Glutamatergic Animal Models of Schizophrenia

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ABSTRACT: Several lines of evidence, including recent genetic linkage studies implicating susceptibility genes for schizophrenia, make a strong case that abnormal NMDA receptor–mediated neurotransmission is a major locus for the pathophysiology of schizophrenia. Animal models that are relevant to putative NMDA dysfunction in schizophrenia have excellent face validity for several symptoms of schizophrenia and are important tools for the design of novel pharmacological intervention in schizophrenia. The present chapter includes a brief review of the utility of these models and the search for new medications that have the potential of normalizing glutamate neurotransmission in schizophrenia.

KEYWORDS: NMDA receptors; schizophrenia; cognitions; dopamine; prefrontal cortex

INTRODUCTION

Schizophrenia remains the most morbid and debilitating of all psychiatric disorders. Current modes of therapy, which are based on the accidental discovery of neuroleptics 50 years ago, do not treat the disease but merely ameliorate the psychotic symptoms in a subgroup of patients. Because all known antipsychotic drugs block at least one type of receptor for the neurotransmitter dopamine, the focus of preclinical and clinical research in this field has been primarily on the dopaminergic system. However, several decades of research focused on the dopaminergic system have made it evident that drugs that specifically target dopamine receptors are not sufficient for treatment of schizophrenia. Specifically, while dopamine receptor blockers, such as haloperidol, are generally effective in reducing the so-called positive symptoms of this disease (e.g., psychosis, hallucinations, and paranoia), these medications generally fail to treat the affective and cognitive symptoms of this disorder. These latter symptoms contribute greatly to the long-term morbidity of schizophrenia and the inability of patients to function in society. Thus, there is an acute need for novel approaches to the pharmacotherapy of this disorder that will, we hope, lead to the design of treatments that target the emotional symptoms and cognitive dysfunctions that are associated with schizophrenia.

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Undoubtedly, development of more effective treatments depends on a better understanding of the underlying functional pathology of schizophrenia and the development of appropriate animal models with similar functional abnormalities so that novel mechanistic ideas and pharmacotherapeutic approaches can be tested. Because of the accumulating evidence that glutamatergic neurotransmission may be abnormal in schizophrenics (chapters by Tamminga and Coyle in this volume), there is growing emphasis on glutamatergic animal models of schizophrenia and their utility for testing novel pharmacological strategies for treatment of schizophrenia. In this chapter, recent studies with these models that have had an impact in our understanding of the disease process and novel directions for pharmacological treatments are reviewed.

THE NMDA ANTAGONIST ANIMAL MODEL OF SCHIZOPHRENIA

Systemic injection of the dissociative anesthetic phencyclidine (PCP) and its analogue ketamine produce a behavioral syndrome in non-schizophrenics that closely resembles endogenous symptoms of schizophrenia and that is frequently misdiagnosed as acute schizophrenia.[1–5] These include positive symptoms such as paranoia, agitation, auditory hallucinations; negative symptoms such as apathy, poverty of thought, and social withdrawal; and cognitive deficits such as impaired working memory.

Despite their multifaceted actions at anesthetic doses, lower doses of PCP and ketamine are thought to selectively produce noncompetitive blockade of NMDA receptors by binding to a site located in the ion channel associated with this receptor.[5] This pharmacological characteristic of ketamine and PCP is generally considered the major contributing factor to the psychotomimetic properties of these drugs and is consistent with the "glutamate deficiency hypothesis of schizophrenia," which was first suggested by Kim *et al.*[6] and later elaborated on by other investigators (e.g., see Refs. 7–10).

In laboratory animals PCP, ketamine, and other NMDA antagonists, such as MK801, produce a complex behavioral profile that is related to the clinical effect of these drugs in humans. In particular, they produce impaired cognitive functions, such as impaired performance in working memory tasks, as well as altered social behavior, hyperactivity, stereotypy, and sensory gating deficits.[11–15] Hence, these drugs are routinely used as pharmacological animal models of schizophrenia.

