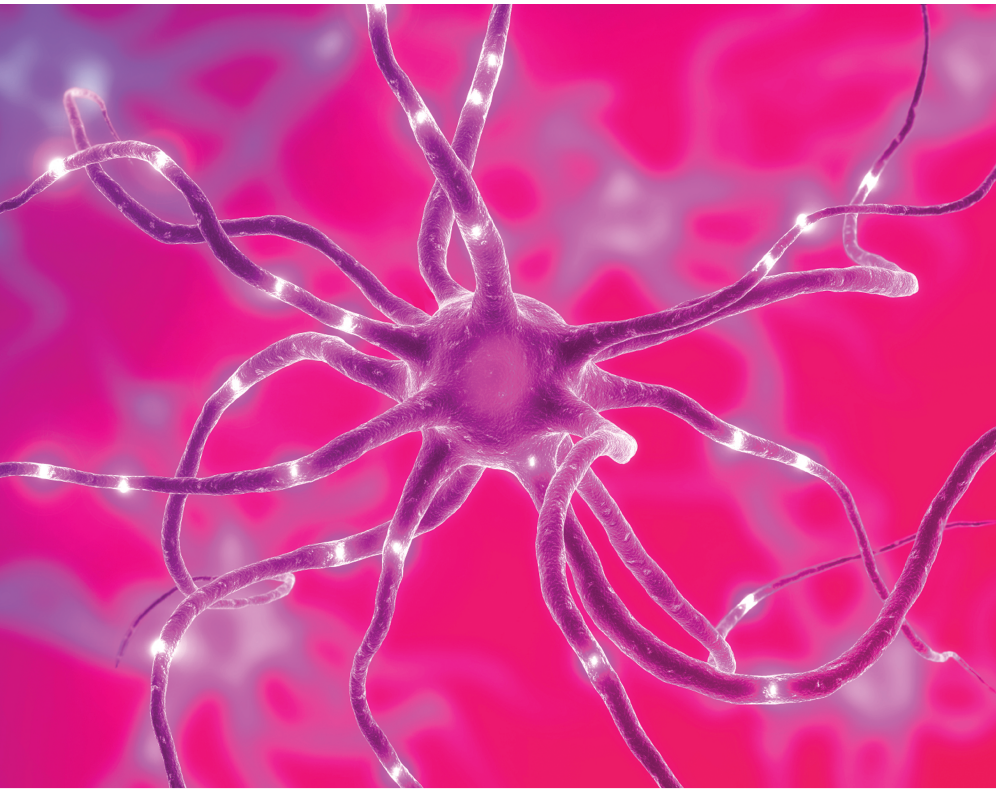
Update on Blood Brain Barrier



THE BLOOD BRAIN BARRIER AND THE ROLE OF RATIOMETRIC MOLECULAR ANALYSIS IN SCHIZOPHRENIA

by Atmaram Yarlagadda, MD; Christiane S. Hampe, PhD; and Anita H. Clayton, MD

Psychiatry (Edgemont) 2010;7(12):20–23

ABSTRACT

The etiology of schizophrenia and other chronic psychotic disorders is complicated considering the multifactorial contribution of developmental, biological, and environmental factors. The role of the blood brain barrier has not yet been established as part of schizophrenia etiology; however, in previous blood brain barrier articles, we discussed potential consequences of various biological abnormalities due to dysregulation of molecular components, such as cofactors,[1] signaling molecules,[2] enzymes,[3] cytokines,[4] and antibodies.[5] In this review, we will discuss the potential use of peripheral ratiometric molecular analysis relevant to the central nervous system for the evaluation of the development of schizophrenia.

