Here, we describe our collaborative efforts to use N-methyl-D-aspartate (NMDA) receptor antagonists as a translational tool to advance our understanding of the pathophysiology of schizophrenia and identify potential new targets for treatment of schizophrenia. We began these efforts in the late 1980s with a keen sense that, in both human and animal studies, we needed to move beyond the dopamine hypothesis of schizophrenia; if the dopamine hypothesis were correct, the existing dopamine antagonists should have cured the disease but they have not. We used NMDA receptor antagonists, not to produce schizophrenia, but as a tool to provide insights into effects of disturbances in glutamate synaptic function in schizophrenia. Our work has provided insights into potential mechanisms that may contribute to disrupted cortical function in schizophrenia and has helped identify potential treatment targets for the disorder. The translational nature of this study made the clinical testing of the first of these targets feasible. Advances in systems neuroscience approaches in animals and humans make new types of translational research possible; however, our concern is that the current obstacles facing translational research funding and academia-industry collaborations threaten the future progress in this field.

Key words: ketamine/NMDA/glutamate

Introduction

In 1959, a group of investigators at Wayne State University published a landmark study of the effects of the arylcyclohexylamine, phencyclidine (PCP). This study described the psychosis and emotional blunting produced by this drug, a pattern of effects described by Elliot Luby, Jacques Gottlieb, and their collaborators as “schizophrenomimetic.” By the mid-1960s, Ed Domino and his colleagues characterized the effects of a less potent and rapidly metabolized derivative of PCP, ketamine, as “dissociative” on an electrophysiological basis. The existence of binding sites for PCP and ketamine emerged in 1979 followed by reports in the early 1980s that they block the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor. By the late 1980s, NMDA receptors were implicated in most of the important processes in the central nervous system (CNS). In the context of schizophrenia, reports that the activity of dopamine neurons and release of dopamine was enhanced by PCP and other NMDA receptor antagonists further solidified the dopamine hypothesis of schizophrenia and led to attempts to unify glutamate hypofunction-dopamine hyperfunction theories, shaping the direction of subsequent basic research.

In parallel, serious concerns emerged by the 1980s as to whether it would be possible to develop antipsychotic medications that acted through mechanisms other than by blocking the dopamine D2 receptor. Now, nearly 60 years after chlorpromazine was first tested in psychiatric patients, the question remains central to the quest for more effective pharmacotherapy for schizophrenia. Although at the time there was hope that clozapine might constitute a transformative treatment advance, in retrospect, clozapine appears to have been an incremental advance over chlorpromazine. The lack of progress was discouraging to many psychiatric investigators. The lack of progress was discouraging to many psychiatric investigators. One of our teachers, for example, called schizophrenia research in the 1980s “the graveyard of psychiatric careers.” Thus, it was becoming apparent that the field of schizophrenia research needed to take a fresh look at the neurobiology and treatment of this disorder. At the time, we were at the beginning of our independent careers. One of us (B.M.) was working on characterizing the impact of psychotomimetic compounds and antipsychotic drugs, especially clozapine, on glutamate neurotransmission and its interaction with dopamine. The other (J.K.) was a young psychiatrist running a clinical and
research program focused on psychotic disorders at the West Haven VA Medical Center, and becoming increasingly frustrated by the obvious limitations in the efficacy of clozapine for many of his patients. On the basic side, we were observing that clozapine was enhancing dopamine release in limbic regions as well as in the prefrontal cortex (PFC). This was counterintuitive to the dopamine (hyperactivity) hypothesis of schizophrenia. Furthermore, while NMDA antagonists played a critical role in regulating corticolimbic function and behaviors that were relevant to schizophrenia, many of these effects were dopamine independent. These findings further reinforced our thinking that mechanisms other than limbic dopamine hyperactivity are critical to the pathophysiology of schizophrenia. Here, we describe our efforts to use NMDA receptor antagonists as a translational tool to advance our understanding of the pathophysiology of schizophrenia and define potential new targets for treatment of schizophrenia. It should be underscored that we did not think that NMDA receptor antagonists would produce schizophrenia. Instead, we hypothesized that NMDA receptor antagonists would provide insights into effects of disturbances in glutamate synaptic function in schizophrenia. We believed that the neurobiology of schizophrenia was complex, poorly understood, and quite heterogeneous across patients; thus, trying to identify novel treatment mechanisms in patients was a daunting task. As a result, we hypothesized that studying NMDA receptor antagonists, an increasingly understood pharmacological tool, would yield scientific traction toward the goal of identifying novel treatments for glutamate synaptic abnormalities associated with schizophrenia.

