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Review

Antidepressants for the new millennium

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Abstract

Despite a remarkable structural diversity, most conventional antidepressants may be viewed as 'monoamine based', increasing the synaptic availability of serotonin, norepinephrine, and/or dopamine. Both preclinical and recent clinical studies indicate that compounds which reduce transmission at *N*-methyl-D-aspartate (NMDA) receptors are antidepressant. Moreover, chronic administration of antidepressants to mice alters both the mRNA levels encoding *N*-methyl-D-aspartate receptor subunits and radioligand binding to these receptors within circumscribed areas of the central nervous system. It is hypothesized that these two different treatment strategies converge to produce an identical functional endpoint: a region-specific dampening of NMDA receptor function. The pathways leading to this convergence provide a rudimentary framework for discovering novel antidepressants.

Keywords: Antidepressant; Monoamine; NMDA receptor; Brain-derived neurotrophic factor; Neurotrophin

1. Introduction

While estimates of the prevalence of depression vary widely (Ohayon et al., 1999), the impact of this major psychiatric disorder has been grossly underestimated by traditional approaches that do not appropriately value disability. If disability rather than death is used as a measure of societal burden, then major depression is the fourth highest source of disability-adjusted life years (based on statistics gathered in 1990), and will rank second only to heart disease by the year 2020.

Antidepressants have been in widespread use during the last four decades of this millennium. During the past decade, refinements in these agents have eliminated many of the serious side effects associated with 'first generation' antidepressants such as the tricyclics and monoamine oxidase inhibitors. Nonetheless, these newer antidepressants are far from ideal, requiring ≥ 2 weeks (with a more conservative estimate of 3–6 weeks) of treatment to produce significant therapeutic effects (a phenomenon that has been termed the 'therapeutic lag'). Moreover, most well controlled double-blind studies indicate that $\sim 30\%$ of the population do not respond to current therapies. New drugs that are more efficacious and rapid acting will address a significant public health need with a worldwide market approaching US$10 billion at millennium's end.

Most antidepressants in current use have well-documented effects on the disposition (i.e., uptake and/or metabolism) of biogenic amines (serotonin, norepinephrine, and dopamine) that are readily demonstrable both in vivo and in vitro. Nonetheless, the delayed onset of action common to all antidepressants has made it difficult to establish a causal relationship between the well-described neurochemical effects produced by these agents and a reduction in the symptoms of depression. Perhaps one of the most controversial issues in contemporary biological psychiatry is whether strategies directed at optimizing synaptic concentrations of biogenic amines will produce an increase in responder rates and eliminate (or diminish) the therapeutic lag (e.g., Artigas et al., 1994; Berman et al., 1997).

During the past decade, converging lines of evidence have led us beyond the monoaminergic synapse for strategies to improve antidepressant therapy. Emerging from these studies is a rapidly changing picture that may provide an entirely new set of potential therapeutic targets. In this review, I summarize studies from several laboratories demonstrating that *N*-methyl-D-aspartate (NMDA) antagonists are antidepressants, and that chronic antidepressant treatments can, in turn, impact NMDA receptor function in circumscribed areas of the central nervous system. The

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molecular mechanism(s) responsible for these changes provide one subset of potential targets that may yield more efficacious and faster acting antidepressants.

2. NMDA antagonists as antidepressants: preclinical and clinical findings

In our initial studies (Trullas and Skolnick, 1990), administration of a competitive NMDA antagonist (2-aminophosphonoheptanoic acid), a use-dependent channel blocker (dizocilpine), and a glycine partial agonist (1-aminocyclopropanecarboxylic acid; ACPC) to mice reduced immobility in the forced swim test. The effects of 2-aminophosphonoheptanoic acid and ACPC exhibited a clear dose-dependence, and the maximum reductions in immobility produced by these agents were comparable to those obtained with imipramine. The reduction in immobility produced by ACPC (but *not* imipramine) was blocked by administration of glycine, indicating that these effects were attributable to a functional blockade of strychnine-insensitive glycine sites. ACPC also produced a dose-dependent reduction in immobility in the tail suspension test (Trullas and Skolnick, 1990). While the forced swim and tail suspension tests are not generally considered animal models of depression, these preclinical procedures will detect many clinically useful antidepressants (Borsini and Meli, 1988; Porsolt and Lenegre, 1992). Moreover, there is a partial overlap in the range of antidepressants detected by these tests (Porsolt and Lenegre, 1992), increasing the likelihood of clinical efficacy for agents testing positive in both procedures. Subsequent studies have confirmed and extended these observations as summarized in Table 1. These studies demonstrate that a range of structurally diverse agents acting at the multiple, allosteric loci on NMDA receptors are active in both mouse and rat variations of the forced swim test.

