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Searching for Unique Endophenotypes for Schizophrenia and Bipolar Disorder Within Neural Circuits and Their Molecular Regulatory Mechanisms

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The endophenotype is a construct that has utility for the study of postmortem brains from patients with psychotic disorders. By identifying networks of genes that show changes in expression within specific neuronal populations implicated in the pathophysiology of schizophrenia and bipolar disorder, it may be possible to move toward understanding these disorders at the cellular and molecular levels. The ultimate goal is to characterize their respective underlying genotypes.

Key words: apoptosis/L-type calcium channel/antioxidation genes/cell viability/gene expression profiling

Introduction

Endophenotypes are ''measurable components unseen by the unaided eye along the pathway between disease and distal genotype.''[1] The field of psychiatry is now faced with the challenge of identifying endophenotypes for each disorder for which there is compelling evidence that a heritable component contributes to susceptibility. In reality, an endophenotype is a rather ill-defined construct that probably involves neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, and even psychological factors.[1] Schizophrenia was the first psychiatric disorder for which compelling evidence for heritability was obtained from comparative studies of first-degree relatives as well as monozygotic and dizygotic twin pairs.[2] It has become apparent that heritability of a disorder, such as schizophrenia, involves many different factors. The concept of the ''endophenotype'' provides a means of operationalizing this process.

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In psychiatric disorders, this is particularly difficult because there are well-known similarities among the clinical phenotypes for very different disorders. For example, in psychotic disorders, the presence of hallucinations and delusions and a beneficial response to neuroleptic drugs often belies the fact that there are distinctly different genotypes associated with schizophrenia and bipolar disorder. Indeed, a single genotype can theoretically give rise to a different clinical phenotypes, if there are different environmental influences associated with one phenotype versus another.[1] An important step toward identifying specific genotypes for schizophrenia and bipolar disorder is to identify underlying endophenotypes for the various psychotic disorders.

A combination of clinical, epidemiological, neurobiological, and genetic studies can be used to select and evaluate different candidate endophenotypes.[3] For example, a defect in the P50 auditory evoked potential gating deficit, reportedly abnormal in schizophrenia, has been associated with a dysregulation in the expression of the alpha 7 nicotinic receptor in hippocampal γ-aminobutyric acid (GABA) cells.[4] At the level of neural circuits and their constituent cells, there are probably many such molecular interactions that can be examined. These may involve the influence of mutated DNA for specific genes on the relative abundance of their transcripts or their post-translationally modified proteins products. Gene expression profiling provides a particularly powerful tool for studying endophenotypes because it provides a broad cross-sectional profile of many different aspects of neuronal cell functioning including receptors, ion channels, transduction, signaling, metabolism, and transcriptional pathways. If the respective molecular profiles in 2 disorders such as schizophrenia and bipolar disorder are fundamentally different from one another, they may potentially be related to complex traits like those observed in psychotic patients. Because a molecular strategy of this type is used in the study of postmortem brain, it may eventually help to point the way toward a molecular basis for defining genotype and rationale treatment strategies.[5] In the discussion that follows, this idea is considered in more detail by reviewing specific findings obtained in microscopic and microarray studies of the limbic lobe in schizophrenia and bipolar disorder.