Human Studies With Ketamine

Emerging neuroimaging and postmortem studies in the late 1980s began to demonstrate that schizophrenia was a disorder of cortical dysfunction. In the days before MRI and modern molecular biology, there were few tools that one could use to probe the neurobiology of the cortex. We gravitated to human experimental psychopharmacology for a few reasons. It was the only strategy available at the time to probe specific receptor mechanisms in the human cortex in vivo. This approach had been used successfully at our home institution to characterize noradrenergic and serotonergic alterations in panic disorder depression. The strength of this approach was its ability to interface productively with Yale basic scientists including George Aghajanian, Benjamin Bunney, Robert Roth, Eugene Redmond, and others whose work both guided the clinical studies and were in turn informed by them. This work also was stimulated by the studies of the PFC conducted by Patricia Goldman-Rakic, who was both an advisor and a collaborator in this study at various stages as it developed. In the case of opiate dependence, the collaboration of basic and clinical neuroscientists led to the discovery of clonidine for the treatment of opiate withdrawal symptoms, perhaps the first instance in psychiatry where a new treatment mechanism was identified through a process that would now be called modern translational neuroscience. We hoped to achieve for schizophrenia what the prior generation of Yale investigators achieved with the clonidine studies for opiate dependence.

Having decided to probe NMDA glutamate receptor function in healthy humans, we next had to choose the agent to study. PCP and MK801 (dizocilpine), the prototypical NMDA receptor antagonists, were not readily available and were poorly suited for human study because their high potency and long plasma half-lives would produce long-lasting cognitive and perceptual changes in subjects. The most acceptable drug from a safety/tolerability perspective was dextromethorphan, a widely available cough suppressant. However, humans vary widely in their metabolism of dextromethorphan and rapid metabolizers of this drug accumulate pharmacologically active levels of a potent and long-lasting NMDA receptor antagonist metabolite, dextrophan. Ketamine met all of our criteria, it had moderate potency, short plasma half-life, an acceptable pharmacological profile with regards to off-target actions in the brain (reviewed in Ref. 13), and it was a Food and Drug Administration (FDA)-approved surgical anesthetic. In addition, it already had been employed in some human studies of cognition and perception. A critical challenge in initiating ketamine research was to obtain approval to administer subanesthetic ketamine doses to human subjects. We remain grateful to Drs Elliot Luby and Ed Domino and Yale anesthesiologists for advising us on how to conduct ketamine research safely and effectively. The collaboration between prior investigators, anesthesiologists, and the IRBs laid the foundation for two decades of safe and informative Yale human studies involving ketamine administration.

Our first ketamine study provided evidence that it produced cognitive, behavioral, and subjective responses that might be relevant to schizophrenia in a dose-related fashion (figure 1). It showed that ketamine produced psychotic symptoms and thought disorders, negative symptoms (blunted affect, withdrawal, amotivation), and executive cognitive impairments. However, it did not significantly reduce scores on the Mini-Mental State Examination, suggesting that the psychosis produced by ketamine did not emerge in the context of delirium. The results from this study were presented for the first time at the American College of Neuropsychopharmacology (ACNP) Annual Meeting in December 1991 and the final study was published in 1994. Within a few years, several research groups replicated and extended our findings (eg, Ref. 16,17).