NMDA antagonists are also active in the chronic mild stress model of anhedonia developed by Willner and his associates (reviewed in Willner, 1997; Willner and Papp, 1997). This procedure has not yet been as extensively validated using as many drugs as the forced swim and tail suspension tests. Nonetheless, chronic treatment with antidepressants will reverse the reduction in both sucrose (or saccharin) consumption and intracranial self stimulation produced by chronic application of inescapable, uncontrollable stressors (Willner, 1997). Antidepressants (including tricyclics and serotonin-specific reuptake inhibitors) typically require ≥ 3 weeks of treatment to reverse stress-induced deficits in (e.g.) sucrose consumption. Papp and Moryl (1993, 1994) examined the effects of NMDA antagonists in this paradigm and demonstrated that both dizocilpine and the competitive NMDA antagonists CGP 37849 and CGP 40116 were as effective as imipramine in restoring stress-induced deficits in sucrose consumption. These investigators subsequently examined the effects of ACPC in this procedure (Papp and Moryl, 1996). ACPC reversed stress-induced deficits in sucrose consumption in a dose dependent fashion, with the higher dose (200 mg kg^{-1} day^{-1}) effective in 2 weeks compared to 5 weeks for a standard dose of imipramine.

There has been one exploratory proof-of-concept study examining the effects of ketamine, a use-dependent channel blocker, in depressed patients (Cappiello et al., 1998). In this study, a cohort of seven patients with a DSM-IV diagnosis of major depression was administered an intravenous infusion of either 0.5 mg kg^{-1} of ketamine or saline over 40 min. These patients, who were unresponsive to conventional antidepressants, exhibited a substantial and sustained improvement in mood for up to 72 h after infusion of ketamine, but did not respond to saline. The results of this pilot study are consistent with preclinical evidence that NMDA antagonists are antidepressant. The efficacy of metapramine (Timaxel®) provides additional, albeit indirect evidence that NMDA antagonists are antidepressant. While no longer in clinical use (because of unacceptable, non-central nervous system related toxicity), early studies with this clinically effective antidepressant reported no obvious or dramatic effects on monoamine transport or metabolism. A recent report (Boireau et al., 1996) demonstrated that metapramine is a low affinity ($IC_{50} \sim 1.4$ μM to inhibit [^{3}H]*N*-[1-(2-thienyl)cyclohexyl]-piperdine binding) NMDA antagonist.

Table 1
NMDA antagonists are active in preclinical models that predict antidepressant efficacy

- AP-7, dizocilpine, and ACPC are active in the forced swim test (mice); ACPC is active in the tail suspension test (mice)[1–3].
- Dizocilpine is active in forced swim test and potentiates 'classical' antidepressants in this test (rats)[4].
- CGP 37849 and CGP 39551 (competitive NMDA antagonists) are active in the forced swim test (rats)[5].
- Memantine (a use dependent channel blocker) is active in the forced swim test (rats)[6].
- SL 82.0715 (eliprodil) is active in the forced swim test (mice)[7].
- ACPC and CGP 37849 are active in the forced swim test (rats)[8].
- CGP 37849, CGP 40116 (competitive NMDA antagonists) and dizocilpine reverse the decreased consumption of sucrose solution (proposed as a model of anhedonia) produced by chronic, mild stress (rats)[9,10].
- ACPC is active in the chronic mild stress model. The onset of effect is dose-dependent, and significantly more rapid than imipramine (rats)[11].

References: 1. Trullas and Skolnick (1990); 2. Trullas et al. (1991); 3. Skolnick et al. (1992); 4. Maj et al. (1992a); 5. Maj et al. (1992b); 6. Moryl et al. (1993); 7. Layer et al. (1995); 8. Przegalinski et al. (1997); 9. Papp and Moryl (1993); 10. Papp and Moryl (1994); 11. Papp and Moryl (1996).

