Impaired Kynurenine Pathway Metabolism in The Prefrontal Cortex of Individuals With Schizophrenia

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The levels of kynurenic acid (KYNA), an astrocyte-derived metabolite of the branched kynurenine pathway (KP) of tryptophan degradation and antagonist of α7 nicotinic acetylcholine and N-methyl-D-aspartate receptors, are elevated in the prefrontal cortex (PFC) of individuals with schizophrenia (SZ). Because endogenous KYNA modulates extracellular glutamate and acetylcholine levels in the PFC, these increases may be pathophysiologically significant. Using brain tissue from SZ patients and matched controls, we now measured the activity of several KP enzymes (kynurenine 3-monooxygenase [KMO], kynureninase, 3-hydroxyanthranilic acid dioxygenase [3-HAO], quinolinic acid phosphoribosyltransferase [QPRT], and kynurenine aminotransferase II [KAT II]) in the PFC, ie, Brodmann areas (BA) 9 and 10. Compared with controls, the activities of KMO (in BA 9 and 10) and 3-HAO (in BA 9) were significantly reduced in SZ, though there were no significant differences between patients and controls in kynureninase, QPRT, and KAT II. In the same samples, we also confirmed the increase in the tissue levels of KYNA in SZ. As examined in rats treated chronically with the antipsychotic drug risperidone, the observed biochemical changes were not secondary to medication. A persistent reduction in KMO activity may have a particular bearing on pathology because it may signify a shift of KP metabolism toward enhanced KYNA synthesis. The present results further support the hypothesis that the normalization of cortical KP metabolism may constitute an effective new treatment strategy in SZ.
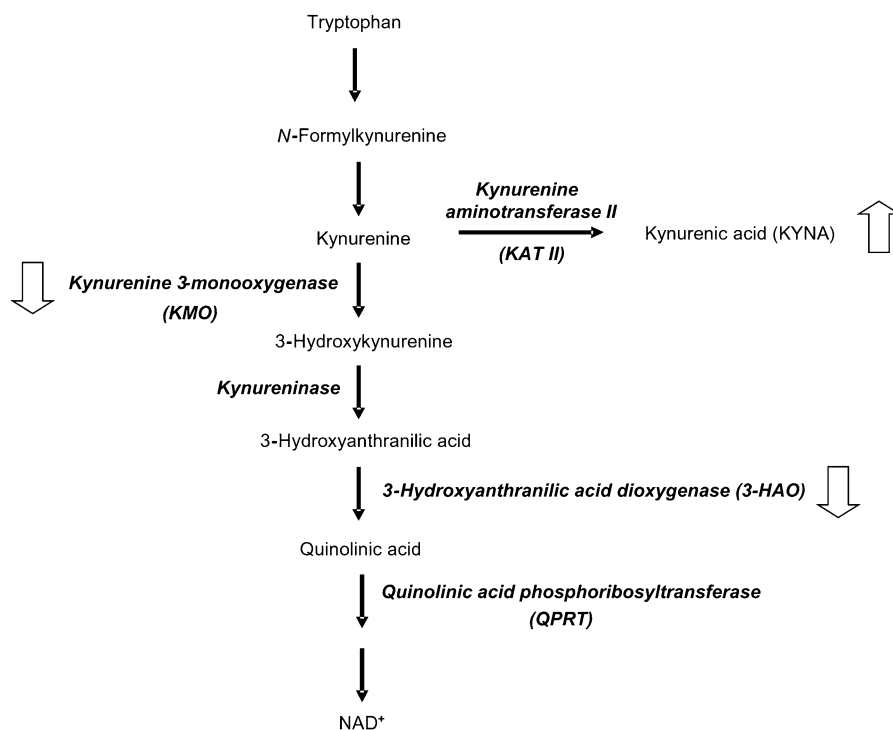
Key words: kynurenine3-monooxygenase (KMO)/ kynurenic acid/α7 nicotinic acetylcholine receptor/ NMDA receptor/prefrontal cortex

Introduction

Neuroanatomical, neurochemical, genetic, and functional studies have provided a large body of evidence linking the pathophysiology of schizophrenia (SZ) to dysfunction in the prefrontal cortex (PFC; see Gur et al[1], Pakkenberg et al[2], and Eisenberg and Berman[3] for recent reviews). These abnormalities are not only believed to play a critical role in psychosis but probably also account for the incapacitating cognitive deficits seen in patients, including poor memory and executive functions.[4–6] Most current thinking regarding the neurochemistry of cognitive impairment in SZ invokes distinct interdependent changes in major neurotransmitter systems within the PFC. Thus, while the precise nature and causes of the abnormalities are not well understood, and although there is an increased awareness of additional factors,[7–9] there is general consensus that changes in cholinergic and glutamatergic function are critically involved in the pathophysiology of SZ.[10,11]

Recent studies suggest that kynurenic acid (KYNA), a metabolite produced in a dead-end side arm of the kynurenine pathway (KP) of tryptophan degradation (figure 1), might also be involved in prefrontal dysfunctions in SZ. Present in the mammalian brain in low (rodents) to high (human) nanomolar concentrations, KYNA is an antagonist of two receptors that are closely linked to cognitive phenomena and psychosis, ie, the α7 nicotinic acetylcholine receptor (α7nAChR)[12] and the N-methyl-D-aspartate (NMDA) receptor.[13] By reducing the function of one or both of these receptors, increases in brain KYNA levels might therefore cause hyponicotinergic and hypoglutamatergic conditions. Acute, KYNA-induced blockade of α7nAChR, in particular, has been shown to have interesting downstream effects in the PFC, including decreases in the extracellular levels

The Kynurenine Pathway of Tryptophan Degradation

Tryptophan

↓

N-Formylkynurenine

↓

Kynurenine → **Kynurenine aminotransferase II (KAT II)** → Kynurenic acid (KYNA) ⇑

Kynurenine 3-monooxygenase (KMO) ⇓

↓

3-Hydroxykynurenine

Kynureninase ↓

3-Hydroxyanthranilic acid

↓ **3-Hydroxyanthranilic acid dioxygenase (3-HAO)** ⇓

Quinolinic acid

↓ **Quinolinic acid phosphoribosyltransferase (QPRT)**

↓

NAD⁺

Fig. 1. The kynurenine pathway of tryptophan degradation, indicating the enzymes measured in the present study. Large open arrows: differences between schizophrenia and control tissues, as described in the text.

of glutamate.[14,15] On a functional level, enhanced brain KYNA has been demonstrated to cause cognitive deficits in animals.[16–19] Interestingly, reductions in brain KYNA levels lead to *increases* in the extracellular concentrations of glutamate and acetylcholine, indicating that endogenous KYNA might function as a bidirectional modulator of glutamatergic and nicotinergic neurotransmission.[14,15,20] Notably, this reduction in brain KYNA formation causes significant cognitive improvements, which can be demonstrated both in behavioral paradigms and using electrophysiological outcome measures.[21] Taken together, these findings underscore the need to understand the dynamics of the disposition and function of KYNA in the mammalian brain.

