The neuronal pathology of schizophrenia: molecules and mechanisms

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Abstract
There is an accumulation of evidence for abnormalities in schizophrenia of both the major neurotransmitter systems of the brain – those of GABA (γ-aminobutyric acid) and glutamate. Initial studies have found deficits in the putative neuronal marker, N-acetylaspartate, in a number of brain regions in schizophrenia. The animal models have provided some interesting correlates and discrepancies with these findings. The deficit in inhibitory interneurons within structures implicated in schizophrenic symptomatology may well have direct functional relevance, and can be induced by animal models of the disease such as subchronic phencyclidine administration or social isolation. Their association with these animal models suggests an environmental involvement. A loss of glutamatergic function in schizophrenia is supported by decreases in markers for the neuronal glutamate transporter in striatal structures that receive cortical glutamatergic projections. Deficits in the VGluT1 (vesicular glutamate transporter-1) in both striatal and hippocampal regions support this observation, and the association of VGluT1 density with a genetic risk factor for schizophrenia points to genetic influences on these glutamatergic deficits. Further studies differentiating neuronal loss from diminished activity and improved models allowing us to determine the temporal and causal relationships between GABAergic and glutamatergic deficits will lead to a better understanding of the processes underlying the neuronal pathology of schizophrenia.

Introduction
From the middle of the last century, research into the brain in schizophrenia has attempted to implicate in the disorder many different neurochemicals, particularly neurotransmitters and other compounds with direct effects on neuronal activity. Few of these theories have lasted long, although for over 30 years the dopamine hypothesis of schizophrenia has been an important stimulus to research. This was first based on the ability of amphetamine and dopamine agonists to induce a schizophreniaiform psychosis, and was strengthened by the finding that almost all antipsychotic drugs are effective antagonists of the dopamine D2 receptor subtype. Subsequently, the higher density of dopamine D2 receptors found in post-mortem brain from schizophrenic patients led to the formulation of a modified dopamine hypothesis in which elevated D2 receptors were proposed to underlie the positive symptoms of schizophrenia. However, an up-regulation of D2 receptors is seen in animals after chronic administration or social isolation. Their association with these animal models suggests an environmental involvement. A loss of glutamatergic function in schizophrenia is supported by decreases in markers for the neuronal glutamate transporter in striatal structures that receive cortical glutamatergic projections. Deficits in the VGluT1 (vesicular glutamate transporter-1) in both striatal and hippocampal regions support this observation, and the association of VGluT1 density with a genetic risk factor for schizophrenia points to genetic influences on these glutamatergic deficits. Further studies differentiating neuronal loss from diminished activity and improved models allowing us to determine the temporal and causal relationships between GABAergic and glutamatergic deficits will lead to a better understanding of the processes underlying the neuronal pathology of schizophrenia.

Key words: γ-aminobutyric acid (GABA), glutamate, isolation rearing, N-acetylaspartate, phencyclidine, schizophrenia.
Abbreviations used: CB, calbindin; CBP, calcium-binding protein; CR, calretinin; GABA, γ-aminobutyric acid; ANS, magnetic resonance spectroscopy; NAA, N-acetylaspartate; NAAAG, N-acetylaspartateglutamate; NAADE, N-methyl-D-aspartate; PEP, phencyclidine; PET, positron emission tomography; PV, parvalbumin; VGluT1, vesicular glutamate transporter 1.
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GABAergic deficits

Post-mortem studies have provided evidence for abnormalities of the GABAergic system in schizophrenia. Deficits in GABA-containing neurons are consistently reported, particularly in the frontal cortex and hippocampus. Benes et al. [3] found reduced density of interneurons in CA2 and CA3 regions in the hippocampus of brains of schizophrenia patients, including drug-free patients, suggesting that deficits in interneurons may play a contributory role in the pathophysiology of schizophrenia. Consistent with this interpretation, neurochemical studies have demonstrated a deficit of GABAergic uptake sites in the hippocampus [4], while other reports showed a widespread compensatory up-regulation of post-synaptic-specific GABAA receptor-binding activity throughout most subfields of the hippocampal formation of schizophrenia patients [5].

However, our understanding of the GABA system in schizophrenia must take into account the fact that there are several different types of non-pyramidal neuron that utilize GABA as a neurotransmitter, and that each subset of neurons may show a unique pattern of change. The CBPs (calcium-binding proteins) PV (parvalbumin), CB (calbindin) and CR (calretinin) can be used as markers for specific subpopulations of GABAergic interneurons in the brain. It is now generally accepted that the major neuronal role of these CBPs is the buffering and transport of calcium ions and the regulation of various enzyme systems. As cellular degeneration is often accompanied by impaired calcium homeostasis, a protective role for CBPs has been postulated [6].

PV is particularly interesting in that it shows a late onset of expression, occurring after GABAergic neurons have been formed [7], imparting a period during which these cells may be particularly susceptible to neurotoxic insult. We have previously reported deficits of PV-immunoreactive cells both in the frontal cortex [8,9] and, more profoundly, in the hippocampus in schizophrenia [10]. These GABAergic deficits in schizophrenia could well be an initial deficit, perhaps of neurodevelopmental origin, that subsequently results in further progressive neuronal dysfunction and the development of schizophrenia in the second or third decade of life. These GABAergic deficits are unrelated to antipsychotic drug treatment, age or duration of illness [10,11].

Deficits in CB have also been reported in schizophrenia [8,12]. In contrast, most of the studies using human post-mortem tissue have reported that CR immunoreactive neurons are unaffected in schizophrenia [10,12,13].