Psychotomimetic side effects are a prominent feature of uncompetitive NMDA antagonists such as phencyclidine and ketamine (Javitt and Zukin, 1991; Krystal et al., 1994), and have also been observed in early stage clinical trials with competitive NMDA antagonists (Sveinbjornsdottir et al., 1993). While these actions would appear to preclude the long term use of NMDA antagonists (e.g., to treat depression), both preclinical and clinical evidence indicates that psychotomimetic side effects are associated with high affinity competitive and uncompetitive NMDA antagonists (Rogawski and Porter, 1990; Kornhuber and Weller, 1997). This principle is perhaps best illustrated with memantine, a low affinity ($IC_{50} \sim 1$ μM) uncompetitive channel blocker that has been used in Europe to treat a variety of chronic neurodegenerative diseases (Kornhuber and Weller, 1997; Parsons et al., 1999). Despite therapeutic blood levels that are well within the range that block NMDA receptors, memantine does not produce the psychotomimetic-like side effects characteristic of higher affinity NMDA antagonists such as phencyclidine (Kornhuber and Weller, 1997; Parsons et al., 1999). Other therapeutic strategies to effect NMDA receptor blockade without producing psychotomimetic side effects include the use of partial glycine agonists, exemplified by ACPC. Despite a variety of preclinical studies (Trullas and Skolnick, 1990; Long and Skolnick, 1994; Zapata et al., 1996) demonstrating a functional blockade of NMDA receptors by ACPC, no phencyclidine-like side effects were observed at high doses (Evoniuk et al., 1991). This is consistent with the absence of side effects noted in a Phase I trial of ACPC (Cherkofsky, 1995) using intravenous infusions of up to 20 mg kg^{-1} (i.e., ~1.4 g). Clinical trials with NMDA antagonists acting at other loci (e.g., eliprodil) have not evidenced psychotomimetic side effects (Patat et al., 1994). Initial studies (reviewed in Scatton et al., 1994) suggested that eliprodil and the related molecule ifenprodil act at the polyamine binding sites associated with NMDA receptors. While polyamine actions at NMDA receptors are far more complex than originally described (Romano and Williams, 1994), eliprodil appears to act at a distinct site (that may be allosterically coupled to polyamine sites) on receptors containing an NR2B subunit (Williams, 1993). Thus, whether it is this subtype selectivity or some other property (e.g., the activity dependence described for ifenprodil) (Kew et al., 1996) that abrogates the emergence of psychotomimetic symptoms, eliprodil does produce antidepressant-like actions in preclinical models (Layer et al., 1995).

3. Modulation of NMDA receptors by chronic antidepressant treatment

3.1. Radioligand binding studies

NMDA antagonists can modulate monoamine turnover in the central nervous system (Loscher et al., 1991; Wedzony et al., 1997). Thus, it could be hypothesized that these actions are responsible for the antidepressant-like properties of NMDA antagonists. An alternative hypothesis is that monoamine-based therapies ultimately affect NMDA receptors, leading to the same functional effects as NMDA antagonists (i.e., a dampening of NMDA receptor function). This latter hypothesis was initially explored by examining radioligand binding to NMDA receptors in mouse brain following acute (1 day) and chronic treatment (14 days) with imipramine (15 mg kg^{-1}, i.p.). The salient effects of chronic treatment with this dose of imipramine (a dose chosen based on activity in the forced swim test) can be summarized as follows: (1) a decrease (~36%) in basal [^{3}H]MK-801 binding that was reversed by the addition of glutamate (100 nM).; (2) a ~2.5-fold reduction (compared to vehicle treated mice) in the potency of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid (DCKA) binding to strychnine-insensitive glycine receptors; no significant change in basal [^{3}H]5,7-dichlorokynurenic acid binding was observed; (3) a 28% reduction in the proportion of high affinity glycine sites inhibiting [^{3}H]CGP 39653 binding to NMDA receptors that was not accompanied by significant alterations in basal [^{3}H]CGP 39653 binding. These effects were observed in cerebral cortex but not in hippocampus, striatum or basal forebrain (Nowak et al., 1993). Based upon the obvious technical limitations of this type of experiment (e.g., gross brain dissection and administering a single drug dose for a fixed time interval), it could be concluded that these effects on radioligand binding to NMDA receptors are restricted to cerebral cortex. However, this conclusion is not supported by more widespread changes in mRNA levels encoding NMDA receptor subunits produced by chronic antidepressant treatment (Boyer et al., 1998). Further, subsequent studies demonstrated that the magnitude of effect on [^{3}H]5,7-dichlorokynurenic acid binding produced by imipramine continues to increase if treatment is continued for 21 days (the longest interval studied) and persists for >5 days following cessation of treatment (Paul et al., 1994). The time-dependent nature of these effects (citalopram and electroconvulsive shock also appear to exhibit time dependence in this measure (Paul et al., 1994; Huang et al., 1997a) may be interpreted as reflecting an adaptive change in NMDA receptors. To determine if alterations in radioligand binding to NMDA receptors are a general consequence of chronic antidepressant treatment, groups of mice were administered antidepressants (i.e., drugs exhibiting efficacy in at least one well, controlled double blind trial) from each principal 'class': tricyclics (e.g. desipramine, amitriptyline), monoamine oxidase inhibitors (e.g., chlorgyline, tranylcypromine), serotonin reuptake inhibitors (e.g., citalopram, sertraline), and 'atypical agents' (e.g., alaproclate, mianserine). Each drug was administered at a fixed dose (dosing was based either on activity in behavioral tests or the ability to produce a downregulation of β-adrenoceptors) for 14 days. These treatments produced

statistically significant reductions in the potency of glycine to inhibit [^{3}H]5,7 dichlorokynurenic acid binding and/or the proportion of high affinity, glycine displaceable [^{3}H]CGP-39653 binding sites in cortical membranes (Paul et al., 1994; Layer et al., 1998). A limited number of non-antidepressant drugs (e.g., chlordiazepoxide, chlorpheniramine, scopolamine) was also examined, and chronic treatment (for fourteen days at a fixed dose) failed to significantly alter [^{3}H]5,7-dichlorokynurenic acid binding to cortical membranes (Paul et al., 1994).