We reported previously that KYNA levels in the PFC are significantly elevated in individuals with SZ.[22] The present study constitutes a first effort to explore the cause(s) of these high KYNA levels. To this end, we used postmortem tissue samples from SZ patients and matched control subjects and determined the activities of several KP enzymes in two topographically defined key regions of the PFC, Brodmann areas (BA) 9 and 10. All enzymes selected, ie, kynurenine aminotransferase II (KAT II), kynurenine 3-monooxygenase (KMO), kynureninase, 3-hydroxyanthranilic acid dioxygenase (3-HAO), and quinolinic acid phosphoribosyltransferase (QPRT), act downstream of the pivotal KP

metabolite kynurenine. These enzymes are located in two physically segregated arms of the pathway, producing KYNA in astrocytes and the free radical generators 3-hydroxykynurenine and 3-hydroxyanthranilic acid, as well as the excitotoxic NMDA receptor agonist quinolinic acid, in microglial cells[23] (figure 1). Possibly signifying a shift toward enhanced KYNA formation, our results revealed significant reductions in enzyme activities in the microglial branch of the KP in the disease.

Methods

Chemicals

L-kynurenine (sulfate form; "kynurenine"), DL-3-hydroxykynurenine, and KYNA were purchased from Sigma Chemical Co. (St Louis, Missouri). All other biochemicals and chemicals were "reagent grade" and were purchased from a variety of commercial suppliers. Risperidone (Risperdal; Janssen-Ortho) was obtained from Global Drugs Direct (Vancouver, British Columbia, Canada). The KMO inhibitor Ro 61-8048 was kindly provided by Dr W. Fröstl (Novartis, Basel, Switzerland).

Radioactive enzyme substrates were custom synthesized by Amersham Corp. (Arlington Heights, Illinois; L-5-³H-kynurenine [12 Ci/mmol] and ³H-quinolinic acid [27 Ci/mmol]) and DuPont/New England Nuclear

(Boston, Massachusetts; 1-^{14}C-3-hydroxyanthranilic acid [6 mCi/mmol]).

Human Brain Tissue

Specimens were obtained from the Maryland Brain Collection, a repository of postmortem tissue maintained in cooperation with the Office of the Chief Medical Examiner of the State of Maryland and housed at the Maryland Psychiatric Research Center. Normal control subjects ($n = 15$) were free of neurological or psychiatric disorders. Patients ($n = 15$) met *Diagnostic and Statistical Manual of Mental Disorders, Third Edition Revised,* criteria for SZ, ascertained by consensus of two research psychiatrists. The diagnosis was based on information from clinical records and family members. The latter were interviewed by phone, using the Diagnostic Evaluation after Death[24] and an informant version of the Structured Clinical Interview. In cases where death was witnessed, the time between death and autopsy was taken as the postmortem interval (PMI). Otherwise, the PMI was defined as the time halfway between the brain donor being found dead and being last seen alive. Only cases with a PMI of less than 24 hours were used for this study.

Two regions of the cerebral cortex (BA 9 and BA 10) were dissected out, and the tissue was stored at $-80°C$ prior to analysis. Brain samples were weighed while frozen and then homogenized (1:5, wt/vol) by sonication (Branson Ultrasonics Corp., Danbury, Connecticut) in ultrapure water. The tissue homogenate was then divided into aliquots for the determination of KYNA levels and KP enzyme activities. For each tissue preparation, all enzyme assays were performed on the same day.

Animals

Adult male Sprague-Dawley rats (200–250 g; Charles River Laboratories, Kingston, New York) were used to assess the effects of chronic risperidone treatment. The animals were group-housed in a temperature-controlled Association for Assessment and Accreditation of Laboratory Animal Care-approved animal facility at the University of Maryland School of Medicine and were kept on a 12/12-hour light/dark cycle with free access to food and water.

Animals received daily intraperitoneal (i.p.) injections of risperidone (3 mg/kg body weight[25]) at 4 PM. Vehicle-treated rats served as controls. After 28 days, rats were deeply anesthetized with chloral hydrate (360 mg/kg, i.p.) and decapitated. Their frontal cortex was rapidly dissected out, frozen on dry ice, and stored at $-80°C$. After thawing, the tissue was processed for KYNA and enzyme analyses, as described below for human brain tissue.

KYNA Measurement

The original tissue homogenate was further diluted (1:1, vol/vol) with ultrapure water. Perchloric acid (6%; 50 µl for human and 25 µl for rat) was then added to 100 µl of the tissue preparation, and the precipitated proteins were removed by centrifugation ($16000g$, 15 min). Twenty microliters of the resulting supernatant were subjected to high-performance liquid chromatography (HPLC) analysis. KYNA was isocratically eluted from a 3-µm C_{18} reverse-phase column (80 mm × 4.6 mm; ESA, Chelmsford, Massachusetts), using a mobile phase containing 250mM zinc acetate, 50mM sodium acetate, and 5% acetonitrile (pH adjusted to 6.2 with glacial acetic acid), using a flow rate of 1.0 ml/minute. In the eluate, KYNA was quantitated fluorimetrically (excitation: 344 nm, emission: 398 nm; Perkin Elmer Series 200 fluorescence detector [Perkin Elmer, Waltham, Massachusetts]). The retention time of KYNA was approximately 7 minutes.

Enzyme Analyses

KMO Activity. After diluting the original homogenate 1:10 (vol/vol) in 100mM Tris-HCl buffer (pH 8.1) containing 10mM KCl and 1mM EDTA, 100 µl of the tissue preparation were incubated for 40 minutes at 37°C in a solution containing 1 mM NADPH (nicotinamide adenine dinucleotide phosphate; reduced form), 3mM glucose-6-phosphate, 1 U/ml glucose-6-phosphate dehydrogenase, 100µM kynurenine, 10mM KCl, and 1mM EDTA in a total volume of 200 µl. For kinetic analyses, because of limited tissue availability, equal tissue aliquots were pooled from five individuals per group (controls and SZ). The pooled tissues were homogenized (1:25, wt/vol), and KMO activity was determined in the presence of 1–100µM kynurenine. The reaction was stopped by the addition of 50 µl of 6% perchloric acid. Blanks were obtained either by adding the tissue preparation at the end of the incubation, ie, prior to the denaturing acid, or by including the specific enzyme inhibitor Ro 61-8048[26] (100µM) in the incubation solution. After centrifugation ($16000g$, 15 min), 20 µl of the supernatant was applied to a 3-µm HPLC column (HR-80; 80 mm × 4.6 mm; ESA), using a mobile phase consisting of 1.5% acetonitrile, 0.9% triethylamine, 0.59% phosphoric acid, 0.27mM EDTA, and 8.9mM sodium heptane sulfonic acid. In the eluate, the reaction product, 3-hydroxykynurenine, was detected electrochemically using either a Coulochem 5100A detector[27] (ESA; oxidation potential: +0.2 V; flow rate: 1.0 ml/min) or an HTEC 500 detector (Eicom Corp., San Diego, California; oxidation potential: +0.5 V; flow rate: 0.5 ml/min). Depending on the flow rate, the retention time of 3-hydroxykynurenine varied between 5.5 and 11 minutes.