In general, the NMDA antagonist model has several advantages over other pharmacological models of schizophrenia. The most important advantage is that this is the only animal model that has a clinical parallel. Using modern diagnostic criteria this model has been extensively characterized in humans. Healthy individuals treated with low doses of ketamine exhibit transient negative and positive symptoms[4] and cognitive deficits similar to those reported in patients with schizophrenia.[16] There are also ongoing clinical studies with ketamine in several clinical laboratories, which is especially useful for translating treatment strategies from animal experiments to the clinic.

Another strength of this model is that, unlike other pharmacological models, such as amphetamine and cannabinoid models, prolonged use of ketamine or PCP is not necessary for expression of psychosis. The first reports of PCP psychosis were dur-

ing its initial use as an anesthetic, and case reports of PCP-precipitated psychosis after recreational use often include individuals who thought they were consuming other substances and had not experienced PCP before.[17] Furthermore, in clinical trials, a single exposure to ketamine or PCP produces schizophrenic-like symptomatology in non-schizophrenics, which may last for several hours or days.[18] Another important aspect of the NMDA antagonist model is that PCP psychosis is not responsive to conventional antipsychotic therapy.[2,17] This is supported by findings that only selective behavioral effects of PCP and ketamine in basic and clinical studies are ameliorated with dopamine antagonists.[19,20] This makes the PCP model especially useful for strategies aimed at developing novel approaches for treatment of schizophrenia.

The schizophrenia-like effects of NMDA antagonists in healthy volunteers suggest that the primary glutamatergic abnormality in schizophrenia may be reduced NMDA receptor function. Basic studies, therefore, have focused mostly on enhancing NMDA receptor function through mechanisms that produce subtle positive modulation of this receptor. The most common approach has been to activate the glycine/D-serine modulatory site on the NMDA receptor. In animal studies glycine, D-serine, or glycine uptake blockers, which increase intrasynaptic levels of glycine, ameliorate some of the behavioral effects of PCP.[21] Limited clinical studies have complemented basic studies by demonstrating that oral administration of D-serine or exogenous ligands, such as D-cycloserine, may be useful for treatment of the cognitive symptoms of schizophrenia.[22,23] One issue with these studies has been that the ligands used either have poor absorption or are not highly specific. However, more selective ligands are under development and are being characterized in basic studies using the NMDA antagonist model.[21]

In our laboratory, we have observed that selective NMDA receptor antagonists, such as AP5, increase the efflux of glutamate.[24] This finding suggested that although this class of drug reduces glutamatergic neurotransmission at the NMDA receptor, this class of drug may also work to stimulate glutamatergic neurotransmission at non-NMDA glutamate receptors. Similar to AP5, a dose-dependent increase in glutamate efflux in response to subanesthetic doses of ketamine and PCP was observed.[25] In addition, recent electrophysiological studies in awake animals suggest that systemic administration of NMDA antagonists increases the spontaneous firing rate of neurons in the prefrontal cortex (FIG. 1). Furthermore, some of the cognitive and dopaminergic effects of these drugs in the rodent are reduced by pretreatment with antagonists of non-NMDA glutamate receptors. Specifically, (1) activation of cortical dopamine release elicited by ketamine and PCP is reduced by local and systemic administration of non-NMDA antagonists.[25] This is important because the hyperdopaminergic state produced by ketamine and PCP is thought to mediate some of the behavioral effects of PCP and ketamine. (2) The performance decrement produced by ketamine and PCP in a delayed alternation task is ameliorated by non-NMDA antagonist pretreatment.[25] Consistent with a hyperglutamatergic mechanism accounting for some of the effects of ketamine, clinical trials also indicate a dramatic reduction in memory-related deficits caused by ketamine in healthy individuals following pretreatment with lamotrigine, a glutamate release inhibitor.[26]