KEY WORDS

Ratiometic molecular analysis (RMA), TNF-alpha, S100B, NMDA and GAD receptor antibodies

INTRODUCTION

Central nervous system (CNS) pathology involved in neuropsychiatric conditions is complex and daunting. Research focused toward the role of the blood brain barrier (BBB) in better understanding of these disorders is emerging rapidly. The term *sickness behavior* has been replaced by the terms *mood lability*, *anxiety,* and *psychosis*. Sickness responses, on the other hand, include fatigue, headaches, fever, and occasionally involve severe clinical and neurological findings like movement disorders. Both sickness behavior and responses have been attributed to direct toxic effects of substances able to cross and disrupt the integrity of the BBB.[6] Toxicity has been linked to activation of signaling molecules, such as calcium, followed by dysregulation of calcium homeostasis.[2] In this review, we will attempt to hypothesize the progression of psychopathology due to toxicity resulting in rupture of neuronal cells and leakage of cytoplasmic enzymes, such as glutamic acid decarboxylase (GAD),[3] and subsequent formation of antibodies.[5] The pathway of interest will target increased glutamate levels at the presynaptic N-methyl-D-aspartate (NMDA) receptor junction of GABAergic neurons due to blockade and its effect on subsequent release of the central inhibitory neurotransmitter gamma-aminobutyric-acid (GABA) at the post-synaptic end and its influence on the cholinergic system.

BACKGROUND

Selective permeability of substances through the BBB is a well-established fact.[7] Lack of differentiation of "self" from "foreign" molecules by the BBB complicates neuropathology further as immune defense mechanisms of an intact CNS are thought to be basically weak. This concept is rapidly changing, and the interactions between inflammatory proteins and brain cells are becoming more evident.[8] The compromised BBB, therefore, becomes vulnerable to exposure to antigens capable of launching an immune response and formation of antibodies. Antibodies to NMDA receptors, such as NR1 and NR2 in particular, block the passage of glutamate entering the GABA-ergic neuron and could potentially initiate a downstream effect of glutamate build up.[9] Glutamate build up is associated with excitotoxicity and positive symptoms commonly seen in early schizophrenia and other psychotic conditions. Excitotoxicity caused by glutamate build up is likely the result of mobilization and activation of free/unbound calcium in the brain.[2]

Altered calcium homeostasis can have major implications in the development of neuropathology depending on the location in the CNS. The cascade of events leading to schizophrenia, however, are thought to be located in the prefrontal cortex (PFC) area of the brain. The central role of altered calcium homeostasis within different areas of the brain is frequently cited in the literature for a wide spectrum of neuropsychiatric conditions.[10]

Cell death induced by exitotoxicity allows the release of GAD65 and GAD67 from the cells; the presentation of these cytoplasmic antigens triggers the development of GAD antibodies. Moreover, glutamate-induced excitotoxicity is associated with NMDA receptor-mediated reduction of GAD protein expression[11] and cleavage of GAD by proteases,[12] further reducing GABA levels. Levels of the inhibitory neurotransmitter GABA are, therefore, reduced by a glutamate-mediated decrease in GAD protein expression or through enzyme activity leading to the formation of GAD antibodies.

Variations in GABA and acetylcholine levels ultimately play an important role in processing of initial signal and subsequent assembly of information in the higher-cortical areas of the brain.[13] Reception and relay of the signal is reportedly a function mediated by the neurotransmitter acetylcholine. This phenomenon is known as synchronization-desynchronization, which is tightly regulated by both neurotransmitters GABA and acetylcholine.[14] Perturbations in synchronization-desynchronization mechanisms as reflected by calcium dysregulation are thought to be crucial in development of schizophrenia pathology.[15] Therefore, in addition to optimization of dopamine function, neurotransmission of both GABA and acetylcholine become relevant considerations in the effective treatment of schizophrenia.

DISCUSSION

Circulation of specific cytokines, proteins, and antibodies with both central and peripheral properties clearly points to free passage of molecules to and from the CNS indicating a compromise in the integrity of the BBB. In an attempt to understand the progression in schizophrenia pathology, one should consider ratiometric molecular analysis (RMA), i.e., quantification of biological markers more specific to the CNS. The first goal is to establish effects of cytotoxicity by cytokines on the CNS (i.e., by identifying pro-inflammatory vs. anti-inflammatory cytokine levels). Cytokines, such as tumor necrosis factor (TNF) alpha, have been described extensively in the literature to have a tremendous impact on the etiology of neuropsychiatric disorders induced by damage to microvascular endothelial vasculature including the BBB.[16] The second goal is to identify a potential marker signaling BBB damage due to cytotoxicity induced by cytokines. Decrease in Notch-4 and ZO-1 expression and release of protein S100B, a calcium-dependent protein, experimentally appear to be specific to CNS microvasculature (astrocytes) signaling the BBB damage and could serve as a biological markers.[17,18] Finally, determination of the presence of antibodies to the NMDA receptor and GAD could provide a quantitative immunological metric of complex schizophrenia etiology.