KMO activity in rat brain was determined in an identical manner except that 80 µl of a 1:10 (wt/vol) homogenate were used in the assay.

Kynureninase Activity. The original tissue homogenate was further diluted (1:100, vol/vol) with 5mM Tris-HCl (pH 8.4) containing 10mM of 2-mercaptoethanol and

50μM pyridoxal-5′-phosphate. Eighty microliters of the tissue preparation were then incubated for 2 hours at 37°C in a solution containing 90mM Tris-HCl buffer (pH 8.4) and 4μM DL-3-hydroxykynurenine in a total volume of 200 μl. The reaction was terminated by adding 50 μl of 6% perchloric acid. To obtain blanks, tissue homogenate was added at the end of the incubation, ie, immediately prior to the denaturing acid. After centrifugation to remove the precipitate (16 000g, 15 min), 25 μl of the resulting supernatant was applied to a 5-μm C_{18} reverse-phase HPLC column (Adsorbosil; 150 mm × 4.6 mm; Grace, Deerfield, Illinois) using a mobile phase containing 100mM sodium acetate (pH 5.8) and 1% acetonitrile at a flow rate of 1.0 ml/minute. In the eluate, the reaction product, 3-hydroxyanthranilic acid, was detected fluorimetrically (Perkin Elmer Series 200) using an excitation wavelength of 322 nm and an emission wavelength of 414 nm. The retention time of 3-hydroxyanthranilic acid was ~4 minutes.

3-HAO Activity   The original tissue homogenate was diluted (1:4, vol/vol) in 60mM 2-(*N*-morpholino)ethane sulfonic acid buffer, pH 6.0, and 100 μl of the tissue preparation were incubated for 1 hour at 37°C in a solution containing 153μM $Fe(NH_4)_2SO_4$, 0.01% ascorbic acid, and 3μM [1-^{14}C]-3-hydroxyanthranilic acid (5 nCi) in a total volume of 200 μl. Blanks were obtained using heat-inactivated tissue. The reaction was terminated by the addition of 50 μl of 6% perchloric acid, and the resulting precipitate was removed by centrifugation (16 000g, 15 min). The supernatant, containing newly formed ^{14}C-quinolinic acid, was applied to a Dowex 50W (H^+-form) cation exchange column, and radioactivity in the eluate was quantitated by liquid scintillation spectrometry.[28]

The same protocol was followed to measure 3-HAO activity in rat brain, using 100 μl of the original tissue homogenate (1:10, wt/vol).

QPRT Activity.   Forty microliters of the original tissue homogenate were incubated for 2 hours at 37°C in a solution containing 50mM potassium phosphate buffer (pH 6.5), 10mM 2-mercaptoethanol, 1mM $MgCl_2$, 1mM phosphoribosylpyrophosphate, and 20nM [^{3}H]-quinolinic acid (30 nCi) in a total volume of 500 μl. Blanks were obtained using heat-inactivated tissue. The reaction was terminated by placing the tubes on ice, and particulate matter was separated by centrifugation (16000g, 10 min). Newly formed ^{3}H-nicotinic acid mononucleotide was recovered from a Dowex AG 1 × 8 anion exchange column and quantitated by liquid scintillation spectrometry.[29]

KAT II Activity.   The original tissue homogenate was further diluted (1:1, vol/vol) in 5mM Tris-acetate buffer, pH 8.0, containing 10mM 2-mercaptoethanol and 50μM pyridoxal-5′-phosphate. Eighty microliters of this

preparation were incubated for 20 hours at 37°C in a reaction mixture containing 150mM Tris-acetate buffer, pH 7.4, 2μM kynurenine, 0.79μM ^{3}H-kynurenine (23 nCi), 1mM pyruvate, and 80μM pyridoxal-5′-phosphate (total volume: 200 μl).[30,31] Blanks were obtained using heat-inactivated tissue. The reaction was terminated by the addition of 20 μl of 50% (wt/vol) trichloroacetic acid and 1 ml of 0.1 M HCl, and the precipitated proteins were removed by centrifugation (16000g, 10 min). Newly produced ^{3}H-KYNA was then purified by cation exchange chromatography (Dowex 50W; H^+-form) and quantitated by liquid scintillation spectrometry as described.[31]

Protein Determination

The protein content of tissue samples was determined by the method of Lowry et al[32] using bovine serum albumin as a standard.

Data Analysis

Data from controls and SZ patients were compared using SAS PROC MIXED to fit a mixed model for repeat measures ANCOVA, using the model: analyte = age + brain region (BA 9 or BA 10) + diagnosis (control or SZ) + diagnosis × brain region, where the repeated factor was multiple samples from two regions of the same brain. Post hoc contrasts from this model were used to estimate age-adjusted differences between groups for each brain region.

Differences in the distribution of analytes were compared between individuals with SZ who were on and off antipsychotic medication using the Wilcoxon statistic, with *P*-values calculated by the exact (permutation) method, as implemented in SAS PROC NPAR1WAY.

Data obtained in control and risperidone-treated rats were compared using Student *t* test.

Results

Demographics

Age, sex, PMI, subtype of SZ, and antipsychotic treatment at the time of death are listed for all brain donors (table 1). The cases were well matched and showed no statistically significant group differences in any of the demographic categories. Moreover, there was no apparent effect of PMI, sex, or drug treatment on any of the biochemical measures described here. Effects of age (<70 years) were similar in both groups and were adjusted for in ANCOVA models comparing the groups.

Kynurenic Acid

Mean KYNA concentrations in control tissue in BA 9 and BA 10 were 1.7 ± 0.2 and 2.2 ± 0.2 pmol/mg protein, respectively (mean ± standard error of the mean [SEM]).

Table 1. Demographics of Brain Donors

| Group	A/G/R	PMI	SubDx	Antipsychotic
Control	61/M/C	17		None
Control	49/M/C	7		None
Control	23/F/C	12		None
Control	45/M/C	6		None
Control	37/M/AA	9		None
Control	31/M/AA	8		None
Control	68/F/C	4		None
Control	46/M/AA	16		None
Control	57/F/C	22		None
Control	74/F/C	5		None
Control	37/M/AA	5		None
Control	35/M/C	4		None
Control	63/M/C	20		None
Control	29/F/AA	19		None
Control	79/M/AA	3		None
SZ	61/F/C	20	Unknown	Off
SZ	54/M/C	24	Paranoid	On
SZ	31/M/AA	14	CUT	On
SZ	51/M/C	2	CUT	On
SZ	41/M/AA	16	CUT	On
SZ	31/M/AA	8	Disorganized	On
SZ	78/F/C	25	Paranoid	On
SZ	37/M/C	3	Paranoid	On
SZ	58/M/AA	3	Paranoid	On
SZ	83/M/C	3	Disorganized	On
SZ	67/F/C	4	CUT	On
SZ	39/M/C	11	Paranoid	On
SZ	56/F/AA	5	CUT	On
SZ	34/M/AA	11	CUT	Off
SZ	30/M/C	16	Unknown	Off

Note: A, age (in y); G, gender; M, male, F, female; R, race; C, Caucasian; AA, African American; PMI, postmortem interval (in h); CUT, chronic undifferentiated type; SubDx, subdiagnosis; SZ, schizophrenia. Age (mean ± SEM)—controls: 46.7 ± 4.2; SZ: 50.0 ± 4.4. PMI (mean ± SEM)—controls: 11.0 ± 1.7; SZ: 11.0 ± 2.0.

