving biogenic amine–based antidepressants include the so-called serotonin augmentation strategy and the broad spectrum agent that simultaneously blocks reuptake at the serotonin, norepinephrine, and dopamine transporters. Two nontraditional approaches employ modulation of glutamate receptor function. At face value, these glutamate-based approaches (*N*-methyl-D-aspartate [NMDA] antagonists and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid [AMPA] receptor potentiators) appear diametrically opposed. However, these 2 mechanisms may ultimately impact similar cellular endpoints.

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Advances in antidepressant treatment have resulted in agents that are safer and easier to use than, for example, nonselective irreversible monoamine oxidase inhibitors (MAOIs) and tricyclics (TCAs). These newer agents are far from ideal. As with the first generation antidepressants, these newer agents have a delayed onset of action, and most studies indicate that approximately 30% of patients do not respond to these agents.[1] Many of these drawbacks—including slow onset, low rate of response, and low rate of remission—may be mechanism related. There are 2 divergent approaches that may abrogate some or all of the limitations associated with today's biogenic amine–based antidepressants. The first is to utilize the biogenic amine synapse while attempting to overcome the limitations of contemporaneous agents. The second is to explore and develop novel (or at least nontraditional) mechanisms.

IMPROVING BIOGENIC AMINE–BASED APPROACHES

One biogenic amine–based mechanism that has been extensively studied on the preclinical and clinical levels is generally termed serotonin augmentation. This strategy is based on the hypothesis that the delayed onset of serotonin-based agents (including selective serotonin reuptake inhibitors [SSRIs] and TCAs such as imipramine) is produced by activation of 5-HT_{1A} autoreceptors located on cell bodies. Addition of a 5-HT_{1A} antagonist to, for example, an SSRI would block this effect, thereby hastening onset of action.

Over the past decades, it was generally thought that serotonin terminal fields were the principal sites of antidepressant action. (Figure 1 shows SSRIs inhibiting the serotonin transporter, thereby increasing the synaptic availability of serotonin, which binds to multiple serotonin receptors.) However, researchers did not appreciate the effects of these agents on somatodendritic autoreceptors when SSRIs were under development. Several years ago, it was demonstrated that neuron cell bodies also contain high densities of serotonin transporters as well as 5-HT_{1A} receptors. SSRIs act on transporters located at both the terminal fields and the cell bodies. When SSRIs inhibit serotonin reuptake in the vicinity of the cell body, 5-HT_{1A} autoreceptors on the cell body are activated, inhibiting the firing of these neurons. Over time, these 5-HT_{1A} autoreceptors adapt through a desensitization process.[2] Because the operant phrase is "over time," this adaptation has been hypothesized as one mechanism responsible for the delayed onset of action observed following SSRI administration.

Preclinical data indicate that when a serotonin 5-HT_{1A} receptor antagonist is given in combination with an SSRI, serotonin increases in the terminal fields are more robust

From DOV Pharmaceutical Inc., Hackensack, N.J.

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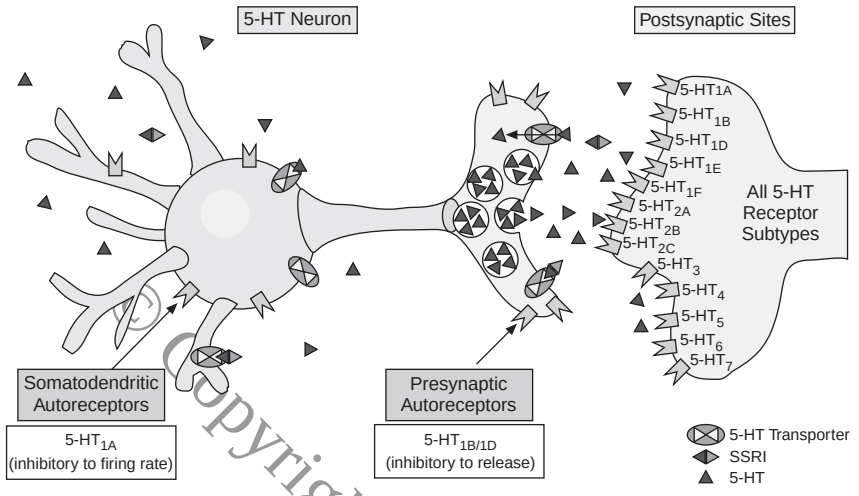
Reprint requests to: Phil Skolnick, Ph.D., DOV Pharmaceutical Inc., 433 Hackensack Ave., Hackensack, NJ 07601 (e-mail: pskolnick@dovpharm.com).