Collectively, these findings suggested that NMDA antagonists may produce some of their adverse behavioral effects by increasing cortical glutamatergic neurotransmission at non-NMDA receptors. Thus, reduction of this excess glutamatergic out-

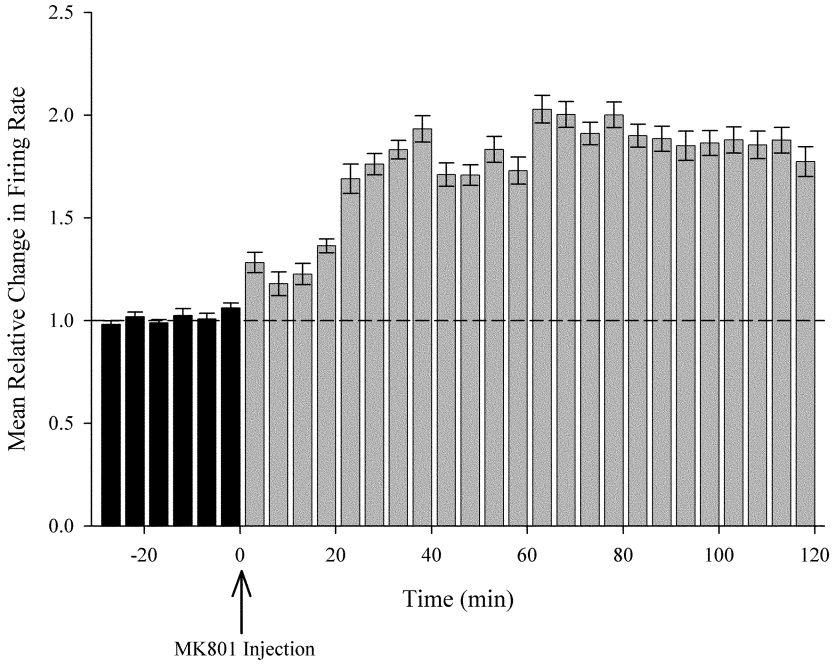

FIGURE 1. Mean firing rate increases of prefrontal cortex neurons after systemic injection of the NMDA antagonist MK801 (0.1 mg/kg). Data is the combined average of a total of 76 single units recorded from 6 different rats. Extracelluar single unit recordings were obtained from awake animals using electrode arrays (eight microwires per array) chronically implanted into the prefrontal cortex. Changes in firing rate for individual units during a 2½ hour period were calculated by determining the average firing rate during 5-min bins and dividing by the average firing rate during the 30-min preinjection baseline period (*black bars*). Thus, a relative change of 1.0 (*dashed line*) indicates no change from baseline firing rates. Following systemic MK801 injection (*gray bars*), there is an immediate and sustained increase in PFC spontaneous firing rates, nearly doubling the average baseline firing rate. (Error bars = SEM).

put may have important clinical implications for the pharmacotherapy of cognitive deficits associated with schizophrenia. To test this mechanism, we pretreated animals with an agonist of group II metabotropic glutamate receptors (mGluR), which are localized presynaptically and have been shown to reduce the stimulated release of glutamate, presumably by presynaptic mechanisms (chapters by Conn and Schoepp in this volume). In these animals, we observed that activation of glutamate efflux by PCP was decreased. We also determined the effect of this drug in some of the behavioral disruptions produced by PCP, including activation of locomotor activity, stereotypical behavior, and disruption of working memory.[27] These basic studies have now been extended to clinical studies using the ketamine model of psychosis in healthy volunteers.[28] These studies so far suggest that pretreatment with a group II mGluR agonist diminishes some of the cognitive impairing effects of

ketamine. Clinical trials in schizophrenia are forthcoming, but, regardless of whether this class of drugs is successful in treating symptoms of schizophrenia, the series of studies outlined above demonstrate the effectiveness of this model in providing novel therapeutic options for treatment of schizophrenia.