Translation to Animal Studies

Our studies with systemic injection of ketamine in behaving animals began shortly after the human infusion studies began as a result of a direct collaboration between

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the authors. Our basic work before that point had focused on dopamine-glutamate interactions in the striatum and PFC.15 We were observing regional differences in NMDA (and AMPA) receptor regulation of dopamine release in PFC compared with the striatum as well as changes in glutamate efflux in response to local application of selective NMDA antagonists such as AP5. At the time, we attributed this latter effect to local presynaptic mechanisms that autoregulated the release of glutamate, although it was later established that presynaptic NMDA autoreceptors are scarce and do not play a critical role in inhibiting glutamate release. Our first published studies with systemic ketamine showed that doses that impair working memory increase dopamine.19 But given the AP5 observation, we soon moved to examining the effect of ketamine on glutamate efflux in the PFC of the awake animal. We saw a dose-dependent increase in extracellular glutamate at subanesthetic doses (figure 2), which also were associated with detrimental behavioral effects, such as impaired working memory. Higher, anesthetic, doses on the other hand, decreased glutamate levels consistent with reduced cortical activity during general anesthesia.20 At this point, two critical experiments had to follow. First, we needed to demonstrate that the increase in extracellular glutamate resulted in enhanced glutamate neurotransmission. A transient increase in extracellular glutamate levels could have been a result of metabolic and not neuronal output. Furthermore, the increased extracellular glutamate levels could have been cleared by glia before glutamate reached receptors at the synapse and thus have no excitatory influence on glutamate-mediated neurotransmission. To address this, we looked to see if local intra-PFC application of an AMPA receptor antagonist could block some of the secondary behavioral and neurochemical effects of systemic ketamine. The rationale was that an increase in synaptic glutamate would activate AMPA receptors causing some of these secondary effects. We observed that the detrimental effects of ketamine on working memory, as well as the increase in dopamine release in PFC, were blocked by AMPA antagonist application,20 supporting the idea that ketamine was increasing synaptic availability of glutamate. The second experiment was to demonstrate that this effect of ketamine generalizes to other NMDA antagonists. Ketamine is not a particularly clean compound and even at low subanesthetic doses it binds, albeit at much lower affinity, to proteins other than the NMDA receptor. We observed similar glutamate-activating effects with other NMDA antagonists.21

Our finding that intra-PFC application of an AMPA antagonist ameliorates the detrimental effects of ketamine suggested that reduced glutamate neurotransmission could have clinical efficacy for schizophrenia. There were two obstacles to pursuing this effort. First, the idea was the opposite of the mainstream thinking with NMDA antagonists, in that the general assumption was that they mimic a state of “glutamate deficiency” in schizophrenia and thus, treatment approaches should aim to enhance glutamate neurotransmission.5,6 Second, reducing glutamate neurotransmission by oral AMPA antagonists was not a feasible option given the widespread involvement of these receptors in mediating fast excitatory neurotransmission throughout the CNS. The only feasible option for chronic treatment would have to involve subtle modulation of activated glutamate release. At the 1997 ACNP Annual Meeting, where some of these data were presented, Darryl Schoeppe from Eli Lilly presented data on a blood-brain barrier permeable agonist of metabotropic glutamate 2/3 (mGlu2/3) receptor LY354740, which, in several in vitro models, reduced activated glutamate release.22 This class of G-protein-coupled glutamate receptors were

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**Fig. 1.** *Left,* Ketamine hydrochloride effects on the brief psychiatric rating scale (BPRS): 4 key positive symptoms in healthy subjects (*n* = 18). Cluster scores (mean ± SEM) are presented for placebo (open circles), ketamine hydrochloride (0.1 mg/kg) (closed circles), and ketamine hydrochloride (0.5 mg/kg) (closed squares) test days. Individual time point increases from baseline, by Dunnett’s test: † † † *P* < .01. All other statistics are presented in the text. *Right,* Ketamine effects on the BPRS: 3 key negative symptoms in healthy subjects (*n* = 18). Cluster scores (mean ± SEM) are presented for placebo (open circles), ketamine hydrochloride (0.1 mg/kg) (closed circles), and ketamine hydrochloride (0.5 mg/kg) (closed squares) test days. Individual time point increases from baseline, by Dunnett’s test: † *P* < .05; † † *P* < .01. All other statistics are presented in the text.

