Cortical Kynurenine Pathway Metabolism: A Novel Target for Cognitive Enhancement in Schizophrenia

Ikwunga Wonodi* and Robert Schwarcz

Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD

*To whom correspondence should be addressed; Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, PO Box 21247, Baltimore, MD 21228; tel: 410-402-6830, fax: 410-402-6023, e-mail: Iwonodi@mprc.umaryland.edu

The brain concentration of kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation and antagonist at both the glycine coagonist site of the N-methyl-D-aspartic acid receptor (NMDAR) and the α7 nicotinic acetylcholine receptor (α7nAChR), is elevated in the prefrontal cortex (PFC) of individuals with schizophrenia. This increase may be clinically relevant because hypofunction of both the NMDAR and the α7nAChR are implicated in the pathophysiology, and especially in the cognitive deficits associated with the disease. In rat PFC, fluctuations in endogenous KYNA levels bidirectionally modulate extracellular levels of 3 neurotransmitters closely related to cognitive function (glutamate, dopamine, and acetylcholine). Moreover, behavioral studies in rats have demonstrated a causal link between increased cortical KYNA levels and neurocognitive deficits, including impairment in spatial working memory, contextual learning, sensory gating, and prepulse inhibition of the startle reflex. In recent human postmortem studies, impairments in gene expression and activity of kynurenine pathway enzymes were found in cortical areas of individuals with schizophrenia. Additional studies have revealed an interesting association between a sequence variant in the gene of one of these enzymes, kynurenine 3-monooxygenase, and neurocognitive deficits seen in patients. The emerging, remarkable confluence of data from humans and animals suggests an opportunity for developing a rational pharmacology by targeting cortical kynurenine pathway metabolism for cognition enhancement in schizophrenia and beyond.

Key words: kynurenic acid/endophenotype/cognition/schizophrenia/kynurenine 3-monooxygenase/prefrontal cortex/genetic association

Cognitive Deficits in Schizophrenia

Kraepelin coined the phrase “dementia praecox” to describe the precocious and global cognitive impairments that characterized the disease later known as schizophrenia.1 A century later, cognitive impairments have emerged as primary determinants of the long-term functional disability characteristic of the disease.2,3 Cognitive deficits occur in the majority of individuals with schizophrenia, display onset in early adolescence preceding psychotic symptoms of young adulthood, are orthogonal to psychotic symptoms, and persist even after pharmacological control of psychosis.4,5 Additionally, the severity of cognitive deficits predicts symptom relapse and poor treatment adherence in first-episode patients.6,7 Cognitive impairments in schizophrenia are now hypothesized to be due to primary neuronal dysfunction rather than chronicity or neurodegeneration.8,9 The current pharmacological treatment of the disease relies on compounds that owe their efficacy to action on dopaminergic neurotransmission.10,11 However, antipsychotic medications fail to alleviate some positive symptoms and exert minimal impact on preexisting cognitive dysfunction. With the increased appreciation that reducing cognitive impairments in schizophrenia would significantly improve functional outcomes and quality of life, the National Institute of Mental Health initiated the large-scale Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) project.12

MATRICS was charged with facilitating the development of targeted treatments for cognitive impairments in schizophrenia. The first MATRICS committee established objective criteria to identify the different areas of cognitive dysfunction. This resulted in the identification of 7 primary domains critical for developing targeted pro-cognitive treatments in the disease: working memory, verbal learning and memory, visual learning and memory, attention and
vigilance, reasoning and problem solving, speed of processing, and social cognition. A second MATRICS committee focused on neuropsychopharmacological approaches to cognitive remediation in schizophrenia and determined pharmacological strategies and promising compounds for the targeted treatment of cognitive dysfunction. The primary molecular targets identified include dopamine receptors in the prefrontal cortex (PFC), serotonin receptors in the PFC and the anterior cingulate cortex, nicotinic and muscarinic acetylcholine receptors in the hippocampus, the excitatory glutamatergic synapse, and the inhibitory \( \gamma \)-aminobutyric acid system.

Extensive research has identified neurocognitive markers in schizophrenia, which represent the output of known neurobiological pathways. These neurobiological traits are highly frequent in patients and their unaffected biological relatives (indicating heritability), enduring, and independent of clinical symptoms or pharmacological treatment. The study of these quantitative traits or “endophenotypes” was initially advanced as an alternative approach to circumvent the problem of clinical heterogeneity in schizophrenia gene discovery efforts. Thus, investigators characterized several schizophrenia endophenotypes, including oculomotor movements (eye tracking), P50 and P300 sensory gating, prepulse inhibition (PPI) of the startle reflex, and working memory deficits. Consequently, endophenotypes with cross-species validity are increasingly being used for hypothesis testing in mutant and pharmacological animal models of the disease and have been suggested as attractive biomarkers to evaluate novel treatments of cognitive deficits. It is anticipated that the convergence of results from these preclinical and clinical studies will help characterize the underlying neurochemical and molecular abnormalities and accelerate progress toward the development of a rational pharmacology for cognitive dysfunction in schizophrenia.