These studies have recently been summarized (Huang et al., 1997a; Layer et al., 1998), and a detailed treatment of these data is beyond the scope of this review. However, one aspect of this work merits additional comment in the context of this review. There appeared to be no clearcut stoichiometric relationship between antidepressant-induced changes in these two neurochemical measures. This point may perhaps best be illustrated by the ability of chronic citalopram treatment to produce only a modest increase ($<$ 2-fold) in the EC_{50} of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid binding while effecting a robust reduction in the proportion of high affinity, glycine-displaceable [^{3}H]CGP 39653 binding sites (Paul et al., 1994; Nowak et al., 1996; Layer et al., 1998). While it could be argued that these measures would be similarly affected by, for example, increasing either the dose or duration of treatment, the apparent lack of concordance between these measures is more likely to reflect distinct but related effects of antidepressants on NMDA receptors (see Section 3.2).

3.2. In situ hybridization studies

Based on studies in recombinant NMDA receptors, subunit composition is the principal determinant of ligand affinity (Wafford et al., 1993; Laurie and Seeburg, 1994). Thus, we hypothesized that antidepressant-induced changes in radioligand binding could reflect region-specific alterations in subunit composition. In preliminary studies (Huang et al., 1997a), chronic antidepressant treatment did not produce consistent changes in mRNA levels obtained from pooled cortical areas and hybridized with cDNA probes on slot blots. However, when this hypothesis was reexamined using in situ hybridization, region-specific effects on mRNA levels encoding NMDA receptor subunits were observed following chronic treatment of mice with both citalopram and imipramine (Boyer et al., 1998). Both antidepressants reduced ξ (the mouse homolog of NMDAR-1) subunit mRNAs in frontal, parietal, and occipital cortices as well as in a number of subcortical structures including striatum and amygdala, but not hippocampus. The failure to observe significant, antidepressant-induced changes in hippocampal ξ subunit mRNA levels is consistent with the reported inability of repeated electroconvulsive shock (a highly effective antidepressant therapy) to change mRNA levels encoding NMDAR-1 (the rat homolog of ξ) in hippocampus (Naylor et al., 1996). While reductions in ξ transcript levels were statistically significant in other brain regions, these changes may be considered modest (i.e., reductions of $<$ 20%). However, the use of a pan probe may have masked a more robust change in a particular ξ subunit splice variant (Kraus et al., 1996; Meshul et al., 1996). Further, this study may be viewed as a 'snapshot', taken at a single time point (and drug dose) that may underestimate the peak effects produced by these antidepressants.

By comparison, the impact of antidepressants on the ε family of subunits (the mouse homolog of NMDAR-2) was more restricted from a neuroanatomical standpoint but also more robust (Fig. 1). With the same caveat applied (i.e., these data are, at best, a snapshot), the effects of citalopram and imipramine on ε subunit mRNA levels provide a hint of treatment dependence. This point is illustrated by comparing the effects of citalopram and imipramine treatment in cerebral cortex. Citalopram produced a large reduction ($\sim$ 40%) in ε1 subunit mRNA in frontal cortex, but did not significantly alter transcript levels in either the parietal or occipital cortices; imipramine did not affect ε1 subunit mRNA levels (Boyer et al., 1998). In contrast, imipramine significantly reduced ($\sim$ 25%) ε2 mRNA levels in frontal, parietal and occipital cortices while citalopram produced small but non-significant changes across these brain regions. Similar hints of a treatment dependent effect can also be observed in hippocampus, with citalopram reducing ε1 mRNA levels by $\sim$ 40% in CA2 without effecting a statistically significant reduction in ε2 mRNA levels. By contrast, imipramine reduced ε2 mRNA levels in CA1, CA2, and CA3–4, but did not significantly affect ε1 mRNA levels. In certain brain regions, most notably amygdala, both antidepressants reduced ε2 mRNA by $\sim$ 40% in amygdala and also reduced ε1 mRNA by 25–30%. These antidepressant-induced changes in mRNA levels are more widely distributed than would be predicted from radioligand binding studies. If there is a relationship between these antidepressant-induced changes in mRNA levels and radioligand binding to NMDA receptors, then the anatomically discrete changes in transcript levels evinced by in situ hybridization would likely be muted or masked in the pooled tissue preparations used in the radioligand binding studies.

