Innovative Approaches for the Development of Antidepressant Drugs: Current and Future Strategies

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Summary: Depression is a highly debilitating disorder that has been estimated to affect up to 21% of the world population. Despite the advances in the treatment of depression with selective serotonin reuptake inhibitors (SSRIs) and serotonin and norepinephrine reuptake inhibitors (SNRIs), there continue to be many unmet clinical needs with respect to both efficacy and side effects. These needs range from efficacy in treatment resistant patients, to improved onset, to reductions in side effects such as emesis or sexual dysfunction. To address these needs, there are numerous combination therapies and novel targets that have been identified that may demonstrate improvements in one or more areas. There is tremendous diversity in the types of targets and approaches being taken. At one end of a spectrum is combination therapies that maintain the benefits associated with SSRIs but attempt to either improve efficacy or reduce side effects by adding additional mechanisms (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2C}, α-2A). At the other end of the spectrum are more novel targets, such as neurotrophins (BDNF, IGF), based on recent findings that antidepressants induce neurogenesis. In between, there are many approaches that range from directly targeting serotonin receptors (5-HT_{2C}, 5-HT_6) to targeting the multiplicity of potential mechanisms associated with excitatory (glutamate, NMDA, mGluR2, mGluR5) or inhibitory amino acid systems (GABA) or peptidergic systems (neurokinin 1, corticotropin-releasing factor 1, melanin-concentrating hormone 1, V1b). The present review addresses the most exciting approaches and reviews the localization, neurochemical and behavioral data that provide the supporting rationale for each of these targets or target combinations. **Key Words:** Monoamines, glutamate, peptides, neurotrophins, GABA, serotonin.

INTRODUCTION

Depression is a prevalent psychiatric disorder with estimates reaching as high as 21% of the world population. Despite the fact it is a psychiatric disorder, the World Health Organization predicts that it will be the second leading cause of death by the year 2020 due to complications arising from stress and the cardiovascular system. Multiple subtypes of depression exist although major depressive disorder or unipolar depression appears to be the predominant diagnosed subtype and has been the primary focus of drug development efforts. Depression is characterized most often by anhedonia or the loss of interest or pleasure in normal daily activities and feelings of sadness. Additional symptoms may include sleep disturbances, a gain or loss of weight accompanied, respectively, by increases or decreases in appetite, recurrent inappropriate feelings of guilt, psychomotor agitation, difficulty concentrating and thinking including indecisiveness and thoughts of death or suicide. The diagnosis as clinically described in the DSM-IV (Diagnostic and Statistical Manual of Mental Disorders –Fourth Edition, 1994) requires that five of the nine major criteria are met during a period persisting for at least 2 weeks.

Although the first drugs discovered to treat depression were serendipitous in nature, the foundation for further development of drugs that modulate monoaminergic neurotransmission was established. The chemical underpinnings of depression for the last 50 years have been referred to as the monoamine hypothesis that postulates that the debilitating and often chronic symptoms of depression result from perturbations in serotonin (5-HT), norepinephrine and/or dopamine transmission. This hypothesis spawns from work done in the late 1950s showing that monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), which elevate levels of monoamines by preventing their metabolism and block-

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ing their reuptake, respectively, were effective antidepressants.[1] Interestingly, further support for the chemical hypothesis of depression is based on clinical data where the side effects of reserpine as an antihypertensive agent in the 1960s suggested that depleting brain monoamines had detrimental effects on mood.[2] In the 1980s and 1990s, the MAOIs and TCAs were superseded by the next generation of antidepressants termed the selective serotonin reuptake inhibitors (SSRIs) including fluoxetine (Prozac), paroxetine (Paxil), and sertraline (Zoloft), which are characterized by their ability to preferentially increase serotonin levels. A more recent class of novel antidepressants blocks the reuptake of both serotonin and norepinephrine. Such dual-acting serotonin/norepinephrine reuptake inhibitors (SNRIs), including venlafaxine (Effexor), are reported to exhibit a faster clinical onset of action[3–5] and be more effective in treating depression that is refractory to other types of antidepressants.[6,7] Bupropion (Wellbutrin), likely by virtue of its ability to block the reuptake of norepinephrine and dopamine, has been touted as an effective antidepressant with less incidence of side effects including sexual dysfunction and weight gain.[8] Notably and regardless of their mechanism of action, there continue to be significant unmet clinical needs with respect both to efficacy and side effect profile.

Over the last 10 years, it has also been revealed that depression may be related to neurodegenerative processes that lead to loss of synaptic connectivity and perhaps neuronal networks in limbic brain structures including the hippocampus and cortex. Several reports have now demonstrated that the volume of the hippocampus and prefrontal cortices are decreased and structural imaging studies have shown reduced gray matter volumes in depressed patients.[9–11] Notably, the amygdala, which may play a role in the neuronal circuitry involved in regulating mood, appears to be enlarged in patients exhibiting their first major episode of depression but subsequently shrinks in volume with prolonged and recurrent depression.[12,13] The significance of this has yet to be determined and raises interesting questions regarding the role of the amygdala in depression. Furthermore, there appear to be functional consequences of this volume loss based upon anatomical correlates related to regional cerebral blood flow and glucose metabolism.[14] Additionally, in terms of loss of function, it has been demonstrated and observed that there is a loss of executive memory in depressed patients. The finding that depressed patients frequently exhibit cognitive deficits is well documented and dates back to work done in the 1960s.[15] The framework in which one can base the comorbidity of depression and cognitive deficits can certainly be related to overlapping and reciprocal neural networks in the brain.[16,17] Nonetheless, it is still not clear if the neurodegenerative aspects play a major role in the etiology of the disease or are secondary as a result of neurotoxicity induced by increased excitatory amino acids such as glutamate, increased cortisol levels, or a decrease in the functioning of neurotrophins.

TABLE 1. *Unmet Clinical Needs for Antidepressant Drug Development*

Unmet Medical Need
● Decrease in side effect profile (sexual dysfunction and gastrointestinal events)
● Efficacy in refractory patients
● Treatment remission (recovery, relapse and recurrence)
● Weight gain
● Faster onset of antidepressant action
● Return of normal sleep patterns
● Reduction of cognitive deficits

Despite the advances that have been made in the development of antidepressants, there are clearly still unmet clinical needs that need to be addressed (Table 1). The future generation of successful antidepressants will need to address multiple efficacy parameters or issues that lead to discontinuation such as sexual dysfunction. The current strategies being undertaken are generally directed at symptomatic and/or disease modifying approaches. Indeed there are multiple new approaches in progress to improve current pharmacological means of modulating serotonin or norepinephrine neurotransmission by either combining mechanisms or alternatively selectively stimulating receptor subtypes that may trigger improved efficacy or fewer side effects (FIG. 1). Overall, these approaches take advantage of the current state of

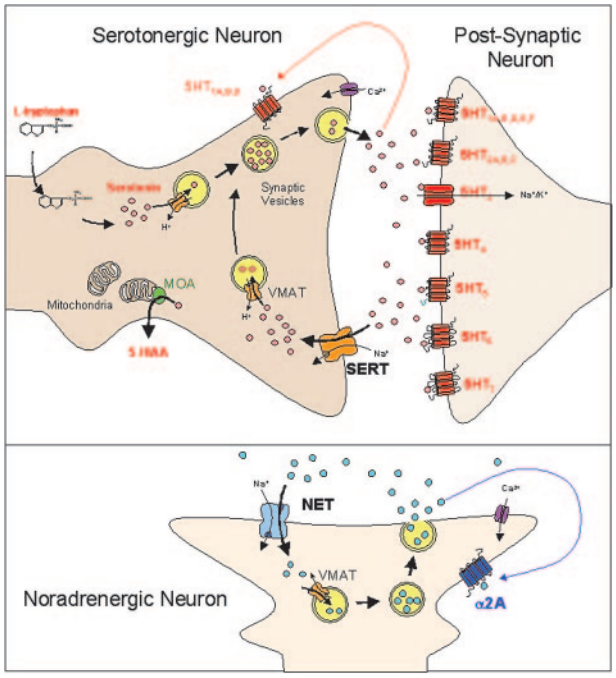

FIG. 1. Potential targets for novel monoaminergic antidepressants: overview of a serotonergic and noradrenergic synapse defining the multiple targets for drug development.

understanding as to the role of monoaminergic enhancement strategies. Other innovative approaches are targeting excitatory or inhibitory amino acids and receptors, peptidergic systems, and neurotrophins. This review will bring to light the current progress in these areas with emphasis on the background, rationale and potential advantages, which may arise from these approaches.

MONOAMINERGIC STRATEGIES

The monoamine hypothesis of depression postulates that the etiology and pathogenesis of depression arises from central deficiencies in serotonin, norepinephrine, and/or dopamine. Correspondingly, current pharmacotherapies have been developed in an effort to amend these alterations in monoaminergic systems (e.g., SSRIs, SNRIs). Regardless of their mechanism of action, however, a drawback of all marketed antidepressants is the 3- to 5-week delay necessary to achieve therapeutic efficacy. This lag time is thought to reflect the time required for desensitization of the receptors regulating monoamine release (e.g., 5-HT_{1A}, 5-HT_{2C}, and α-2 adrenergic receptors). To potentially accelerate the onset of antidepressant action—as well as limit unwanted side effects—current drug development strategies are focusing on designing new antidepressants with dual and/or triple modes of action. These approaches, along with examples of preclinical and clinical studies, will be highlighted in the following sections.