In summary, from a diagnostic point of view, we propose clinical studies aimed at stepwise RMA to correlate specific markers with various stages of the multifactorial, polygeneic pathology of schizophrenia. Quantitative analysis includes examining the ratios of peripherally circulating molecules such as the following: 1) pro-inflammatory versus anti-inflammatory cytokines to explore the impact of inflammatory reactions on the CNS, 2) levels of S100 protein to assess BBB damage caused by cytokines, 3) levels of antibodies to the NMDA receptor and GAD to estimate immune responses to antigen presentation by the CNS, which in turn impact and influence the release of 4) GABA and/or acetylcholine, thus explaining the imbalance of synchronization-desynchronization mechanisms. The results of these ratiometric molecular analyses can then be used to target

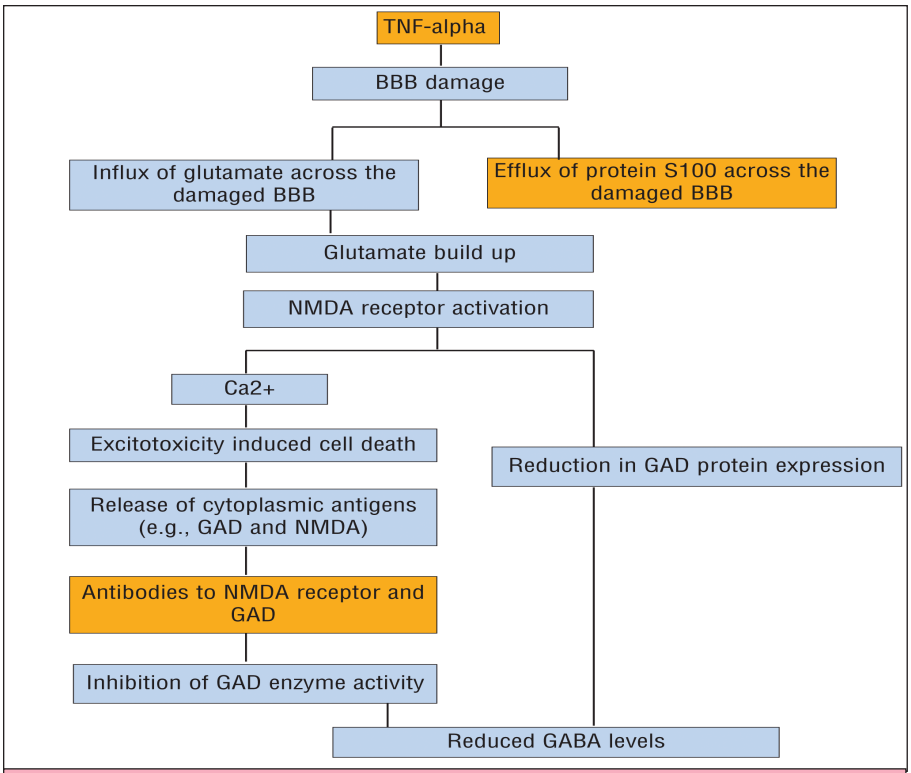


Figure 1. Simplified schematic outline. Th1-inflammatory cytokines (e.g., TNF-alpha) induce damage of the BBB, resulting in the influx of glutamate to the brain and the efflux of protein S100. Glutamate levels build up in the synapse, leading to NMDA receptor activation and subsequent excitotoxicity. NMDA receptor activation is also associated with a reduction in GAD protein expression, leading to reduced GABA levels. Another pathway resulting in reduced GABA levels is the release of cytoplasmic GAD from neurons following excitotoxicity-induced cell death. GAD release induces the development of GAD antibodies that inhibit GAD enzyme activity. Excitotoxicity can trigger autoimmune production of NMDA receptor antibodies leading to NMDA receptor hypofunction. Steps that can be determined with RMA are indicated in orange.