Figure 1. Serotonin Neuron[a]



[a]Used with permission from David L. Nelson, Ph.D., Lilly Neurosciences Research, Indianapolis, Ind. Abbreviations: 5-HT = serotonin, SSRI = selective serotonin reuptake inhibitor.

than when an SSRI is given alone.[3,4] However, the clinical data are not as definitive. There have been at least 10 controlled trials to date. The trials, which used pindolol as an augmenting agent, reported some benefit in terms of efficacy (30% of the trials) and a more rapid onset of action (60% of the trials).[5,6] There were significant differences in both the protocol and design among these studies, including dose and type of antidepressant, selection criteria for patients, and endpoint criteria. However, far more problematic is that pindolol, the only available 5-HT_{1A} antagonist that can be used clinically, is not a selective and specific 5-HT_{1A} antagonist. The β-adrenoceptor antagonist properties of pindolol have long been recognized. Pindolol has also been reported to act as a 5-HT_{1A} partial antagonist.[7] Further, a recent positron emission tomography study[8] showed that, in healthy volunteers, the doses of pindolol used in most augmentation strategies were not sufficient to occupy a significant proportion of 5-HT_{1A} receptors in vivo. On the basis of these findings, the hypothesis that a 5-HT_{1A} receptor blockade combined with an SSRI will be more effective (i.e., more efficacious and/or rapid acting) has not yet been adequately tested in the clinic. The ability to more appropriately test the serotonin augmentation strategy must await a clinically acceptable 5-HT_{1A} receptor antagonist, for example, or a molecule that has the properties of both a 5-HT_{1A} receptor antagonist and, for example, an SSRI.

Another biogenic amine–based approach is a broad spectrum antidepressant capable of inhibiting amine reuptake of the serotonin, norepinephrine, and dopamine transporters. Both preclinical and clinical studies indicate that dopaminergic pathways are integral to reward, or hedonic, processes. Anhedonia has long been recognized as a core symptom of depression. It is hypothesized that an increase in synaptic concentrations of dopamine, when combined with conventional antidepressants, will produce a rapid reduction in anhedonia, in effect jump-starting the onset of antidepressant action. Consistent with this hypothesis, preclinical studies demonstrate that chronic treatment with antidepressants (e.g., imipramine)[9] sensitizes dopamine receptors, indicating that this may be important for an antidepressant action. Further, increasing synaptic dopamine levels (or introducing a dopamine antagonist) may also increase the efficacy of conventional antidepressants. Thus, adjunctive use of pergolide (a mixed D_1/D_2 receptor agonist) with conventional antidepressants appears to improve the Clinical Global Impressions scale ratings in approximately 40% of patients refractory to these conventional agents.[10] Increasing synaptic dopamine concentrations could be accomplished by combining a dopamine reuptake blocker with a dual uptake inhibitor, for example. However, treatment with multiple drugs may introduce pharmacokinetic confounds. The ideal drug would be a single molecule that inhibits the reuptake of serotonin, norepinephrine, and dopamine. While the clinical efficacy of such a broad spectrum antidepressant has not yet been demonstrated, we have recently completed a first in-human safety study with a molecule that inhibits the reuptake of all 3 transmitters (B. Beer, Ph.D.; A. Lippa, Ph.D.; P.S.; et al., unpublished data, July 2001).

