Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 68 (2004) 1507–1514
Commentary
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

The GABA-glutamate connection in schizophrenia: which is the proximate cause?

Joseph T. Coyle

Department of Psychiatry, Harvard Medical School, McLean Hospital, 115 Mill St., Belmont, MA 02478, USA

Abstract

Schizophrenia is a chronic, disabling psychiatric disorder that genetic studies have shown to be highly heritable. Although the dopamine hypothesis has dominated the thinking about the cause of schizophrenia for 40 years, post-mortem and genetic studies have provided little support for it. Rather, post-mortem studies point to hypofunction of subsets of GABAergic interneurons in the prefrontal cortex and the hippocampus. Furthermore, clinical pharmacologic, post-mortem and genetic studies have provided compelling evidence of hypofunction of a subpopulation of NMDA receptors in schizophrenia. In support of this inference, agents that directly or indirectly activate the glycine modulatory site on the NMDA receptor (the Glycine B receptor) reduce symptoms in chronic schizophrenia, especially negative symptoms and cognitive impairments. Electrophysiologic and pharmacologic studies suggest that the vulnerable NMDA receptors in schizophrenia may be concentrated on cortico-limbic GABAergic interneurons, thereby linking these two neuropathologic features of the disorder.

Keywords: Schizophrenia; GABA; Glutamate; NMDA receptor; Glycine B receptor; D-Serine

1. Introduction

The role of dopamine has dominated thinking about the pathophysiology of schizophrenia for over four decades because of the therapeutic effects of antipsychotics on the positive symptoms of the disorder, especially psychosis [1]. However, the growing appreciation that negative symptoms and cognitive impairments are more robust predictors of outcome than psychosis has prompted interest in the potential role of dysfunction of other neuronal systems in this disorder [2]. Indeed, the persistence of negative symptoms and cognitive impairments, their correlation with reduced cortical volume as well as the evidence that children and adolescents, who go on to develop schizophrenia, often exhibit impairments in cognition, social awkwardness and visual-motor clumsiness suggest that these may be core features of schizophrenia and represent its endophenotype [3]. Notably, post-mortem studies carried out over the last 30 years have not revealed consistent alterations in pre- or post-synaptic markers for the dopaminergic neurons in schizophrenia [4]. In contrast, abnormalities in markers for GABAergic neurons in prefrontal cortex and hippocampus have been documented. Furthermore, research over the last decade has provided evidence of hypofunction of cortical–limbic glutamatergic neurotransmission.

In the prefrontal cortex and in the hippocampal formation, GABAergic cells are exclusively interneurons, which play a dominant role in regulating the activity of the projecting glutamatergic pyramidal cells [5]. In turn, the activity of GABAergic interneurons is determined by glutamatergic afferents arising from projecting neurons or recurrent glutamatergic collaterals. An important question in trying to clarify pathophysiology of schizophrenic is what alterations are etiologic and what alterations are down-stream consequences. The dichotomous nature of such questions, however, must be suspect because of the now compelling genetic evidence that schizophrenia, while highly heritable, reflects the impact of a number of risk genes of small effect [6]. Furthermore, epidemiologic studies link genetic risk to perinatal insults such as second trimester influenza infection, seasonality of birth and low birth weight, which significantly affect the expression of the phenotype [3]. Accelerating findings from linkage and association studies are identifying potential risk genes that

The Eben S. Draper. Professor of Psychiatry. Tel.: +1 617 855 2101; fax: +1 617 855 2705.
E-mail address: joseph_coyle@hms.harvard.edu.

0006-2952/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2004.07.034

will undoubtedly aid in identifying processes which disrupt synaptic function in selective ways that result in the endophenotype and the psychosis.

This paper will review the evidence of alterations in GABAergic and glutamatergic function in the frontal cortex and hippocampus in schizophrenia. It will then examine possible ways in which these alterations may be related to each other that could account for cognitive impairments that characterize the endophenotype of the disorder.

2. GABAergic neurons

Initial studies, a quarter of a century ago, suggested reductions in the activity of glutamic acid decarboxylase (GAD) in cortex and GABA in the nucleus accumbens and thalamus in post-mortem studies of individuals diagnosed with schizophrenia [7]. However, the findings were not consistent and subsequent studies suggested that reduction in GAD activity may be an artifact of a slow death, to which the schizophrenic subjects were prone [8,9]. However, more recent studies from several laboratories that utilized either immunocytochemical or mRNA expression methods have developed compelling evidence of reduced expression of pre-synaptic markers in subpopulations of GABAergic interneurons in the frontal cortex and the hippocampal formation (for review, see [5,10]).

Two isoforms for GAD have been distinguished on the basis of molecular weight—the 65 and 67 kDa isoforms, respectively [5]. The 67 kDa isoform is preferentially expressed in perikarya and dendrites whereas the 65 kDa isoform is more prominently expressed in axons and terminals. No changes in the immunoreactivity of the 65 kDa isoform were observed in the anterior cingulate cortex of schizophrenic subjects although marked decreases were observed on pyramidal neurons in layers and 2 and 3 in bipolar disorder [5]. Generally, no overall differences between normals and schizophrenics were detected in the density of GAD 65—immunoreactive terminals on pyramidal or non-pyramidal cells in any subregion of the hippocampus. Although in a smaller cohort of patients, who were neuroleptic free for at least a year, immunoreactivity was reduced in sectors CA2, 3 and 4 but not CA1 [11]. On the other hand, several studies have reported reduced numbers of GAD 67 mRNA expressing neurons in prefrontal cortex and overall reduced expression of GAD 67 as compared to GAD 65 [12–16]. In laminar analysis, Volk et al. [13] found that the density of neurons with detectable GAD 67 mRNA was significantly decreased in the intermediate layers of the prefrontal cortex but the level of GAD 67 mRNA expression per neuron did not differ from control subjects. Thus, they proposed that approximately 65–75% of the dorsolateral prefrontal cortical GABAergic neurons express normal levels of GAD 67 mRNA in schizophrenia but a minority (25–35%) fail to express detectable levels of the mRNA.