A fundamental issue emerging from these studies centers on the functional consequences of reducing (in a region-specific manner) mRNA levels encoding these NMDA receptor subunits. At face value, a loss in NMDAR-2 subunits would be predicted to result in fewer (and/or perhaps different) receptors, reducing the efficiency of signal transduction (for NMDA receptors, a decrease in $[Ca^{+2}]_i$ following binding of the co-agonists glutamate and glycine). This hypothesis is consistent with the observation that in primary neuron culture, a reduction (induced by incubation with the neurotrophins brain derived neurotrophic factor (BDNF) and basic fibroblast growth factor) in NMDAR-2A and -2C mRNA levels

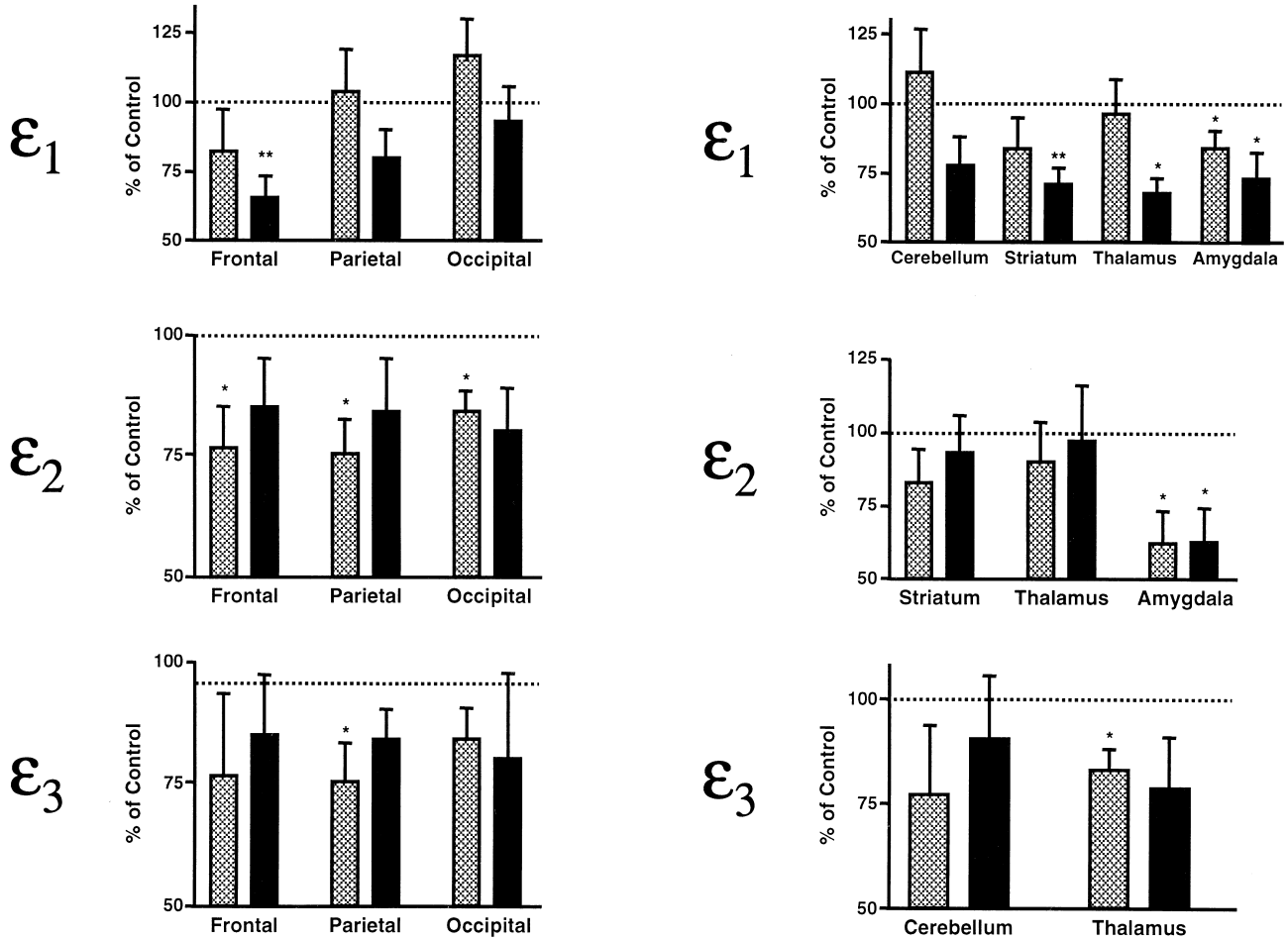

Fig. 1. Differential effects of chronic imipramine and citalopram on expression of ε subunit mRNA levels. Mice were injected daily (16 days) with saline, imipramine (15 mg kg^{-1}, stippled bars) or citalopram (20 mg kg^{-1}, solid bars). Twenty-four hours after the final injection, animals were sacrificed and mRNA levels quantified by in situ hybridization. Films contained images from one mouse from each of three treatment groups; mRNA levels measured in each region were expressed as a percentage of those obtained in the parallel processed saline treated animal. Left panel: cortical subfields; right panel: subcortical structures and cerebellum as indicated. Values represent the $X \pm$ S.E.M. for six to eight mice. Symbols: $^{*}P < 0.05$; $^{**}P < 0.025$ versus saline treated mice, Wilcoxon matched-pairs signed-ranks test. These figures were modified from Boyer et al. (1998).