### Kynurenic Acid: A Unique, Astrocyte-Derived Neuromodulator

Kynurenic acid (KYNA), a product of the kynurenine pathway of tryptophan degradation that is present in the mammalian brain in nanomolar to low micromolar concentrations, may play an important role in cognition. This emerging hypothesis is based on evidence showing that endogenous levels of KYNA antagonize the \( \alpha 7 \) nicotinic acetylcholine receptor (\( \alpha 7nAChR \)) and the glycine coagonist (glycine\( _{GABA} \)) site of the \( N \)-methyl-D-aspartic acid receptor (NMDAR), both of which are critically involved in physiological processes underlying learning, memory, and other manifestations of synaptic plasticity. Experiments in rodents have demonstrated that even relatively minor elevations in KYNA levels in the PFC cause a decrease in the extracellular levels of 3 neurotransmitters known to be associated with cognitive functions, ie, glutamate, acetylcholine, and dopamine. Interestingly, these effects are bidirectional, ie, a selective reduction in KYNA formation substantially enhances the extracellular presence of these classic neurotransmitters. It follows that fluctuations in endogenous KYNA might tonically modulate and therefore control neurochemical events closely linked to cognitive processes. These insights stimulated considerable interest in the neurobiology of KYNA and prompted a series of studies, which revealed unique characteristics of the metabolite.

In the brain as in the periphery, KYNA is produced by the irreversible transamination of L-kynurenine, the first major catabolic product of tryptophan (figure 1). The conversion of L-kynurenine to KYNA is catalyzed by distinct kynurenine aminotransferases (KATs), which in the brain are preferentially localized in astroglial cells. KYNA synthesis in astrocytes is driven by the availability of substrate (L-kynurenine) and cosubstrate (2-oxoacids) and further regulated by cellular energy status. Newly formed KYNA is then rapidly released into the extracellular milieu where it has access to neuronal \( \alpha 7nAChRs \) and NMDARs. Subsequently, KYNA is not degraded enzymatically or removed by reuptake but is slowly eliminated from the brain by a nonspecific acid transporter. These unorthodox features suggest that astrocytes, by liberating KYNA and thereby controlling its extracellular concentration, might exert substantive influence over cognitive functions.

In addition to KYNA, the kynurenine pathway generates 2 other neuroactive compounds—the free radical generator 3-hydroxykynurenine and the excitotoxic NMDAR agonist quinolinic acid. As illustrated in figure 1, these metabolites are components of a competing branch of the enzymatic cascade, eventually leading to the ubiquitous enzyme cofactor nicotinamide adenine dinucleotide (NAD\( ^{+} \)). In the mammalian brain, this segment of the metabolic chain is localized in microglial cells and thus physically segregated from the dead-end arm that produces KYNA in astrocytes. Compelling data indicate that the 2 branches of the pathway do not interact functionally under normal physiological conditions or following an acute rise of the central metabolite, L-kynurenine. However, cross talk might occur during sustained stimulation of upstream events in the kynurenine pathway, especially when micro- and/or astroglial functions are compromised in pathological situations. The nature and consequences of these adaptive changes are currently being investigated in several laboratories. One of the most tantalizing questions is whether persistent or repeated up- and downregulation of the microglial branch of the pathway impacts cognitive function by indirectly modulating astrocytic KYNA formation.

The enzymes directly responsible for the production and degradation of L-kynurenine are best positioned to control the levels of this pivotal metabolite. The synthesis of L-kynurenine is regulated by 2 separate enzymes,
Fig. 1. (A and B): Kynurenine pathway metabolism is initiated by the oxidative ring opening of tryptophan by both indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase. In the brain, the pivotal metabolite L-kynurenine is enzymatically converted in microglial cells and astrocytes, respectively. In schizophrenia, a persistent reduction of microglial kynurenine 3-monooxygenase activity, possibly accompanied by increased L-kynurenine influx from the circulation, results in increased kynurenic acid formation in astrocytes. See text for further details.
Forms, 38 is a major immunomodulator, which shows increased activity and expression in the brain in association with macrophage infiltration and microglial activation. 39 However, the activities of both TDO and IDO in the brain are normally very low. Under regular physiological conditions, their role(s) in the cerebral production of l-kynurenine are in fact difficult to ascertain. Thus, brain L-kynurenine derives to a significant extent from the peripheral circulation so that fluctuations in peripheral L-kynurenine formation have significant influence on brain KYNA formation and function. 40

