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Building models for postmortem abnormalities in hippocampus of schizophrenics

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ABSTRACT

Postmortem studies have suggested that there is abnormal GABAergic activity in the hippocampus in schizophrenia (SZ). In micro-dissected human hippocampal slices, a loss of interneurons and a compensatory upregulation of $GABA_A$ receptor binding activity on interneurons, but not PNs, has suggested that disinhibitory GABA-to-GABA connections are abnormal in stratum oriens (SO) of CA3/2, but not CA1, in schizophrenia. Abnormal expression changes in the expression of kainate receptor (KAR) subunits 5, 6 and 7, as well as an inwardly-rectifying hyperpolarization-activated cationic channel (Ih3; HCN3) may play important roles in regulating GABA cell activity at the SO CA3/2 locus. The exclusive neurons at this site are GABAergic interneurons; these cells also receive direct projections from the basolateral amygdala (BLA). When the BLA is stimulated by stereotaxic infusion of picrotoxin in rats, KARs influence axodendritic and presynaptic inhibitory mechanisms that regulate both inhibitory and disinhibitory interneurons in the SO-CA3/2 locus. The rat model described here was specifically developed to extend our understanding of these and other postmortem findings and has suggested that GABAergic abnormalities and possible disturbances in oscillatory rhythms may be related to a dysfunction of disinhibitory interneurons at the SO-CA3/2 site of schizophrenics.

1. Introduction

Over the past 30 years, it has become increasingly clear that GABA neuron dysfunction contributes to the pathophysiology of schizophrenia. Key postmortem findings have included abnormalities related to interneuron numbers (Benes et al., 1991), GABAA receptor binding activity on pyramidal neurons (Benes et al., 1992) and GAD67 expression (Akbarian et al., 1993). Additionally, decreases of interneurons expressing a variety of subtype markers, such as NADPH diaphorase (Akbarian et al., 1993), parvalbumin and calbindin [PVB], markers for GABAergic interneurons (Woo et al., 1997; Reynolds et al., 2001; Beasley et al., 2002; Lewis et al., 2005) have also been reported. PVB is a calcium binding peptide (CBP) associated with fast-spiking (FS) inhibitory interneurons (Kawaguchi et al., 1987; Gulyas et al., 2010), a class of interneuron believed to play a central role in inhibitory dysfunction. Although there is evidence of GABAergic abnormalities in several brain regions, such as the prefrontal cortex, anterior cingulate region and hippocampus (Lewis et al., 2005) (Benes and Berretta, 2001), it cannot be assumed that the basic cellular and molecular mechanisms in each region are similar they all show significant differences in their respective cytoarchitectonics, cellular constituents and connectivity. For example, the PFC is a true 6 layered neocortex, while the hippocampus is a 3-layered, the primitive precursor (archicortex) of virtually all other cortical areas seen in the mammalian brain. In comparing the PFC (Jones et al., 1978) to hippocampus (Rosene and Van Hoesen, 1987), their respective cytoarchitectonics reflect profound differences in their cellular constituents and connectivity.

The hippocampus is composed of a relatively simple circuit, the so-called trisynaptic pathway, that has been remarkably well-preserved across mammalian evolution, even primates and man (Rosene and Van Hoesen, 1987). This consistency lends itself to cross-species identification of homologous subregions that have been characterized in terms of their cellular constituents and molecular mechanisms associated with neuronal activation. The apparent consistency in the organization of the trisynaptic pathway facilitates the testing of hypotheses regarding circuitry abnormalities in schizophrenia and other neuropsychiatric disorders. This pathway relays information in a very stylized manner and consists of 1) the perforant path fibers originating in the entorhinal cortex that terminate in the dentate gyrus, 2) mossy

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fibers from dentate granule cells that synapse on apical dendrites of pyramidal neurons (PNs) in sector CA3 and 3) Schaffer collaterals from these projection neurons that, in turn, project to apical dendrites of CA1 PNs that project to other regions, such as the subiculum, amygdala and prefrontal cortex (Rosene and Van Hoesen, 1987). Hippocampal subregions can also be distinguished on the basis of subregional distribution of GABA cell subtypes, peptide content and synaptic connectivity (Gulyas et al., 1996). This latter concept can been particularly important for understanding how changes in the regulation of GAD_{67} expression (Benes et al., 2007, 2008) may be linked to dysfunction of specific subtypes of GABA neurons in schizophrenia (Heckers and Konradi, 2010; Konradi et al., 2011).

Most investigators agree that postmortem studies are limited in terms of the information that can be obtained from tissue exposed to perverse agonal events and/or autolytic changes occurring during the postmortem period. To understand if cellular and molecular abnormalities detected in postmortem tissue are related to schizophrenia or to confounding factors, parallel studies in rodent models are essential and provide a powerful tool for making this distinction. By manipulating the activity of a neural circuit within a region like the hippocampus where the cytoarchitecture and basic connectivity are remarkably similar to that seen in the human brain (Rosene and Van Hoesen, 1977, 1987; Saunders et al., 1988), it is possible to detect cellular and molecular changes that may be of pathophysiological significance to the disease process at work in schizophrenia.

In the discussion that follows, the principle focus is on the development of a rodent model to determine whether abnormalities seen in GABA cells of SO-CA3/2 in postmortem tissues are attributable to the influence of excitatory inputs from the basolateral amygdala on the intrinsic activity of interneurons (Benes, 2009). This locus plays a central role in social memory (Hitti and Siegelbaum, 2014) which is known to be defective in schizophrenia (Kuperberg and Heckers, 2000). Postmortem studies from this laboratory have repeatedly demonstrated abnormalities in cellular and molecular composition at this site in schizophrenia [for a recent review, see (Benes, 2010)]. These findings have raised important questions regarding the functional interconnectivity of GABA cells in the SO, and have raised questions as to how these changes might be influenced by the activity of basolateral amygdalar projections from the amygdala may be integrated within the GABA microcircuitry of SO-CA3/2. This rodent model is unique because it is based on our postmortem findings at a discrete site and is being used to test hypotheses specifically derived from microscopic, cellular and molecular postmortem findings (Benes and Berretta, 2000; Berretta and Benes, 2006).