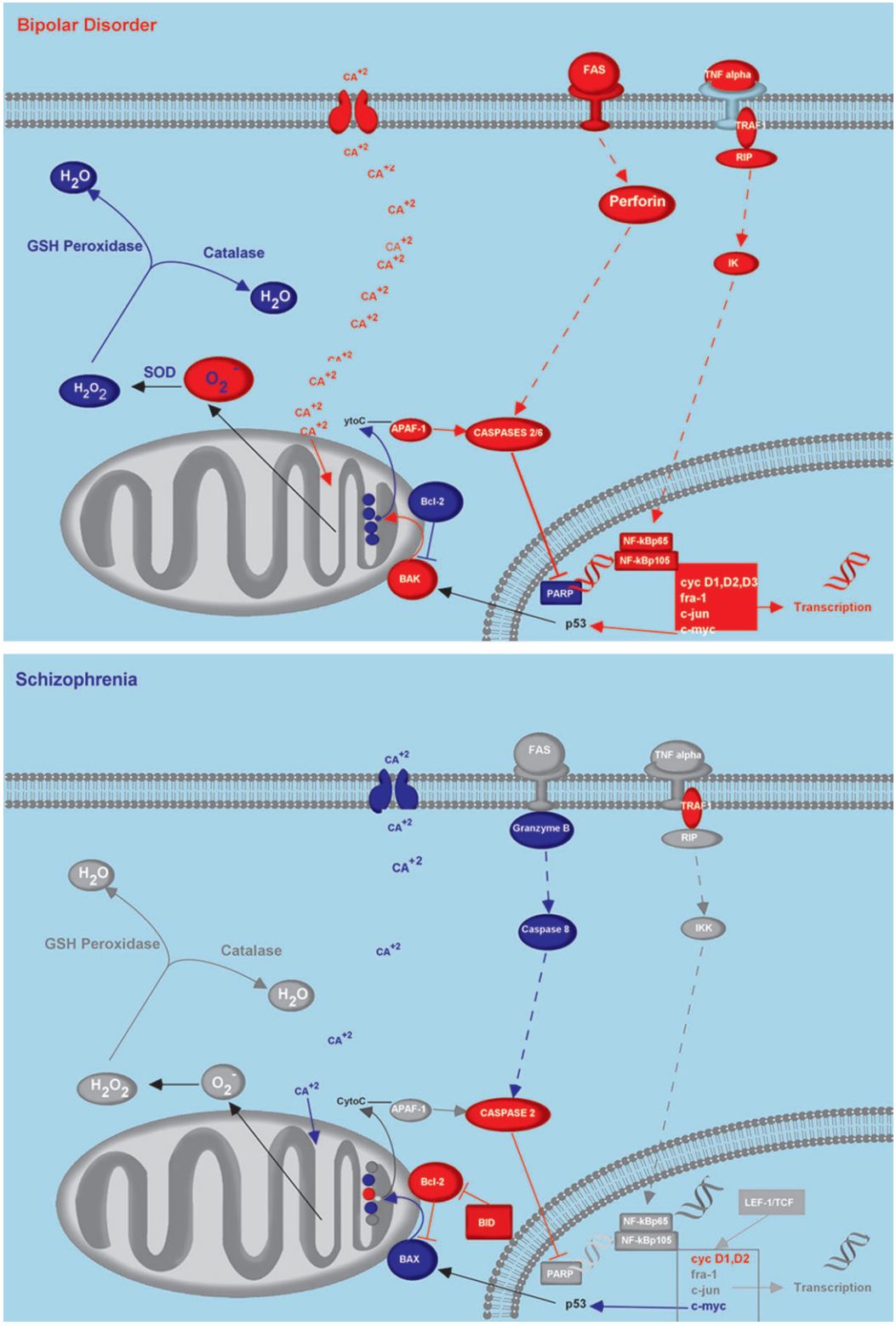

Fig. 1. Schematic diagrams depicting changes in the expression of genes associated with a mitochondrial oxidation, anti-oxidation regulation, and an L-type calcium channel in bipolar disorder (left) and schizophrenia (right). There are fundamental differences in the regulation of these various genes in the two disorders, with bipolars showing an upregulation (red) of apoptosis, the L-type calcium channel, and mitochondrial oxidation. Taken together with the down-regulation of the anti-oxidation enzymes, these changes would mitigate toward dysfunction or even death of hippocampal neurons in this disorder. In schizophrenia, the profile of expression changes is anti-apoptotic. These respective patterns in the two disorders may reflect the presence of uniquely different cellular endophenotypes and reflect differences in the genotype for each.

Preferential Abnormalities in Discrete Aspects of Limbic Lobe

Over the past 20 years, postmortem studies have suggested that the limbic consisting of the cingulate gyrus, hippocampal formation, entorhinal cortex, and amygdala may play a central role in the pathophysiology of schizophrenia.[6–11] Specific abnormalities in these regions have been reported using a variety of quantitative microscopic approaches (for a review, see Benes[12]). Noteworthy is the fact that significant changes have been preferentially found within certain loci, such as layer II of the anterior cingulate cortex (ACCx-II) and sectors CA3/CA2 of the hippocampus in schizophrenics. These 2 sites receive robust projections from the basolateral amygdala,[13] and based on this observation, it has been postulated that the projections from the basolateral nucleus (BLn) of the amygdala to these 2 loci may play a pivotal role in the induction of abnormalities. Such changes may be related to the stress response and other emotional reactions mediated through the amygdala.[14,15] Based on the subregional distribution of findings described above, we have developed a "partial" rodent model for neural circuitry abnormalities in postmortem studies of schizophrenia and bipolar disorder.[16] With this model, picrotoxin, an antagonist of the $GABA_A$ receptor, is stereotaxically infused into the BLn.[17] Within 2 hours, a reduction of GABAergic terminals visualized with antibodies against glutamic acid decarboxylase (GAD)65 was observed in sectors CA3 and CA2 but not CA1.[16] This subregional distribution is remarkably similar to that described above for our postmortem studies. We postulated that the changes observed in CA3/2 in our postmortem studies could potentially be related to increased glutamatergic activity originating in the BLn.[16]