GABAergic interneurons co-express calcium binding proteins including parvalbumin, calretinin and calbindin [5]. Parvalbumin has been shown to be expressed predominantly in chandelier and basket cells in prefrontal cortex with calbindin 28 kDa localized to double bouquet cells and calretinin expressed in double bouquet and bipolar cells. Notably, the parvalbumin expressing GABAergic neurons have their synaptic contacts concentrated on proximal axon and soma of the pyramidal cell, thereby exerting a major influence over pyramidal cell firing. The expression of parvalbumin but not calretinin was reduced in the prefrontal cortex in schizophrenia [17], In contrast to the findings with GAD 67 expression, the density of neurons with detectable parvalbumin mRNA in the prefrontal cortex was unchanged in subjects with schizophrenia whereas the level of mRNA per neuron was significantly decreased. These combined results suggest that the chandelier afferents innervating pyramidal cells may be the subpopulation of GABAergic interneurons specifically affected in schizophrenia.

Another informative presynaptic marker for GABAergic neurons is the GABA transporter, GAT 1. Early studies reported significant reductions in GAT 1 expression in the prefrontal cortex, amygdala and hippocampus as assessed by ligand binding [18,19]. A later study did not confirm such abnormalities in the prefrontal and temporal cortex [20]. In an in situ hybridization study, Volk et al. observed that GAT 1 expression was decreased below the level of detectability in a subpopulation of GABAergic interneurons in the intermediate layers of the dorsolateral prefrontal cortex [21]. Exploiting GAT 1 immunohistochemistry, a marked reduction was observed in immunoreactive GABAergic terminal boutons innervating pyramidal cell proximal axons, which represent the chandelier neuron axon ‘‘cartridge’’ [22,23]. Taken together, the results with parvalbumin, GAD 67 and GAT 1 point to a rather selective defect in the chandelier cells innervating the mid-layer pyramidal neurons in prefrontal cortex.

Decreased expression of GAT 1 and decreased expression of GAD 67 would appear to have opposing effects with the former enhancing and the latter attenuating GABAergic neurotransmission. For this reason, studies of the GABA A receptor could be informative in resolving this contradiction. Early studies by Hanada et al. [24] revealed a 40% increase in the B_{max} but no change in affinity in the specific binding of [^{3}H] muscimol in the prefrontal cortex of patients with schizophrenia. Subsequent studies demonstrated significant increases in [^{3}H] muscimol binding to the GABA A receptor in subfields of the hippocampal formation, the anterior cingulate cortex and the prefrontal cortex [25–27]. Interestingly, benzodiazepine binding exhibited only modest elevation in the CA1 and in the subiculum in schizophrenia [28]. Higher resolution analysis indicated that the increase in a receptor

binding was concentrated in the intermediate layers of the prefrontal cortex, restricted to the pyramidal cells. A similar analysis in the hippocampal formation indicated a preferential increase of GABA A receptors on pyramidal cells in CA1 whereas the CA3 receptor increase appeared to be localized to interneurons. Consistent with the selective vulnerability of chandelier cells, Volk et al. [23] reported a 100% increase of immunoreactive alpha 2 subunit of the GABA A receptor on the pyramidal neuron initial axon segments. Impagnatiello et al. [29] also found increased alpha 1 and alpha 5 subunits of the GABA A receptor in prefrontal cortex. Thus, taken together the alterations in pre- and post-synaptic expression of GABAergic markers strongly support the notion of a net hypofunction of subpopulations of GABAergic intemeurons in prefrontal cortex and in the hippocampal formation.

Mechanisms that may account for these localized GABAergic deficits remain unclear. Akbarian et al. [30] carried out a study on a small number of subjects with schizophrenia and controls examining the distribution of NADPH diaphorase positive neurons in prefrontal cortex. NADPH diaphorase expression is co-localized to a subpopulation of GABAergic intemeurons. A significant difference in the distribution of these neurons were noted between the schizophrenic subjects and controls with a relative enrichment in the deep layers and a relative paucity in the outer layers of the cerebral cortex in the schizophrenic subjects, consistent with impaired migration in schizophrenia. Notably, migration of these interneurons occurs during second trimester, a period associated with increased risk for schizophrenia in association with maternal influenza, infection or famine [3]. However, a subsequent study suggested that this specific migratory defect may occur only in a distinct subpopulation of schizophrenics [12].