As illustrated in figure 2 using individual data points, average KYNA values in individuals with SZ were elevated compared with controls (by 46.8% and 83.4%, respectively), with the difference approaching significance in BA 9 [$t(27) = 1.98$, $P = .058$] and attaining significance in BA 10 [$t(28) = 2.73$, $P = .011$].

Enzyme Activities

KMO. Control KMO activities in BA 9 and BA 10 were 25.7 ± 2.9 and 28.9 ± 3.3 pmol/hour/mg protein, respectively (mean ± SEM). Compared with controls, enzyme activity in SZ patients was reduced by 36.0% [$t(27) = 2.62$, $P = .014$] and 38.3% [$t(28) = 2.55$, $P = .017$], respectively, in these brain areas (figure 3A). Kinetic analyses, obtained from single tissue preparations using equal aliquots from five control and five SZ brain homogenates, indicated that the reductions in enzyme activity in SZ in both BA 9 (figure 3B) and BA10 (figure 3C) appeared to be a reflection of decreased v_{max} values rather than
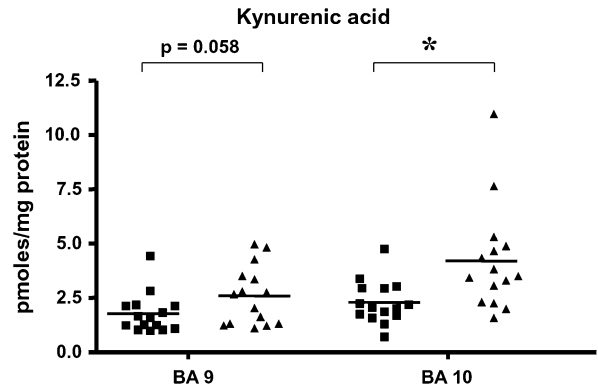
Fig. 2. Individual case representation of kynurenic acid levels in BA 9 and BA 10 in controls (squares) and schizophrenia patients (triangles). Horizontal lines indicate mean values. *$P < .05$ (ANCOVA post hoc test).

changes in substrate affinity (BA 9—control: v_{max} = 2.1 pmol/hour/mg tissue, K_m = 37.1μM; SZ: v_{max} = 1.7 pmol/hour/mg tissue, K_m = 49.7μM; BA 10—control: v_{max} = 4.6 pmol/hour/mg tissue, K_m = 73.6μM; SZ: v_{max} = 1.3 pmol/hour/mg tissue, K_m = 30.8μM).

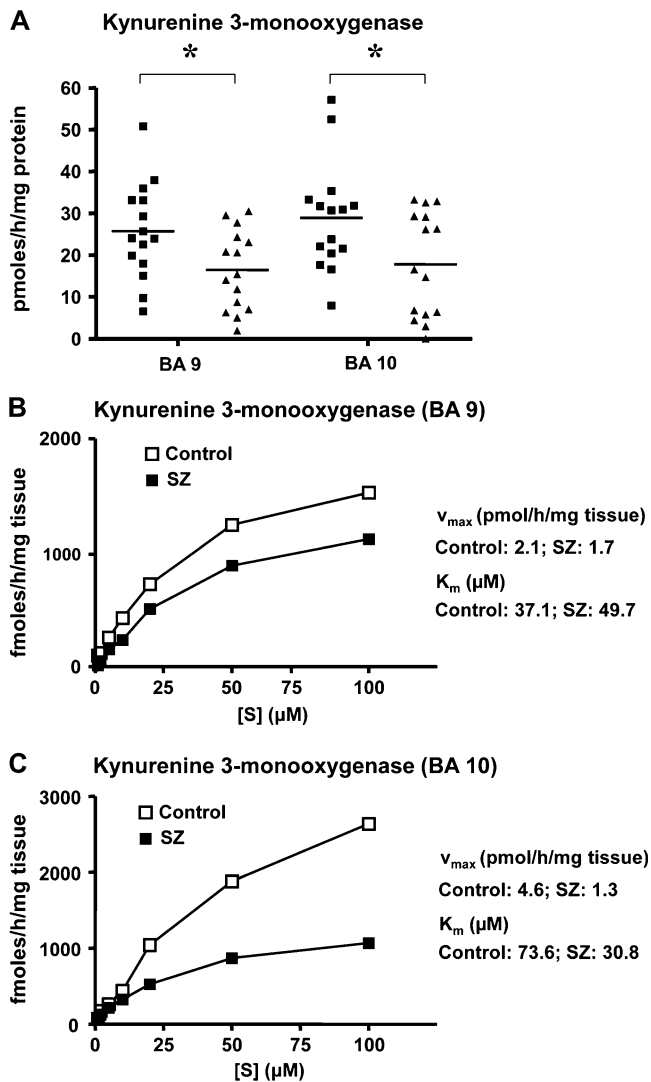*3-HAO.* Control 3-HAO activities in BA 9 and BA 10 were 297.9 ± 53.8 and 127.7 ± 33.6 pmol/hour/mg protein, respectively (mean ± SEM). Compared with controls, decreases in enzyme activity were observed in SZ patients in both BA 9 and BA 10 (figure 4). This reduction was statistically significant in BA 9 [$t(27) = 2.76$, $P = .01$] but not in BA 10 [$t(27)=0.85$, $P = .40$].

Kynureninase, QPRT, and KAT II. Control activities for kynureninase, QPRT, and KAT II in BA 9 and BA 10 were 86.1 ± 14.3 and 55.3 ± 10.3 pmol/hour/mg protein, 66.8 ± 9.6 and 63.5 ± 10.0 fmol/hour/mg protein, and 0.21 ± 0.02 and 0.16 ± 0.02 pmol/hour/mg protein, respectively (means ± SEM). None of these enzyme activities showed differences between controls and SZ patients in BA 9 and BA 10 (minimum $P > .22$; figures 5A–C).

Chronic Risperidone Treatment in Rats

In order to control for possible effects of antipsychotic medication, the tissue levels of KYNA and the activities of the two KP enzymes that had shown significant changes in SZ (KMO and 3-HAO) were determined in the frontal cortex of rats that had been treated for 28 days with risperidone or vehicle ($n = 8$ per group). Control levels of KYNA were 181.3 ± 59.5 fmol/mg protein, and control enzyme activities were 13.2 ± 3.7 (KMO) and 198.0 ± 15.6 (3-HAO) pmol/hour/mg protein, respectively (mean ± SEM). Neither KYNA levels [$t(14)$ = 0.33, $P = .74$] nor KMO [$t(14) = 0.81$, $P = .43$] or 3-HAO [$t(14) = 0.14$, $P = .89$] activity differed significantly between risperidone- and vehicle-treated animals (figure 6).