SSRI/5-HT_{1A} antagonists

The delayed clinical efficacy of SSRIs is believed to result, to a large extent, from the indirect activation of somatodendritic 5-HT_{1A} autoreceptors. A profound body of preclinical literature indicates that acute SSRI treatment increases serotonin levels in various brain regions including the dorsal raphe nuclei. This elevation in serotonin engages inhibitory 5-HT_{1A} autoreceptors residing in the dorsal raphe to inhibit 5-HT cell firing and dampen subsequent 5-HT release in terminal serotonergic brain regions.[18] However, following long-term SSRI treatment (14–21 d) 5-HT_{1A} autoreceptors desensitize resulting in more pronounced elevations in serotonin levels compared to acute treatment.[19,20] These data suggest that a strategy combining SSRIs with 5-HT_{1A} receptor antagonists would produce robust and more rapid increases in central serotonin levels and likely yield an antidepressant with an accelerated onset of activity. This neurochemical hypothesis is supported by a plethora of microdialysis studies demonstrating that pretreatment with selective 5-HT_{1A} antagonists such as WAY-100635 augments SSRI- and SNRI-induced changes in cortical serotonin levels.[21] Preclinical models sensitive to the behavioral effects of serotonergics corroborate these findings as 5-HT_{1A} antagonism is reported to potentiate the antidepressant-like effects of SSRIs in the rodent resident-intruder, social interaction, and schedule-induced polydipsia assays.[22–24] Clinical data using this combination strategy demonstrate that the antidepressant activity of SSRIs is accelerated and/or enhanced when combined with the mixed 5-HT_{1A}/β-adrenoceptor antagonist, pindolol[25]. While the results of these clinical studies remain somewhat equivocal, there is enough collective evidence suggesting that a strategy combining SSRIs with 5-HT_{1A} antagonists may be useful in the clinical management of depression. Correspondingly, several companies have recently published on the synthesis of such dual-acting compounds possessing potent activity as both SSRIs and full/partial 5-HT_{1A} receptor antagonists, some of which are reported to be active in preclinical models of depression.[26–28] As some of these dual-acting SSRI/5-HT_{1A} compounds begin their clinical evaluation, it may only be a matter of time to determine whether this approach will represent the newest generation of antidepressants.

SSRI/5-HT_{2C} antagonists

Desensitization of 5-HT_{2C} receptors is routinely reported following chronic SSRI treatment. However, the overall contribution of this molecular change to the antidepressant effects of SSRIs is not well understood. Recent data suggest that 5-HT_{2C} receptor inactivation may play a role in augmenting the neurochemical and behavioral effects of antidepressants. Using *in vivo* microdialysis techniques in rats, Cremers et al. and others showed that the selective 5-HT_{2C} antagonists, SB 242084 and RS102221, and the nonselective 5-HT_{2C} receptor antagonists, ketanserin and irindalone, potentiate the neurochemical effects of SSRIs on hippocampal and cortical serotonin levels.[29,30] Despite the robust neurochemical effects when these agents are combined, 5-HT_{2C} receptor antagonism alone has no significant effects on extracellular serotonin.[30,31] Similar to the reported neurochemical effects, this serotonergic combination produces marked augmentation of the antidepressant-like effects of SSRIs in behavioral models of depression and anxiety including the mouse tail suspension test (TST) and schedule-induced polydipsia assay.[29,31] Complementary studies done in 5-HT_{2C} receptor null mutant mice show enhanced neurochemical and behavioral (TST) responses to fluoxetine compared to their wild-type littermates.[31] Although the precise neural mechanisms mediating these enhanced behavioral and neurochemical responses are unclear, it likely occurs at the postsynaptic level via modifying negative feedback mechanisms. This is evidenced by studies showing that local infusion of RS102221 into serotonergic nerve terminals produces effects on SSRI neurochemistry similar to that of systemically administered 5-HT_{2C} antagonists.[29] Overall, these preclinical data show that 5-HT_{2C}

antagonism augments the neurochemical and behavioral effects of SSRIs. Moreover, these data highlight a novel strategy of combining both targets, either in a single molecular entity or as adjunctive therapy to already marketed SSRIs, for the potential treatment of depressive disorders.

SSRI/α-2 adrenergic antagonists

The success of SNRIs in the clinic underscores the importance of elevating both norepinephrine and serotonin in the treatment of depression. Ways to enhance the effects of SSRIs via concomitant blockade of serotonin receptors have already been discussed. However, a strategy that targets noradrenergic autoreceptors may have merit in augmenting the neurochemical effects of conventional antidepressants. Several classes of antidepressants, particularly norepinephrine reuptake inhibitors such as reboxetine (Edronax) and the SNRIs, acutely elevate extracellular levels of norepinephrine. The release of norepinephrine can activate presynaptic α-2 adrenergic autoreceptors located on both norepinephrine and dopamine cells causing blunted noradrenergic and dopaminergic responses, respectively. Furthermore, because serotonin efferents from the dorsal raphe are inhibited by local α-2 adrenergic receptors, blocking α-2 receptors may also influence serotonergic.[32] Thus, antidepressants, when given in combination with agents that "turn off " α-2 autoreceptors, can potentially elevate levels of all three monoamines. Neurochemical validation of this hypothesis comes from microdialysis studies showing that α-2 adrenergic antagonists markedly potentiate the ability of antidepressants to increase extracellular levels of norepinephrine, serotonin, and dopamine, depending on the brain region examined.[32] Although there are essentially no published data showing that this particular combination strategy is efficacious in preclinical behavioral models of depression, the data from microdialysis studies suggest that α-2 adrenergic antagonism may strengthen the neurochemical effects of antidepressants, and may improve the efficacy of antidepressants in humans. Several arguments support this notion because coadministration of antidepressants with α-2 adrenergic antagonists results in an accelerated down regulation of cortical β-adrenergic receptors.[33] In addition, nonselective α-2 adrenergic receptors antagonists such as mirtazapine (Remeron) are reported to possess modest antidepressant activity in their own right.[34] Finally, clinical studies emphasize that combining SSRIs with nonselective α-2 receptor antagonists actually shortens the time required to achieve antidepressant activity.[35,36] Collectively, these data have ignited considerable chemistry efforts to design and synthesize novel antidepressant molecules that combine monoamine reuptake inhibition with α-2 adrenergic receptor antagonism.[37,38]

Triple monoamine uptake blockers

Whereas the monoamine hypothesis of depression primarily focuses on the role of norepinephrine and serotonin, a critical role of dopamine in mediating the action of antidepressants was postulated nearly three decades ago.[39] Adjunctive treatment with dopamine receptor agonists augments the effects of antidepressants in the rodent forced swim test, whereas, clinically, dopamine agonists have been shown to improve depressive symptomatology in patients refractory to conventional antidepressants.[40–43] This evidence, taken together with the clinical success of SSRIs and SNRIs, provides considerable rationale for targeting all three monoamine reuptake sites in the treatment of depression. Moreover, this "broad-spectrum" approach has gained momentum recently as growing clinical and preclinical evidence links core symptoms of depression (i.e., anhedonia) to deficits in dopaminergic transmission. Recently, a single molecule possessing nanomolar inhibition of uptake of all three monoamines has been described and shown to be active *in vivo*.[44] Thus, DOV 21,947 produces antidepressant-like activity in the rodent forced swim and tail suspension tests.[44] The success, however, of this compound—as well as the strategy and benefit of combing inhibition of all three monoamines into a single molecule—is still awaiting evaluation in human patients.

Additional multitarget, monoamine strategies

Both transporter and inhibitory autoreceptor mechanisms strictly control the release of biogenic amines into the extracellular environment. For instance, 5-HT_{1A} and 5-HT_{1B} receptors are somatodendritic and terminal autoreceptors, respectively, regulating levels of central serotonin levels. Blockade of 5-HT_{1B} receptors alone has been shown to acutely increase levels of serotonin in the guinea pig frontal cortex and hippocampus as well as augment the effects of SSRIs on serotonin levels.[45] Combining the selective 5-HT_{1A} antagonist, WAY-100635, with the 5-HT_{1B} receptor antagonist, SB-224289, produced marked elevations in serotonin levels in the guinea pig.[46] These latter results curiously suggest that combining 5-HT_{1A} and 5-HT_{1B} receptor antagonism can elevate serotonin and, consequently, potentially be an effective strategy to treat depression. Additional examples of targeting multiple postsynaptic receptors as putative antidepressant agents include the 5-HT_{1A} agonist/α-2 antagonist, sunepitron, the 5-HT_{1A} agonist/dopamine D2 agonist, roxindole, and α-2 adrenergic antagonist/5-HT_2 antagonist, mirtazapine.[47] It is also noteworthy that, in addition to their strict regulation of monoamine release, these monoaminergic receptors (e.g., 5-HT_{1A} and α-2 adrenergic receptors) also influence the release of other neurotransmitters such as acetylcholine, which is thought to be procognitive.[48] Therefore, targeting the aforementioned receptor systems could yield an effective antide-

pressant with an added benefit of improving cognitive dysfunction, for example, which is commonly reported in patients suffering from major depressive disorders.

In summary, these strategies seem to efficiently "tweak" the monoaminergic systems in the hopes of developing a more rapid acting antidepressant. However, much needed clinical data regarding the efficacy, safety, and tolerability of such "dual-acting" compounds is eagerly awaited. Perhaps newer approaches targeting convergent, down stream components of the monoamine system (e.g., neurotrophins) and/or nonmonoaminergic systems including GABA and glutamate may ultimately prove beneficial in the clinical management of depression.