A second line of evidence supporting a developmental defect is the observed abnormalities in reelin expression in schizophrenia. Reelin is a high affinity ligand for integren receptors. In frontal cortex, reelin is synthesized by virtually every GABAergic neuron where it is secreted into the extracellular matrix and binds to dendritic spines of pyramidal neurons [31]. Reelin has also been implicated in neuronal migration because of its expression in Retzius cells in fetal cortex. Several post-mortem studies indicate a highly significant reduction in reelin protein and mRNA in the prefrontal cortex, especially in the outer layers, temporal cortex, hippocampus, caudate nuclei and cerebellum [15,29,32]. Reelin expression is also reduced in the dendate gyrus in schizophrenia [33]. Fatemi et al. [32] found significant reduction in reelin positive adjusted cell densities in the dentate molecular layer, total hippocampal area and C4 area of schizophrenics as compared to controls. Eastwood and Harrison [34] found an increased density of interstitial white matter neurons that express less reelin in schizophrenic subjects than controls. Fatemi et al. [35] reported altered levels of isoforms of reelin in the blood obtained from patients suffering from schizophrenia although bipolar and depressed patients exhibited similar but less robust alterations. Given the important role of reelin in the laminar formation of the cortex and in synaptogenesis, along with its relatively restricted expression to GABAergic interneurons, it is a plausible risk gene that could account for the cortical volumetric reductions in schizophrenia and the selective abnormalities in GABAergic neuronal function in the prefrontal cortex and hippocampal formation. However, an association analysis of the polymorphic CGG repeat in the 5′ untranslated region of reelin did not reveal a significant linkage to schizophrenia [36].

Thus, studies from several different research groups using a variety of techniques and brain material from diverse sources have revealed fairly replicable findings consistent with hypofunction and reduced gene expression of proteins associated with a subpopulation of GABAergic interneurons in the prefrontal cortex and in the temporal lobe. However, genetic studies to date have not pointed to potential risk genes that would be specifically associated with GABAergic neuronal development or function. Furthermore, pharmacologic studies directed at enhancing GABAergic function, primarily through the use of benzodiazepines, have not yielded convincing results of specific effects in schizophrenia [37].

3. NMDA receptor hypofunction in schizophrenia

The potential involvement of impaired glutamatergic neurotransmission in the pathophysiology of schizophrenia was initially based on the clinical observations of the psychotic effects of dissociative anaesthetics, first reported over 40 years ago [38]. The demonstration that these drugs act as use dependent non-competitive inhibitors of NMDA receptors at the concentration associated with their psychotomimetic properties pointed to inhibition of NMDA receptors as a potential pathologic mechanism [39]. The growing appreciation of the importance of negative symptoms and cognitive impairments as critical components of the endophenotype of schizophrenia prompted a more careful analysis of the psychologic effects of ketamine, another NMDA receptor antagonist. Nonnal controls in an experimental setting administered intravenously low doses of ketamine that does not cause impairments in consciousness, developed negative symptoms (blunted affect, withdrawal) and the rather selective impairments in memory and cognition that are specifically associated with schizophrenia [40,41]. Positive symptoms with auditory hallucinations and fully formed delusions do not typically occur with acute administration of ketamine to normal subjects but are seen in neuroleptic free schizophrenics [42]. In addition, low dose ketamine produces several physiologic abnormalities associated with schizophrenia including abnormal eye tracking [43], enhanced subcortical dopamine release ([44]; but see [45]), impaired prepulse

inhibition in experimental animals [46], hypofrontality and abnormal cortical event related potentials [47].

The neuroanatomic organization and functional features of the forebrain glutamatergic systems implicate them in pathophysiology of schizophrenia. Specifically, glutamatergic pyramidal cells are projecting systems that interconnect prefrontal cortex, temporal cortex/hippocampus and thalamus, regions of the brain which structural and functional brain imaging have demonstrated to exhibit abnormalities in schizophrenia. Secondly, multiple roles of the NMDA receptor in regulating neuronal migration, neuronal differentiation, response to trophic factors, functional plasticity such as long term potentiation and long term depression and finally the elaboration of synaptic spines are consistent with the multiple functional and structural abnormalities documented in schizophrenia.

Post-mortem studies combined with genetic findings have generated compelling evidence that disruption in the modulation of subtypes of NMDA receptors contribute to the psychopathology of schizophrenia [48]. The NMDA receptor has a second site to which D-serine or glycine bind that must be occupied in order for glutamate to gate the NMDA ion channel [49]. D-Serine is a full agonist at this "glycine B receptor" on the NMDA receptor. Tissue levels of D-serine are determined by the catabolic enzyme D-amino acid oxidase (DAAO) since the levels of the former are inversely correlated with the activity of the latter [50]. A mutation in a protein designated G72, which results in robust activation of DAAO, has been described in three genetic studies of schizophrenia [51–53]. Single nucleotide polymorphisms (SNPs) of DAAO have been associated with increased risk for schizophrenia [51]. Furthermore, the levels of D-serine in plasma, which appears to come primarily from the CNS, are decreased in schizophrenic subjects [54].

Glutamate carboxy peptidase II (CGP II) degrades the neuropeptide, *N*-acetylaspartyl glutamate (NAAG), which is co-localized and released by glutamatergic neurons as well as other neuronal systems (cholinergic motor neurons, noradrenergic locus coeruleus neurons) [55]. NAAG is an agonist at the mGluR 3 receptor, which reduces glutamate release, and a glycine reversible antagonist at hippocampal NMDA receptors [56]. Post-mortem studies with cohorts from three different brain banks have documented significant reductions in its enzyme activity and in its mRNA levels in prefrontal cortex and temporal cortex/hippocampus [57,58]. A translocation associated with the risk for schizophrenia affects the locus on chromosome 11q13 in close proximity to the gene encoding GCP II [59]. Kynurenic acid is another endogenous antagonist at the glycine B receptor on the NMDA receptor. The levels of kynurenic acid are significantly elevated in prefrontal cortex but not motor cortex and in the cerebral spinal fluid in schizophrenic patients as compared too controls, an alteration which appears to be unrelated to antipsychotic treatment [60,61]. Tryptophan-2,3-dioxygenase, an upstream enzyme in kynurenic acid synthesis, is also up-regulated in the cortex in schizophrenic patients [62]. Individually and together these alterations would non-competitively attenuate NMDA receptor function.