Identifying Potential Cellular Endophenotypes in Schizophrenia and Bipolar Disorder

Increased glutamatergic activity was postulated to be present in neural circuits within the brains of schizophrenics.[18] Increased excitatory activity emanating from glutamatergic neurons can promote oxidative stress and apoptosis in psychotic disorders.[19] To test the hypothesis that oxidative stress might be present in schizophrenia and possibly also in bipolar disorder, a microarray-based gene expression profiling study was conducted in the hippocampus of normal controls, schizophrenics, and bipolars.[20] As shown in figure 1, the results demonstrated a striking difference in the pattern of expression of 24 different genes associated with this signaling pathway in schizophrenics and bipolars. While the bipolar subjects showed an increased expression of proapoptotic genes, such as FAS ligand and its receptor, tumor necrosis factor alpha, perforin, several caspases, c-myc, and BAK, schizophrenics showed the opposite pattern, ie, many proapoptotic genes, such as granzyme B and BAX, were either downregulated or showed no change in regulation. Conversely, the antiapoptotic gene, Bcl-2, was found to be upregulated in schizophrenics but downregulated in bipolars. Additionally, bipolars showed a highly significant downregulation of several different antioxidation genes, including superoxide dismutase, glutathione peroxidase, glutathione synthase, and catalase, changes that can lead to the accumulation of reactive oxygen species and cellular toxicity.[21] Overall, the results in bipolars were consistent with a previous study in which genes involved in the regulation of the electron transport chain were also found to be markedly downregulated.[22] It is noteworthy that a study of DNA damage in the anterior cingulate cortex demonstrated a marked reduction in schizophrenics but no change in bipolars.[23] Subsequently, a double localization of single-stranded DNA breaks in cells expressing GAD67 messenger RNA demonstrated a significant increase in bipolars.[24] Of course, it is important to consider whether neuroleptic drugs may have played a role in these changes in the expression of genes involved in the regulation of oxidation reactions. A careful analysis of the regulation of both pro- and antiapoptotic genes in patients with schizophrenia and bipolar disorder who were receiving low versus high doses of these drugs during the year prior to death are not consistent with this possibility.

Taken together, the results described above support the idea that schizophrenia and bipolar disorder involve a common cellular phenotype, one in which dysfunctional GABAergic interneurons contribute to abnormal information processing in the limbic lobe. As suggested by others,[1] the endophenotypes for such cells may nevertheless be quite different in the 2 different forms of psychotic disorder. It might be concluded then that the mechanisms responsible for the decreased amount of GABAergic activity may be fundamentally different in schizophrenia and bipolar disorder. In bipolars, the gene expression profiling findings clearly point to molecular changes, such as activation of the apoptosis cascade and the L-type calcium channel 1D, but suppression of the antioxidant pathways, that could play a central role in the pathophysiology of this disorder. In schizophrenia, on the other hand, it is unlikely that GABA cell dysfunction involves oxidative mechanisms because similar changes were not observed. Indeed, the regulation of genes associated with apoptosis was suppressed to a large extent. It is important to emphasize, however, that reductions of interneuronal numbers have been found to be reduced in sector CA2 of subjects with both disorders bipolar disorder and schizophrenia. If the apoptotic cascade were not activated in schizophrenia, why would reduced numbers of GABA cells be found in these patients? One possible explanation for this paradox is that interneurons in the hippocampus of schizophrenics

