Increased Levels of Kynurenine and Kynurenic Acid in the CSF of Patients With Schizophrenia

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Background: The kynurenic acid (KYNA) hypothesis for schizophrenia is partly based on studies showing increased brain levels of KYNA in patients. KYNA is an endogenous metabolite of tryptophan (TRP) produced in astrocytes and antagonizes *N*-methyl-ᴅ-aspartate and α7* nicotinic receptors. ***Methods:*** The formation of KYNA is determined by the availability of substrate, and hence, we analyzed KYNA and its precursors, kynurenine (KYN) and TRP, in the cerebrospinal fluid (CSF) of patients with schizophrenia. CSF from male patients with schizophrenia on olanzapine treatment (*n* = 16) was compared with healthy male volunteers (*n* = 29). ***Results:*** KYN and KYNA concentrations were higher in patients with schizophrenia (60.7 ± 4.37nM and 2.03 ± 0.23nM, respectively) compared with healthy volunteers (28.6 ± 1.44nM and 1.36 ± 0.08nM, respectively), whereas TRP did not differ between the groups. In all subjects, KYN positively correlated to KYNA. ***Conclusion:*** Our results demonstrate increased levels of CSF KYN and KYNA in patients with schizophrenia and further support the hypothesis that KYNA is involved in the pathophysiology of schizophrenia.

*Key words:* psychosis/kynurenate/olanzapine/ cerebrospinal fluid/tryptophan

Introduction

The kynurenic acid (KYNA) hypothesis of schizophrenia is based on findings that patients with schizophrenia have elevated levels of KYNA in the cerebrospinal fluid (CSF)[1,2] and in the postmortem prefrontal cortex.[3] KYNA is an endogenous tryptophan (TRP) metabolite (figure 1) synthesized in and released by astrocytes in the brain. In millimolar concentrations, it blocks the α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate and kainate receptors, but in lower, micromolar concentrations, it blocks the glycine site of the *N*-methyl-ᴅ-aspartate receptor (NMDAR)[4] as well as the cholinergic α7* nicotinic receptor (α7nAChR).[5]

The exceptional receptor-binding profile of KYNA is particularly interesting given that hypoglutamatergia, possibly induced by NMDAR hypofunction, is a prominent theory as part of the pathophysiology of schizophrenia.[6] In addition, the importance of an intact α7nAChR signaling in cognitive functions has during the last decade been suggested in numerous studies.[7] In further support of the KYNA hypothesis of schizophrenia, animal studies show that pharmacologically elevated levels of brain KYNA impair contextual learning and working memory[8–10] and disrupt prepulse inhibition,[11] a behavioral model measuring sensory motor gating. Interestingly, these domains are affected in patients with schizophrenia.[12–15]