NMDA receptors are anchored in the post-synaptic density (PSD) in a protein complex with which over four score other proteins have been associated. Post-mortem studies have examined the expression of the subunits of the NMDA receptor as well as components of the PSD. In the thalamus, the NR1 and NR2 B subunits were decreased in schizophrenia and PSD 95, SAP 102 and NF-L were also significantly reduced although the latter was also affected in bipolar disorder [63]. Other studies have shown significant reductions in the expression of SAP 97, SAP 102 and PSD 95 in prefrontal cortex in schizophrenia [64]. Decreased phosphorylation of NR1 at serine 97, which impairs NMDA receptor function, has been found in prefrontal cortex in two cohorts of schizophrenic subjects [65]. That the post-synaptic density complex may be an important target for the pathophysiology of schizophrenia is reinforced by the highly significant association of SNPs for distobrevin and dysbinden, two proteins in the PSD complex, with the risk for schizophrenia [6]. Notably, dysbinden expression is reduced in prefrontal cortex [66].

Other components of the glutamatergic signaling system are also affected. For example, the glutamate transporters excitatory amino acid transporter (EAAT1 and 2) are elevated in the thalamus, which should further compromise glutamatergic neurotransmission [63]. Consistent reductions in the kainic acid receptor have been documented by ligand binding, in situ hybridization of its subunits and DNA microarray studies in prefrontal cortex and hippocampus [67]. SNPs for the mGluR 3 receptor gene, which down-regulates glutamate release, have been linked to the risk for schizophrenia in three different studies although the effects on its expression or function remain unclear at present [6].

Finally, neuregulins are cell–cell signaling proteins that are ligands for the tyrosine kinase receptor, ErB. There are at least three separate studies of associated allelic variants of neuregulin 1 gene and the risk for schizophrenia [68]. Neuregulin has complex effects on glial differentiation, astrocyte function and synapse stabilization. Post-mortem studies have revealed relative reductions in neuregulin isoforms II and III expression in cortex in schizophrenia [69]. Mice homozygous for a null mutation of neuregulin or its ErB 4 receptor display hyperactivity that is responsive to clozapine and exhibit reduced numbers of NMDA receptors [68].

4. Effects of agonists at the glycine B receptor in schizophrenia

A compelling line of evidence supporting the hypothesized hypofunction of NMDA receptors in schizophrenia comes from clinical trials demonstrating the therapeutic effectiveness of agents that enhance NMDA receptor

function, particularly with regard to the endophenotypic symptoms (for review, [48]). These interventions have focused on agents that would activate the glycine B receptor on the NMDA receptor, thereby avoiding the potential excitotoxic effects of direct agonists at the glutamate recognition site. Preclinical studies have demonstrated that such agents can reverse the behavioral effects of MK801 and have cognitive enhancing effects. In these studies, the agents were used as ''add ons'' in a placebo control design to existing antipsychotic treatments, primarily typical antipsychotics in patients with chornic schizophrenia and prominent negative symptoms.

One of the first agents examined, D-cycloserine, is a partial agonist at the glycine B receptor with approximately 50% efficacy [70]. The initial dose finding study revealed a U-shaped dose response curve for D-cycloserine with significant reductions in negative symptoms and significant improvement on a cognitive task that engages the prefrontal cortex. Subsequent placebo control studies confirmed the effects on negative symptoms although in no case were positive symptoms affected [71,72]. Trials with glycine, with doses ranging from 30 to 60 g per day, also revealed significant reductions in negative symptoms and improvement in cognitive functions without effect on positive symptoms [73–75]. A trial with the full agonist, D-serine at 2 g per day demonstrated improvement not only in negative symptoms and cognition but also in positive symptoms [76]. This impact on positive symptoms in contrast to D-cycloserine and glycine may reflect better brain access and greater efficacy of D-serine at the glycine B receptor. Finally, sarcosine, an endogenous antagonist at GlyT 1 at doses of 2 g per day, was also reported to reduce negative symptoms, improve cognitive and reduce positive symptoms in patients suffering from chronic schizophrenia receiving neuroleptic treatment [77].

Clozapine, the first atypical antipsychotic, has some unusual properties in terms of being particularly efficacious in a subgroup of patients who respond poorly to typical antipsychotics and also reduces substance abuse and suicidality in schizophrenic patients. Trials with the full agonists, glycine and D-serine added to clozapine, revealed that they had no additional effects on negative symptoms or cognition in contrast to their effects on these symptoms in patients receiving typical antipsychotics [78–80]. Furthermore, the partial agonist D-cycloserine in two trials actually exacerbated negative symptoms in clozapine responders [81,82]. The most parsimonious explanation of these effects is that clozapine causes markedly enhanced occupancy of the glycine B receptor, thereby causing the partial agonist D-cycloserine to behave as an antagonist and the full agonists to have no added benefit.