MUTANT MODELS

In addition to pharmacological models, recent mutant models have been introduced that may be relevant to putative genetic abnormalities of glutamate systems in patients with schizophrenia. Although only limited phenotypic characterization has been performed in these models, they are important models for understanding the processes that lead to the vulnerability to develop schizophrenia. One of the most interesting and clinically promising of these models are mice that are hypomorphic for neuregulin 1 (*NRG1)*. Recent genome-wide scans of schizophrenia families in five distinct populations show that schizophrenia maps to chromosome 8p, and extensive fine-mapping of the 8p locus have identified *NRG1* as a candidate gene for schizophrenia.[29] *NRG1* is expressed at central nervous system synapses and has a clear role in the expression and activation of neurotransmitter receptors, in particular the NMDA receptors. Specifically, *NRG1* regulates expression of glutamate receptor subunits and directly activates ErbB4 receptors.[30] This member of the ErbB family of tyrosine kinases is colocalized with the NMDA receptor and is thought to regulate the kinetic properties of the NMDA receptor by phosphorylating the NR2 subunit of the NMDA receptor. Mutant mice heterozygous for either *NRG1* or its receptor, *ErbB4,* show a behavioral phenotype of impaired prepulse inhibition and hyperlocomotion that overlaps with the NMDA antagonist model of schizophrenia.[29] Furthermore, *NRG1* hypomorphs have fewer functional NMDA receptors than wild-type mice.

Another model with relevance to schizophrenia is the NMDAR1 (NR1) "knockdown" line of mutant mice.[31] These animals display exaggerated spontaneous locomotion and stereotypy, as well as deficits in social and sexual interactions. Although genetic or postmortem abnormalities in the NR1 subunit of NMDA receptors have not been found in schizophrenia, this is an interesting model that may add to our understanding of the long-term effects of congenital NMDA receptor hypofunction.

CONCLUSIONS

Recent lines of work suggest that abnormal NMDA receptor–mediated neurotransmission may contribute to the ontogeny and the pathophysiology of schizophrenia. Pharmacological and genetic animal models that are based on this mechanism appear to have excellent face validity for symptoms of schizophrenia and are likely to provide important tools for design of novel therapeutic options for schizophrenia.

REFERENCES

1. LUBY, E. *et al.* 1959. Study of a new schizophrenomimetic drug-sernyl. Am. Med. Assoc. Arch. Neurol. Psychiatry **81:** 363–369.