treatment options for schizophrenia pathology at a given time or specific stage of illness. While the identification of certain neurotransmitters and cytokines may correspond with initial psychosis, decrease in Notch-4 and ZO-1 expression and release of S100 and GAD may be markers for progression of the disease, followed by the formation of GAD antibodies correlated with degeneration and dementia seen toward the terminal stage.

Depending on the stage of illness, (e.g., excess acetylcholine in early vs. diminished levels due to atrophy during later stages of schizophrenia) one could also consider the use of psychotropics, such as clozapine, early followed by donepezil later to regulate/supplement acetylcholine. Therefore, modulation of acetylcholine levels should be seriously considered to correlate with GABA levels for maintenance of optimal synchronization-desynchronization mechanisms for successful treatment of schizophrenia.

Extrapyramidal symptoms (EPS) seen early in the disease process are reported as a result of increased sensitivity to medications due to disruption of the BBB frequently seen in human immunodeficiency virus (HIV)-related acquired immune deficiency syndrome (AIDS) dementia complex (ADC).[19] Tardive dyskinesia (TD), however, is seen much later in schizophrenia and may be an irreversible complication of treatment with antipsychotics. Clozapine appears to have preventive effects on both EPS as well as remission of TD.[20] Its use, however, is not considered first line due to the potentially serious side effect of agranulocytosis. On the other hand, the importance of iatrogenic causes of EPS and TD with the use of antipsychotics and other neurotoxic medications[19] cannot be underestimated given the variations in treatment outcomes in schizophrenia with typical and atypical agents.[21]

The addition of neuroprotective chemicals, such as anti-inflammatory agents, antioxidants, and calcium channel blockers, early in the disease process may decrease BBB sensitivity to psychotropics by preventing endothelial oxidative stress.[22] Experimental studies comparing the stage of illness with a specific molecule (i.e., neurotransmitter or antibodies related to the enzymes involved), become an important factor in determining schizophrenia treatment. Currently, epitopal classification of antibodies (GAD or NMDA) specific to either CNS or schizophrenia is unavailable. Crossreactivity with tissue elsewhere in the body (e.g., the presence of GAD65 in pancreatic islet cells) further complicates the pursuit of CNS epitopal specificity. See Figure 1.

CONCLUSION

Goals of RMA are to aggressively target etiology, course of the pathologic process, and most importantly, treatment outcomes in chronic neuropsychiatric disorders. As practicing psychiatrists, the

importance of expansion of conventional treatment options directed toward regulating GABA and acetylcholine levels is also becoming apparent in schizophrenia treatment. Research-oriented clinical trials in this direction, therefore, would provide clues to better understanding of the complex pathology of neuropsychiatric conditions.