5. GABAergic–glutamatergic interactions

This review of post-mortem studies, genetic studies and pharmacologic trials provides convincing evidence of hypofunction of a subset of GABAergic intemeurons in the frontal cortex and temporal lobe, on the one hand, and hypofunction of NMDA receptors in these same regions, on the other hand, in schizophrenia. Are these two separate and distinct pathologic mechanisms or can they be related to each other in some meaningful fashion? A review of the evidence from genetic studies implicating neuregulin, disbindin, and G72 as risk genes suggests that they may be more closely related to NMDA receptor dysfunction than a presynaptic abnormality in GABAergic neurons. In vivo dialysis studies with dissociative anaesthetics point to a disinhibition of frontal cortical glutamatergic neurotransmission with secondary elevation in dopamine release [83]. These findings would suggest a particular vulnerability of GABAergic interneurons to the effects of NMDA receptor antagonists. In electrophysiologic studies of the CA1 region, Grunze et al. [84] found that GABAergic interneurons were 10-fold more sensitive to the canonical NMDA antagonist amino-phosphonovaleric acid and to NAAG than were the pyramidal neurons receiving the same Shaffer collateral input In rodents, the pyramidal neurons in the limbic cortex, especially the retrosplinal cortex, are vulnerable to the cytotoxic effects of the dissociative anaesthetics resulting in marked expression of heat shock proteins. Notably, these effects can be reversed by GABA A receptor agonists [85]. Exploring the GABAergic basis of this vulnerability, Li et al. [86] demonstrated in acute slices prepared from the rat limbic cortex that the NMDA receptors on the GABAergic neurons were disproportionately more sensitive to the antagonistic effects of MK 801 as compared to the NMDA receptors on the pyramidal neurons. Moreover, these effects appeared to be restricted to limbic GABAergic interneurons.

It must be emphasized that the doses of dissociative anaesthetics such as ketamine that reproduce syndromic features of schizophrenia do so without causing gross disturbance of consciousness or significant effects on the Mini Mental Status Exam, an instrument for detecting delirium or dementia [40]. This feature indicates that the effects of the dissociative anaesthetics must be restricted to a class of neurons with a distinctly more sensitive subpopulation of NMDA receptors. Based on the findings from the electrophysiologic and in vivo dialysis studies, it is not unreasonable to speculate that the NMDA receptors on frontal cortical and limbic GABAergic interneurons are most sensitive to these antagonists and therefore may be an important site of pathology resulting in NMDA receptor dysfunction. Thus, the down-regulation of presynaptic markers for the GABAergic interneurons including GAD67, GAT and parvalbumin may reflect a chronic loss of the trophic influences of NMDA receptor activation. In support of this hypothesis, Paulson et al. [87] recently reported that chronic treatment with MK 801 resulted in down-regulation in the expression of GAD and GAT 1 in the frontal cortex of rats.

Computational models indicate that the loss of the NMDA receptor component of the EPSC on hippocampal GABAergic neurons disrupts memory and cognitive processing in a fashion analogous to that in schizophrenia [83]. A functional imaging study of the hippocampus in a region specific memory task lends support for this model. Schizophrenic subjects as compared to controls performed the task poorly and did not increase hippocampal blood flow. But, their baseline blood flow was as high as that of the controls when they were performing the task, consistent with a persistent mild disinhibition of hippocampal neurons in the schizophrenic subjects [88]. Given this evidence of GABAergic hypofunction in schizophrenia, it is surprising that drugs increase GABA A receptor function, such as benzodiazepines have negligible therapeutic effects in schizophrenia. Perhaps their relative non-specificity produces sedation that adumbrates any cognitive enhancing effects.

A novel pharmacologic strategy to enhance phasic GABAergic activity would be the use of drugs that increase glycine B receptor agonist occupancy at the GABAergic NMDA receptors such as a full agonist like D-serine, inhibitors of GlyT 1 or inhibitors of D-AAO. Such interventions could have particular therapeutic effect on the cognitive impairments and negative symptoms, the most disabling components of schizophrenia that are relatively insensitive to existing antipsychotics. The results from preliminary clinical trials with four agents that directly or indirectly enhance the function of glycine B provide convincing evidence of the efficacy of this approach.

Acknowledgments

Some of the research described in this article was supported by a National Institute of Mental Health Conte Center on the Neurobiology of Schizophrenia (MH60450) and MH-572901 and grants from the Stanley Foundation and the National Alliance for Research on Schizophrenia and Affective Disorders (NARSAD) to Joseph T. Coyle.