2. BURNS, R. & L.S. LERNER. 1976. Perspectives: acute phencyclidine intoxication. Clin. Toxicol. **9:** 477–501.
3. PEARLSON, G. 1981. Psychiatric and medical syndromes associated with phencyclidine (PCP) abuse. Johns Hopkins Med. J. **148:** 25–33.
4. KRYSTAL, J.H. *et al.* 1994. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans: psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch. Gen. Psychiatry **51:** 199–214.
5. JAVITT, D.C. & S.R. ZUKIN. 1991. Recent advances in the phencyclidine model of schizophrenia. Am. J. Psychiatry **148:** 1301–1308.
6. KIM, J. *et al.* 1980. Low cerebrospinal fluid glutamate in schizophrenic patients and a new hypothesis on schizophrenia. Neurosci. Lett. **20:** 379–382.
7. ULAS J., C.W. COTMAN. 1993. Excitatory amino acid receptors in schizophrenia. Schizophr. Bull. **19:** 105–117.
8. OLNEY, J. & N. FARBER. 1995. Glutamate receptor dysfunction and schizophrenia. Arch. Gen. Psychiatry **52:** 998–1007.
9. COYLE, J. 1996. The glutamatergic dysfunction hypothesis for schizophrenia. Harvard Rev. Psychiatry **3:** 241–253.
10. TAMMINGA, C.A. 1998. Schizophrenia and glutamatergic transmission. Crit. Rev. Neurobiol. **12:** 21–36.
11. GREENBURG, B.D. & D.S. SEGAL. 1985. Acute and chronic behavioral interactions between phencyclidine (PCP) and amphetamine: evidence for a dopaminergic role in some PCP-induced behaviors. Pharmacol. Biochem. Behav. **23:** 99–105.
12. SCHMIDT, H.W. *et al.* 1989. Effects of intrastriatal blockade of glutamatergic transmission on the acquisition of T-maze and radial maze tasks. J. Neural Transm. **78:** 29–41.
13. STEINPREIS, R. *et al.* 1994. The effects of haloperidol and clozapine on PCP and amphetamine induced suppression of social behavior. Pharmacol. Biochem. Behav. **47:** 579–585.
14. VERMA, A. & B. MOGHADDAM. 1996. NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine. J. Neurosci. **16:** 373–279.
15. MANSBACH, R.M. & M. GEYER. 1989. Effects of phencylidine and phecylidine biologs on sensorimotor gating in the rat. Neuropsychopharmacology **2:** 299–308.
16. ADLER, C.M. *et al.* 1999. Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia. Am. J. Psychiatry **156:** 1646–1649.
17. RAINEY, J.J. & M. CROWDER. 1975. Prolonged psychosis attributed to phencyclidine: report of three cases. Am. J. Psychiatry **10:** 1076–1078.
18. BAKKER, C.B. & F.B. AMINI. 1961. Observations on the psychotomimetic effects of sernyl. Comp. Psychiatry **2:** 269–280.
19. KEITH, V.A., R.S. MANSBACH & M.A. GEYER. 1991. Failure of haloperidol to block the effects of phencyclidine and dizocilpine on prepulse inhibition of startle. Biol. Psychiatry **30:** 557–566.
20. KRYSTAL, J. *et al.* 1995. Modulating ketamine-induced thought disorder with lorazepam and haloperidol in humans. Schizophr. Res. **15:** 156a.
21. JAVITT, D.C. 2002. Glycine modulators in schizophrenia. Curr. Opin. Investig. Drugs **3:** 1067–1072.
22. GOFF, D.C. *et al.* 1999. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. [see comments]. Arch. Gen. Psychiatry **56:** 21–27.
23. HERESCO-LEVY, U. *et al.* 2002. Placebo-controlled trial of D-cycloserine added to conventional neuroleptics, olanzapine, or risperidone in schizophrenia. Am. J. Psychiatry **159:** 480–482.
24. LIU, J. & B. MOGHADDAM. 1995. Regulation of glutamate efflux by excitatory amino acid receptors: evidence for tonic inhibitory and phasic excitatory regulation. J. Pharmacol. Exp. Ther. **274:** 1209–1215.
25. MOGHADDAM, B. *et al.* 1997. Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic

and cognitive disruptions associated with the prefrontal cortex. J. Neurosci. **17:** 2921–2927.
26. ANAND, A. *et al.* 2000. Attenuation of the neuropsychiatric effects of ketamine with lamotrigine: support for hyperglutamatergic effects of N-methyl-D-aspartate receptor antagonists. Arch. Gen. Psychiatry **57:** 270–276.
27. MOGHADDAM, B. & B. ADAMS. 1998. Reversal of phencyclidine effects by a group II metabotropic glutamate receptor agonist in rats. Science **281:** 1349–1352.
28. Krystal, J.H., W. Abi-Saab, E. Perry, *et al.* 2003. Interaction of ketamine and an mGluR2/3 agonist in healthy volunteers. Biol. Psychiatry **53:** 312.
29. STEFANSSON, H. *et al.* 2002. Neuregulin 1 and susceptibility to schizophrenia. Am. J. Hum. Genet. **71:** 877–892.
30. OZAKI, M. *et al.* 1997. Neuregulin-beta induces expression of an NMDA-receptor subunit. Nature **390:** 691–694.
31. MOHN, A.R. *et al.* 1999. Mice with reduced NMDA receptor expression display behaviors related to schizophrenia. Cell **98:** 427–436.