**Fig. 2.** Ketamine increases glutamate efflux in the prefrontal cortex (PFC).20
The mechanism for NMDA receptor antagonist-induced activation of glutamate efflux remains inconclusive. Currently, the prevalent model is the so-called disinhibition mechanism whereby NMDA antagonists block the NMDA-mediated drive on GABA interneurons that normally inhibit principal (pyramidal) cells. This leads to enhanced activity of pyramidal cells, which then results in increased glutamate release in adjacent cortical or afferent regions (figure 3). Consistent with this model, systemic injection of NMDA antagonist produces excitatory responses in a subpopulation of PFC pyramidal neurons in behaving rodents at the same time that they inhibit putative GABA interneurons. This model fits nicely with the proposed GABA deficiency in schizophrenia as the putative diminished GABA neurotransmission in the PFC would result in disinhibition of pyramidal cell activity similar to treatment with NMDA receptor antagonists. There is, however, a practical weakness with this theory. According to the disinhibition hypothesis, enhancing GABA neurotransmission with benzodiazepines, including novel subunit-specific ligands, should be effective in treating schizophrenia or at least blocking the effects of ketamine in healthy subjects. As described below, and as demonstrated in recent studies, this is not the case.

Translation Back to Human Studies

The first human ketamine study yielded results that were robust and fit so well with our experimental hypotheses that we assumed it would be easy to find drugs that would attenuate ketamine effects in humans. However, the next several years were invested in conducting studies that failed to support some of the prevalent ideas that had suggested that benzodiazepines and first-generation antipsychotics would reduce ketamine effects. Perhaps the most important finding of these negative studies was that a dose of haloperidol that produced complete occupancy of dopamine D2 receptors and that produced clear antipsychotic effects in patients had no beneficial effect on psychosis in the ketamine model. A subsequent study also demonstrated that the ketamine psychosis was not worsened by amphetamine coadministration. These findings were consistent with emerging animal findings and demonstrated conclusively that the ketamine effects on psychosis were not dependent upon overstimulation of D2 receptors. In retrospect, the “failed” translational studies could have been predicted by studies of ketamine-antipsychotic interactive effects on the specific cognitive processes that we were translating from animals to humans. From these negative studies, we learned painfully that translational neuroscience requires (1) careful attention to the construct that one is trying to translate from an animal model to a human model and (2) even with carefully matched constructs or processes, differences may emerge in the pharmacology in animals and humans. For example, the findings emerging from the animal and human laboratories related to the interplay of NMDA receptor antagonists and D2 receptor antagonists related to executive cognitive functions seemed consistent. However, several laboratories predicted that haloperidol would have antipsychotic effects in humans administered NMDA receptor antagonists based on the capacity of haloperidol to attenuate the motor effects of NMDA receptor antagonists. Instead, haloperidol increased the sedative effects of ketamine without reducing the psychotogenic effects of ketamine. The first novel hypothesis that we tested was that the ketamine psychosis could be attenuated by drugs that reduced glutamate release. It was shown that lamotrigine, a voltage-gated cation channel that contributes to glutamate release, attenuated the cognitive and psychotogenic effects. Although lamotrigine was clinically effective as an adjunctive treatment in combination with clozapine in schizophrenia in an initial study, it was not an

Fig. 3. NMDA receptor deficits on GABA interneurons disinhibit glutamate release in PFC and hippocampus promoting disorganized cortical activity.
effective adjunctive treatment in larger studies of patient population.35