Kynurenine 3-monoxygenase (KMO), which converts L-kynurenine to 3-hydroxykynurenine, is increasingly viewed as a major gatekeeper of the kynurenine pathway. This enzyme, too, shows much higher activity in peripheral tissues than in the brain. Because of its low capacity in the brain and relatively low Km for L-kynurenine (approximately 20 μM), this microglial enzyme is more rapidly saturated by rising brain L-kynurenine concentrations than astrocytic KATs (Km values: ~1 mM). 41 It follows that cerebral KMO exerts preferential control over the fate of L-kynurenine within the brain. Thus, in certain physiological or pathological situations, increased L-kynurenine influx from the blood or enhanced intracerebral synthesis of L-kynurenine will eventually exceed the catabolic capacity of KMO in microglia, raising L-kynurenine levels in astrocytes and, secondarily, promoting KYNA production. This effect can be expected to be aggravated by a persistent reduction of brain KMO activity (figure 1B).

Does KYNA Cause Cognitive Deficits in Schizophrenia?

The distinct neuromodulatory effects of KYNA might be pertinent to the pathophysiology of cognitive deficits in schizophrenia. 42–44 This is supported by studies in animals, which demonstrated that stimulation of cortical KYNA synthesis reliably causes deficits in (1) visuospatial working memory, (2) contextual learning and memory, and (3) PPI and habituation of auditory evoked potentials. 45–48 These neurophysiological measures, which are critically dependent on glutamatergic, nicotinergic, and dopaminergic transmission, are increasingly being used to examine cognitive functions known to be impaired in schizophrenia. Induction of these neurocognitive deficits, which were identified as primary domains for procognitive drug development by MATRICS (working memory, contextual learning) or as schizophrenia endophenotypes (PPI, sensory gating), 16,21 suggests that cortical KYNA may be an attractive new target for cognition enhancement.

Studies in humans provide more substantive evidence favoring a pathophysiological important role of impaired kynurenine pathway metabolism in schizophrenia (table 1). Whereas several earlier studies, including measurements of metabolite levels in urine (not referenced here), failed to reveal consistent pathway dysfunctions. 49,50 2 independent publications in 2001 reported that KYNA concentrations are significantly elevated in PFC and cerebrospinal fluid of schizophrenia patients. 51,52 These increases were probably unrelated to treatment with antipsychotic medications. Supporting evidence from studies in rats suggest that brain KYNA levels are in fact reduced after prolonged administration of conventional or second-generation antipsychotic medications. 53 Notably, the upregulation of KYNA levels in schizophrenia was accompanied by increases in the tissue levels of L-kynurenine, KYNA’s immediate bioprecursor. 51

The increased cortical L-kynurenine content in schizophrenia might be a direct consequence of enhanced synthesis catalyzed by either TDO or IDO (figure 1), both of which could be dysregulated during inflammatory and immune sensitization states. In individuals with schizophrenia, TDO gene (TDO2) expression and the density of TDO-immunopositive cells are indeed significantly elevated in 2 pivotal brain regions, ie, the PFC and the anterior cingulate cortex. 53,56 Furthermore, the effects of TDO2 gene dysregulation appear to be exacerbated in individuals with additional risk genes. 61 Notably, pathologically elevated TDO activity either in the periphery or in the brain is likely to generate even more L-kynurenine during infections or other insults to the immune system in schizophrenia. 62–64 On the other hand, and inconsistent with the idea that immune-activated IDO plays a significant role in the disease, 65 brain IDO gene (IDO1) expression appears to be normal in schizophrenia patients. 53

Recent evidence suggests that an alternate mechanism, namely a reduction in KMO activity in the periphery and/or in the brain, may underlie the abnormally high cortical L-kynurenine and KYNA levels in schizophrenia. The first indication came from Aoyama et al, 55 who reported an association between a single nucleotide polymorphism (SNP) (rs2275163) in the KMO gene and schizophrenia in an initial Japanese sample of 465 probands and 440 control subjects. However, the authors failed to replicate this finding in a similarly powered second sample, possibly due to genetic or clinical heterogeneity across both samples. In our own cohort of 248 schizophrenia and 228 healthy control subjects, we recently found the same KMO SNP to be significantly associated with predictive pursuit and visuospatial working memory deficits in schizophrenia. 58 Motivated by this endophenotype link, we conducted a follow-up study using tissue from the frontal eye field, a cortical brain region associated with abnormal eye tracking in schizophrenia patients. These postmortem analyses revealed significant
and highly correlated decreases in KMO gene expression (−33%) and KMO enzyme activity (−30%) in individuals with schizophrenia. Quantitatively very similar reductions in KMO activity were detected in the PFC (Brodmann areas 9 and 10). It is unlikely that these changes were related to medication exposure because cerebral KMO expression and KMO activity in rats were not altered by prolonged antipsychotic treatment (K.V. Sathyasaikumar, PhD, I.W., and R.S., unpublished data, 2009). Taken together, these results raise the possibility that decreased KMO activity, effected by genetic, environmental, or other causes and driving kynurenine pathway metabolism toward enhanced KYNA production, might be causally related to cognitive deficits in persons with schizophrenia (figure 1B).

