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Modulation by kynurenine of extracellular kynurenate and glutamate in cerebral cortex of rats with acute liver failure

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ABSTRACT

Background: Kynurenic acid (KYNA) modulates the glutamatergic tone by controlling neuronal glutamate (GLU) release. The present study tested the potential of the KYNA precursor, kynurenine (KYN) to counter increased extracellular GLU associated with the pathogenesis of hepatic encephalopathy accompanying acute liver failure (ALF).
Methods: ALF was induced in adult rats by administration of a hepatotoxin, thioacetamide. KYNA and GLU were measured in the cerebral cortical microdialysates of control (saline-treated) and ALF rats using HPLC. The expression of mRNA coding for kynurenine aminotransferase II (KAT-II), the astrocytic enzyme converting KYN to KYNA, was assayed by real-time PCR.
Results: Cerebral cortical extracellular KYNA was increased in ALF rats not treated with KYN, consistent with a previously observed increase of cerebral cortical KATII activity in this ALF model. Single intraperitoneal administration of KYN (50 mg/kg, 120 min before microdialysate collection), produced a further substantial increase of extracellular KYNA, paralleled by a decrease of extracellular GLU. In cultured cerebral cortical astrocytes, the cells which *in situ* are the primary target of blood-derived ammonia and other toxins liberated due to ALF, elevation of KAT-II mRNA expression was noted upon their incubation with KYN and the KYN precursor, tryptophan (Trp), which is normally elevated by ALF.
Conclusions: Administration of exogenous KYN to stimulate KYNA synthesis may help correcting excessive extracellular accumulation of GLU in cerebral cortex caused by ALF. The therapeutic potential of KYN in ALF appears to be fostered by increased expression of KAT-II in astrocytes upon exposure to KYN or Trp.

Introduction

Hepatic encephalopathy (HE) is a complex neurological syndrome of which many pathophysiological manifestations are due to impaired excitatory glutamatergic (GLUergic) transmission [1,2]. Increased GLUergic tone resulting from overactivation of ionotropic glutamate (GLU) receptors (mainly N-methyl-D-aspartate receptors (NMDAR) is observed at the onset of HE associated with acute liver failure [3,4], giving way to receptor downregulation and decreased GLUergic transmission in more advanced stages of the disease [5,6]. The above sequence of events is causally related, among other factors, to excessive accumulation of GLU in the extracellular (perisynaptic) space of the brain, seen both in experimental HE animals [7–9] and human HE patients [10]. Two mutually not exclusive mechanisms have been implicated in the increase of extracellular GLU in HE: (i) impaired reuptake of released GLU to astrocytes associated with downregulation of astrocytic GLU transporters [11,12] and/or of the inward rectifying astrocytic potassium channel Kir4.1 [13]; (ii) increased synaptic [14] or astrocytic GLU release [15,16], partly related to excessive activation of NMDA receptors [3,4]. While the relative contribution of mechanisms (i) and (ii) at different stages of HE has not been accurately assessed, investigations reported up-to-date unambiguously underscore the role of increased NMDA receptor-mediated neuronal GLU release in its acute phase [3,17].

Kynurenic acid (KYNA) is a tryptophan (Trp) metabolite along the kynurenine (KYN) pathway, and KYN is its direct precursor [18]. KYNA synthesis occurs mainly in astrocytes where it is

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catalyzed by kynurenine aminotransferase II (KAT-II) [19]. KYNA is the only well documented endogenous ligand of α7 nicotinic acetylcholine receptor (α7nAChR) and NMDAR in the brain [20], with KYNA affinity to α7nAChR being an order of magnitude higher than to NMDAR (for a recent review see [21]). Studies of the last decade have unambiguously documented the negative modulatory effect of KYNA interaction with α7nAChR on GLU release in the cerebral cortex ([22,23] and references therein). A significant reduction of extracellular GLU in the rat cerebral cortex has been achieved by local intracerebral administration of KYNA [23,24], or, under conditions of inefficiently operating blood-brain-barrier (BBB), its peripheral administration [25]. KYNA synthesis in the brain has also been found stimulated by intraperitoneal (i.p.) administration of KYN supported or not by other pharmacological manipulations [22,26–30].

To our knowledge, no successful attempt to decrease GLU release to the synaptic space in HE-affected brain has been reported before, except for the use of a high, relatively unsafe dose of an NMDA receptor antagonist memantine [9]. Therefore, we decided to test whether evoking an increase the brain KYNA content would ameliorate HE-induced GLU accumulation in the extrasynaptic space of the brain, paving the way to a desired therapeutic effect. To this point we compared the effect of i.p. administration of KYN on the extracellular KYNA and GLU levels in cerebral cortical microdialysates of control rats and rats with ALF induced with a hepatotoxin – thioacetamide (TAA) [17,31,32]. The concept of the potential advantage of treating HE rats with KYN was born from the recent observation that TAA treatment increases KATII expression in the rat cerebral cortex and the effect can be reproduced in cerebral cortical astrocytes in culture treated with pathophysiologically relevant concentrations of ammonia [33]. Here we extended the *in vitro* study by analyzing the KATII expression in astrocytes treated with KYN and its direct precursor, Trp, the blood-to-brain transport and brain level of which are elevated in HE-affected brain [34].