The concentration of KYNA found in rodent or human brain and CSF or rodent extracellular fluid is below that required to affect either the *N*-methyl-ᴅ-aspartate/ glycine site (half maximal inhibitory concentration [IC_{50}] = 8–15μM)[4] or the a7nAChR (IC_{50} = 7μM).[5] Thus, the physiological role of KYNA as a modulator of neural transmission has been questioned ever since it was first discovered in the brain.[16,17] However, a variety of animal studies have clearly confirmed a physiological role of KYNA in neural transmission.[18–23] This discrepancy might be related to the production and release of KYNA by astrocytes, closely connected to the synapse and known to adjoin glutamatergic boutons.[24] Such an appropriate location puts newly synthesized KYNA in an excellent position to interact with the receptors.
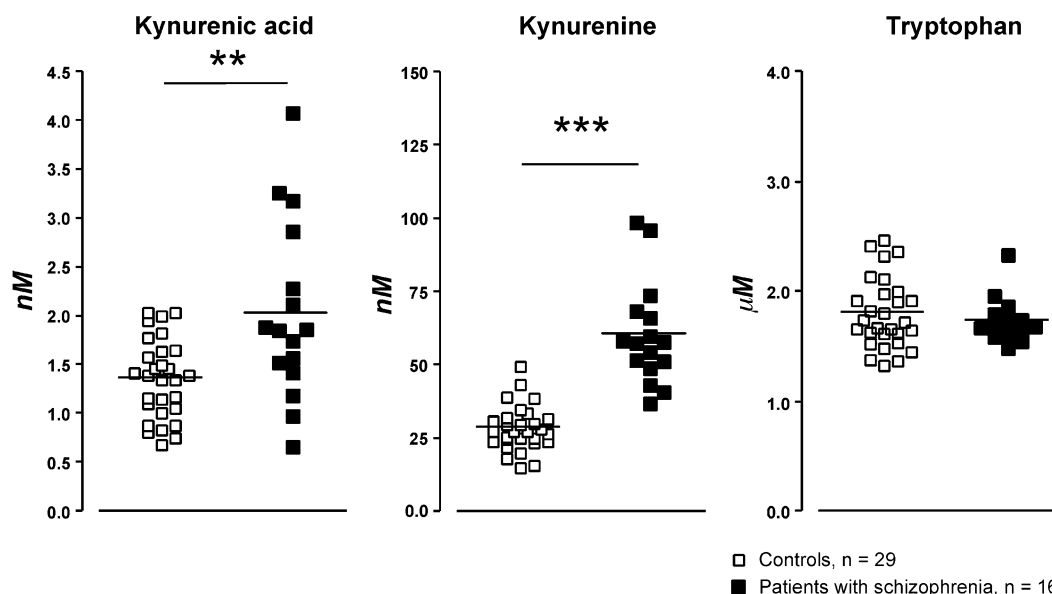
Fig. 1. Kynurenic acid, kynurenine, and tryptophan in cerebrospinal fluid (CSF) of healthy male volunteers and male patients with schizophrenia. Each point represents the concentration in a single CSF sample and the horizontal line the mean for each group. Statistics: **$P < .01$, ***$P < .001$ (Mann-Whitney U test).

This interaction has been confirmed by studies showing a tonically modulatory role of endogenous KYNA on midbrain dopamine firing. Thus, pharmacologically elevated levels of KYNA increase midbrain dopamine firing,[25–31] whereas lowered levels of KYNA dampen the activity of these neurons.[28,29] Pharmacologically altered levels of KYNA have also been shown to influence brain dopaminergic, cholinergic, and glutamatergic terminal efflux[18,19,23,32] as well as the pharmacological response to several drugs, eg, nicotine, clozapine, and amphetamine.[27,29,31,33,34] Furthermore, several studies during the last years also suggest a critical role of KYNA in cognitive functions.[8–11,21] Interestingly, mice with a targeted deletion of kynurenine aminotransferase II resulting in low levels of endogenous KYNA display increased performance in cognitive tests.[35]

Brain levels of KYNA heavily depend on the availability of its immediate precursor, kynurenine (KYN).[18] Thus, the aim of the present study was not only to confirm elevated CSF KYNA levels in schizophrenia investigating well-controlled outpatients and age-matched healthy volunteers but also, in these cohorts, to analyze CSF levels of TRP and KYN.

Methods

Patients and Healthy Volunteers

TRP, KYN, and KYNA concentrations were measured in CSF from *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (*DSM-IV*) verified male patients with schizophrenia ($n = 16$, age $36.8 ± 7.9$ y [mean ± SD], range 23–49 y). Diagnosis was based on clinical interviews by an experienced psychiatrist. All

patients were stable, chronically ill outpatients, and all had been prescribed antipsychotic drugs for a minimum of 6 months. Symptom severity was rated with Brief Psychiatric Rating scale (BPRS)[36] and global assessment of functioning (GAF).[37] At the time of lumbar puncture, all patients were prescribed and taking the antipsychotic drug olanzapine as the only antipsychotic treatment. Patients had been medicated with olanzapine between 0.1 and 10 years (median 2 y) and received the same dose (2.5–25 mg/d) for at least 14 days before lumbar puncture. CSF concentrations of TRP, KYN, and KYNA from patients were compared with those of healthy male volunteers ($n = 29$, age $25.4 ± 7.3$ y [mean ± SD], range 18–51 y).

All controls were in good physical health and mainly recruited among medical students, hospital staff members, and their relatives from the same community and with a similar socioeconomic background. Controls and patients were subjected to a medical checkup including routine laboratory tests and a physical examination and were all free from any form of substance abuse. All controls were medication free for at least 1 month prior to lumbar puncture, although smoking and coffee were allowed. All controls were also subjected to a semistructured interview using the Structured Clinical Interview of *DSM-IV* Axis I disorders (SCID-I),[38] completed the SCID-II questionnaire for personality disorders,[39] and considered to be eligible for the study. None of them had a family history of major psychosis or suicide in first or second-degree relatives, and they were all found to be free from current signs of psychiatric morbidity or difficulties in social adjustment at the time of sampling.

