Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia

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Abstract

Kynurenic acid is an endogenous glutamate antagonist with a preferential action at the glycine-site of the N-methyl D-aspartate-receptor. Mounting evidence indicate that the compound is significantly involved in basal neurophysiological processes in the brain. In the present investigation, cerebrospinal fluid (CSF) level of kynurenic acid was analyzed in 28 male schizophrenic patients and 17 male healthy controls by means of high pressure liquid chromatography and fluorescence detection. Schizophrenic patients showed elevated CSF levels of kynurenic acid (1.67 ± 0.27 nM) compared to the control group (0.97 ± 0.07 nM). Furthermore, CSF levels of kynurenic acid in schizophrenic patients were also found to correlate with age. The present finding is indicative of a contribution of kynurenic acid in the pathogenesis of schizophrenia.

Keywords: Schizophrenia; Kynurenate; Glutamate; Cerebrospinal fluid; Pathogenesis; N-methyl D-aspartate-receptor

In recent years the original dopamine hypothesis of schizophrenia, formulated nearly 40 years ago and based on dopaminergic hyperactivity, has been modified into a more diversified view where an attenuated glutamatergic neurotransmission is believed to participate in the pathogenesis of this disease [2]. Thus, phencyclidine (PCP, angel dust) and ketamine, compounds with a preferential antagonistic action on a subgroup of glutamate receptors, i.e. the N-methyl D-aspartate (NMDA)-receptors, induces schizophrenia-like positive and negative symptoms as well as cognitive dysfunction in healthy volunteers [11,13]. In addition, these drugs aggravate psychotic symptoms in schizophrenic patients [11]. Kynurenic acid is a naturally occurring NMDA-receptor antagonist in the human brain and increasing evidence suggest that this compound physiologically interact with glutamatergic neurotransmission in the brain [5,17]. In the present study, we investigate a putative involvement of endogenous kynurenic acid in the pathogenesis of schizophrenia by analyzing the concentration of the compound in the cerebrospinal fluid (CSF) of schizophrenic patients.

Kynurenic acid was measured in the CSF of 28 male patients with a DSM-III-R verified schizophrenia (mean age 27.4 years, range 18–47) and 17 healthy male controls (mean age 27.3 years, range 22–44). Twenty-five of the patients were first episode schizophrenics, and drug naive, i.e. they had never been treated with antipsychotic drugs. CSF was obtained by lumbar puncture (L4–L5). Twelve to eighteen milliliters of CSF were collected with a 0.9 mm needle and the samples were immediately frozen, coded and sent blindly to the Karolinska Institute. The analysis of kynurenic acid, a stable compound without degradation in spite of repeated thawing [10], was performed as previously described [5]. Briefly, an isocratic reversed-phase high pressure liquid chromatography system was used, including, an Eclipse XDB-C18 column and a fluorescence detector. A mobile phase of 50 mM sodium acetate pH 6.20 (adjusted with acetic acid) and 6.9% acetonitrile was used and assays were injected with a single sample loop of 50 μl. 0.5 M zinc acetate was delivered post column by a peristaltic pump. The signals from the fluorescence detector were passed through a MacLab analogue to a digital converter and transferred to a Macintosh computer. Precise acetoniitrele concentration of the mobile phase (6.9%) and pH (6.20) was essential to separate the kynurenic acid peak from an
unknown peak with nearly the same retention time (about 9 min at a flow rate of 0.5 ml/min). The sensitivity of this method was 0.125 pmol (signal: noise ratio 5:1).

Kynurenic acid was detected in all healthy volunteers, with little inter-individual variation (0.97 ± 0.07 nM). In patients suffering from schizophrenia, CSF levels of kynurenic acid levels were higher (1.67 ± 0.27 nM; P = 0.038, Mann–Whitney U-test) than in healthy volunteers and the variation between individuals was larger, with a maximum value of 6.8 nM (Fig. 1). A correlation between age and CSF kynurenic acid was observed in schizophrenics (r = 0.51, P = 0.0054) in contrast to healthy volunteers (r = 0.08).