A Kynurenine 3-monooxygenase

B Kynurenine 3-monooxygenase (BA 9)

v_{max} (pmol/h/mg tissue)
Control: 2.1; SZ: 1.7

K_m (μM)
Control: 37.1; SZ: 49.7

C Kynurenine 3-monooxygenase (BA 10)

v_{max} (pmol/h/mg tissue)
Control: 4.6; SZ: 1.3

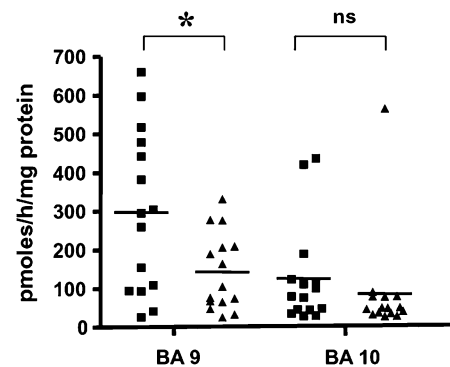K_m (μM)
Control: 73.6; SZ: 30.8

Fig. 3. (A) Individual case representation of kynurenine 3-monooxygenase (KMO) activity in BA 9 and BA 10 in controls (squares) and schizophrenia (SZ) patients (triangles). Horizontal lines indicate mean values. *$P < .05$ (ANCOVA post hoc test). (B and C) Kinetic characteristics of KMO activity in BA 9 and BA 10, respectively. [S], kynurenine concentration.
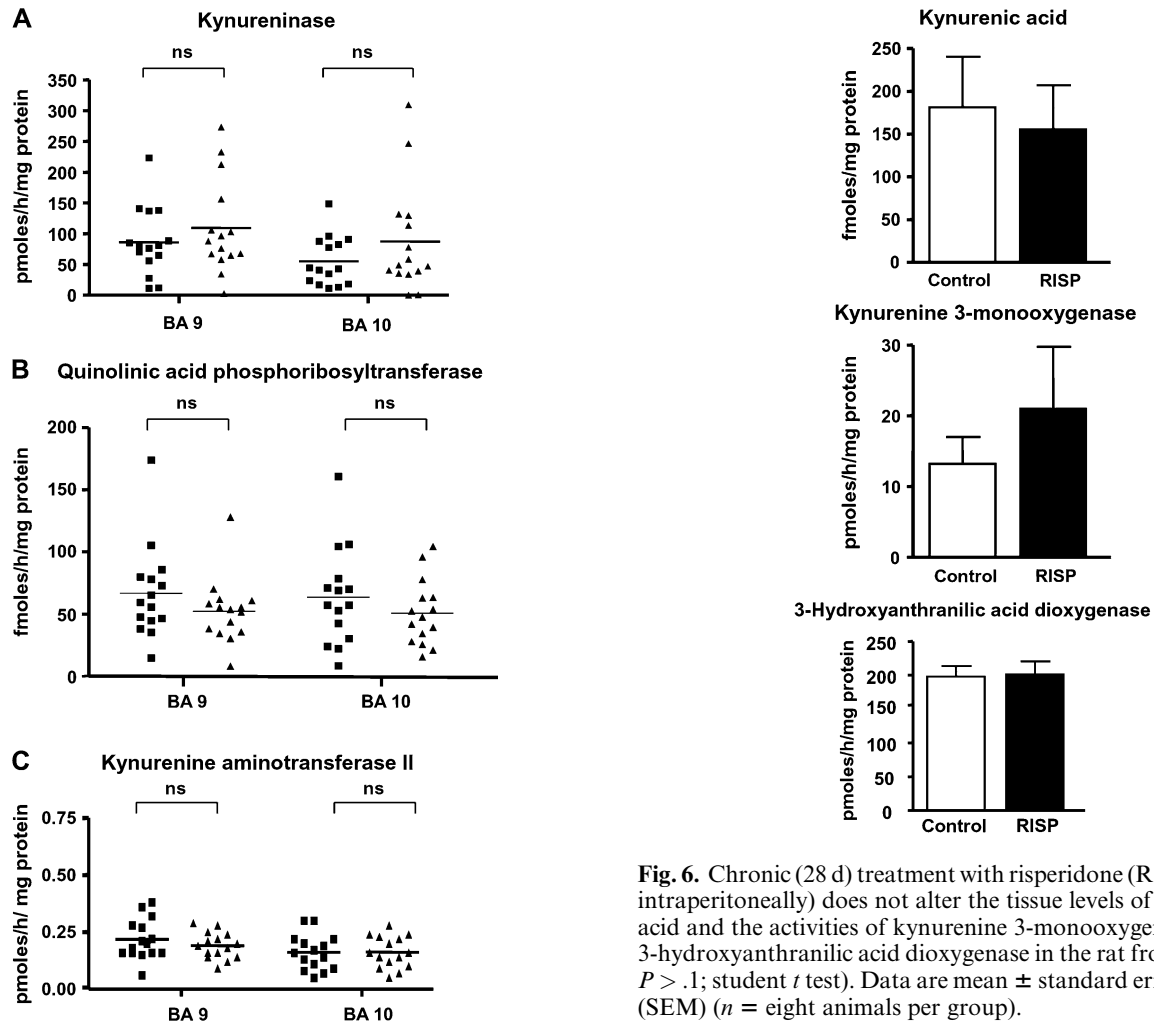
3-Hydroxyanthranilic acid dioxygenase

Fig. 4. Individual case representation of 3-hydroxyanthranilic acid dioxygenase activity in BA 9 and BA 10 in controls (squares) and schizophrenia patients (triangles). Horizontal lines indicate mean values. *$P < .05$ vs controls (ANCOVA post hoc test). ns, not significant.

The tryptophan metabolite kynurenine occupies a central position in the KP (figure 1). In the brain, kynurenine gives rise to two physically segregated branches of the pathway, producing 3-hydroxykynurenine and its downstream metabolites 3-hydroxyanthranilic acid and quinolinic acid in microglial cells and KYNA in astrocytes (cf Introduction).[23] Excessive formation of the three microglial compounds, which are neurotoxins and generators of highly reactive free radicals, may play significant roles in brain pathology.[33–37] Astrocyte-derived KYNA, in contrast, has neuroprotective properties due to its ability to block neuronal excitation and scavenge free radicals.[37–39]

Of the enzymes that use kynurenine as a substrate, KMO is the most specific and has the lowest K_m, and is therefore rate limiting. Reduced KMO activity will decrease the flux of the KP toward quinolinic acid and might therefore indirectly provide a degree of neuroprotection. We have previously proposed that this can be exploited for the treatment of Huntington's disease and other neurodegenerative disorders by cautiously targeting KMO with specific enzyme inhibitors.[40]