Animal findings regarding the efficacy of mGluR2/3 agonists in the ketamine model guided the evaluation of LY354740 in the human ketamine model. Obtaining access to this drug for experimental purposes was a formidable task in the early 2000s as relatively few novel agents were made available to investigators for translational research studies at that time. The strong and persistent support from leaders at Lilly made this study possible. It found that, as with the animal studies, LY354740 attenuated the ketamine-induced disruption in human working memory in a dose-related fashion.36 Also, there was a trend for this drug to reduce the ketamine psychosis. These data, along with the growing preclinical findings, provided a rationale for the promising mGluR2/3 agonist study in schizophrenia.37 Nonetheless, the clinical progress with this drug illustrated that a drug acting through a novel mechanism with antipsychotic efficacy could be identified through a translational neuroscience process.

Some novel compounds tested in this way by us did not yield signs of clear clinical benefit, including cannabidiol, nicotine, and N-acetylcysteine. However, a recent study of a glycine transporter (GLYT1) antagonist found that it reduced the behavioral effects produced by ketamine in healthy human subjects.38 These data are consistent with recently reported, but yet unpublished, encouraging results from a clinical trial of a Roche GLYT1 inhibitor as an adjunctive agent in schizophrenia.

Current Status

The studies outlined here helped identify a potential novel mechanism that may contribute to the pathophysiology of schizophrenia: transient increases in extracellular levels of glutamate that disrupt information processing by enhancing disorganized activity or “noise.”24,39,40 The ketamine model was instrumental in establishing the validity of this mechanism by showing that an mGlu2/3 agonist, which reduces activated glutamate release and aberrant PFC firing,41 ameliorates some of the behavioral effects of ketamine that have relevance to schizophrenia. The translational nature of these findings was instrumental in moving this target to clinical trials in patients with schizophrenia. The results of the initial placebo-controlled trials with an mGlu2/3 agonist were promising, demonstrating comparable efficacy with olanzapine in treating negative and positive symptoms of schizophrenia.37 The mGlu2/3 agonist also was devoid of motor and metabolic side effects associated with current antipsychotic drugs. These findings were, in essence, the first demonstration of laboratory-based discovery of a novel target for treatment of schizophrenia. A follow-up study, however, led to a failed trial in that the efficacy of established antipsychotic drugs or mGlu2/3 agonist did not differ from the placebo.42 A more recent safety trial comparing individuals with schizophrenia receiving standard care or an mGlu2/3 agonist has been more encouraging in that, after 6 months of treatment, there were no differences in seizure incidents or PANSS positive and negative scores between the two groups (Bruce Kinon, personal communication). After 3 months, however, symptoms of a subgroup of individuals receiving the mGlu2/3 agonist worsened. This could be due to the fact that the compound used is a direct agonist causing receptor down-regulation; thus, higher doses or approaches using positive allosteric modulation of the receptor may have to be used to maintain efficacy. Nevertheless, the best-case scenario is that we will have a target that provides similar efficacy as current treatment in the absence of metabolic or extrapyramidal side effects. While this will provide a significant improvement in the quality and duration of life of patients, the fact that this target does not provide superior efficacy highlights the need to focus on alternative targets. Since the late 1990s, when the first series of animal studies outlined the potential antipsychotic efficacy of mGlu2/3 agonists, several other potentially superior targets have been identified.43,44 One example is positive allosteric modulators of metabotropic glutamate group 5 (mGlu5) receptors, which are quite effective in reducing the adverse effects of NMDA antagonists on PFC activity and behavior.45