results in a concomitant decrease in NMDA-induced Ca^{+2} entry (Brandoli et al., 1998). The magnitude of these reductions in mRNA levels observed in primary neuron culture are in the same range as those produced by chronic treatment of mice with citalopram and imipramine (Boyer et al., 1998). Further, the modest but statistically significant reductions in the potency of glycine to inhibit [^{3}H]5,7-dichlorokynurenic acid binding observed after a variety of antidepressants may also be viewed as an effect producing a 'reduction in function' given that strychnine-insensitive glycine receptors are not saturated in situ (Bergeron et al., 1998). A more direct test of this hypothesis awaits electrophysiological studies on NMDA receptor containing neurons (in, for example, frontal cortex) following chronic antidepressant treatment. Nonetheless, preclinical studies demonstrating the antidepressant-like actions of NMDA antagonists (Table 1) provide additional, albeit circumstantial support for the hypothesis that chronic antidepressant treatments dampen NMDA receptor function within circumscribed areas of the central nervous system.

4. Molecular links between conventional antidepressants and NMDA receptors: a framework for the development of novel therapies

If an adaptation of NMDA receptors is necessary for the action of conventional (i.e., monoamine based) antidepressants (Huang et al., 1997a), then understanding the molecular mechanism(s) governing this process may provide a rudimentary framework for developing novel agents (Fig. 2). Most currently used antidepressants increase synaptic concentrations of norepinephrine and/or serotonin, stimulating adenylyl cyclase via G protein (Gs) coupled recep-

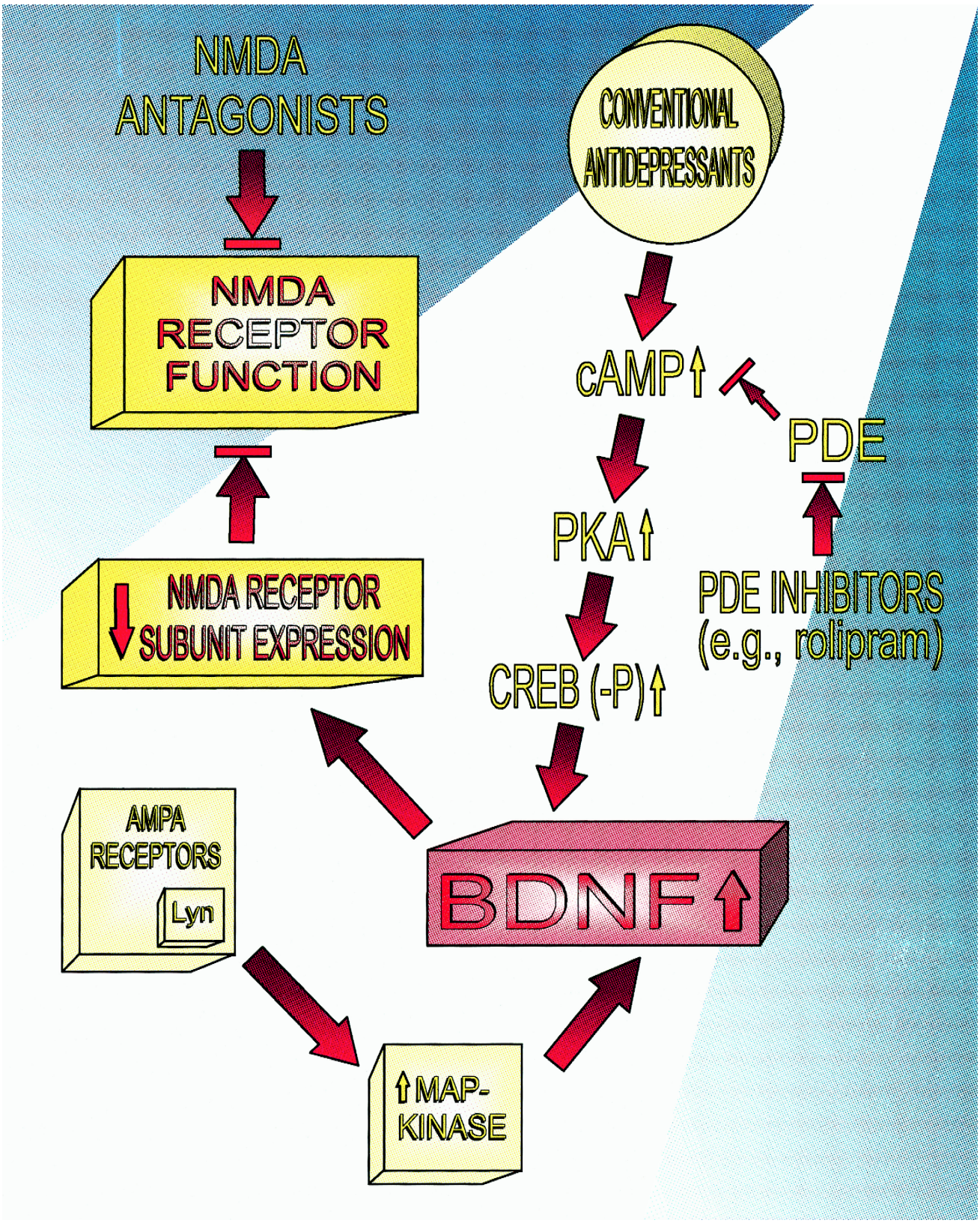

Fig. 2. Linking conventional antidepressants to reductions in NMDA receptor function: a framework for the development of novel therapies. A solid bar preceding an arrow denotes inhibition. See text for details.