Future Prospects: Envisioning Kynurenergic Interventions in Schizophrenia

Irrespective of the nature of the signaling and receptor impairments that underlie cognitive deficits, therapeutic strategies targeting more than one molecular mechanism are likely to be synergistic. Treatment approaches used to stimulate nicotinic receptors, such as the partial α7nAChR agonist DMXB-A or galantamine, which acts as an agonist at an allosteric potentiating site of the α7nAChR, have only yielded partly positive results. Similarly inconclusive results have been obtained using NMDAR agonists as cognition enhancers. Specific remediation of α7nAChR and NMDAR hypofunction by manipulating KYNA through rational pharmacology could be a crucial therapeutic innovation. This would be akin to the treatment of pathological conditions in other medical disciplines. For instance, in the treatment of hypertension, a single patient may be on a diuretic (to lower blood volume) and a beta-blocker (to reduce stroke volume) or a calcium channel blocker (to achieve arterial dilatation). These medications affect the cardiovascular system synergistically to attain/maintain blood pressure control.

The optimal approach to limit the effects of elevated brain KYNA levels is by direct pharmacological manipulation of kynurenine pathway metabolism. In principle, such "kynurenergic" agents do not need to penetrate into the brain but could be efficacious by reducing the peripheral levels of circulating L-kynurenine. This would lower brain uptake of the precursor and decrease cerebral KYNA formation. Examples in this category include inhibitors of TDO and/or IDO. Such compounds are available and, in the case of IDO inhibitors, are being developed for the treatment of a variety of diseases ranging from cancer to asthma and infectious diseases. Of note, IDO inhibitors are also beginning to attract attention for possible applications in psychiatry but are yet to be adequately tested by schizophrenia researchers.

Interventions resulting in the activation of KMO, too, might diminish peripheral and central L-kynurenine levels and consequently attenuate KYNA formation indirectly. While the rational design of enzyme activators is generally difficult, KMO is known to be stimulated in situations of immune sensitization. Insights into the molecular mechanisms underlying this increase in enzyme activity could conceivably provide structural hints for the design of specific KMO activators. Eventually, however, the potential clinical merit of this approach will have to be weighed against possible detrimental consequences of inducing increased kynurenine pathway flux toward the neurotoxins 3-hydroxykynurenine and quinolinic acid. This might be especially relevant in light of the observation that the cortical levels of another highly reactive—and potentially neurotoxic—pathway intermediate, 3-hydroxyanthranilic acid (figure 1), are elevated in individuals with schizophrenia.

The most prudent strategy to reduce brain KYNA is to target KATs, the proximal enzymes of KYNA synthesis. Although the mammalian brain contains at least 4 distinct KATs, it appears that one of these enzymes, now termed KAT II (=α-aminoisoadipate aminotransferase), preferentially controls a pool of KYNA that can...
be rapidly mobilized in the brain. Importantly, and in stark contrast to the other 3 KATs, KAT II is also distinguished by its substrate specificity, ie, abundant, common amino acids do not interfere with the enzyme’s ability to convert L-kynurenine to KYNA. Selective inhibition of KAT II is therefore a rational approach to attenuate KYNA formation in the brain with direct implications for the enhancement of cognitive processes. This hypothesis recently received strong support from experiments in rats, which were given a focal intra-PFC infusion of the specific KAT II inhibitor (S)-4-(ethylsulfanyl) benzoylalanine. This treatment not only caused expected, prompt reduction in extracellular KYNA but also resulted in substantial increases in the extracellular levels of glutamate, dopamine, and acetylcholine.

In complementary studies, mutant mice with a genomic deletion of KAT II revealed marked cognitive enhancement ex vivo, compared with wild-type controls. Thus, it appears that KAT II inhibition may indeed constitute a promising molecular strategy for cognitive improvement. This approach may not only be useful under normal physiological conditions but could be especially effective for improving cognition in individuals with schizophrenia, who present with elevated levels of KYNA in the PFC. Proof of concept studies are needed to test this novel hypothesis in human subjects using tolerable, brain-penetrable KAT II inhibitors.

Funding
National Institute of Mental Health (grants MH-77852 and MH-83729); National Institutes of Health (grant NIH-K12RR023250).

Acknowledgments
We are indebted to our colleagues at the Maryland Psychiatric Research Center, who contributed in many ways to the evolution of the concepts described in this article.

References