Lumbar punctures of patients with schizophrenia and healthy controls were performed under the same conditions, by the same physicians, after a minimum of 8 h in fasting state. There were no restrictions concerning posture or rest during the preceding 8 h. At about 8 AM, a disposable needle (BD Whitacre Needle 0.7 × 90 mm) was inserted at the lumbar vertebrae 4–5 level with the subject in the right decubitus position. Intraspinal pressure was measured using a disposable spinal fluid manometer (Optidynamic, Mediplast) before and after the collection of two 6-ml CSF fractions. The CSF was allowed to drip into a plastic test tube. The 6-ml fractions of CSF were protected from light and centrifuged at 3500 rpm for 10 min within 30 min after the puncture. Each 6-ml sample was divided into 2 plastic tubes and stored at −70°C for 2–4 years until analyzed. CSF from the second fraction (7–12 ml) was used for analysis throughout the present study. Data from a subset of the control samples (23) have previously been published,[40] whereas those from patients with schizophrenia have not been published previously.

Policy and Ethics

The work described in the present study was carried out in accordance with "The code of ethics of the world medical association" (Declaration of Helsinki) for experiments including humans: http://www.wma.net/e/policy/b3.htm. The study was approved by the Ethics Committee of the Medical Faculty of Linköping University, Sweden, the Swedish Medical Products Agency, and the Swedish Data Inspection Board. All patients and healthy volunteers received verbal and written information and gave their written informed consent.

Analysis of KYNA

The analysis of KYNA was performed utilizing an isocratic reversed-phase high-performance liquid chromatography (HPLC) system as previously described.[41] Fifty-microliter samples were manually injected, and some samples were analyzed in duplicates, and the interindividual variation was less than 5%.

Analysis of KYN and TRP

To analyze KYN and TRP, samples were thawed in 4°C and 50 µl manually injected immediately (Rheodyne, Cotati, California) into a HPLC system. Separation of KYN and TRP was achieved by reversed-phase liquid chromatography using a 20mM NaH_2PO_4 buffer (not pH adjusted) with 5.0% acetonitrile. The mobile phase was delivered by an HPLC pump (Bischoff Chromatography, Leonberg, Germany) through a ReproSil-Pur C18 column (4 × 150 mm, Dr Maisch GmbH, Ammerbuch, Germany) at a rate of 0.5 ml/min. Following separation, the analysate was first passed through a guard cell with an oxidizing potential of 50 mV. Samples were then quantified by sequential oxidation and reduction in a high-sensitivity analytical cell (ESA 5011; ESA Inc, Chelmsford, Massachusetts) controlled by a potentiostat (Coulochem III; ESA Inc) with an applied potential of 600 mV for detection of KYN and TRP. The signals from the detector were transferred to a computer for analysis (Datalys Azur, Grenoble, France). The retention time of KYN was approximately 8–9 min and approximately 15–16 min for TRP. The sensitivity of the system was verified by analysis of standard mixtures of KYN, with concentrations from 5 to 100nM, and TRP, with concentrations from 0.5 to 5µM, resulting in a linear standard plot.

Statistical Analysis

KYNA, KYN, and TRP values are given as mean ± standard error of the mean. Differences regarding CSF concentrations were established using Kruskal-Wallis analysis of variance followed by Mann-Whitney U test. Linear regression analysis was performed to study the relation between age and CSF KYNA levels, CSF KYN levels, or CSF TRP levels. The association between CSF KYNA levels, CSF KYN levels, and CSF TRP levels, respectively, and with prescribed dose of olanzapine, smoking status, and total BPRS and GAF scores were analyzed using Spearman rank correlation. Significance was assumed for all comparisons with $P < .05$.