REFERENCES

1. Yarlagadda A, Clayton AH. Blood brain barrier: the role of pyridoxine. *Psychiatry (Edgmont)*. 2007;4(8):58–60.
2. Yarlagadda A. Role of calcium regulation in pathophysiology model of schizophrenia and possible interventions. *Med Hypotheses*. 2002;58(2):182–186.
3. Yarlagadda A, Helvink B, Chou C, Clayton AH. Blood brain barrier: The role of GAD antibodies in psychiatry. *Psychiatry (Edgmont)*. 2007;4(6):57–59.
4 Yarlagadda A, Alfson E, Clayton AH. The blood brain barrier and the role of cytokines in neuropsychiatry. *Psychiatry (Edgmont)*. 2009;6(11):18–22.
5. Yarlagadda A, Helvink B, Chou C, Gladieux K, et al. Glutamic acid decarboxylase (GAD) antibodies in tardive dyskinesia (TD) as compared to patients with schizophrenia without TD and normal controls. *Schizophr Res*. 2008;105(1-3):287–288.
6. Aubert A, Vega C, Dantzer R, Goodall G. Pyrogens specifically disrupt the acquisition of a task involving cognitive processing in the rat. *Brain Behav Immun*. 1995;9(2):129–148.
7. Kronfol Z, Remick DG. Cytokines and the brain: implications for clinical psychiatry. *Am J Psychiatry*. 2000;157(5):683–694.
8. Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today*. 1992;13(12):507–512.
9. Kambe Y, Nakamichi N, Takarada T, Fukumori R, et al. Induced tolerance to glutamate neurotoxicity through down-regulation of NR2 subunits of N-methyl-D-aspartate receptors in cultured rat striatal neurons. *J Neurosci Res*. 88(10):2177–2187.
10. Yarlagadda A, Kaushik S, Clayton AH. Blood brain barrier: the role of calcium homeostasis. *Psychiatry (Edgmont)*. 2007;4(12):55–59.
11. Monnerie H, Le Roux PD. Reduced dendrite growth and altered glutamic acid decarboxylase (GAD) 65- and 67-kDa isoform protein expression from mouse cortical GABAergic neurons following excitotoxic injury in vitro. *Exp Neurol*. 2007;205(2):367–382.
12. Baptista MS, Melo CV, Armelao M, Herrmann D, et al. Role of the proteasome in excitotoxicity-induced cleavage of glutamic acid decarboxylase in cultured hippocampal neurons. *PLoS One*. 5(4):e10139.
13. Tojima T, Ito E. Bimodal effects of acetylcholine on synchronized calcium oscillation in rat cultured cortical neurons. *Neurosci Lett*. 2000;287(3):179–182.
14. Yarlagadda A, and Clayton AH. Role of cholinergic system and calcium synchronization in schizophrenia. *Psychiatry (Edgmont)*. 2009;6(4):37–41.
15. Gupta S, Bisht SS, Kukreti R, Jain S, et al. Boolean network analysis of a neurotransmitter signaling pathway. *J Theor Biol*. 2007;244(3):463–469.
16. Pan W, Xiang S, Tu H, Kastin A. Cytokines interact with the blood-brain barrier. In: Derietzel R, DCS, Nedergaard M, eds. *Blood-Brain Barriers: From Ontogeny to Artificial Interfaces*. Weinheim: Wiley-VCH, 2006:247–264.
17. Manda VK, Mittapalli RK, Geldenhuys WJ, Lockman PR. Chronic exposure to nicotine and saquinavir decreases endothelial Notch-4 expression and disrupts blood-brain barrier integrity. *J Neurochem*. 2010;115(2):515–525.
18. Sen J, Belli A. S100B in neuropathologic states: the CRP of the brain? *J Neurosci Res*. 2007;85(7):1373–1380.
19. Grigorian A, Hurford R, Chao Y, Patrick C, et al. Alterations in the Notch4 pathway in cerebral endothelial cells by the HIV aspartyl protease inhibitor, nelfinavir. *BMC Neurosci*. 2008;9:27.
20. Bassitt DP, Louza Neto MR. Clozapine efficacy in tardive dyskinesia in schizophrenic patients. *Eur Arch Psychiatry Clin Neurosci*. 1998;248(4):209–211.
21. Meyer JM, Marsh J, Simpson G. Differential sensitivities to risperidone and olanzapine in a human immunodeficiency virus patient. *Biol Psychiatry*. 1998;44(8):791–794.
22. Packer L, Tritschler HJ, Wessel K. Neuroprotection by the metabolic antioxidant alpha-lipoic acid. *Free Radic Biol Med*. 1997;22(1-2):359–378.

AUTHOR AFFILIATIONS: Dr. Yarlagadda is Assistant Professor, Department of Psychiatry and Neurobehavioral Sciences, Charlottesville, Virginia; Dr. Clayton is Professor, Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, Charlottesville, Virginia; and Dr. Hampe is Research Associate Professor, Department of Medicine, University of Washington, Seattle, Washington.

ADDRESS CORRESPONDENCE TO:
Atmaram Yarlagadda, MD; E-mail: atma@golfolks.com