The present study revealed a significant decrease in KMO activity in the PFC of individuals with SZ. This reduction, which was tentatively linked to a lower v_{max} rather than to a K_m change, was not accompanied by a decrease in the activity of kynureninase, the next enzyme in the metabolic cascade. On the contrary, kynureninase activity in SZ tended to be higher than in controls, though the difference did not attain statistical significance in either of the two prefrontal regions studied. It therefore appears that the observed reduction in KMO activity is not a reflection of a generalized microglial abnormality, which has been invoked to play a significant role in SZ and interpreted as an indication of a compromised immune system in the disease.[41–45] In light of recent studies, it is more likely that the impairment of KMO activity

Discussion

The present study, which was designed to explore mechanisms underlying the increase in brain KYNA levels in the PFC of individuals with SZ, revealed distinct abnormalities in KP enzymes in both cortical regions studied, ie, BA 9 and BA 10. These changes in SZ, which were not related to demographics, were restricted to enzymes in the main branch of the KP, whereas the activity of KAT II, in the side arm of the pathway, was in the normal range. Moreover, both the patients' medication history and complementary studies in chronically risperidone-treated rats indicated that the results of our study were not affected by antipsychotic drug use. Our data therefore provide insights into the pathophysiology of SZ and also suggest new treatment strategies.

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A Kynureninase

B Quinolinic acid phosphoribosyltransferase

C Kynurenine aminotransferase II

Fig. 5. Individual case representation of kynureninase (A), quinolinic acid phosphoribosyltransferase (B), and kynurenine aminotransferase II (C) activity in BA 9 and BA 10 in controls (squares) and schizophrenia (SZ) patients (triangles). Horizontal lines indicate mean values. None of the enzymes showed differences between controls and SZ patients (all $P > .05$; ANCOVA post hoc tests). ns, not significant.

Kynurenic acid

Kynurenine 3-monooxygenase

3-Hydroxyanthranilic acid dioxygenase

Fig. 6. Chronic (28 d) treatment with risperidone (RISP; 3 mg/kg/d, intraperitoneally) does not alter the tissue levels of kynurenic acid and the activities of kynurenine 3-monooxygenase and 3-hydroxyanthranilic acid dioxygenase in the rat frontal cortex (all $P > .1$; student t test). Data are mean ± standard error of the mean (SEM) (n = eight animals per group).

in SZ is selective, possibly due to functional sequence variants in the *KMO* gene.[46,47]

The activity of 3-HAO, which catalyzes the formation of the NMDA receptor agonist quinolinic acid from 3-hydroxyanthranilic acid, was found to be reduced in BA 9, ie, the dorsolateral subdivision of the PFC that is preferentially involved in sustaining attention and working memory.[48] A tendency toward lower 3-HAO activity was also observed in BA 10, though the results were not statistically significant. Decreased 3-HAO activity might account for the elevation in the tissue levels of 3-hydroxyanthranilic acid in SZ, which was recently demonstrated in the anterior cingulate cortex[49] and might affect the redox status of neurons and glial cells in the area (see above). In addition, reduced 3-HAO activity

will translate into lower quinolinic acid formation and may thus possibly contribute to NMDA receptor hypofunction.

No disease-related changes were seen in the activity of the next enzyme in the cascade, QPRT, in either region of the PFC. This further supports the notion that distinct, rather than generalized, KP impairments exist in the brain of patients with SZ. Studies currently in progress in our laboratory are designed to elucidate the genetic underpinnings and molecular mechanisms of the discrete anomalies in KP metabolism reported here. In particular, we are investigating the possible role of cosubstrates, cofactors, and regulators of the two impaired oxygenases, ie, KMO and 3-HAO, such as molecular oxygen, metal ions, and the endogenous anti-oxidant glutathione,[50,51] all of which are established risk factors in SZ (see Brown and Susser[52] and Do et al[53] for recent reviews).

The question then arises whether and how specific impairments in KP enzymes might account for the significant increases in prefrontal KYNA levels in SZ, which were originally described in 2001.[22] The most parsimonious explanation would be that a reduction in KMO activity eventually triggers a shift in cerebral KP metabolism

toward enhanced KYNA formation in SZ. As demonstrated in a recent in vivo study in rats, such a redirection of KP metabolism toward increased KYNA synthesis does not occur in the normal brain when KMO activity is acutely reduced by pharmacological means.[54] However, KYNA production is indeed enhanced under these conditions when the experiment is performed in injured brain tissue where glial functions are abnormal.[54] This mechanism may therefore also operate in SZ, where microglial and astrocytic anomalies in the PFC have been repeatedly described (see above).[42,55–57] Moreover, it is quite conceivable that *prolonged* downregulation of KMO, as opposed to the effects of *acute* enzyme inhibition studied by Amori et al,[54] will eventually favor KYNA synthesis over the synthesis of 3-hydroxykynurenine.

The dynamics of the pivotal metabolite kynurenine deserve special consideration in a discussion of possible functional interactions between the two KP branches in the brain of individuals with SZ. Postmortem analysis reveals that kynurenine levels are elevated in the PFC of patients, and this increase is correlated with KYNA levels in the same tissue.[22] The explanation for this nexus seems unambiguous because the high K_m of KAT II and all other cerebral kynurenine aminotransferases allows for a proportional increase in KYNA formation when kynurenine levels rise.[58] The *cause* of increased kynurenine levels in the brain of SZ patients is less clear. This elevation, which is also seen in the cerebrospinal fluid[59] and must therefore include changes in the extracellular milieu, may be directly related to reduced KMO activity, ie, to an accumulation of the enzyme's substrate. Alternatively or quite possibly in addition, kynurenine levels in the SZ brain might be elevated due to increased activity of the biosynthetic enzymes tryptophan 2,3-dioxygenase[60] or indoleamine-2,3-dioxygenase.[43] Notably, these two enzymes, like the entire cerebral KP pathway, are preferentially localized in glial cells,[23,60–62] and newly produced kynurenine is readily liberated into the extracellular compartment.[63]

Irrespective of the underlying enzymatic and cellular mechanism(s), there are reasons to assume that the observed increase in prefrontal KYNA levels plays a role in the pathophysiology of SZ.[22,64] Within the PFC, astrocyte-derived KYNA controls the levels of acetylcholine and glutamate[14,15,20] by initially targeting and thus reducing the activity of α7nAChRs.[12] Thus, increased KYNA levels trigger or exacerbate the nicotinergic and glutamatergic deficits, which have been credibly linked to both cognitive dysfunctions and psychotic manifestations in humans (cf Introduction).[1–3,65,66]

The demonstration of distinct impairments in cerebral KP metabolism in SZ, which are also observed in the basal ganglia,[67] raises the prospect that more than one KP enzyme could be targeted to provide clinical benefits in the disease. This idea, which is an extension of our recently proposed strategy to use selective KAT II inhibitors as cognition enhancers in SZ,[68] includes interventions that are aimed specifically at normalizing KMO and 3-HAO activity in the brain of patients. We are currently testing this concept in relevant animal models of SZ.