Results

Demographics from patients with schizophrenia are summarized in table 1. CSF KYNA levels in male patients with schizophrenia (2.03 ± 0.23nM, $n = 16$, $P < .01$) were found to be elevated compared with CSF KYNA levels in healthy male volunteers (1.36 ± 0.08nM, $n = 29$). Additionally, patients with schizophrenia displayed a 2-fold increase in CSF KYN levels (60.7 ± 4.37nM, $n = 16$, $P < .001$) compared with CSF KYN levels in healthy volunteers (28.6 ± 1.44nM, $n = 29$). Concentrations of CSF TRP revealed no significant difference between patients with schizophrenia (1.73 ± 0.05µM, $n = 16$) and healthy volunteers (1.80 ± 0.06µM, $n = 29$). Data from patients with schizophrenia and healthy volunteers are summarized in figure 1.

When all subjects were included, a positive correlation was found between the CSF levels of KYNA and KYN ($P < .05$, Spearman's $r = 0.360$, $n = 45$, figure 2). This correlation was not present in either patients or healthy controls when analyzed separately. No correlation was found between CSF TRP and KYNA or KYN levels. Further, no correlations were found between CSF concentrations (KYNA, KYN, or TRP) and BPRS and GAF scores, age, prescribed dose of olanzapine, or smoking status.

Table 1. Demographics of Male Patients With Schizophrenia

| Patient	KYNA (nM)	KYN (nM)	TRP (μM)	BPRS	GAF	Age (y)	Olanzapine dose (mg)	Smoking
SZ 1	1.57	52.27	1.96	29	63	27	2.5	N
SZ 2	3.27	37.55	1.48	25	68	41	7.5	N
SZ 3	2.29	66.51	1.68	33	62	49	25	Y
SZ 4	2.12	96.31	1.79	29	68	34	10	N
SZ 5	1.87	41.23	1.55	32	53	23	10	N
SZ 6	1.75	54.81	1.62	33	58	34	10	N
SZ 7	4.08	68.81	1.67	33	49	48	20	N
SZ 8	1.53	43.41	1.65	36	48	30	10	Y
SZ 9	2.87	60.17	1.87	46	52	38	12.5	N
SZ 10	1.89	74.22	1.74	46	45	43	20	N
SZ 11	0.67	49.38	2.33	36	65	42	15	N
SZ 12	0.98	51.91	1.72	35	48	33	10	Y
SZ 13	1.86	99.27	1.59	27	65	29	2.5	N
SZ 14	1.42	58.82	1.61	30	58	46	10	N
SZ 15	3.19	57.88	1.74	38	49	31	20	Y
SZ 16	1.19	58.36	1.69	31	61	38	7.5	N

Note: BPRS, Brief Psychiatric Rating scale; GAF, global assessment of functioning; KYN, kynurenine; KYNA, kynurenic acid; N, no; TRP, tryptophan; Y, yes.

Discussion

Present results show that CSF KYNA concentrations are elevated in male patients with schizophrenia. These results are in line with previous studies showing increased CSF KYNA concentrations in first episode, drug naive patients, or patients receiving various antipsychotic treatments[1,2] and thus provide further support of a role of KYNA in the pathophysiology of schizophrenia. In the present study, we have extended the analysis to include also TRP and KYN. Hence, in line with the previously observed increase in CSF KYNA levels in schizophrenia, we found elevated levels also of its immediate precursor KYN. CSF TRP was, however, not altered. In the present study, we did not find any correlation between CSF KYN or KYNA and psychiatric symptom scores (BPRS and GAF). However, our relatively small cohort included well-controlled and stable patients, making such an assessment difficult.

Although the levels of CSF KYNA found in the present study are relatively low (cf, Introduction), its concentration at critical sites of action, ie, within the synapses, should be sufficient to interact with glutamatergic/cholinergic receptors as demonstrated in animal studies (cf, Introduction). Interestingly, both NMDAR and α7nAChR hypofunction is suggested to be widely implicated in the pathophysiology of psychiatric disorders, and besides elevated levels of KYNA in patients with schizophrenia,[1–3] also suicide attempters with major depressive disorder[42] as well as patients with bipolar disorder[40] display elevated CSF KYNA levels. It is thus tempting to speculate that cognitive dysfunctions, a common denominator in symptomatology of these diseases, are related to elevated brain KYNA levels, as also indicated from animal studies (cf, Introduction).