Subtype selective serotonergic approaches: 5-HT_{2C} and 5-HT_6 agonists

While there are many strategies targeted toward improving the effects of SSRIs by the addition of another mechanism (norepinephrine reuptake inhibition, 5-HT_{1A} antagonist, 5-HT_{2C} antagonist; α-2A antagonist; see above), an alternative strategy involves directly targeting the postsynaptic serotonergic receptors that may more directly mediate the antidepressant and/or anxiolytic effects of SSRIs. SSRIs increase levels of synaptic serotonin that act at 14 different serotonin receptors. The antidepressant and/or anxiolytic effects of SSRIs are likely mediated by one or more of these receptors, but it is unlikely that all 14 play a critical role. The undesired side-effects of SSRIs are also likely mediated via the activation of one or more of these receptors that may be distinct from those that mediate antidepressant or anxiolytic actions. Due to feedback regulation by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors, chronic administration of SSRIs is necessary to sustain increases in serotonin levels in brain regions associated with depression such as frontal cortex, consistent with the 2- to 3-week delay required for antidepressants to become effective.[21,49] Therefore, by specifically targeting a postsynaptic serotonin receptor, it may be possible to improve onset of action.

Two potential candidate serotonin receptors for mediating either the antidepressant or anxiolytic effects of SSRIs are the 5-HT_{2C} receptor and the 5-HT_6 receptor. Over the last few years, a number of novel 5-HT_{2C} receptor agonists including Org 37684,[50] Ro 60-0175,[51] WAY-161503,[52] YM348,[53] WAY-629,[54] and WAY-163909[55] have been identified and characterized for multiple indications including depression and obsessive-compulsive disorder.[55] Ro 60-0175, WAY-161503 and WAY-163909, which are highly structurally diverse, have been more extensively characterized in depression models than the other recently identified 5-HT_{2C} receptor agonists. The first 5-HT_6 receptor agonists (LY586713 and WAY-466) have also been identified and are being evaluated as potential treatments for depression and/or anxiety.[56,57]

5-HT_{2C} receptor agonists show antidepressant-like effects in multiple animal models of depression. For example, 5-HT_{2C} receptor agonists decrease immobility time and increase swimming time in the forced swim test in rats in a manner comparable with SSRIs.[58] The effects of the 5-HT_{2C} receptor agonists and SSRIs in the rat forced swim test are antagonized by 5-HT_{2C} receptor antagonists consistent with the role for 5-HT_{2C} receptors in mediating antidepressant-like effects of 5-HT_{2C} receptor agonists and SSRIs.[58] 5-HT_{2C} receptor agonists are also effective in additional models of antidepressant action including the DRL-72 s (DRL = differential reinforcement of low rate) model, the resident-intruder model, the olfactory bulbectomy model and the chronic mild stress model.[51,59,60] In both the chronic mild stress model and in the olfactory bulbectomy model, antidepressants typically require 2–3 weeks of administration to show effectiveness. 5-HT_{2C} receptor agonists are effective by day 3 in the chronic mild stress model and are effective following short-term treatment (<1 week) in the olfactory bulbectomy model, consistent with rapid onset antidepressant-like effects (Rosenzweig-Lipson, S., unpublished observation).[51,59,61] Taken together, these results suggest that 5-HT_{2C} receptor agonists have therapeutic potential as rapid onset antidepressants.

5-HT_{2C} agonists may also be beneficial in the treatment of some anxiety disorders such as obsessive-compulsive disorder and panic anxiety. Several lines of evidence suggest that 5-HT_{2C} receptor agonists may be effective treatments for obsessive-compulsive disorder. 5-HT_{2C} knockout mice exhibit compulsive-like behaviors.[62] 5-HT_{2C} receptor agonists are effective in animal models of compulsive behavior, such as schedule-induced polydipsia (SIP), 8-hydroxy-2-dipropyl-aminotetralin-induced scratching in squirrel monkeys, marble burying, and excessive eating of palatable foods.[51,63] The acute effects of 5-HT_{2C} agonists in SIP contrast with the effects of serotonin reuptake inhibitors that typically require chronic administration in this model.[24,51,64] It has been suggested that SIP may also be an appropriate model for detecting the onset of activity of antidepressant compounds insomuch as combining the activity of 5-HT_{1A} or 5-HT_{1B} antagonists with SSRIs results in an acute effect in this model.[24] 5-HT_{2C} agonists are also effective in a model of panic anxiety.[65] Taken together, these results suggest that 5-HT_{2C} agonists may be effective rapid onset treatments for obsessive-compulsive disorder and panic anxiety.

The introduction of novel 5-HT_6 receptor agonists has allowed for the investigation of their potential in anxiety and depression. Current therapeutic agents for the treatment of anxiety disorders include benzodiazepines and SSRIs that act either directly or indirectly to modulate

GABAergic neurotransmission. Benzodiazepines, which act as positive allosteric modulators of the $GABA_A$ receptor/Cl^- ion channel complex, enhance GABA signaling following receptor stimulation. SSRIs may enhance levels of GABA as predicted from recent imaging studies in humans.[66] Interestingly, immunohistochemical studies suggest that 5-HT_6 receptors are colocalized on GABAergic neurons.[67] In neurochemical studies, both WAY-181187 and WAY-466 consistently elevate levels of GABA in multiple brain regions associated with anxiety including frontal cortex and amygdala (Schechter, L., unpublished observations). The neurochemical effects of 5-HT_6 agonists can be blocked by 5-HT_6 receptor antagonists, indicative of 5-HT_6 receptor activation in the mediation of these effects. Additional supporting neurochemical evidence is based on the effects of 5-HT_6 receptor agonists on stimulated glutamate release. Both WAY-181187 and WAY-466 attenuate stimulated glutamate release in brain slices (Schechter, L., unpublished observations). Under stressful situations, glutamatergic neurotransmission may increase in cortical and limbic systems and may be associated with anxiety symptoms as well as hippocampal atrophy. These hypoglutamatergic effects may also be beneficial in obsessive-compulsive disorder, which may involve increased levels of glutamate and dopamine.[68,69] To that end, in electrophysiological studies, chronic administration of a 5-HT_6 receptor agonist decreases the basal firing rate of the A9 dopaminergic cell body (substantia nigra compacta) that would be expected to decrease stereotypic behavior, which is one of the cardinal symptoms of the disease. Moreover, 5-HT_6 agonists are effective acutely in a schedule-induced polydipsia model, indicative of a rapid onset anti-OCD-like effect (Schechter, L., unpublished observations). Taken together, these results are suggestive of potential anxiolytic-like activity for 5-HT_6 agonists.

5-HT_6 receptor agonists may also play a role in depression. Antidepressants, including SSRIs, upregulate BDNF gene expression.[70] One candidate 5-HT receptor for mediating these changes in BDNF is the 5-HT_6 receptor. The 5-HT_6 agonist LY586713 upregulates BDNF mRNA in the hippocampus following either acute or short-term (4 day) treatment; effects which can be blocked by a 5-HT_6 antagonist.[56] Additional studies investigating the role of 5-HT_6 agonists in depression will be required to further elucidate the role of this receptor.

STRATEGIES TO TARGET EXCITATORY AMINO ACIDS

The NMDA receptor is an ionotropic glutamate receptor with highest densities located in cortico-limbic regions of the brain. Extracellular glutamate concentrations are enhanced by various stressors, like tail pinch and restraint,[71,72] and an involvement of the NMDA receptor became apparent in the modulation of stress-induced glutamate responses. Furthermore, chronic antidepressant administration can influence NMDA receptor function and receptor binding profiles, as well as generate regional alterations in mRNA expression that encodes multiple NMDA receptor subunits.[73,74] The NMDA receptor is composed of an oligomer of neuromodulatory subunits including at least one NR1 entity necessary for channel function and several other sites termed NR2A-NR2D that associate to form the channel.[75] The regulatory subunits include a glycine-sensitive binding site, two voltage-sensitive magnesium and zinc sites (involved in blocking NMDA responses) and two polyamine sites (either stimulatory or inhibitory). With this in mind, the direction of major research efforts for the treatment of depression and affective disorders now encompasses the development of compounds that regulate the target-rich environment within the NMDA receptor complex.

An extensive library of noncompetitive NMDA antagonists (e.g., MK-801, memantine, ketamine) that reduce glutamatergic transmission at the NMDA receptor have demonstrated antidepressant-like effects in animal models, including forced swim and tail suspension tests, inescapable stressors, and in learned helplessness.[76,77] In clinical trials, Berman et al. reported the first placebo-controlled, double-blind study assessing the therapeutic effects of a single infusion dose of ketamine in unipolar depressives.[78] Their observations indicated that the antidepressant effects of ketamine appeared only after plasma clearance of the drug and supported a rebound hypothesis in which NMDA receptor blockade possibly triggers therapeutic neurogenesis. There is evidence from preclinical studies in rats that subanesthetic doses of ketamine applied subchronically can enhance neurogenesis in the hippocampal subgranular zone.[79] The potential antidepressant efficacy of another NMDA antagonist, felbamate is under phase II evaluation for treatment-resistant bipolar depression.[80] Felbamate, a 2-phenyl-1, 3-propanediol dicarbamate, is a potent nonsedative anticonvulsant whose clinical effect may be related to the inhibition of NMDA currents through NR1-2B subunits, with additional inhibitory activity at AMPA/kainate and dihydropyridine-sensitive calcium channels; however, its precise mode of action remains unclear.