Where to Go From Here

In the future, our prediction is that the NMDA receptor antagonist model of schizophrenia will remain useful for mechanistic studies and drug discovery efforts, albeit in different contexts than their initial applications. Genetic and postmortem studies do not support a fundamental disruption in the static structure of the NMDA receptor in schizophrenia. Thus, the earlier approach that these antagonists are modeling an “etiology” of diminished NMDA receptor activity is not well supported today. On the other hand, these antagonists provide a useful tool for modeling the “pathophysiology” of disrupted cortical network dynamics in schizophrenia. With animal studies, 10–15 years ago, we would have predicted that mutant models would have replaced pharmacological models by now, but NMDA receptor antagonists have remained in active use because of the difficulties in the recreation of susceptibility alleles in the mouse genome. Knocking out (or down) the genes that contain these alleles is teaching us a great deal about the function of the gene in question, but the relevance of specific susceptibility alleles to the etiology of schizophrenia remains unclear. Given this, pharmacological “challenges” with NMDA antagonists and other psychotomimetic compounds are routinely used in mutant models to confer schizophrenia-like phenotypes. Moreover, mechanistic data reported from mutant models so far have focused on establishing glutamate/NMDA (or dopamine) abnormalities in schizophrenia,36,47 as
opposed to defining novel mechanisms of pathology. More recent genetic studies pointing to the prevalence of rare de novo mutations in schizophrenia\textsuperscript{46} may prove easier to model in rodents than susceptibly alleles. It will be interesting to see if animal models of these mutations converge on the glutamate synapse as a common locus of pathophysiology or identify completely different targets. For clinical studies, a new generation of “pharmacointaging” studies are combining experimental pharmacology with various imaging and cortical electrophysiology methods.\textsuperscript{49,50} These studies show substantial translational potential, as they enable investigators to examine the interactive effects of agents on the function of particular brain circuits in humans in a manner that is somewhat analogous to studies that can be conducted intracortically in animals. A particularly promising new translational link is emerging from pharmacogenomic studies of ketamine effects in humans. These studies hold the promise of evaluating the impact of genetic variation in humans that parallels informative genetic manipulations in animals. While these studies present many methodological challenges, the resulting explanatory power may open new windows to probing molecular mechanisms regulating brain function from a translational perspective and potentially lead to identification of novel targets for treatment of schizophrenia.

Despite our optimism that these progresses will occur, there is concern that the field of psychiatry currently is in a state of paralysis for advancing laboratory-generated novel concepts to the clinic. With our mGlu2/3 agonist studies, a series of circumstances allowed for the feasibility of translation and clinical testing of a novel target. First, there was the intellectual will and the funds that were critical for initiating a truly translational collaboration. At Yale, where the authors received their postdoctoral and residency training, there was an exceptional tradition of spontaneous and informal interaction and exchange of ideas between basic laboratory and clinical researchers. The clinicians were genuinely interested in keeping up with basic science and the basic scientists were conscious of the fact that their research must be clinically relevant. The funding for the initial phases of the study came from VA, where we both were located (VA Merit award to J.K. and the West Haven VA Schizophrenia Center). The VA Center grants gave a fair amount of leeway on the approach taken by each laboratory. Unlike the NIHs translational centers (eg, NIMHs Conte centers) that require polished and well-established “single” idea, also known as “central hypothesis,” and are essentially super R01s that aim to spend 5 years to accumulate evidence for the same concept, the VA Center funds, albeit limited, afforded us the freedom to test novel concepts. Second, the pharma-academia relationship was practical and conducive to collaboration. For example, material transfer from Lilly for a novel compound in active investigation (LY354740) to our basic lab took some effort but was feasible and occurred in a timely fashion. Currently, these transfers have become nearly impossible. Similarly, the nature of collaboration between the Krystal group and Lilly scientists allowed for a human proof of concept study that paved the way for future clinical trials with this compound. Third, there was the allocation of resources by Lilly to develop clinically suitable ligands for the mGlu2/3 receptor and to follow through with trials in patients with schizophrenia. The unique progress with this target illustrated that all these elements—(1) intellectual and monetary resources that facilitate translational neuroscience in academia, (2) academia-industry collaboration to support proof of concept studies, and (3) industry resources for clinical trials—are critical if a drug acting through a novel mechanism with antipsychotic efficacy can be identified. Notwithstanding the advances we have made so far, elegant translational studies and discovery of novel mechanisms and treatment targets in our animal or human laboratories will have no impact on the life of the patients if they are not advanced to the clinic.

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