References

1. Gur RE, Keshavan MS, Lawrie SM. Deconstructing psychosis with human brain imaging. *Schizophr Bull*. 2007;33: 921–931.
2. Pakkenberg B, Scheel-Krüger J, Kristiansen LV. Schizophrenia; from structure to function with special focus on the mediodorsal thalamic prefrontal loop. *Acta Psychiatr Scand*. 2009;120:345–354.
3. Eisenberg DP, Berman KF. Executive function, neural circuitry, and genetic mechanisms in schizophrenia. *Neuropsychopharmacology*. 2010;35:258–277.
4. Tan HY, Callicott JH, Weinberger DR. Dysfunctional and compensatory prefrontal cortical systems, genes and the pathogenesis of schizophrenia. *Cereb Cortex*. 2007;17(suppl 1):171–181.
5. Kerns JG, Nuechterlein KH, Braver TS, Barch DM. Executive functioning component mechanisms and schizophrenia. *Biol Psychiatry*. 2008;64:26–33.
6. Ragland JD, Laird AR, Ranganath C, Blumenfeld RS, Gonzales SM, Glahn DC. Prefrontal activation deficits during episodic memory in schizophrenia. *Am J Psychiatry*. 2009;166:863–874.
7. Clarke HF, Walker SC, Crofts HS, Dalley JW, Robbins TW, Roberts AC. Prefrontal serotonin depletion affects reversal learning but not attentional set shifting. *J Neurosci*. 2005;25:532–538.
8. Friedman JI, Stewart DG, Gorman JM. Potential noradrenergic targets for cognitive enhancement in schizophrenia. *CNS Spectr*. 2004;9:350–355.
9. Lewis DA, Hashimoto T, Morris HM. Cell and receptor type-specific alterations in markers of GABA neurotransmission in the prefrontal cortex of subjects with schizophrenia. *Neurotox Res*. 2008;14:237–248.
10. Sarter M, Nelson CL, Bruno JP. Cortical cholinergic transmission and cortical information processing in schizophrenia. *Schizophr Bull*. 2005;31:117–138.

11. Lewis DA, Moghaddam B. Cognitive dysfunction in schizophrenia: convergence of gamma-aminobutyric acid and glutamate alterations. *Arch Neurol*. 2006;63:1372–1376.

12. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. *J Neurosci*. 2001;21:7463–7473.

13. Kessler M, Terramani T, Lynch G, Baudry M. A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. *J Neurochem*. 1989;52:1319–1328.

14. Konradsson-Geuken Å, Wu H-Q, Gash CR, et al. Changes in cortical kynurenic acid bi-directionally modulate prefrontal glutamate levels as assessed by microdialysis and rapid electrochemistry. *Neuroscience*. 2010;169:1848–1859.

15. Wu H-Q, Pereira EF, Bruno JP, Pellicciari R, Albuquerque EX, Schwarcz R. The astrocyte-derived alpha7 nicotinic receptor antagonist kynurenic acid controls extracellular glutamate levels in the prefrontal cortex. *J Mol Neurosci*. 2010;40:204–210.

16. Shepard PD, Joy B, Clerkin L, Schwarcz R. Micromolar brain levels of kynurenic acid are associated with a disruption of auditory sensory gating in the rat. *Neuropsychopharmacology*. 2003;28:1454–1462.

17. Erhardt S, Schwieler L, Emanuelsson C, Geyer M. Endogenous kynurenic acid disrupts prepulse inhibition. *Biol Psychiatry*. 2004;56:255–260.

18. Chess AC, Simoni MK, Alling TE, Bucci DJ. Elevations of endogenous kynurenic acid produce spatial working memory deficits. *Schizophr Bull*. 2007;33:797–804.

19. Chess AC, Landers AM, Bucci DJ. L-kynurenine treatment alters contextual fear conditioning and context discrimination but not cue-specific fear conditioning. *Behav Brain Res*. 2009;201:325–331.

20. Zmarowski A, Wu HQ, Brooks JM, et al. Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release. *Eur J Neurosci*. 2009;29:529–538.

21. Potter MC, Elmer GI, Bergeron R, et al. Reduction of endogenous kynurenic acid formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior. *Neuropsychopharmacology*. 2010;35:1734–1742.

22. Schwarcz R, Rassoulpour A, Wu HQ, Medoff D, Tamminga CA, Roberts RC. Increased cortical kynurenate content in schizophrenia. *Biol Psychiatry*. 2001;50:521–530.

23. Guillemin GJ, Kerr SJ, Smythe GA, et al. Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection. *J Neurochem*. 2001;78:842–853.

24. Zalcman S, Endicott J. *Diagnostic Evaluation After Death*. New York, NY: New York State Psychiatric Institute; 1983.

25. Choi YK, Gardner MP, Tarazi FI. Effects of risperidone on glutamate receptor subtypes in developing rat brain. *Eur Neuropsychopharmacol*. 2009;19:77–84.

26. Röver S, Cesura AM, Huguenin P, Kettler R, Szente A. Synthesis and biochemical evaluation of N-(4-phenylthiazol-2-yl)benzenesulfonamides as high-affinity inhibitors of kynurenine 3-hydroxylase. *J Med Chem*. 1997;40:4378–4385.

27. Heyes MP, Quearry BJ. Quantification of 3-hydroxykynurenine in brain by high-performance liquid chromatography and electrochemical detection. *J Chromatogr*. 1988;428:340–344.

28. Foster AC, White RJ, Schwarcz R. Synthesis of quinolinic acid by 3-hydroxyanthranilic acid oxygenase in rat brain tissue in vitro. *J Neurochem*. 1986;47:23–30.

29. Foster AC, Whetsell WO Jr, Bird ED, Schwarcz R. Quinolinic acid phosphoribosyltransferase in human and rat brain: activity in Huntington's disease and in quinolinate-lesioned rat striatum. *Brain Res*. 1985;336:207–214.

30. Guidetti P, Okuno E, Schwarcz R. Characterization of rat brain kynurenine aminotransferases I and II. *J Neurosci Res*. 1997;50:457–465.

31. Schmidt W, Guidetti P, Okuno E, Schwarcz R. Characterization of human brain kynurenine aminotransferases using [3H]kynurenine as a substrate. *Neuroscience*. 1993;55:177–184.

32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem*. 1951;193:265–275.

33. Dykens JA, Sullivan SG, Stern A. Oxidative reactivity of the tryptophan metabolites 3-hydroxyanthranilate, cinnabarinate, quinolinate and picolinate. *Biochem Pharmacol*. 1987;36:211–217.

34. Rios C, Santamaria A. Quinolinic acid is a potent lipid peroxidant in rat brain homogenates. *Neurochem Res*. 1991;16:1139–1143.

35. Okuda S, Nishiyama N, Saito H, Katsuki H. Hydrogen peroxide-mediated neuronal cell death induced by an endogenous neurotoxin, 3-hydroxykynurenine. *Proc Natl Acad Sci U S A*. 1996;93:12553–12558.