Glycine and D-serine are endogenous ligands that potentiate NMDA receptor-mediated neurotransmission by association with the NMDA/glycine-sensitive binding site. The site is activated by the presence of endogenous glycine and is required for receptor activation by L-glutamate. Chronic exposure to imipramine and ECS treatment was reported to induce adaptive changes at the glycine-sensitive site.[81] D-Cycloserine is a partial agonist at the glycine-sensitive site and exhibits anxiolytic-like

activity in the fear-potentiated startle response,[82] and the Vogel conflict drinking test at relatively high doses (200–300 mg/kg) in rats.[83] However, attempts to synthesize glycine and/or D-serine amino acid analogs with selective agonist activity at the NMDA/glycine binding site have so far remained unsuccessful.

The importance of the polyamine site in NMDA receptor function and especially its relevance to animal behavior is subject to debate. Polyamines can alter the function of NMDA receptors via the polyamine site(s) and the NR2B subunit renders NMDA receptors particularly sensitive to potentiation by polyamines. At the stimulatory polyamine site, both spermine and spermidine can increase the binding of open channel blockers, such as MK-801, and of L-glutamate and glycine to their respective sites. Ifenprodil, a competitive antagonist at the stimulatory polyamine site has an overall negative modulatory effect on the receptor, decreasing the binding of MK-801 and its analogs.[84] Ifenprodil, at a relatively low dose, has a favorable anxiolytic-like profile without associated deficits in working memory in mice using the plus-maze paradigm.[85] This suggests that selective modulators of the polyamine site to promote anxiolytic effects might provide distinct therapeutic advantages over other noncompetitive NMDA antagonists by avoiding loss of NMDA-dependent working memory and operating through a different NMDA-dependent circuitry.

AMPA and kainate receptors mediate the majority of fast excitatory glutamatergic transmission in the brain and their distribution is similar to that observed for NMDA receptors with highest densities present in the cerebral cortical and hippocampal areas, septum and striatum. The AMPA receptors work in concert with NMDA receptors to mediate the primary depolarization required to unblock NMDA receptors and to trigger synaptic strengthening. The receptor is composed of combinations of subunits, termed, GluR1-4, forming allosteric modulatory sites that represent targets for fine-tuning glutamatergic activity by pharmacologic means. Because these receptors are prone to rapid desensitization following stimulation, direct AMPA agonists are unlikely to be therapeutically useful. One class of compounds, termed AMPA receptor-positive modulators or AMPAkines, have been developed that potentiate AMPA receptor transmission in the presence of agonist (e.g., glutamate, AMPA) and reduce the rate of receptor desensitization and/or deactivation.[86,87] Several chemical classes of AMPAkines have been reported, including pyrrolidones (piracetam, aniracetam), benzothiadiazides (cyclothiazide), benzoylpiperidines (CX516, CX546), and more recently, the biarylopropylsulfonamides (LY392098, LY404187, LY451646). AMPAkines, such as LY392098 and LY451646, exhibit dose-dependent antidepressant-like effects in the forced-swim and tail suspension tests.[88–90]

The AMPA receptor has been functionally associated to a variety of signal transduction events involving G proteins, Src-family kinases, and the neurogenic MAP kinase (MAPK) pathway.[91] The dentate gyrus region appears to be particularly sensitive to AMPAkines and raises the possibility that subtle modulation of AMPA receptors may provide a useful strategy to activate neurotrophic MAPK cascades. *In vivo* studies have demonstrated the ability of AMPAkines to promote BDNF levels. For example, short-term chronic (5 days) treatment with LY451646 and LY404187 increased BDNF protein levels in neurons of the dentate gyrus.[92] Furthermore, effective antidepressant doses of LY451646 were well within the range of those required to raise BDNF protein and mRNA expression in hippocampus.[88] At present, it is unknown if the antidepressant-like actions of AMPAkines are causally related to changes in BDNF expression. However, if BDNF is proven to be a major contributor to antidepressant effects, AMPAkines may represent a faster onset of action versus biogenic-amine based antidepressants which increase BDNF mRNA following long-term chronic (2–3 weeks) treatment.[93] An investigation of CNS-penetrant AMPAkines in the treatment of depression is clearly warranted and could provide additional advantages over current therapies such as an alleviation of cognitive dysfunction and improved working memory.

The metabotropic glutamate receptors (mGluRs) exert longer-term, modulatory effects on glutamatergic neurotransmission compared to the ionotropic receptors. They consist of a family of eight G protein-coupled receptors separated into three groups (group 1: mGluR1 and mGluR5; group II: mGluR2 and mGluR3; group III: mGluR4, mGluR6-mGluR8) on the basis of effector coupling, ligand sensitivity and molecular homologies.[94] The discovery of compounds that selectively modulate the heterogeneous family of G protein-coupled mGluRs is still in its infancy. Targeting of the glutamate binding site has provided limited success in the development of small molecules with high-affinity, selectivity, potency, and bioavailability. An alternative approach has relied upon the identification of mGluR allosteric modulators; these bind in a noncompetitive manner to site(s) distal to the glutamate recognition site and can profoundly influence glutamate-induced responses.

Group I negative allosteric modulators (NAMs) block glutamate-induced signaling, and in general, have proven attractive as potential pharmacological agents for the treatment of depression and anxiety disorders. 2-Methyl-6-(phenylethynyl)-pyridine (MPEP) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) represent the very first mGluR5 NAMs to be described with high selectivity and potency, and good systemic activity.[95] mGluR5 NAMs demonstrate antidepressant-like activity in the tail suspension test in mice, and in passive-avoid-

ance learning in the olfactory bulbectomy model of depression in rats, as well as anxiolytic-like effects in several animal models of anxiety including elevated plus maze, fear potentiated startle, Vogel conflict drinking, ultrasonic vocalization, four-plate and social interaction in mice.[96–98] Additional support for the involvement of mGluR5 receptors in anxiety came from mGluR5 knockout mice that displayed an anxiolytic-like phenotype in several stress-induced hyperthermic paradigms.[99] The mechanism of mGluR5 NAMs to alleviate anxiety and depression is under investigation. In a recent *in vivo* microdialysis study, MPEP attenuated stress-induced increases in prefrontal cortical noradrenaline levels in rats,[100] suggesting that the anxiolytic-like effects of mGluR5 NAMs may be influenced by a noradrenergic component.

mGluR1 receptors also belong to the group I family, yet have been studied much less intensively than the mGluR5 subtype. The selective mGluR1 antagonist, JNJ16259685, exerted anxiolytic-like properties after acute administration in the Vogel conflict drinking test but not in the zero maze, and after a 2-week dosing period in lick suppression studies.[101] Furthermore, direct injection of the mGluR1 antagonist, CPCOOEt, into the hippocampus induced anticonflict effects in the Vogel conflict drinking study, which suggested that blockade of mGluR1 in the hippocampus may be an important glutamatergic site for anxiolytic activity.[102] Notably, the anxiolytic-like effects seen following mGluR1 antagonism appear task-dependent whereby prominent effects seen in a conflict procedure are not observed in a task based on spontaneous exploration.[101]

Group II receptor subtypes are negatively linked to adenylyl cyclase through Gi-protein coupling and serve to inhibit endogenous glutamate release, particularly during conditions of glutamate excess from the synaptic cleft. Group II mGluRs are highly localized to the forebrain regions and limbic structures. Preclinical antidepressant effects were reported for group II mGluR antagonists. MGS0039 and LY341495 are new, potent, and selective group II mGluR antagonists that exhibit antidepressant-like effects in rodent models of depression such as the forced swim and tail suspension tests.[103] Moreover, microdialysis studies demonstrated that MGS0039 can elevate serotonin levels in the mPFC,[104] and that both MGS0039 and LY341495 can increase the firing rate of serotonin neurons within the dorsal raphe nucleus.[105] These observations suggest that mGluR2/3 antagonists elicit antidepressant-like effects in part by facilitating serotonergic neurotransmission. It has also been reported that stimulation of postsynaptic AMPA receptors plays a role in mediating the pharmacological effects of LY341495 and MGS0039. Whereas group II mGluR antagonists are potential candidates as antidepressants, preclinical anxiolytic effects have been reported for group II mGluR agonists. Clinical data suggest that the group II agonist LY354740 was effective in reducing fear-of-shock-induced startle potentiation and subjective anxiety in normal volunteers without being sedative.[106] To the extent that group II agonists ultimately prove effective as anxiolytics, it would suggest that preclinical glutamatergic hyperactivity might be more relevant to anxiety disorders.