BEYOND THE MONOAMINERGIC SYNAPSE: IDENTIFYING NEW TARGETS

Over the past decade, new targets for antidepressant treatment have been identified through an increased understanding of the signal transduction pathways impacted by monoamine-based therapies. For example, most monoamine-based antidepressants stimulate adenylyl cyclase through G protein (G_s)–coupled receptors, such as β-adrenergic and 5-$HT_{4,6,7}$. Elevated levels of cyclic adenosine monophosphate (cAMP) are known to activate a cAMP-dependent protein kinase A, which regulates the phosphorylation of specific proteins like the transcription factor cAMP response element binding protein (CREB).[11] Duman et al.[11] hypothesized that activated CREB resulting from chronic antidepressant treatments in turn increases levels of mRNA encoding brain-derived neurotrophic factor (BDNF) in the hippocampus. BDNF is a member of the

nerve growth factor family that, when applied to neurons, has been shown to protect serotonin and dopamine neurons against insult (reviewed in Altar[12] and Skolnick et al.[7]). This protective quality may mean that BDNF induction dampens the ability of chronic stressors to damage valuable neurons.[11] The transcriptional control of BDNF mRNA in the mammalian central nervous system is complex (e.g., Timmusk et al.[13]). However, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor activation has been known for more than a decade to be an effective means of increasing BDNF mRNA.[14]

Recently, my colleagues and I[15] tested whether a novel AMPA receptor potentiator, LY392098, would increase the expression of BDNF. We found that adding either AMPA or LY392098 to cortical neurons elicited a time- and concentration-dependent increase in mRNA encoding BDNF. Further, the co-addition of subeffective concentrations of AMPA (e.g., 1 μM) and LY392098 (1 μM) resulted in a 25-fold increase in BDNF mRNA levels and in an approximately 7-fold increase in protein levels. The results of this research show that this druglike molecule can dramatically increase BDNF mRNA expression in vitro. It is not known if increasing BDNF expression is either necessary or sufficient for an antidepressant action of conventional agents. However, we now know that these AMPA receptor potentiators produce antidepressant-like actions in models of behavioral despair such as the forced swim and tail suspension tests.[16,17] This type of mechanism may circumvent some of the limitations inherent in biogenic amine–based agents.[1]

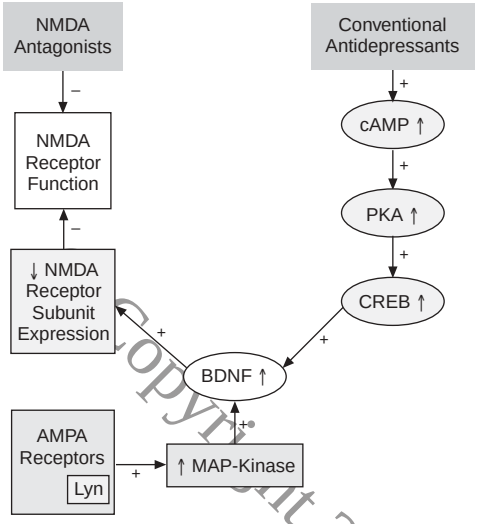
One potential mechanism that would explain the antidepressant-like effects of AMPA receptor potentiators in behavioral despair models is that such compounds release biogenic amines (e.g., norepinephrine). My colleagues and I[7] have begun studies to explore this possibility. Initially, we compared the effects of fluoxetine and LY392098 on norepinephrine, dopamine, and serotonin release (using microdialysis) in prefrontal cortex. Fluoxetine administered at 3 mg/kg, 10 mg/kg, and 20 mg/kg significantly increased dialysate concentrations of serotonin (and at the highest dose norepinephrine and dopamine). In some behavioral despair models, these doses of fluoxetine are pharmacologically active. A dose of LY392098 (1 mg/kg) that was active in behavioral despair models did not produce changes or increases in dialysate concentrations of these biogenic amines.[7] These data indicate, but by no means prove, that the effects of LY392098 in behavioral despair models are independent of any effects on biogenic amines.

Another potential target for new antidepressants is a glutamate-based mechanism, which is, from a cellular standpoint, diametrically opposed to the actions of AMPA receptor potentiators. Thus, *N*-methyl-D-aspartate (NMDA) receptor antagonists will hyperpolarize the cell membrane while AMPA receptor potentiators in the presence of glutamate will depolarize it. Over a decade ago, Ramon Trullas and I[18] examined functional NMDA antagonists in animal behavioral despair models. We found that a competitive NMDA antagonist (2-amino-7-phosphonoheptanoic acid [AP-7]), a noncompetitive NMDA antagonist (dizolcipine [MK-801]), and a partial agonist at strychnine-insensitive glycine receptors (1-aminocylopropanecarboxylic acid [ACPC]) mimicked the effects of clinically effective antidepressants in these models. On the basis of these findings, we proposed that compounds capable of reducing neurotransmission at the NMDA receptor complex may represent a new class of antidepressants. Since that time, a number of studies have confirmed and extended the observation that functional NMDA antagonists are active in behavioral despair models. The NMDA receptor has many different loci at which transmission can be reduced, and structurally diverse compounds with NMDA antagonist properties produce antidepressant actions in these models.