References

[1] Snyder SH. Dopamine receptors, neuuroleptics, and schizophrenia. Am J Psychiatr 1981;138:460–4.
[2] Carpenter Jr WT, Heinrichs DW, Wagman AM. Deficit and nondeficit forms of schizophrenia: the concept. Am J Psychiatr 1988;145: 578–83.
[3] Cannon TD, van Erp TG, Bearden CE, Loewy R, Thompson P, Toga AW, et al. Early and late neurodevelopmental influences in the prodrome to schizophrenia: contributions of genes, environment, and their interactions. Schizophr Bull 2003;29:653–69.
[4] Meltzer HY, Deutch AY. Neurochemistry of schizophrenia. In: Siegel GJ, editor. Basic neurochemistry: molecular, cellular and medical aspects, 6th ed., Philadelphia: Lippicott-Rowen; 1998. p. 1053–72.
[5] Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. 2001;25:1–27.
[6] Harrison PJ, Owen MJ. Genes for schizophrenia? Recent findings and their pathophysiological implications Lancet 2003;61:417–9.
[7] Bird ED, Spokes EG, Barnes J, Mackay AV, Iversen LL, Shepherd M. Glutamic-acid decarboxylase in schizophrenia. Lancet 1978;1:156.
[8] Spokes EG, Garrett NJ, Iversen LL. Differential effects of agonal status on measurements of GABA and glutamate decarboxylase in human post-mortem brain tissue from control and Huntington's chorea subjects. J Neurochem 1979;33:773–8.
[9] Cross AJ, Crow TJ, Woen F. Gamma-aminobutyric acid in the brain in schizophrenia. Lancet 1979;1:560–1.
[10] Lewis DA, Volk DW, Hashimoto T. Selective alterations in prefrontal cortical GABA neurotransmission in schizophrenia: a novel target for the treatment of working memory dysfunction. Psychopharmacology 2003;174:143–50.
[11] Todtenkopf MS, Benes FM. Distribution of glutamate decarboxylase65 immunoreactive puncta on pyramidal and nonpyramidal neurons in hippocampus of schizophrenic brain. Synapse 1998;29:323–32.
[12] Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney Jr WE, et al. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatr 1995;52:258–66.
[13] Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatr 2000;57:237–45.
[14] Mimics K, Middleton FA, Marquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. Neuron 2000;28:53–67.
[15] Guidotti A, Auta J, Davis JM, Di-Giorgi-Gerevini V, Dwivedi Y, Grayson DR, et al. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch Gen Psychiatr 2000;57:1061–9.
[16] Vawter MP, Crook JM, Hyde TM, Kleinman JE, Weinberger DR, Becker KG, et al. Microarray analysis of gene expression in the prefrontal cortex in schizophrenia: a preliminary study. Schizophr Res 2002;58:11–20.
[17] Hashimoto T, Volk DW, Eggan SM, Mimics K, Pierri JN, Sun Z, et al. Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. J Neurosci 2003;16:6315–26.
[18] Reynolds GP, Czudek C, Andrews HB. Deficit and hemispheric asymmetry of GABA uptake sites in the hippocampus in schizophrenia. Biol Psychiatr 1990;27:1038–44.
[19] Simpson MD, Slater P, Deakin JF, Royston MC, Skan WJ. Reduced GABA uptake sites in the temporal lobe in schizophrenia. Neurosci Lett 1989;107:211–5.
[20] Simpson MD, Slater P, Deakin JF. Comparison of gl7utamate and gamma-aminobutyric acid uptake binding sites in frontal and temporal lobes in schizophrenia. Biol Psychiatr 1998;44:423–7.
[21] Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. GABA transporter-1 mRNA in prefrontal cortex in schizophrenia: decreased expression in a subset of neurons. Am J Psychiatr 2001;158: 256–65.
[22] Woo TU, Whitehead RE, Melchitzky DS, Lewis DA. A subclass of prefrontal gama-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci USA 1998;28:5341–6.
[23] Volk DW, Pierri JN, Fritschy JM, Auh S, Sampson AR, Lewis DA. Reciprocal alterations in pre- and postsynaptic inhibitory markers at chandelier cells inputs to pyramidal neurons in schizophrenia. Cereb Cortex 2002;12:1063–70.
[24] Hanada S, Mita T, Nishino N, Tanaka C. [^{3}H]Muscimol binding sites increased in autopsied brains of chronic schizophrenics. Life Sci 1987;40:259–66.
[25] Benes FM, Vincent SL, Marie A, Khan Y. Up-regulation of GABAA receptor binding on neurons of the prefrontal cortex in schizophrenic subjects. Neuroscience 1996;75:1021–31.