Material and methods

The thioacetamide (TAA) model of acute liver failure (ALF)

Animals used in this study have been acquired and cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals and all animal protocols used have been approved by the authors' institutional animal experimentation committee. ALF evolving to acute HE including GLUergic dysfunction and brain edema was induced in adult male Sprague–Dawley rats (180–220 g) by i.p. administration of TAA (a 200–250 mg/kg dose, in physiological saline solution) three times at 24-h intervals, as earlier described in this laboratory [17].

Brain microdialysis and determination of GLU and KYNA in the microdialysates

The microdialysis of the cerebral cortex was carried out in rats 72 h after the 1st TAA administration. KYN was administered i.p. at a dose of 50 mg/kg, 120 min after the onset of microdialysis. The microdialysis of animals anesthetized with halothane was performed exactly as described by Węgrzynowicz et al. [35]. Briefly, concentric microdialysis probes (dialyzing membrane: diameter 0.5 mm; length 3 mm) were implanted into left prefrontal cerebral cortex (coordinates from bregma: AP + 3.2; ML − 0.6; DV − 4.0, after [36]). The probes were perfused with artificial cerebrospinal fluid, pH 7.4, containing: 150 mM NaCl, 3 mM KCl, 1.2 mM $CaCl_2$, 0.8 mM $MgCl_2$, 31 mM KH_2PO_4 at a rate of 2.5 μl/min. Fractions were collected every 40 min.

GLU content was assessed using HPLC with fluorescence detection after derivatization with o-phthalaldehyde (OPA) plus mercaptoethanol, exactly as described earlier [7].

KYNA concentration was likewise measured fluorometrically. Samples were deproteinated with 6% perchloric acid, centrifuged, subjected to HPLC (Dionex HPLC system; ESA catecholamine HR-80, 3 μm, C18 reverse-phase column) and quantified fluorometrically (excitation 344 nm, emission 398 nm). The mobile phase consisted of 50 mM sodium acetate and 50 mM zinc acetate (pH-4.9), containing 4% of acetonitrile. The flow rate was 1.0 ml/min.

Culturing and treatment of cerebral cortical astrocytes

The rat primary cerebral cortical astrocytes were cultured as described by Hertz et al. [37]. Briefly, cerebral cortex was dissected from brains of 1-day-old Sprague-Dawley rats, passed through Nitex nylon netting (pore size 80 μm) into Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 20% fetal bovine serum (FBS; Gibco). Cells were cultured at standard conditions (37 °C; 95% air and 5% CO_2). Medium was changed twice a week, gradually decreasing FBS content to 10%. From the second week of culture, dibutyryl cAMP (Sigma–Aldrich) was added to the medium to promote morphological differentiation of astrocytes. Astrocytes were exposed, for 72 h, to Trp (1 mM) or KYN (50 or 100 μM).

RNA isolation and reverse transcription

Total RNA from astrocytes was isolated using Tri Reagent (Ambion). The quantity and purity of RNA (samples with OD260/OD280 > 1.7 were used) were measured with NanoDrop ND-1000 spectrophotometer. 1 μg RNA was reverse-transcribed using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's protocol.

Real-time PCR

The transcription of KAT-II (Aadat) and β-actin (Actb), used as the endogenous control, was determined by TaqMan Gene Expression Assay (Applied Biosystems). The efficiency of Taqman probes amplification was validated. 1.5 μl of cDNA was used in a reaction volume of 10 μl. The TaqMan assays' IDs were Rn00567882_m1 for KAT-II, and Rn00667869_m1 for β-actin. The fold change in mRNA level was determined by the $2^{-\Delta\Delta Ct}$ method [38].

Statistical analysis

A one-way ANOVA analysis of variances with Bonferroni's (for statistical analysis of real-time PCR results) or Dunnett's *post hoc* tests (for microdialysis results) was performed, using Prism 3.0 software. A p-value < 0.05 was considered statistically significant.

Results

Induction of HE with TAA was associated with both a pronounced elevation KYNA level (~330%) (Fig. 1A), and a significant increase above control (~200%) of GLU accumulation in the cerebral cortical microdialysates (Fig. 1B). I.p. administration of KYN has led to a robust accumulation of extracellular KYNA in the cerebral cortex, the effect being more pronounced in HE rats (~970% increase) than in control rats (~730% increase) (Fig. 1A). KYN administration led to a moderate, albeit statistically significant decrease of the extracellular GLU content in both control (~35%) and HE rats (~16%) (Fig. 1B).