tors (e.g., β-adrenergic, 5-$HT_{4,6,7}$) (reviewed in Duman et al., 1997a,b; Rossby and Sulser, 1997). The resulting elevations in cyclic AMP levels activate cyclic AMP dependent protein kinase A which can initiate a series of

events regulating gene transcription. For example, this enzyme may be translocated to the nucleus and phosphorylate the transcription factor cyclic AMP response element binding protein (CREB), increasing transcription of genes containing a cyclic AMP response element (Montminy et al., 1990). A pivotal series of studies by Duman and his colleagues (Nibuya et al., 1995, 1996; reviewed in Duman et al., 1997a,b) demonstrate that chronic antidepressant treatments elevate levels of both CREB mRNA and protein in rat hippocampus. Thus, these antidepressants may be viewed as effective regulators of gene transcription by dint of both increasing levels of CREB and inducing the activation of protein kinase A; the latter action provides a means of further increasing the transcription of genes containing cyclic AMP response elements because the phosphorylated form of CREB is a more effective transcription factor than its dephosphorylated form (Armstrong and Montminy, 1993).

Duman et al. (1997a,b) have hypothesized that increases in CREB may be responsible for the elevations in hippocampal levels of brain-derived neurotrophic factor (BDNF) observed after chronic antidepressant treatment (Nibuya et al., 1995). BDNF is a member of a structurally related family of trophic factors, which includes nerve growth factor, neurotrophin-3 and neurotrophin-4. Because of its trophic and neuroprotective properties (Mamounas et al., 1995; Tong and Perez-Polo, 1998), Duman et al. (1997a,b) and more recently, Altar (1999) have hypothesized that BDNF induction is a pivotal step in blunting the ability of chronic stressors (which in turn reduce BDNF and increase glucocorticoids) to damage vulnerable neurons. Since NMDA antagonists are protective against a wide variety of neuronal insults (Choi, 1988; Kornhuber and Weller, 1997), it could be hypothesized that by promoting the formation of BDNF, conventional antidepressants and NMDA antagonists reach a similar cellular endpoint: *protection of vulnerable neurons*. However, the data of Brandoli et al. (1998) indicate these two different treatment strategies reach an identical functional endpoint: *dampening NMDA receptor function* (Fig. 2) Thus, these investigators demonstrated that long term (>6 h) exposure of cerebellar granule cell neurons to BDNF reduced mRNA and protein levels of NMDAR-2A and -2C. The magnitude of the reduction in NMDAR-2A mRNA produced by BDNF in these primary neuron cultures is in the range ($\sim 40\%$) produced by chronic imipramine treatment in frontal cortex, the CA2 layer of hippocampus, and amygdala (Boyer et al., 1998) [Fig. 1]. Moreover, this BDNF-induced reduction in NMDA receptor subunit mRNA and protein is accompanied by a robust reduction in NMDA-evoked increases in $[Ca^{+2}]$ (Brandoli et al., 1998), an effect that can readily be mimicked by direct application of NMDA antagonists (Dildy and Leslie, 1989).

This rapidly emerging body of data describes only one subset of pathways (Fig. 2) that may be used both to target antidepressants acting beyond the monoaminergic synapse (Duman et al., 1997a,b; Rossby and Sulser, 1997) and optimize conventional therapies. For example, in view of the critical role of cyclic AMP in the action of conventional agents (Fig. 2), it may not be viewed as surprising that rolipram, a selective inhibitor of phosphodiesterase (PDE) IV (Hughes et al., 1997), is a clinically effective antidepressant (Horowski et al., 1985). While this compound has an unacceptable side effect profile (reports of nausea precluded further clinical development), phosphodiesterase inhibitors represent a potential monotherapy and a means of augmenting conventional agents. Consistent with the idea that limiting the degradation of cyclic AMP represents a viable monotherapy, Nibuya et al. (1996) have demonstrated that chronic (21 days) administration of either rolipram or the nonselective phosphodiesterase inhibitor papaverine elevated hippocampal mRNA levels of CREB and BDNF. Further, when combined with imipramine, rolipram produced a more rapid increase in hippocampal BDNF and CREB mRNA levels than either compound alone (Duman et al., 1997a). The hypothesis that phosphodiesterase inhibition may be used to augment conventional antidepressants is also consistent with an anecdotal report (Malison et al., 1997) that in a female patient refractory to a number of antidepressants, addition of papaverine to a (previously ineffective) regimen of venlafaxine resulted in dramatic clinical improvement. The recent report of Suda et al. (1998) further strengthens the rationale for this augmentation strategy. Thus, these investigators observed that chronic electroconvulsive shock and imipramine treatment increase the expression of phosphodiesterase IV in rat frontal cortex. If this increased expression of phosphodiesterase IV can be confirmed and extended to other antidepressants, then it could be hypothesized that the increased degradation of cyclic AMP may contribute to both the therapeutic lag and the response rate associated with conventional antidepressants.