In the present study, all patients were treated with olanzapine, providing a homogenous, well-controlled cohort. Clearly, treatment with antipsychotic drugs should be taken into consideration as a confounding factor when evaluating biological aberrations in brain of patients with schizophrenia. However, previous animal studies have shown that treatment with antipsychotic drugs rather reduce endogenous concentrations of KYNA while KYN levels are unaffected.[3,43] Furthermore, recent unpublished data from our laboratory show that chronic treatment with olanzapine does not affect rat brain KYNA. Additionally, the concentration of KYNA tended to
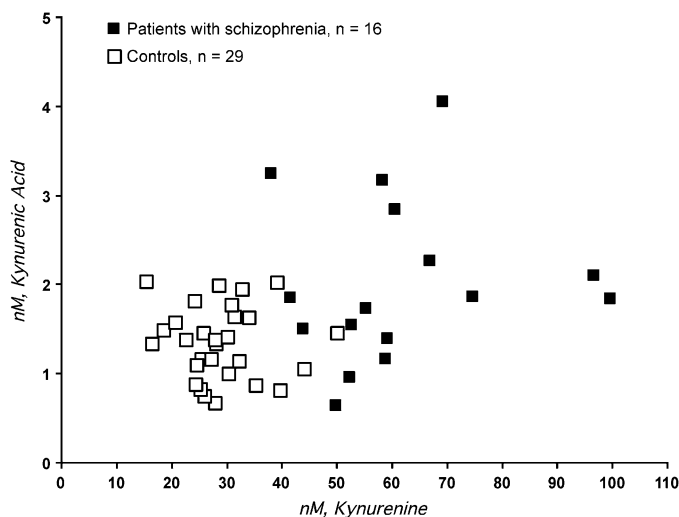
Fig. 2. Correlation between cerebrospinal fluid (CSF) kynurenine and CSF kynurenic acid ($n = 45$) in all subjects. Statistics: $P < .05$, Spearman's $r = 0.360$.

be decreased in the postmortem brain of patients with schizophrenia on antipsychotic treatment compared with patients without treatment.[44] Thus, the presently shown elevation of CSF KYN and KYNA in male patients with schizophrenia is in all probability not related to the olanzapine treatment.

While TRP levels were not changed in patients with schizophrenia, KYN, the immediate precursor of KYNA, was markedly elevated in the CSF. This is in line with previous postmortem studies,[44,45] further supporting an induction of the KYN pathway in schizophrenia. The elevated CSF KYN levels may arise from an increased synthesis of KYN from TRP or a decreased synthesis of 3-hydroxykynurenine from KYN (see figure 1). An increased synthesis of KYN might result from an induction of indoleamine 2,3-dioxygenase (IDO) and/or tryptophan 2,3-dioxygenase (TDO), enzymes responsible for the rate-limiting step of the KYN pathway. Notably, these enzymes are induced during infections or immune activation,[46–48] and numerous studies suggest that KYNA is a biological marker of neuroinflammation.[49,50] Supporting an activation of the brain immune system in schizophrenia, it was recently found that the CSF concentration of the proinflammatory cytokine interleukin-1β is elevated in first-episode patients.[51] Indeed, gene expression of TDO as well as the density of TDO-immunopositive cells are found to be elevated in postmortem brain of patients with schizophrenia, whereas IDO expression is unaffected.[44,52]

A decreased synthesis of 3-hydroxykynurenine from KYN, resulting in increased KYN levels, is supported by experimental as well as genetic studies. It is well known that pharmacological blockade of the enzyme converting KYN to 3-hydroxykynurenine, kynurenine 3-monooxygenase (KMO), results in elevated brain KYNA levels.[19,34,53–55] In addition, we recently reported that a nonsynonymous polymorphism in the *KMO* gene results in higher CSF KYNA levels in both healthy volunteers and patients with schizophrenia.[56] Also, a postmortem study shows decreased *KMO* gene expression as well as decreased KMO enzyme activity in individuals with schizophrenia.[57] These latter findings also imply that levels of quinolinic acid, a neurotoxic NMDAR agonist synthesized in the alternative branch of the KYN pathway, are not elevated in patients with schizophrenia, in agreement with previous reports.[58]

In conclusion, present results show that CSF concentrations of KYN and KYNA are elevated in patients with schizophrenia. Because the availability of KYN critically determines the formation of KYNA,[18] an elevation of KYN should explain the increased formation of brain KYNA in patients with schizophrenia. The precise mechanism responsible for the elevation remains to be revealed; however, present results further support an involvement of metabolites of the KYN pathway in the pathophysiology of schizophrenia.

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