[26] Benes FM, Khan Y, Vincent SL, Wickramasinghe R. Differences in the subregional and cellular distribution of GABAA receptor binding in the hippocampal formation of schizophrenic brain. Synapse 1996;22: 338–439.
[27] Dean B, Hussain T, Hayes W, Scarr E, Kitsoulis S, Hill C, et al. Changes in serotonin2A and GABA(A) receptors in schizophrenia: studies on the human dorsolateral prefrontal cortex. J Neurochem 1999;72:1593–9.
[28] Benes FM, Wickramasinghe R, Vincent SL, Khan Y, Todtenkopf M. Uncoupling of GABA(A) and benzodiazepine receptor binding activity in the hippocampal formation of schizophrenic brain. Brain Res 1997;755:121–9.
[29] Impagnatiello F, Guidotti AR, Pesold C, Dwivedi Y, Canmcho H, Pisu MG, et al. A decrease of reeling expression as a putative vulnerability factor in schizophrenia. Proc Natl Acad Sci 1998;95:15718–23.
[30] Akbarian S, Vinuela A, Kim JJ, Potkin SG, Bunney Jr WE, Jones EG. Distorted distribution of nicotinamide–adenine dinucleotide phosphate–diaphorase neurons in temporal lobe of schizophrenics implies anomalous cortical development. Arch Gen Psychiatr 1993;50:178–87.
[31] Rodriguez MA, Caruncho HJ, Costa E, Pesold C, Liu WS, Guidotti A. In Patas monkey, glutamic acid decarobxylase-67 and reelin mRNA coexpression varies in a manner dependent on layers and cortical areas. J Comp Neurol 2002;451:279–88.
[32] Fatemi SH, Earle JA, McMenomy T. Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. Mol Psychiatr 2000;5:654–63.
[33] Knable MB, Bard BM, Webster MJ, Meador-woodruff J, Torrey EF. Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley neuropathology Consortium. Mol Psychiatr 2004;9:609–20.
[34] Eastsood SL, Harrison PJ. Interstitial white matter neurons express less reelin and are abnormally distributed in schizophrenia: towards an integration of molecular and morphologic aspects of the neurodevelopmental hypothesis. Mol Psychiatr 2003;8:821–31.
[35] Fatemi SH, Kroll JL, Stary JM. Altered levels of Reelin and its isoforms in schizophrenia and mood disorders. Neuroreport 2001;12:3209–15.
[36] Akahane A, Kunugi H, Tanaka H, Nanko S. Association analysis of polymorphic CGG repeat in 5′ UT‰ of the reelin and VLDLR genes with schizophrenia. Schizophr Res 2002;58:37–41.
[37] Wolkowitz OM, Pickar D. Benzodiazepines in the treatment of schizophrenia: a review and reappraisal. Am J Psychiatr 1991; 148:714–26.
[38] Luby ED, Cohen BD, Rosenbaum G, Gottlieb JS, Kelley R. Study of a new schizophrenomimetic drug; sernyl. AMA Arch Neurol Psychiatr 1959;81:363–9.
[39] Javitt DC, Zukin SR. Recent advances in the phencyclidine model of schizophrenia. Am J Psychiatr 1991;148:1301–8.
[40] Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Arch Gen Psychiatr 1994;51:199–214.
[41] Newcomer JW, Farber NB, Jevtovic-Todorovic V, Selke G, Melson AK, Hershey T, et al. Ketamine-induced NMDA receptor hypofunction as a model of memory impairment and psychosis. Neuropsychopharmacology 1999;20:106–18.
[42] Malhortra AK, Adler CM, Kennison SD, Elman I, Pickar D, Breier A. Clozapine blunts *N*-methyl-D-aspartate antagonist-induced psychosis: a study with ketamine. Biol Psychiatr 1997;42:664–8.
[43] Avila MT, Weiler MA, Lahti AC, Tamminga CA, Thaker GK. Effects of ketamine on leading saccades during smooth-pursuit eye movements may implicate cerebellar dysfunction in schizophrenia. Am J Psychiatr 2002;159:1490–6.
[44] Vollenweider FX, Vontobel P, Oye I, Hell D, Leenders KL. Effects of 9S-ketamine on striatal dopamine: a [11C] raclopride PET study of a model psychosis in humans. J Psychiatr Res 2000;34:35–43.
[45] Kegeles LS, Martinez D, Kochan LD, Hwang DR, Huang Y, Mawlawi O, et al. NMDA antagonist effects on striatal dopamine release: positron emission tomography studies in humans. Synapse 2002;43:19–29.
[46] Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychophannacology 2001;156:234–58.
[47] Umbricht D, Roller R, Vollenweider FX, Schmid L. Mismatch negativity predicts psychotic experiences induced by NMDA receptor antagonist in healthy volunteers. Biol Psychiatr 2002;1:400–6.
[48] Coyle JT, Tsai G. The NMDA receptor glycine modulatory site: a therapeutic target for improving cognition and reducing negative symptoms in schizophrenia. Psychopharmacology 2004;174:32–8.
[49] Bergeron R, Meyer TM, Coyle JT, Greene RW. Modulation of *N*-methyl-D-aspartate receptor function by glycine transport. Proc Natl Acad Sci USA 1998;95:15730–4.
[50] Mothet JP, Parent AT, Wolosker H, Brady Jr RO, Linden DJ, Ferris CD, et al. D-Serine is an endogenous ligand for the glycine site of the *N*-methyl-D-aspartate receptor. Proc Natl Acad Sci USA 2000;97: 4926–31.
[51] Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H, et al. Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. Proc Natl Acad Sci USA 2002;99:13675–80.
[52] Wang X, He G, Gu N, Yang J, Tang J, Chen Q, et al. Association of G72/G30 with schizophrenia in the Chinese population. Biochem Biophys Res Commun 2004;319:1281–6.
[53] Hall D, Gogos JA, Karayiorgou M. The contribution of three strong candidate schizophrenia susceptibility genes in demographically distinct populations. Genes Brain Behav 2004;4:240–8.
[54] Hashimoto K, Fukushima T, Shimizu E, Komatsu N, Watanabe H, Shinoda N, et al. Decreased serum levels of D-serine in patients with schizophrenia: evidence in support of the *N*-methyl-D-aspartate receptor hypofunction hypothesis of schizophrenia. Arch Gen Psychiatr 2003;60:572–6.
[55] Coyle JT. The nagging question of the function of *N*-acetylaspartylglutamate. Neurobiol Dis 1997;4:231–8.
[56] Bergeron R, Coyle JT, Tsai G, Greene RW. NAAG reduces NMDA receptor current in CA1 hippocampel pyramidal neurons of acute slices and dissociated neurons. Neuropsychopharmacology, in press.
[57] Tsai G, Passani LA, Slusher BS, Carter R, Baer L, Kleinman JE, et al. Abnormal excitatory neurotransmitter metabolism in schizophrenic brains. Arch Gen Psychiatr 1995;52:829–36.
[58] Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci USA 2001;98:4746–51.
[59] Semple CA, Devon RS, Le Hellard S, Porteous DJ. Identification of genes from a schizophrenia-linked translocation breakpoint region. Genomics 2001;73:123–6.
[60] Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. Biol Psychiatr 2001;50:521–30.
[61] Erhardt S, Blennow K, Nordin C, Skogh E, Lindstrom LH, Engberg G. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neurosci Lett 2001;313:96–8.
[62] Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. Neurobiol Dis 2004;15:618–29.
[63] Meador-Woodruff JH, Clinton SM, Beneyto M, McCullumsmith RE. Molecular abnormalities of the glutamate synapse in the thalamus in schizophrenia. Ann NY Acad Sci 2003;1003:75–93.
[64] Toyooka K, Iritani S, Makifuchi T, Shirakawa O, Kitamura N, Maeda K, et al. Selective reduction of a PDZ protein, SAP-97, in the prefrontal cortex of patients with chronic schizophrenia. J Neuroochem 2002;83:797–806.
[65] Emamian ES, Karayiorgou M, Gogos JA. Decreased phosphorylation of NMDA receptor type 1 at serine 897 in brains of patients with Schizophrenia. J Neurosci 2004;24:1561–4.