Group III mGluRs have been the least investigated and documented of the three groups, because pharmacological tools with the requisite receptor subtype selectivity are lacking. Group III receptors are typically presynaptic, localized to limbic-system nuclei, and are associated with inhibition of glutamate and/or GABA within the hippocampus and hypothalamus.[107] Like the group II receptor subtypes, they are coupled to the Gi-protein adenylyl cyclase pathway. To date, all group III agonists synthesized have proven to be systemically inactive relying on direct central injection of compounds and/or the characterization of knockout animals for the elucidation of their functional properties. Intrahippocampal injection of the selective group III agonists ACPT-1 (also known as L-serine-O-phosphate, L-SOP) and HomoAMPA produced a dose-dependent anxiolytic-like effect in the Vogel conflict drinking test in rats and their actions were reversed by CPPG, a group III antagonist.[108] An antidepressant-like effect of ACPT-I after intraventricular injections was also described in the forced swim test.[109] Recently, N-phenyl-7-(hydroxyimino) cyclopropa[b] chromen-1a-carboxamide was identified as an mGluR4 positive allosteric modulator, and demonstrated dose-dependent anticonflict effects in Vogel conflict drinking in rats after administration into the basolateral amygdala, a key structure in the regulation of anxiety.[110]

The generation of specific mGluR7 and mGluR8 receptor knockout animals has enabled further elucidation of the putative physiological and pathological roles for these group III receptor subtypes. mGluR7 is the most widely distributed presynaptic auto- and heteroreceptor in the brain being abundant in regions such as the hippocampus, amygdala, and the locus coeruleus. The mGluR7 receptor is thought to provide mainly negative feedback, limiting L-glutamate release at synapses in a frequency-dependent manner.[111] In mice, the targeted gene deletion of mGluR7 generated a phenotypic antidepressant-like behavior in the forced swim and tail suspension tests as well as anxiolytic-like activity in the light-dark box, elevated plus maze, and stress-induced hyperthermia.[112] mGluR7 blockade might prevent glutamate-mediated inhibition of GABAergic release resulting in elevated GABA levels,[113] thus facilitating the anxiolytic phenotype in mGluR7 knockout mice. In contrast, mGluR8-deficient mice demonstrated an increase in anxiety-related behavior in the elevated plus maze and subtle performance deficits in some learning tasks.[114,115]

The development of selective mGluR8 receptor compounds should help dissect out the relevant contribution of this receptor subtype to key limbic-system pathways associated with anxiogenesis and the potential clinical benefits of mGluR8 agonists.

At the present time, only a minority of glutamate-based approaches is currently being exploited as novel pharmaceutical therapies. Drug development for glutamatergic neurotransmitters is substantially behind that for other systems (e.g., monoamine-based approaches) and was initially focused on the small molecular synthesis of direct agonists and antagonists of the various ionotropic glutamate receptors. Unfortunately, the therapeutic utility of competitive and uncompetitive NMDA receptor antagonists is greatly hampered by an adverse side effect profile such as psychosis and memory loss leaving unresolved the ideal therapeutic role of NMDA receptor antagonist treatment in the management of persistent depression and anxiety.

THE ROLE OF GABA IN DEPRESSION—TARGETS FOR NOVEL ANTIDEPRESSANTS?

GABA is the primary inhibitory neurotransmitter in the CNS. GABA has been implicated in a number of psychiatric disorders including schizophrenia and affective disorders. A number of studies have been carried out to assess the concentration of GABA in CSF or plasma in patients suffering from psychiatric disorders. The most consistent results are from studies in depressed patients. A number of research groups have reported CSF levels of GABA to be significantly decreased in depressed patients.[116–118] Furthermore, studies of plasma levels of GABA in depressed patients concur with these findings.[119] Using proton magnetic resonance spectroscopy, Sanacora and colleagues[120] have measured cortical GABA concentrations *in vivo*. Occipital cortex GABA concentrations in depressed patients were found to be significantly lower than in healthy controls. Subsequent studies demonstrated that these low levels of GABA were normalized after SSRI treatment. Interestingly, low levels of GABA in plasma of depressed patients were not reversed by desipramine treatment.[121] The decreases in GABA observed in depressed patients do not appear to be associated with changes in GABA uptake binding sites, in the frontal cortex and cingulate gyrus at least.[122] Neither $GABA_B$ receptors nor glutamic acid decarboxylase (GAD; biosynthetic enzyme for GABA) activity have been found to be altered in depressed suicide victims, whereas $GABA_A$ receptor binding in frontal cortex was increased in depressed suicide victims.[123,124]

Collectively, these findings have raised interest in the manipulation of GABA as a potential target for treating depression. Levels of GABA in the CNS are controlled by the activity of the biosynthetic enzyme for GABA (GAD), the catabolic enzyme, GABA transaminase (GABA-T), and GABA transporters. GABA transporters exist on neuronal and non-neuronal cells; these transporters are the primary means of terminating the activity of GABA in the synapse.

There are two subtypes of GABA receptor, ionotropic $GABA_A$ receptors and metabotropic $GABA_B$ receptors. Activation of $GABA_A$ receptors causes rapid membrane hyperpolarization. $GABA_A$ receptors are the predominant inhibitory neurotransmitter receptor in the CNS. As such these receptors represent an important target for a variety of clinically used drugs (including anxiolytics, sedatives, and anticonvulsants). Benzodiazepines act as indirect agonists of $GABA_A$ receptors through their activity at the benzodiazepine binding site on this receptor. $GABA_B$ receptors are G protein-coupled receptors which, upon activation, cause hyprepolarization of postsynaptic neurones and inhibition of neurotransmitter release from presynaptic nerve terminals. Selective agonists and antagonists exist for both types of GABA receptor. $GABA_B$ receptor agonists, such as baclofen, act presynaptically to inhibit the release of excitatory amino acids such as glutamate.

Neuroactive steroids, such as the 3α-reduced metabolites of progesterone, 3α,5α-tetrahydroprogesterone (3α,5α-THP or allopregnanolone), can positively modulate $GABA_A$ receptors.[125] Interestingly, CSF allopregnanolone levels are significantly lower in depressed patients, and normalized by treatment with fluoxetine or fluvoxamine.[126] Similarly, plasma levels of allopregnanolone are reported to be decreased in depression and levels restored by effective antidepressant treatment.[127] Fluoxetine and paroxetine have been shown to increase the concentration of allopregnanolone in rat brain.[128] Allopregnanolone itself has been shown to have an antidepressant-like effect in rat and mouse forced swim tests.[129,130] These effects are thought to be mediated by $GABA_A$ receptors as they are blocked by $GABA_A$ receptor antagonists.[129] Levels of allopregnanolone are also reduced in the frontal cortex of mice that have undergone protracted isolation—-a behavioral manipulation that induces depression-like behaviors. In the rat olfactory bulbectomy model of antidepressant-like activity, removal of the olfactory bulbs caused a significant decrease in frontal cortex levels of allopregnanolone which was reversed by chronic treatment with antidepressants of various classes.[131] Interestingly, various SSRIs have been shown to increase allopregnanolone production through increasing the rate of synthesis of allopregnanolone.[132]

There is a wealth of data implicating $GABA_B$ receptors in depression. There are a number of reports in the literature of chronic administration of antidepressants or electroconvulsive therapy increasing the binding and

function of $GABA_B$ receptors in the frontal cortex of mice and rats.[133–137] However, these findings have not been repeated by some groups.[138,139] Furthermore, in the olfactory bulbectomy model of depression, removal of the olfactory bulbs was associated with a significant reduction in $GABA_B$, and a significant increase in $GABA_A$ receptor densities.[140,141] The increase in $GABA_A$ receptors was normalized by chronic treatment with antidepressants.[142] In the learned helplessness model of antidepressant activity, GABA release is diminished in the hippocampus in helpless rats and this is reversed by desipramine treatment in line with the effects on helpless behavior.[141] Interestingly, the selective $GABA_B$ receptor antagonist, CGP36742, has been shown to be efficacious in the rat learned helplessness model of antidepressant activity.[143] Another $GABA_B$ antagonist, CGP56433, also shows antidepressant-like effects in the rat forced swim test.[144] Interestingly, $GABA_{B1}$ knockout mice have an antidepressant-like phenotype in the forced swim test.[145] Furthermore, $GABA_B$ receptor antagonists, CGP 36742, CGP 56433A, and CGP 56999A, produce rapid increases in nerve growth factors, NGF and BDNF in neocortex and hippocampus, an effect also seen after chronic administration of antidepressants (see Neurotrophins).[146]

The putative role of GABA, $GABA_A$, and $GABA_B$ receptors in depression could be mediated directly by GABA or via other neurotransmitter systems. There are pieces of evidence linking $GABA_B$ receptors to noradrenergic and serotonergic systems. For example, administration of $GABA_B$ receptor antagonists has also been demonstrated to cause downregulation of β-adrenoceptors—an effect common to chronic administration of a number of types of antidepressants.[135,147] The $GABA_B$ antagonist, phaclofen, as well as the $GABA_A$ receptor antagonist, bicuculline, increased norepinephrine release in the median preoptic nucleus *in vivo*. Conversely, locally applied agonists of $GABA_A$ and $GABA_B$ receptors (muscimol and baclofen, respectively) decreased dialysate levels of norepinephrine in the same area. These data indicate that $GABA_A$ and $GABA_B$ receptors are involved in the control of norepinephrine release in this part of the rat brain.[148] Given the established role of norepinephrine in depression and the treatment thereof, such effects on the noradrenergic system could contribute to the antidepressant effects of GABA receptor ligands.