Although current animal models of schizophrenia are inevitably limited, behavioural and pharmacological studies concentrate on two approaches with substantial validity. These may be pharmacological [subchronic administration of an NMDA (N-methyl-D-aspartate) antagonist such as PCP (phencyclidine)] or non-pharmacological (isolation rearing from weaning) animal models.

PCP has been shown to induce a psychosis in humans that closely resembles schizophrenia in both positive and negative symptoms and also exacerbates the symptoms in stabilized patients [14]. Thus it has been proposed that NMDA receptor hypofunction may be viewed as a model for the disease mechanism, which could explain the symptoms and course of schizophrenia [15]. In reviewing the neuropsychopharmacology of PCP, Jentsch and Roth [16] have concluded that long-term repeated administration of PCP to both humans and animals models the symptoms of schizophrenia better than acute treatment. In animals long-term repeated administration of PCP has been shown to mimic certain aspects of the disorder. Utilizing a subchronic or chronic intermittent treatment regime, our laboratory and others have found that, compared with vehicle-treated controls, animals receiving PCP exhibit reductions in PV-immunoreactive cells in the frontal cortex and hippocampus [17,18].

Isolation rearing of rats is a non-lesion manipulation that leads to deficits in PPI (prepulse inhibition) of the startle reflex and other behavioural and neurochemical alterations reminiscent of schizophrenia [19].

Compared with socially housed rats, isolated rats exhibited reductions in PV- and CB-immunoreactive cells in the hippocampus, with no significant change in CR [20]. These findings demonstrate selective abnormalities of subpopulations of GABAergic interneurons in the hippocampus of isolation reared rats, which resemble the neuronal deficits seen in this region in schizophrenia.

In conclusion, we find a deficit of PV-immunoreactive hippocampal neurons in animals receiving subchronic PCP [18] as well as in those reared in isolation [20]. These findings mimic the pathology of the disease in their specificity; no deficits of CR-containing neurons are observed in these animal models.

NAA (N-acetylaspartate) and glutamatergic deficits

NAA is an amino acid that is present in high concentrations in the CNS (central nervous system), with intraneuronal concentrations between 10 and 14 mM. NAA is synthesized in neuronal mitochondria from acetyl-coA and aspartate by the enzyme NAA transferase. In the adult rat brain, NAA is found almost exclusively in neurons, being absent from mature glial cells [21]. Although it is 50 years since NAA was first discovered [22] there is still no consensus on its principal metabolic or neurochemical function. It has been shown to be involved in myelin synthesis [23], osmoregulation [24], and a recent study suggests a possible role as an anti-inflammatory [25]. NAA is also a precursor for the biosynthesis of NAAG (N-acetylaspartylglutamate), a neuropeptide with actions at NMDA and metabotropic glutamate receptors [26]. NAA correlates with neuronal density and as such it has been widely used as a neuronal marker, with deficits in NAA thought to reflect neuronal loss. However, previous studies demonstrating the reversibility of NAA following acute brain injury [27,28] indicate that NAA may be a marker of both neuronal loss and cellular dysfunction [29].

The current focus on NAA dysfunction in neuropsychiatric disorders stems from the prominence of the NAA signal in MRS (magnetic resonance spectroscopy). This has led to
a wide number of MRS studies looking at NAA in brain regions in neurodegenerative and psychiatric diseases, notably schizophrenia. Neuronal dysfunction, determined by MRS measurement of NAA deficits \textit{in vivo}, is seen in cortical and medial temporal structures in schizophrenia [30]. We and others have shown that these deficits are unlikely to be due to treatment with antipsychotic medication [31–33] and may in fact be related to the disease process.

In our laboratory we use a sensitive HPLC method to determine levels of NAA in post-mortem tissue. In the first of these studies we demonstrated a significant deficit in NAA in the temporal cortex in schizophrenia, with no change in the frontal cortex [34]. Interestingly, we also find selective deficits in NAA in the temporal cortex both in the PCP animal model [35] and in the isolation rearing model [36]. There was no change in NAA in any other region examined in these models, namely the frontal cortex, striatum or hippocampus (Table 1).

Specific temporal cortex deficits in NAA can be induced by both pharmacological and non-pharmacological effects in animals. This raises the question whether temporal cortical NAA deficits might reflect an environmental aetiology of schizophrenia.

In our recent studies, we have found that in schizophrenia the NAA deficits extend to regions of the medial temporal lobe, the striatum, the hippocampus and amygdala (L.M. Reynolds, personal communication). It is of interest to note that the NAA deficits that we see in the striatum and hippocampus in the human post-mortem tissue are not seen in either of the environmental animal models mentioned above (Table 1). Thus, in contrast with the findings in temporal cortex, this leads us to speculate whether hippocampal (and subcortical) NAA deficits reflect a non-environmental, perhaps genetic, aetiology of schizophrenia.

NAA has been suggested to reflect glutamate concentrations and thus may provide a marker of glutamatergic neuronal function [37]. In support of this are several observations implicating abnormalities of glutamate in the brain in schizophrenia. We have found a deficit in the number of glutamate uptake sites in the caudate nucleus, putamen and nucleus accumbens in schizophrenia [38], which we interpret as a deficit of cortico-striatal glutamatergic innervation. Consistent with this is the increase in the density of NMDA receptors in the putamen from schizophrenic patients when compared with controls [39]. Such increases in glutamate receptors have been interpreted as compensatory and reflect-

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References


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