36. Goldstein LE, Leopold MC, Huang X, et al. 3-Hydroxykynurenine and 3-hydroxyanthranilic acid generate hydrogen peroxide and promote alpha-crystallin cross-linking by metal ion reduction. *Biochemistry*. 2000;39:7266–7275.

37. Schwarcz R, Pellicciari R. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. *J Pharmacol Exp Ther*. 2002;303:1–10.

38. Goda K, Hamane Y, Kishimoto R, Ogishi Y. Radical scavenging properties of tryptophan metabolites. Estimation of their radical reactivity. *Adv Exp Med Biol*. 1999;467:397–402.

39. Hardeland R, Zsizsik BK, Poeggeler B, Fuhrberg B, Holst S, Coto-Montes A. Indole-3-pyruvic and -propionic acids, kynurenic acid, and related metabolites as luminophores and free-radical scavengers. *Adv Exp Med Biol*. 1999;467:389–395.

40. Schwarcz R, Guidetti P, Sathyasaikumar KV, Muchowski PJ. Of mice, rats and men: revisiting the quinolinic acid hypothesis of Huntington's disease. *Prog Neurobiol*. 2010;90:230–245.

41. Wierzba-Bobrowicz T, Lewandowska E, Kosno-Kruszewska E, Lechowicz W, Pasennik E, Schmidt-Sidor B. Degeneration of microglial cells in frontal and temporal lobes of chronic schizophrenics. *Folia Neuropathol*. 2004;42:157–165.

42. Foster R, Kandanearatchi A, Beasley C, et al. Calprotectin in microglia from frontal cortex is up-regulated in schizophrenia: evidence for an inflammatory process? *Eur J Neurosci*. 2006;24:3561–3566.

43. Müller N, Schwarz M. Schizophrenia as an inflammation-mediated dysbalance of glutamatergic neurotransmission. *Neurotox Res*. 2006;10:131–148.

44. van Berckel BN, Bossong MG, Boellaard R, et al. Microglia activation in recent-onset schizophrenia: a quantitative (R)-[11C]PK11195 positron emission tomography study. *Biol Psychiatry*. 2008;64:820–822.

45. Bernstein HG, Steiner J, Bogerts B. Glial cells in schizophrenia: pathophysiological significance and possible consequences for therapy. *Expert Rev Neurother*. 2009;9:1059–1071.

46. Aoyama N, Takahashi N, Saito S, et al. Association study between kynurenine 3-monooxygenase gene and schizophrenia in the Japanese population. *Genes Brain Behav*. 2006;5:364–368.

47. Sathyasaikumar KV, Stachowski E, Wonodi I, Roberts RC, Thaker GK, Schwarcz R. Impairment of kynurenine 3-monooxygenase in the frontal cortex of individuals with schizophrenia: association with the eye tracking endophenotype. *Soc Neurosci Abstr*. 2009;34:747.15.

48. Duncan J, Owen AM. Common regions of the human frontal lobe recruited by diverse cognitive demands. *Trends Neurosci*. 2000;23:475–483.

49. Miller CL, Llenos IC, Cwik M, Walkup J, Weis S. Alterations in kynurenine precursor and product levels in schizophrenia and bipolar disorder. *Neurochem Int*. 2008;52:1297–1303.

50. Stevens CO, Henderson LM. Beef liver 3-hydroxyanthranilic acid oxidase. *J Biol Chem*. 1959;234:1188–1190.

51. Dang Y, Xia C, Brown OR. Effects of oxygen on 3-hydroxyanthranilate oxidase of the kynurenine pathway. *Free Radic Biol Med*. 1998;25:1033–1043.

52. Brown AS, Susser ES. Prenatal nutritional deficiency and risk of adult schizophrenia. *Schizophr Bull*. 2008;34:1054–1063.

53. Do KQ, Cabungcal JH, Frank A, Steullet P, Cuenod M. Redox dysregulation, neurodevelopment, and schizophrenia. *Curr Opin Neurobiol*. 2009;19:220–230.

54. Amori L, Guidetti P, Pellicciari R, Kajii Y, Schwarcz R. On the relationship between the two branches of the kynurenine pathway in the rat brain in vivo. *J Neurochem*. 2009;109:316–325.

55. Radewicz K, Garey LJ, Gentleman SM, Reynolds R. Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. *J Neuropathol Exp Neurol*. 2000;59:137–150.

56. Matute C, Melone M, Vallejo-Illarramendi A, Conti F. Increased expression of the astrocytic glutamate transporter GLT-1 in the prefrontal cortex of schizophrenics. *Glia*. 2005;49:451–455.

57. Steiner J, Bernstein HG, Bielau H, et al. S100B-immunopositive glia is elevated in paranoid as compared to residual schizophrenia: a morphometric study. *J Psychiatr Res*. 2008;42:868–876.

58. Han Q, Cai T, Tagle DA, Li J. Structure, expression, and function of kynurenine aminotransferases in human and rodent brains. *Cell Mol Life Sci*. 2010;67:353–368.

59. Linderholm KR, Skogh E, Olsson SK, et al. Increased levels of kynurenine and kynurenic acid in the CSF of patients with schizophrenia. *Schizophr Bull*. August 20, 2010; doi:10.1093/schbul/sbq086.

60. Miller CL, Llenos IC, Dulay JR, Barillo MM, Yolken RH, Weis S. Expression of the kynurenine pathway enzyme tryptophan 2,3-dioxygenase is increased in the frontal cortex of individuals with schizophrenia. *Neurobiol Dis*. 2004;15:618–629.

61. Kwidzinski E, Bunse J, Kovac AD, et al. IDO (indolamine 2,3-dioxygenase) expression and function in the CNS. *Adv Exp Med Biol*. 2003;527:113–118.

62. Guillemin GJ, Cullen KM, Lim CK, et al. Characterization of the kynurenine pathway in human neurons. *J Neurosci*. 2007;27:12884–12892.

63. Wang Y, Lawson MA, Dantzer R, Kelley KW. LPS-induced indoleamine 2,3-dioxygenase is regulated in an interferon-gamma-independent manner by a JNK signaling pathway in primary murine microglia. *Brain Behav Immun*. 2010;24:201–209.

64. Erhardt S, Schwieler L, Nilsson L, Linderholm K, Engberg G. The kynurenic acid hypothesis of schizophrenia. *Physiol Behav*. 2007;92:203–209.

65. Simosky JK, Stevens KE, Freedman R. Nicotinic agonists and psychosis. *Curr Drug Targets CNS Neurol Disord*. 2002;1:149–162.

66. Coyle JT. Glutamate and schizophrenia: beyond the dopamine hypothesis. *Cell Mol Neurobiol*. 2006;26:365–384.

67. Rassoulpour A, Guidetti P, Conley RR, Roberts RC, Schwarcz R. Kynurenine pathway metabolism in the neostriatum of individuals with schizophrenia. *Soc Neurosci Abstr*. 2006;32:588.11.

68. Wonodi I, Schwarcz R. Cortical kynurenine pathway metabolism: a novel target for cognitive enhancement in schizophrenia. *Schizophr Bull*. 2010;36:211–218.