Clinical evidence for the antidepressant action of NMDA antagonists can be found in a study by Berman et al.[19] that examined the antidepressant effects of ketamine, an NMDA antagonist. Seven patients who met DSM-IV criteria for major depression underwent 2 treatment days separated by at least 1 week in randomized, double-blinded conditions. Patients were randomly assigned to receive either a saline solution or a saline solution with ketamine (a total dose of 0.5 mg/kg). The solutions were infused over a 40-minute period. The 4 patients who received the intravenous ketamine treatment had significant improvement in their depressive symptoms, which was evidenced by reduced Hamilton Rating Scale for Depression scores; the effects were rapid and maintained for at least 72 hours after ketamine infusion. These results indicate the preclinical data concerning NMDA antagonists as antidepressants are correct and predictive.

Convergent evidence that NMDA receptors are valid targets for developing novel agents comes from reports indicating that chronic antidepressant treatment affects NMDA receptors. In one such study,[20] citalopram and imipramine were given chronically to mice. Using in situ hybridization, the researchers observed region-specific effects on mRNA levels encoding NMDA receptor subunits. The antidepressants modestly reduced ζ subunit expression mRNAs in the cortex, cerebellum, thalamus, and striatum, but the drugs did not produce any substantial effects in the hippocampus. In contrast, citalopram and imipramine produced distinct, region-specific effects on mRNA levels encoding the ε family of subunits. For example, citalopram reduced ε1 subunit mRNA levels in CA2 of hippocampus by approximately 40%, while imipramine produced widespread reductions in ε2 subunit mRNA levels in CA1–4 of hippocampus. In this study, the changes in mRNA levels were not bidirectional:

Figure 2. Linking Conventional Antidepressant Treatment to the Down-Regulation of NMDA Receptor Function[a]



[a]Adapted with permission from Skolnick.[1] Abbreviations: AMPA = α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid, BDNF = brain-derived neurotrophic factor, cAMP = cyclic adenosine monophosphate, CREB = cAMP response element binding protein, Lyn = a member of the Src family protein tyrosine kinases, MAP = mitogen activated protein, NMDA = *N*-methyl-D-asparate PKA = protein kinase A. Symbols: + = stimulates, – = inhibits, ↑ (internal) = elevated, ↓ (internal) = reduced.

mRNA levels either were unchanged or reduced (in several instances rather dramatically) by chronic antidepressant treatment. This research[20] supports the notion that chronic antidepressant treatment with conventional biogenic amine–based antidepressants can reduce NMDA receptor function. It may be hypothesized that chronic treatment with conventional antidepressants results in the same functional endpoint as administration of NMDA antagonists.[1]

CONCLUSIONS

The hypothesis that biogenic amine–based antidepressants, AMPA receptor potentiators, and NMDA antagonists converge on common cellular and molecular targets is illustrated in Figure 2. The ability of chronic antidepressant treatment to increase mRNA levels encoding BDNF in hippocampus has been described.[21] Evidence is mounting that this effect is produced via a CREB-dependent mechanism.[22,23] In this figure, BDNF is shown as dampening NMDA receptor function. Although this has not been directly demonstrated in vivo, Brandoli et al.[24] have reported that incubation of primary neuron cell cultures with BDNF produces a robust reduction in mRNA levels encoding ε subunits (in some cases, similar to those produced in vivo following chronic antidepressant treatment) and a dampening of NMDA receptor function. Since NMDA antagonists produce this same dampening, it is hypothesized that a reduction in NMDA receptor function may represent a common mechanism through which structurally diverse compounds produce an antidepressant action. Whether more efficacious or rapid antidepressants will emerge from any of these strategies is unknown at this time. However, given the need to improve on our current armamentarium of drugs to treat depression, these strategies are worth pursuing in the clinic.

Drug names: citalopram (Celexa), fluoxetine (Prozac and others).

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