Our results, showing that the CSF levels of endogenous kynurenic acid are elevated in schizophrenic patients, are in consonance with the hypothesis of a deficiency of glutamate function in schizophrenia [2,12]. Furthermore, our data are in line with a post-mortem study demonstrating an increase in endogenous cortical kynurenic acid in schizophrenic patients [16]. Kynurenic acid is a metabolite of tryptophan and the only known naturally occurring antagonist of glutamatergic receptors in the human brain [18]. At low concentration, it specifically acts at the glycine co-agonist site of the NMDA-receptor (K_D, approximately 8 μM) [15] whereas at higher concentrations the compound is also antagonizing other glutamate receptors like AMPA- and kainate receptors [18]. Kynurenic acid, which is synthesized in brain astrocytes [17] is a polar compound poorly passing through the blood–brain barrier under normal conditions [7]. The presently revealed differences in kynurenic acid levels may therefore reflect changes in either brain synthesis or elimination of the compound, although CSF concentrations of the compound in man or in rat (3.7 nM) [4] are considerably lower than brain concentrations (man: about 1 mM [14,19]; rat: about 20 nM [5,14], respectively). The mechanism responsible for maintaining a large gradient between brain tissue and CSF is however, unclear [10].

The question of the physiological role of kynurenic acid in brain glutamatergic neurotransmission has been a matter of controversy. Thus, as revealed from in vitro electrophysiological studies, endogenous whole brain levels of kynurenic acid are below those necessary to antagonize glutamate receptors [18]. However, recent studies indicate that physiological levels of brain kynurenic acid at specific sites of action, e.g. within the synapses, are sufficient to antagonize NMDA receptors [17]. In concordance, in vivo electrophysiological experiments demonstrate that a moderate (3–5-fold) increase in whole brain kynurenic acid, as induced by a kynurenine 3-hydroxylase inhibitor, is associated with a marked activation of rat midbrain dopamine neurons, including increased firing rate and burst firing activity [4,5]. These effects per se indicate a physiological role of kynurenic acid, and also suggest the compound to be involved in pathophysiological processes related to dopaminergic mechanisms. Thus, the effects of elevated kynurenic acid on midbrain dopaminergic activity show striking similarities to the actions of systemically administered psychotomimetic agents e.g. PCP and ketamine on these neurons [6] and are thought to be induced by an inadequate balance of afferent regulation by GABAergic and glutamatergic projections, e.g. from prefrontal cortex and/or subcortical areas. Similarly, a dysregulation of midbrain dopamine neurons has been hypothesized to mediate symptoms of schizophrenia [8,20], and recently, an elevated phasic activity of dopaminergic neurons was suggested to mediate increased striatal dopamine-receptor occupancy in patients with schizophrenia [1]. Thus, a dysfunction of the neuronal circuitries of the basal ganglia and frontal cortex, induced by elevated kynurenic acid levels and reflected by a hyper-activity of midbrain dopamine neurons, might contribute to the pathophysiology of schizophrenia.

In the present study, the majority (25/28) of patients were drug naive, first episode patients. Thus, the increased levels of kynurenic acid are not caused by treatment with neuroleptics. At any rate, chronic treatment of rats with antipsychotics does not result in increased levels of kynurenic acid, but rather a decrease [3]. Further, since the increase was found in first-episode schizophrenic patients, it is not likely to be a secondary consequence of the disease process, found only at the end stage of the disease.

Previous studies have shown an age-related increase of endogenous kynurenic acid in rats [9]. In the present study, no such relation was observed in healthy volunteers. However, in schizophrenic patients a positive correlation between CSF kynurenic acid levels and age was found. This finding may suggest different pathogenic mechanisms in older first episode schizophrenics resulting in the late onset of the disease.

In summary, the findings of the present study show that CSF levels of kynurenic acid are elevated in schizophrenia, indicating a contribution of the compound in the pathogen-

Fig. 1. Kynurenic acid in human CSF. Each point represents the concentration of kynurenic acid in a single CSF sample in units of nM. Open circles represents values from schizophrenic patients under neuroleptic treatment (n = 3).
esis of the disease. Our findings warrant replication in additional cohorts including correlations to psychiatric symptoms. Nevertheless, the observed difference in CSF kynurenic acid between schizophrenic patients and healthy volunteers suggest novel therapeutic strategies to be directed towards brain kynurenic acid synthesis or elimination.

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