2. Circuitry changes in schizophrenia

2.1. Postmortem changes in the hippocampal GABA system

The first evidence of GABAergic dysfunction in schizophrenia came from early neurochemical studies showing decreases in high affinity uptake of GABA in the hippocampus and other regions (Simpson et al., 1989; Reynolds et al., 1990). Subsequent studies employing a broad array of technologies provided compelling evidence supporting GABAergic dysfunction in schizophrenia. These studies demonstrated a decrease in the numerical density of interneurons (Benes et al., 1991) and a very significant increase of high resolution $GABA_A$ receptor binding activity (RBA) (Benes et al., 1992) on pyramidal neurons (PNs) in the anterior cingulate cortex (ACCx) of schizophrenics; both findings were particularly striking in layer II. PNs receive abundant GABAergic inputs via symmetric axosomatic synapses (Ribak et al., 1990) and the increase of $GABA_A$ RBA was thought to represent a *compensatory* upregulation of this receptor, one that occurs in the setting of GABA cell loss and/or activity (Benes et al., 1991, 1992). Additional evidence for a loss of GABA neurons in schizophrenia has come from an important study showing a decrease of GAD_{67} mRNA expressing interneurons in the prefrontal cortex (PFCx) (Akbarian et al., 1993) and an upregulation of $GABA_A$ RBA in this same region as well (Benes et al., 1996b). Like ACCx (Woo et al., 1997; Reynolds et al., 2001; Beasley et al., 2002; Lewis et al., 2005), the PFCx region also showed a decrease of parvalbumin (PVB)-containing neurons (Woo et al., 1997) and other calcium binding peptides (CBPs), such as calbindin (CB) that are associated with interneurons in the prefrontal cortex (Reynolds et al., 2001; Beasley et al., 2002; Chance et al., 2005; Sakai et al., 2008).

When the loss of interneurons discussed above was also reported in the hippocampus (HIPP), further credence was given to the idea that a GABA defect was contributing to the pathophysiology of schizophrenia. High resolution analyses of $GABA_A$ RBA in specific subregions of the hippocampal formation showed significant changes as well, although in this region, the pattern was quite different from that seen in ACCx and PFCx (Benes et al., 1998). As shown in Fig. 1, a decrease in the density of interneurons was observed preferentially in sector CA2. $GABA_A$ RBA was significantly decreased (80–100%) in sectors CA4 (hilum), CA3 and CA2 (Fig. 1B), while sector CA1 showed very little change in RBA (10%). Within sectors CA3 and CA2 (Fig. 1B), however, *low resolution* analyses demonstrated preferential increases in this RBA in the stratum oriens [SO] and stratum radiatum [SR]; the stratum pyramidale (SP) showed more modest changes. *High resolution* analyses of $GABA_A$ RBA, however, demonstrated that this receptor was not increased in PNs, as it was in ACCx and PFCx. Rather, it was significantly upregulated on only interneurons. A study of the subtypes of interneurons that may be involved in schizophrenia has demonstrated that a reduction in the expression of GAD1(or GAD 67) mRNA occurred in parallel with decreases in the numbers of somatostatin (SST) containing cells and the expression of this peptide in sector CA3/2 of schizophrenics (Konradi et al., 2011). Parvalbumin expression was also significantly decreased at this locus.

Other neurotransmitter systems and their markers have also been found to be abnormal in postmortem schizophrenia brains. Of relevance to the discussion below regarding GABA and its integration with glutamatergic elements, a microarray-based study employed laser microdissection (LMD) to examine the subregional and laminar distribution of gene expression changes in the SO-CA3/2 site. Decreases in the expression of GAD_{67} [GAD1] (Benes et al., 2007), as well as markers for kainate receptor (KAR) subunits GluR5, 6 and 7 (GRIK1, 2 and 3) and an inwardly rectifying hyperpolarization-activated channel (Ih or HCN3) showed significant changes in expression in SO-CA3/2, whereas sector CA1 showed little or no change in SR, SP or SO (Benes et al., 2007, 2008).

2.2. Rodent modeling

2.2.1. General considerations

It became clear that learning more about the precise ways in which various subtypes of GABA cells are functionally integrated within the microcircuitry at the SO-CA3/2 site would be a critical step for understanding abnormal functioning in schizophrenia. The KARs showing significant changes were also found to be an integral part of a GAD_{67} regulatory network of genes and seemed to be a potentially important target for studies in which a rodent model was being employed to study the flow of excitatory activity from the basolateral amygdala (BLA) to SO-CA3/2. Anterograde tracing had demonstrated that at specific coordinates the BLA sends subregion specific projections to the SO and to a lesser degree the SR of sector CA2/3 (see Fig. 2). These fibers originate in the accessory basal nucleus (Berretta et al., 2001). It appeared that this projection could be used to experimentally manipulate the interneuronal circuitry contained therein by using stereotaxic infusions of selective pharmacological agents.

As depicted in Fig. 3, upper panel it was also essential to understand how excitatory afferent fibers, in this case, those emanating from the BLA, may normally influence the functional integrity of GABAergic interneurons in the CA3/2 circuitry (Berretta et al., 2001; Benes, 2010) and

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