There are also considerable data supporting a relationship between GABAergic and serotonergic systems in the CNS. Allopregnanolone has been demonstrated to directly affect the serotonin system. In rats treated with allopregnanolone for 7 days, the firing rats of serotonergic neurones in the dorsal raphe were increased.[149] Given the hypothesis that serotonergic neurotransmission is reduced in depression, such an effect could contribute to the antidepressant-like effects of allopregnanolone. Administration of the $GABA_B$ receptor agonist, baclofen, increases serotonin release from the dorsal raphe as well as, to a lesser extent, striatum.[150] Local infusion of the $GABA_A$ receptor antagonist, bicuculline, increases serotonin release in the dorsal raphe, indicating that GABA afferents exert a tonic inhibitory influence on serotonin neurones in the dorsal raphe.[151] Given the serotonin hypothesis of depression, the efficacy of $GABA_A$ receptor agonists, and $GABA_B$ receptor antagonists could, at least in part, be attributed to these effects on serotonergic transmission. In terms of behavioral effects of GABAergic drugs, the profile of the $GABA_B$ antagonist, CGP56433 in the forced swim test indicates a serotonin-mediated effect; CGP56433 decreases immobility and increases swimming, a profile comparable with fluoxetine.[144] Depletion of serotonin prevents the effects of $GABA_B$ receptor antagonists in this model, further suggesting that the effect involves an interaction with serotonin.[144] Reports in the literature also support an interaction between GABAergic and dopaminergic systems. Local application of a $GABA_A$ receptor antagonist (bicuculline) increases striatal dopamine release, whereas the $GABA_A$ receptor agonist muscimol has the opposite effect.[152] Administration of a $GABA_B$ receptor agonist (baclofen) has no effect on striatal dopamine levels alone; however, it has been shown to attenuate nicotine-, morphine-, and cocaine-evoked dopamine release in the shell of the nucleus accumbens.[153]

In summary, GABA is strongly implicated in depression such that GABA receptors are potential targets for the development of novel antidepressants. As the GABAergic system is closely linked to monoaminergic neurotransmitter systems, manipulations of the GABAergic system are very likely to affect other neurotransmitter systems and *vice versa*. To what extent the effects of antidepressants on the GABAergic system contribute to the efficacy of these drugs as antidepressants remains to be determined.

CENTRAL PEPTIDERGIC SYSTEMS AS TARGETS FOR NOVEL ANTIDEPRESSANT DEVELOPMENT

In the area of depression research, interest in central peptide systems has focused on the high-profile efforts targeting receptors of the central substance P [neurokinin 1 (NK1)] and corticotropin-releasing factor (CRF1) systems. This has led to the development of numerous compounds now in clinical trials for depression. In addition to NK1 and CRF1, however, interest has also fallen on receptors involved in mediating the effects of other central peptidergic systems. These include examples such as melanin-concentrating hormone (MCH) and arginine vasopressin, which are discussed below. Whereas interest in peptidergic systems as platforms for antidepressant development has grown, it is important to note the frus-

TABLE 2. *Compounds Targeting Peptidergic Receptors with Potential Utility as Antidepressants*

Peptide System	Receptor Target	Compound	Receptor Pharmacology	Probable Clinical Phase
SP	NK1	GW823296	Antagonist	I
		GW679769	Antagonist	II
		GW597599 (Vestipitant)	Antagonist	II
		R673	Antagonist	II
		CP-122,721	Antagonist	II
		L-759274	Antagonist	II
	NK2	SR48968	Antagonist	III
CRF	CRF1	DMP696	Antagonist	I
		DMP904	Antagonist	I
		GW876008	Antagonist	I
		AAG561	Antagonist	I
		TS-041	Antagonist	I
AVP	V1b	SSR149415	Antagonist	I

tration recognized by the lack of success in the clinic to date. In this section, we will provide a brief overview of several central peptidergic systems, which have been implicated in the pathophysiology of depression, and are currently considered as emerging targets for novel antidepressant development.

Substance P

Substance P (SP) is an undecapeptide member of the tachykinin family of mammalian neuropeptides, which also include neurokinin A and neurokinin B. SP is the most abundantly expressed of the tachykinins in the CNS, and had originally been shown to modulate pain transmission in the spinal cord.[154] SP also acts as a neuromodulator in the CNS acting to regulate an array of stress-related behaviors, autonomic control of cardiovascular and respiratory function, as well as emetic reflexes.[155–157]

The rationale behind the SP system as a target for depression has been reiterated frequently and is assembled from several lines of evidence. Firstly, the expression profile of SP and its receptors is observed within regions of the CNS that are traditionally associated with the regulation of stress responses (e.g., amygdala, hypothalamus, hippocampus, and frontal cortex). Secondly, both acute and chronic stressors (e.g., immobilization, foot shock, maternal separation) have been shown to increase SP content (synthesis/release) in these areas.[158–160] Central administration of SP or NK1 agonists induces stress-related behaviors in animal models.[160–162] Elevated levels of SP in plasma and CSF are observed in patients with depression.[163]

The effects that SP exerts are primarily mediated through the NK1 receptor, and subsequently this receptor has emerged as a target for antidepressant development. NK1 is a member of the class-A GPCR family of receptors that also include NK2 and NK3.[164] In the CNS, autoradiographic and immunohistochemical techniques have revealed extensive expression of NK1 receptors in regions involved in modulating affective behaviors, and the neurochemical response to stress (e.g., hypothalamus, hippocampus, nucleus accumbens, raphe nucleus).[165] SP exhibits inhibitory effects on monoaminergic neurotransmission under physiological conditions that are mediated through NK1, and NK1 antagonists have been shown to enhance firing rates of dopaminergic, noradrenergic, and serotonergic neurons.[166] Collectively, these data have helped support the rationale behind the antidepressant hypothesis of NK1 receptor antagonism, and has resulted in the development of numerous NK1-selective antagonists as potentially novel antidepressants. To date, numerous NK1-selective antagonists have been developed and reported by drug companies to demonstrate antidepressant-like profiles in a range of preclinical animal models.[167]

Despite the preclinical evidence, the track record of NK1 antagonism in humans has been one of disappointment. This has been largely influenced by the rather high-profile failure of aprepitant in the clinic. In initial phase II studies, aprepitant had shown promising antidepressant results in a small population of depressed patients, but fell short of reaching efficacy in five larger phase III studies, thus failing to provide Merck a proof-of-concept for NK1 antagonism.[168] Skepticism in NK1 antagonism as a novel mechanism of action following these results, although widespread in the Pharmaceutical Discovery community, has not slowed interest in the continued clinical development of other NK1 antagonists. This is evidenced by appearance of numerous other NK1 antagonists in clinical development (Table 2).

In contrast to NK1, comparatively less interest has fallen on the NK2 receptor as target for depression. Clinical development of NK2 antagonists has historically focused on inflammatory conditions such as obstructive airways disease.[169] However, support from preclinical

findings in rodent models has suggested potential for novel antidepressant-like activity for different, receptor-specific, approach of the same system.[170] Adding to the antidepressant rationale, NK2 antagonism has also been shown to attenuate stress induced increases in locus coeruleus firing and norepinephrine release in the prefrontal cortex.[171] Compounds such as SR48968 (Saredutant) have now entered clinical trials for depression where this approach will to be tested.

Novel approaches that target the central SP systems have also been contemplated, which feature molecules that combine NK1 antagonism with selective serotonin reuptake inhibition (SSRI). This has been based in large part by observations from neurochemical evidence. For instance, increases in extracellular serotonin levels elicited with SSRI (paroxetine) treatment are significantly more robust in NK1−/− mice when compared with wild-type controls.[172] Preclinical evidence appears to support this approach as coadministration of an NK1 antagonist (GR205171 or L733060) and an SSRI (paroxetine) produced similar increases in cortical extracellular serotonin, as well as antidepressant-like activity in preclinical models of depression.[173] Together, the evidence implies that the antidepressant-like effects of NK1 antagonism may be mediated through modulation of serotonergic activity, which may be potentiated by also including a SSRI component. Small molecules that combine NK1 antagonism with serotonin reuptake inhibition (NK1/SSRI) have now been reported, and these compounds exhibit antidepressant-like activity in animal models sensitive to either SSRI and NK1 antagonists.[174]

CRF

CRF is a 41-amino acid peptide.[175] The CRF system extends throughout the CNS and plays an important role integrating the body's endocrine, autonomic, immune, and behavioral responses to stress.[176,177] Through neurosecretory terminals in the median eminence, CRF-synthesizing neurons of the hypothalamic paraventricular nucleus (PVN) release CRF into portal circulation of the anterior pituitary, where CRF stimulates release of ACTH from corticotrophs into peripheral circulation. This positions CRF as an important mediator of hypothalamic-pituitary-adrenal (HPA) axis activity. CRF projections are also observed in numerous extrahypothalamic sites, including key limbic areas (e.g., amygdala, bed nucleus of the stria terminalis), consistent with its involvement in affective behavioral responses to stress.[177] CRF neurons are also found in several brain stem nuclei (e.g., locus coeruleus, nucleus of solitary tract) involved in controlling autonomic components of the stress response.[177]

Several lines of evidence have linked hyperactivity of the central CRF system with depression in humans. The best understood link is drawn from the well-described role of CRF as the principle mediator of ACTH release from the pituitary, which is central to regulation of the HPA axis. This is relevant to depression as hyperactivity of the HPA axis is one of the most consistent clinical findings in depressed patients, and can be normalized after successful antidepressant treatment.[178,179] Increased levels of CRF are directly associated with HPA disturbances in subpopulations of depressed patients.[179,180] Moreover, elevated levels of CRF in CSF, decreased CRF receptor binding in the frontal cortex, and increased numbers of CRF neurons in the PVN are all observed in depressed patients.[181,182]

The biological effects of CRF are mediated by two class-B GPCRs, CRF1 and CRF2. CRF1 has emerged as the target of interest for antidepressant development based on the following lines of evidence. CRF1 is the receptor subtype expressed on corticotrophs of the anterior pituitary, and thus responsible for mediating CRF effects on ACTH release and the HPA axis. CRF1 antagonists exhibit the ability, in preclinical animal models, to block many of the behavioral and endocrine responses to stress. For example, CP-154,526 (antalarmin), one of the first CRF1 antagonists to reach clinical trials in humans in depression, produces antidepressant-like activity in learned helplessness (rats) and chronic mild stress (mice) paradigms, as well as attenuation of stress-induced hyperthermia, distress vocalizations, and cortical norepinephrine release.[183–185] DMP696, SSR125543, and R278995/CRA0450 are examples of other CRF1 antagonists that have been developed which also have antidepressant-like activity reported in preclinical rodent models, helping to further support the proof of concept for this mechanism of action.[186,187]

Despite the preclinical picture for CRF1 antagonism as a novel mechanism of action for antidepressant development, only one compound to date, R121919 (Janssen), has demonstrated antidepressant efficacy in clinical trials.[188] Unfortunately, clinical development of this compound was ultimately discontinued (believed to be because of hepatotoxicity), and the initial report was never confirmed in larger studies. This has left the field to wait for the outcome of clinical evaluation of the numerous other CRF1 antagonists that have undergone development as antidepressants.