are, indeed, being subjected to oxidative stress but only during an earlier phase of the illness. If cells drop out in the schizophrenics, but the apoptotic cascade is subsequently downregulated, the overall numbers of GABA cell could remain stable. If this were the case, it would be difficult to explain the results of a study in which the numerical density of interneurons in CA2 of schizophrenics and bipolars were found to be the same.[25] If apoptosis is indeed killing GABA cells in CA2 of bipolars, these cells would be expected to drop out of the neuronal population in that sector. If the regulation of apoptosis genes continues to be upregulated, as it appears to be in bipolar disorder, then one might expect to find that there is an ongoing process of cell loss in these patients as the illness continues. How then can the observation that the number of interneurons is the same in sector CA2 of the bipolars and the schizophrenics? One hypothesis that could account for this apparent discrepancy is that neurons in the bipolars that undergo apoptotic cell death are continually being replaced through active neurogenesis. In this setting, newly generated cells and cells that are dying would coexist in a relative steady state, such that the overall numbers in CA2 would not appear to be changing. An argument in favor of this hypothesis is that evidence for ongoing apoptosis comes from a study of DNA fragmentation in the anterior cingulate cortex. Specifically, increased DNA damage was observed in GABA cells of the anterior cingulate cortex of bipolar subjects but not schizophrenics.[24] Analogous data for the hippocampus, particularly sector CA2, is not available and it is not necessarily the case that a similar pattern would be observed in this latter subregion.

Conclusions

The above discussion has explored the possibility that endophenotypes for schizophrenia and bipolar disorder may exist at the level of specific circuits, neuronal cell types, and neuronal cell mechanisms. The circuitry inherently present within the limbic lobe, ie, the anterior cingulate cortex, hippocampus, and basolateral amygdala, together with their reciprocal interconnections, could be part of a central substrate involved in the mediation of the clinical phenotype for each disorder. Presumably, similar substrates may exist in other regions of the brain, such as the dorsolateral prefrontal cortex, that have also been implicated in the pathophysiology of psychotic disorders.[26,27] The GABA cell may be a focus for abnormal expression of many different but functionally interrelated genes.

Despite the apparent similarities between schizophrenia and bipolar disorder observed in our postmortem studies cited above, specific molecular mechanisms may be probably quite different in the 2 disorders. When not functioning appropriately, alterations of oxidative mechanisms could have the ability to induce dysfunction in bipolars and could potentially explain the observation that mood-stabilizing anticonvulsant medications help to stabilize bipolar disorder symptoms.[28,29] For schizophrenics, the underlying mechanism for GABA cell dysfunction appears to be fundamentally different. Taken together, these findings are consistent with the hypothesis that a common cellular phenotype (ie, GABA neuron dysfunction) could theoretically occur through very different cellular mechanisms in 2 different psychiatric disorders.

It is becoming increasingly clear that our understanding of the nature of endophenotypes for psychotic disorders will require a careful delineation of brain, regions, circuits, neuronal subtypes, and the associated cellular mechanisms that underlie the clinical manifestations of these illnesses. In clinical investigations, complex markers, such as temporal stability of antisaccades,[30] event-related potentials,[31] and working memory,[32] have also been used to distinguish endophenotypes for schizophrenia versus bipolar disorder. As with postmortem studies in which a notable downregulation of GAD67 expression has been observed in both diagnostic groups,[26,33] significant similarities among some of these markers, such as working memory, have also been observed in studies of schizophrenia and bipolar disorder.[32] Such endophenotypic similarities may well indicate the presence of common environmental influences occurring within the life cycle of such individuals. Nevertheless, the fact that there now appears to be discrete differences in the pattern of gene expression in the hippocampus of schizophrenics and bipolars makes it increasingly likely that the endophenotypes for these 2 disorders may also include specific cellular and molecular substrates. An understanding of what constitutes an endophenotype within the brain requires that we learn more about the ways in which candidate neurons are being acted upon by specific afferents projecting to specific downstream brain regions, and how, in turn, neurons comprising complex circuits may or may not respond to either intrinsic or extrinsic afferent within larger circuits. Ultimately, neurobiological information of this type will eventually provide a precise understanding of differences in the cellular and molecular regulation of neurons within affected circuits will bring us closer to understanding the underlying genotype for each disorder.

The fact that there are similarities in the clinical phenotype for schizophrenia and bipolar disorder suggests that the respective endophenotypes may also show areas of overlap with respect to the circuitry involved and the nature of the cellular and molecular changes present. Contrariwise, the fact that these 2 disorders show prominent differences in their clinical phenotypes implies that other aspects of limbic lobe circuitry and GABA cell integration may show abnormalities that are unique to each disorder. The data presented above provide support for this hypothesis. Having similarities and differences in

clinical phenotype and, by inference, endophenotype makes the process of defining the respective neural substrates quite difficult and time consuming. Toward this end, the use of postmortem tissues in combination with molecular strategies, such as gene expression profiling, high-density haplotyping, and other forms of genetic analysis, will be a critical element in the overall strategy to use 2-factor modeling to uncover heritable and environmental components of a complex psychiatric endophenotype.

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