If increased levels of BDNF are essential for the antidepressant actions of conventional agents (Fig. 2), then it may be possible to develop novel therapies bypassing the monoaminergic synapse. An interesting test of this hypothesis stems from the recent report of Hayashi et al. (1999). These investigators demonstrated that Lyn, a member of the Src-family protein tyrosine kinases, is physically associated with AMPA receptors in primary cerebellar cultures. AMPA receptor stimulation (independent of ion flux) was reported to directly activate Lyn which, in turn, activates the mitogen-activated protein (MAP) kinase signalling pathway. Activated MAP kinase can be translocated into the nucleus and regulate gene expression (Treisman, 1996). Hayashi et al. (1999) have linked this activation of MAP kinase to increased gene expression of BDNF. Given the coexistence of AMPA and NMDA receptors at many central synapses (e.g., He et al., 1998) and the pivotal role AMPA receptors play in NMDA receptor activation, corresponding in vivo experiments will provide a interesting (and, arguably stringent) test of the importance of both

BDNF formation and dampening NMDA receptor function in antidepressant action.

Fig. 2 provides a very superficial view of potential targets for new antidepressants. Thus, this scheme illustrates the impact of elevating synaptic monoamine levels on Gs coupled pathways and how this may impact NMDA receptor function. However, it does not touch upon other well described actions of conventional antidepressants on signal transduction pathways. For example, occupation of 5-HT_2 receptors can activate an inositol phosphate cascade which in turn stimulates protein kinase C and ultimately, calcium linked protein kinases (reviewed in Rossby and Sulser, 1997). These events may affect transcription of an additional set of target molecules that can synergize with, and in some cases antagonize, the sequence of events illustrated in Fig. 2. Ironically, the activation of multiple signal transduction pathways (that can produce opposing actions at downstream targets) may form the basis for both the therapeutic lag and incomplete efficacy of conventional antidepressants.

Grounding the search for novel agents on the 'primary' (i.e., well described) transduction pathways used by traditional antidepressants may, by definition, provide only an incremental therapeutic benefit. However, it has been established that conventional agents can affect gene transcription unrelated to modulation of monoamine levels, and understanding these events may form the basis for significantly improved therapies. For example, Rossby et al. (1995) have demonstrated that desmethylimipramine can increase hippocampal mRNA levels of glucocorticoid type II receptors in the absence of presynaptic noradrenergic terminals. While the relationship between changes in glucocorticoid receptor mRNA and an antidepressant action remains unclear, this study can be viewed as a proof of principle that a 'specific norepinephrine reuptake inhibitor' can affect gene transcription by a mechanism independent of its action at the monoaminergic synapse. Based on this principle, it may be possible to find novel targets by identifying genes that are expressed or repressed following traditional antidepressants. Using differential display PCR (Liang and Pardee, 1992), Huang et al. (1997b) reported chronic imipramine and electroconvulsive shock increased levels of mRNA encoding the mitochondrial enzyme, cytochrome b. Based on analysis of Northern blots, these increases were restricted to cerebral cortex and not observed following chronic treatment with either haloperidol or morphine. While this work requires further elaboration, it may provide clues to a novel action that can serve as a target for drug development.

5. Concluding remarks

Breakthrough therapies to treat depression are most likely to develop from research beyond the monoaminergic synapse. An emerging set of tools and concepts may provide the means to identify these novel targets. While there is cause for optimism, there are also challenges that must be considered. Thus, most contemporary experimental strategies and hypotheses rely heavily on preclinical tests that have been validated with conventional antidepressants. True departures from iterations on monoaminergic themes may not be possible within this framework. Further, many of the 'new' targets described in this and other (Duman et al., 1997a,b; Rossby and Sulser, 1997) reviews impact an even greater array of cellular processes than conventional antidepressants. Designing agents that modulate these processes may improve on the therapeutic profile of conventional agents but may also bring a host of unacceptable side effects. Ideally, agents would be targeted to affect these processes in specific brain regions, a task that presents yet another set of challenges. In the absence of either significant clinical advances in our understanding of depression or the serendipitous discovery of a novel class of antidepressants, animal models with greater face and construct validity (Willner and Papp, 1997) will be an important tool for exploring new hypotheses and drugs.

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