MCH receptors

MCH is a 19-amino acid cyclic neuropeptide synthesized by neurosecretory cells of the mammalian lateral hypothalamus and zona incerta.[189] MCH-synthesizing neurons of these nuclei project throughout the CNS comprising a broad circuitry of innervation, modulating areas involved in regulating energy homeostasis, feeding and mood-related behaviors, arousal, sensorimotor integration, and autonomic control.[190,191]

Two GPCRs mediate the effects MCH in primates, MCH1-R and MCH2-R. To date, MCH1-R (also known as somatostatin-like receptor 1 or SLC-1) is the only subtype identified in rats. Drug discovery interest in the central MCH system has historically focused on targeting the effects of this peptide on feeding behavior (orexigenic) and energy homeostasis (metabolic), with MCH1-R antagonism emerging as a novel approach for development of anorectic and anti-obesity compounds. Evidence, however, has also implicated the MCH system in regulating mood and the stress response. Local administration of MCH into the nucleus accumbens shell has been reported to produce depressant-like behavioral effects in the rat forced swim test.[191] MCH also produces stimulatory effects on HPA axis reactivity, as evidenced by the increases in circulating ACTH and cortisol levels reported following central administration of MCH or direct infusion of MCH into the hypothalamic paraventricular nucleus.[192] MCH also increases CRF release from hypothalamic explants, an effect that could be blocked by a selective SLC-1 (rat ortholog of MCH1-R) antagonist.[192]

MCH1-R antagonists have been the preferred approach for targeting the central MCH system for antidepressant development. Although T-226296 was the first MCH1-R selective antagonist to be reported, SNAP-7941 was the first compound to have behavioral effects in preclinical models of depression reported. SNAP-7941 produced antidepressant-like effects in the rat forced swim test similar to those observed with an SSRI (fluoxetine).[193] Several other companies have since reported the synthesis of other MCH1-R antagonist compounds (see Table 2) exhibiting similar antidepressant-like profiles in preclinical models.[194,195] Collectively, the results from preclinical profiling of MCH1-R antagonists support a rationale for this novel mechanism of action for depression. As is evident with other peptidergic receptor targets, despite a complete preclinical rationale, the clinical utility of MCH1-R antagonists as antidepressants awaits evaluation.

Arginine vasopressin

Arginine vasopressin (AVP) is a cyclic nonapeptide synthesized exclusively by neurosecretory cells of the CNS with a diverse array of biological functions based on differences in sites of release. When released into peripheral circulation from the posterior pituitary, AVP is responsible for the classic endocrine functions described for this neurohormone (e.g., vasoconstriction, glycogen metabolism, antidiuresis). In the CNS, AVP acts as a neuromodulator/neurotransmitter regulating a range of CNS-mediated functions that include learning and memory, social behaviors, circadian rhythmicity, thermoregulation, and autonomic function. AVP released into the portal circulation from the median eminence is also known to directly modulate CRF effects on ACTH release and the HPA axis.

The central vasopressinergic system has been examined as a platform for psychiatric drug development, including depression.[196] The central vasopressinergic system acts on several key neural substrates underlying aspects of the depression endophenotype, including monaminergic systems and those regulating memory, pain sensitivity, synchronization of biological rhythms, the timing/quality of R.E.M. sleep, and regulation of fluid and electrolyte homeostasis.[197] Disturbances (hyperactivity) in vasopressinergic activity have also been reported clinically in patients with depression.[198,199] Together, this has led many to hypothesize the utility of central vasopressinergic receptor antagonism as a potentially novel antidepressant strategy.

The biological activity of AVP is mediated through a phylogenetically related family of class-A GPCRs; V1a, V2, and V1b, and the oxytocin receptor (OTR) to a lesser extent. Of these receptors, the V3R has emerged as the most tractable candidate for antidepressant development. V1b mediates AVP regulation of corticotroph function in the anterior pituitary, and plays an important role in regulating stress responsiveness of CRF-mediated ACTH release and the HPA axis. Receptors are also present throughout key areas (e.g., hippocampus, lateral septum, frontal cortex) involved in regulation of the behavioral responses to stress.[200] The lack of truly selective V1b ligands has historically complicated efforts to elucidate the roles of this receptor in the behavioral responses to stress implied by its central distribution. The relevance of this was revealed following the report of the first V1B-selective antagonist, SSR149415.[201] Subsequent behavioral profiling of SSR149415 in a wide range of preclinical models of depression and stress behavior provided the evidence needed to support the hypothesis of V3R antagonism as a novel mechanism of action for antidepressant activity. V1b blockade with SSR149415 has also been shown to produce antidepressant-like activity in both acute (forced swim) and chronic (chronic mild stress, subordination stress) rodent behavioral paradigms.[202] SSR149415 also demonstrates the ability to attenuate stress-induced changes in endocrine (ACTH release from anterior pituitary), neurochemical (tail pinch norepinephrine release), and autonomic (hyperthermia) activity.[202,203] Although these observations have helped to support the hypothesis of V1b antagonism as a novel antidepressant strategy, they are based on the preclinical profile of a single compound, SSR149415, which is currently in clinical trials. More confidence in this mechanism of action awaits the development and preclinical profiling of additional V1b antagonists.

NEUROTROPHINS AS NOVEL ANTIDEPRESSANT DRUGS

The majority of currently marketed antidepressant drugs act directly on serotonergic or noradrengeric neurotransmission. An alternate (or possibly complementary), hypothesis is that neurotrophic factors are involved in or mediate the mechanism of antidepressant drugs. This theory comes from converging lines of data. First, antidepressant drugs require at least 2 weeks administration to see clinical efficacy.[204] This time lag may represent a necessity for long-term adaptations and alterations in downstream signaling pathways, such as neurotrophic pathways, before a therapeutic effect is seen.[70] Secondly, many researchers have hypothesized that depression can arise from the failure of the CNS to exhibit the appropriate synaptic plasticity in response to stress, which may be offset or reversed by neurotrophic support induced by antidepressant treatments. In support of this are multiple reports, detailed below, that chronic antidepressant treatments activate long-term changes in neurotrophic factor expression and neurotrophic signaling pathways, and that the activation of these factors is a common mechanism of effect of antidepressants.[205] Finally, the recent interest in the idea that neurogenesis, or the birth and survival of new neurons, is involved in antidepressant action again leads to the investigation of neurotrophic factors as mediators of antidepressant action.[206] Targets of neurotrophic factors or their pathways may represent a novel treatment for depression.

The NGF family of neurotrophins consists of NGF, BDNF, and neurotrophins-3 and -4 (NT-3 and NT-4). These have been grouped as a family due to the high homology of their receptors. There are two classes of neurotrophin receptors; a low-affinity p75 receptor, which is common to all neurotrophins, and the high-affinity trk receptors, which are associated with specific neurotrophins and encode transmembrane receptor tyrosine kinases (RTKs) that mediate multiple signaling pathways. It has been shown that trkA is the receptor for NGF, trkB is the receptor for BDNF and NT-4, and trkC is the receptor for NT-3. However, there is cross talk between some of these receptors; NT-3 can also bind to trkA and trkB, but with lower affinity than to trkC. To date, NGF, NT-3, and NT-4 and their receptors have not been implicated in antidepressant action, and antidepressant research has centered on BDNF and trkB.

An important regulator of BDNF gene expression is the transcription factor cAMP response element-binding protein (CREB). CRE elements in the promoter region of BDNF indicate that it is a downstream target of CREB, although BDNF may induce CREB phosphorylation as well.[207] BDNF and CREB are involved in multiple CNS functions that all fall under the umbrella of "synaptic plasticity."[70] Given that one hypothesis of depression is a dysfunction in synaptic plasticity, either by genetic, biochemical, or environmental factors, both CREB and BDNF have been implicated in antidepressant action.