[66] Weickert CS, Straub RE, McClintock BW, Matsumoto M, Hashimoto R, Hyde TM, et al. Human dysbindin (DTNBPl)gene expression in noimal brain and in schizophrenic prefrontal cortex and midbrain. Arch Gen Psychiatr 2004;61:544–55.

[67] Harrison PJ, Law AJ, Eastwood SL. Glutamate receptors and transporters in the hippocampus in schizophrenia. Ann NY Acad Sci 2003;1003:94–101.

[68] Stefansson H, Steinthorsdottir V, Thorgeirsson TE, Gulcher JR, Stefansson K. Neuregulin 1 and schizophrenia. Ann Med 2004;36: 62–71.

[69] Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol Psychiatr 2004;9:299–307.

[70] Goff DC, Tsai G, Manoach DS, Coyle JT. Dose-finding trial of D-cyctoserine added to neuroleptics for negative symptoms in schizophrenia. Am J Psychiatr 1995;152:1213–5.

[71] Goff DC, Tsaig G, Levitt J, Amico E, Manoach D, Schoenfeld DA, et al. A placebo-controlled trial of D-cycloserine added to conventional neuroleptics in patients with schizophrenia. Arch Gen Psychiatr 1999;56:21–7.

[72] Heresco-Levy U, Ermilov M, Shimoni J, Shapira B, Silipo G, Javitt DC. Placebo-controlled trial of D-cycloserine added to conventional neuroleptics, olanzapine, or risperidone in schizophrenia. Am J Psychiatr 2002;159:480–2.

[73] Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Horowitz A, Kelly D. Double-blind, placebo-controlled, crossover trial of glycine adjuvant therapy for treatment-resistant schizophrenia. Br J Psychiatr 1996;169:610–7.

[74] Javitt DC, Silipo G, Cienfuegos A, Shelley AM, Bark N, Park M, et al. Adjunctive high-dose glycine in the treatment of schizophrenia. Int J Neuropsychopharmacol 2001;4:385–91.

[75] Heresco-Levy U, Javitt DC, Ermilov M, Mordel C, Silipo G, Lichtenstein M. Efficacy of high-dose glycine in the treatment of enduring negative symptoms of schizophrenia. Arch Gen Psychiatr 1999;56:29–36.

[76] Tsai G, Yang P, Chung LC, Lange N, Coyle JT. D-Serine added to antipsychotics for the treatment of schizophrenia. Biol Psychiatr 1998;44:1081–9.

[77] Tsai G, Lane HY, Yang P, Chong MY, Lange N. Glycine transporter I inhibitor, *N*-methylglycine (sarcosine), added to antipsychotics for the treatment of schizophrenia. Biol Psychiatr 2004;5:452–6.

[78] Evins AE, Fitzgerald SM, Wine L, Rosselli R, Goff DC. Placebo-controlled trial of glycine added to clozapine in schizophrenia. Am J Psychiatr 2000;157:826–8.

[79] Tsai GE, Yang P, Chung LC, Tsai IC, Tsai CW, Coyle JT. D-Serine added to clozapine for the treatment of schizophrenia. Am J Psychiatr 1999;156:1822–5.

[80] Potkin SG, Jin Y, Bunney BG, Costa J, Gulasekaram B. Effect of clozapine and adjuynctive high-dose glycine in treatment-resistant schizophrenia. Am J Psychiatr 1999;156:145–7.

[81] Goff DC, Tsai G, Manoach DS, Hood J, Darby DG, Coyle JT. D-Cycloserine added to clozapine for patients with schizophrenia. Am J Psychiatr 1996;153:1628–30.

[82] Goff DC, Henderson DC, Evins AE, Amico E. A placebo-controlled crossover trial of D-cycloserine added to clozapine in patients with schizophrenia. Biol Psychiatr 1999;45:512–4.

[83] Moghaddam B, Jackson ME. Glutamatergic animal models of schizophrenia. Ann NY Acad Sci 2003;1003:131–7.

[84] Grunze HG, Rainnie DG, Hasselmo ME, Barkai E, Hearn EF, McCarley RW, et al. NMDA-dependent modulation of CAl local circuit inhibition. J Neurosci 1996;16:2034–43.

[85] Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA. NMDA antagonist neurotoxicity: mechanism and prevention. Science 1991;254:1515–8.

[86] Li Q, Clark S, Lewis DV, Wilson WA. NMDA receptor antagonists disinhibit rat posterior cingulated and retrosplenial cortices: a potential mechanism of neurotoxicity. J Neurosci 2002;22:3070–80.

[87] Paulson L, Martin P, Persson A, Nilsson CL, Ljung E, Westman-Brinkmalm A, et al. Comparative genome- and proteome analysis of cerebral cortex from MK-801-rreated rats. J Neurosci Res 2003;71:526–33.

[88] Heckers S, Rauch SL, Goff D, Savage CR, Schacter DL, Fischman AJ, et al. Impaired recruitment of the hippocampus during conscious recollection in schizophrenia. Nat Neurosci 1998;4:318–23.