This hypothesis is supported by the findings that chronic, but not acute, antidepressant treatment increases mRNA and protein levels of BDNF and CREB levels in the rat hippocampus and cortex, indicating that upregulation of these factors is one mechanism by which antidepressants may exert their effects.[205] Importantly, the chronic time course corresponds to the time course necessary for clinical efficacy. Stress has been shown to downregulate BDNF, and this is reversed by chronic antidepressant treatment. In addition, rolipram, a phosphodiesterase-IV inhibitor that activates the cAMP cascade and increases BDNF, has been shown to have antidepressant-like effects in animal models.[208] Clinically, increased hippocampal BDNF levels have been observed in patients taking antidepressants, along with decreased serum BDNF levels in untreated depressed subjects.[209] Postmortem studies have shown decreased hippocampal trkB and BDNF mRNA in suicides compared with controls.[210]

BDNF acutely administered directly into the lateral ventricles or hippocampus has been shown to produce antidepressant-like effects in the forced swim and learned helplessness paradigms.[211,212] Interestingly, these antidepressant-like effects may be localized to the hippocampus, given that infusion of BDNF into the VTA produces prodepressive effects.[213] A similar effect of anatomical specificity has been seen with CREB; acute overexpression of CREB in the dentate gyrus produces antidepressant-like responses, whereas CREB expression in the nucleus accumbens produces the opposite effect.[214,215] These data indicate that the antidepressant-like effects of neurotrophic factors and their pathways may be anatomically restricted or localized.

The finding that antidepressants increase hippocampal BNDF has received much attention. Although not all laboratories are able to demonstrate fluoxetine-induced increases in BDNF, possibly due to variability in experimental protocols, other antidepressant classes, and ECS continue to provide links between antidepressant action and increased BDNF.[216,217] The net effect of increased BDNF expression after chronic antidepressant treatment is not merely the effect of simple activation of the BDNF gene. The BDNF gene has four differentially regulated promoters, all of which have the net effect of increased BDNF expression. Recent research has shown that chronic and acute ECS, desipramine, tranylcypromine, and fluoxetine regulate BDNF mRNA via regulation of different exon-specific promoters.[217] Although the physiological significance of this is currently unknown, future drug development may center on more specific activation of the four BDNF transcripts.

Animal models of neurotrophic and antidepressant

function have yielded varied results. A number of lines of BDNF heterozygous (+/−) knockouts display a reduction in BDNF, but without any accompanying changes in baseline behavior or reactivity to stress.[218] Other lines of +/− BDNF mice display altered synaptic responses but no specific phenotype has been identified.[219] These animal lines reveal that a partial loss of BDNF is not enough to affect baseline behavior, but this interpretation is compromised by the fact that these transgenic animals had a loss of BNDF from birth and may have evolved compensatory responses as adults.

The animal studies have also investigated the necessity for an intact neurotrophic system to produce an antidepressant response. Saarelainen et al.[220] have reported that a line of trkB heterozygous knockout mice, as well as a line of BDNF heterozygous knockout mice, had no change in baseline behavior, but were resistant to the effects of antidepressants. However, Conti et al.[216] reported that a line of CREB-deficient mutant mice respond normally to antidepressants but without the expected increase in BDNF. This indicates that there may be BDNF and CREB-dependent as well as BDNF and CREB-independent pathways for some of the pharmacologic actions of antidepressants.

The ability to generate conditional knockout animals is a powerful new technology that has been used in recent studies. The use of an inducible knockout system was employed to delete forebrain BDNF. These knockout mice did not demonstrate a depressive-like phenotype that might have been predicted with the loss of BDNF but had an attenuated response to antidepressant administration in the forced swim test.[221] Taken together, the animal studies point to a role of BDNF in antidepressant action and provide evidence that antidepressants may work via a neurotrophic mechanism.

Recently, there has been a focus on GABAergic drugs as putative antidepressants, given that ECS and fluoxetine have been shown to activate the $GABA_B$ receptor. In addition, $GABA_B$ receptor antagonists have been shown to be antidepressant-like in animal models of depression, and also increase BNDF mRNA and protein.[222] This increase in BDNF occurs after acute administration of these compounds, in contrast to the chronic treatment needed with clinically used antidepressants. Although this is still an emerging field, the data indicate that $GABA_B$ may represent an alterative downstream pathway by which to activate BDNF.

Given that a common mechanism of effect of antidepressants is to increase CREB and neurotrophic factors, and that neurotrophic factors are antidepressant in the hippocampus, the hypothesis is that a drug that produces increased levels of BDNF would be an effective antidepressant. However, there are no current therapies that directly use neurotrophic factors for depression or any other neurological diseases. One of the difficulties in designing a neurotrophic factor therapy for CNS disorders is the inability of these factors to cross the blood-brain barrier, which prevents entrance into the brain.[223] In the 1990s, multiple phase III clinical trials were conducted using subcutaneous injection of BNDF, IGF-I, or CNTF to treat symptoms of amyotrophic lateral sclerosis. None of these neurotrophic factors crossed the blood brain barrier and all three clinical trials failed due to lack of efficacy.[224]

Despite the failure of these studies, given the great therapeutic potential of the neurotrophic factors, alternate drug delivery systems to the brain are currently being investigated. The use of small molecule peptidomimetics that would bind to specific neurotrophin domains has been hypothesized to be a potential approach for addressing this issue,[225] although there is no current clinical evidence that these will yield any effective therapy.

In contrast to directly targeting the neurotrophic factors, the multiple factors in the signal transduction cascades activated by either neurotrophic factors or antidepressant drugs represent another avenue of research toward drug discovery. These include, but are not limited to, the phosphotidylinositol-3-kinase-Akt pathway and the extracellularly regulated kinase (ERK)/MAPK cascade.[226] These pathways are involved in cell signaling, synaptic plasticity and cell survival; the MAPK cascade itself regulates the transcription factor CREB, and also activates the antiapoptotic factor Bcl-2 and inhibits the proapoptotic factor Bcl-xL/Bcl-2-associated death promoter. The ERK/MAPK cascade is activated by neurotrophins and activated by mood stabilizers and antidepressants.[227,228] Delineation of the role of these factors as well as their specificity for antidepressant-like effects may represent another avenue for drug discovery.

It has recently been demonstrated that chronic antidepressant treatments increase cell proliferation and neurogenesis, and that animal models of depression such as the learned helplessness paradigm and restraint stress decrease cell proliferation.[229,230] The direct physiological significance of antidepressant-induced neurogenesis has yet to be demonstrated, although blockade of hippocampal cell proliferation via irradiation prevents the behavioral actions of antidepressant drugs.[231] These findings have led to the neurogenenic hypothesis of antidepressant activity, which posits that one mechanism of effect of antidepressant drugs is to increase hippocampal cell proliferation and neurogenesis.

These hypotheses have led to the identification of other neurotrophic factors as putative antidepressant compounds. IGF-I is one such recent example. IGF-I administered centrally or peripherally increases adult hippocampal cell proliferation and neurogenesis, and also increases the differentiation of immature cells into neurons.[232] To determine whether the neurotrophic fac-

tor hypothesis would indeed yield a novel antidepressant-like compound, rats were administered IGF-I via intracerebroventricular administration. In agreement with the hypothesis, IGF-I produced an antidepressant-effect in the forced swim test, which lasted 6 days after infusion.[233] Although the mechanism by which the IGF-I induced antidepressant activity occurs is unknown, this finding indicates that the search for drug targets by looking for the neurogenic potential of compounds may provide novel antidepressants.

IGF-I signals though the IGF-I receptor, via a multiprotein signaling complex that activates the MAPK pathway and PI3 kinase pathways.[234] These pathways may be related to the *in vivo* neuorogenic effect of IGF-I, because *in vitro*, IGF-I has a direct proliferative effect on adult hippocampal progenitor cells via MAPK activation.[235]

In addition to IGF-I's direct neurogenic effects on hippocampal cells, there may be interactions between the IGF-I, BDNF, and serotonin pathways to induce neurogenesis and antidepressant-like effects. Although the G protein-coupled serotonin receptors are independent from the trkB and IGF-I receptors, their intracellular signaling pathways have been shown to act cooperatively to regulate genes involved in synaptic plasticity. Serotonin activates receptors coupled to cAMP production, providing a link between drugs that increase serotonergic neurotransmission and enhanced synaptic plasticity. The effect of agonism or antagonism of serotonin receptor subtypes on cell proliferation and neurogenesis is being investigated and the contribution of neurotrophins to this will enhance our understanding of the mechanisms underlying antidepressant-induced neurogenesis and antidepressant action.[236]

SUMMARY

It is an exciting time for drug development in depression as there are many new and novel approaches on the horizon. Over the next decade, proof-of-concept studies will be performed in the clinic for a wide array of mechanisms and the true validity of these novel strategies will be enlightening. This will not only include combination molecules taking further advantage of monoaminergic approaches but novel mechanisms yet to be tested in humans. Clearly, the current array of animal models for determining antidepressant activity has been useful in predicting therapeutic efficacy of multiple monoaminergic mechanisms. Notably, mechanisms that do not directly or indirectly modulate monoaminergic mechanisms remain to be fully validated and the development of further animal models may be necessary. To this end, many of the systems described in this review such as glutamatergic, GABAergic, neurotrophic, and peptidergic systems have links to the serotonergic system and modulate serotonin levels or alter serotonergic firing. The question of whether a compound that does not affect monoaminergic neurotransmission can be an effective antidepressant remains to be answered.

In conclusion, the most successful novel approaches will be those that not only demonstrate preclinical antidepressant-like effects, but those that target the unmet clinical needs and lead to long-term disease modification. For many of the approaches described in this review, clinical testing will determine the extent to which these approaches show distinct advantages over existing therapies and finally demonstrate the true innovation associated with these novel mechanisms.

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