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SHORT COMMUNICATION

D-cycloserine lowers kynurenic acid formation—New mechanism of action

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Abstract
D-Cycloserine, known as a partial agonist at the glycine modulatory site of the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor, exerts anticonvulsive activities and improves cognitive function. The present study evaluates the action of D-cycloserine with respect to the biosynthetic machinery of kynurenic acid (KYNA) synthesis e.g. the activity of enzymes synthesizing KYNA, kynurenine aminotransferases I, kynurenine aminotransferase II and kynurenine aminotransferase III (KAT I, KAT II and KAT III) in the rat liver and brain, and human frontal cortex in the presence of the anti-mycobacterial drug D-cycloserine, in an in vitro study. We found that D-cycloserine blocked dose-dependent and significantly KAT I, II and III activities in rat liver and brain homogenates. Furthermore, the inhibitory effect of KYNA formation was observed in the frontal cortex homogenate of human post mortem tissue, as well. D-Cycloserine, at 63.7 μM concentration blocked significantly KAT II, I and III (53.2, 66.1 and 71.3% of control, $P<0.001$) activities in the human frontal cortex homogenate. Obtained data indicate that D-cycloserine exerts notable biochemical properties to block KYNA synthesis. Lowering of KYNA content due to D-cycloserine inhibition of KATs activities can free up more glycine sites for the actions of D-cycloserine. On the other site, it needs to be clarified, if the postulated mechanism for D-cycloserine to act as a partial agonist at the glycine site of the NMDA receptor could be mainly due to KAT's inhibition. We propose that this mechanism (s) might play a role in the improvement of memory, cognition and/or delusion in Alzheimer's, HIV-1 infected patients and schizophrenia patients.

Abbreviations: KAT, kynurenine aminotransferase; KYNA, kynurenic acid; NMDA, *N*-methyl-D-aspartate; AMPOL, 2-amino-2-methyl-1-propanol; HIV-1, human immunodeficiency virus type-1; HIV, CO control; *N*, number; EAA, excitatory amino acid

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1. Introduction

Kynurenic acid (KYNA), an intermediate metabolite of L-kynurenine, is a competitive antagonist of ionotropic excitatory amino acid (EAA) receptors (Birch et al., 1988; Stone, 1993) and a non-competitive antagonist of alpha 7 nicotine cholinergic receptors (Hilmas et al., 2001). Alterations of KYNA metabolism in the brain under various degenerative and inflammatory conditions have been previously demonstrated and accumulated data indicate that increased KYNA formation in the brain is involved in the development of neurologic and psychiatric diseases, such as Downs syndrome, Alzheimer's (Baran et al., 1996, 1999), schizophrenia (Erhardt et al., 2001) and in HIV encephalopathy (Baran et al., 2012) or during the aging process (Kepplinger et al., 2005). On the other side cerebrolysin, drug able to lower KYNA synthesis (Baran and Kepplinger, 2009) offers significant therapeutic strategy to improve memory and cognition (Álvarez et al., 2000). An animal experimental study also provides significant evidence that increased levels of KYNA in the brain enhances memory impairment (Chess et al., 2007). D-Cycloserine, a partial agonist at the glycine modulatory site of the NMDA receptor, diminishes negative symptoms and improves cognitive function in experimental animal study as well in clinical trials (Schwartz et al., 1996; Goff et al., 1999; Goff, 2012) and exerts anticonvulsive activities (Baran et al., 1994; Löscher et al., 1994; Wlaź et al., 1994). The aim of the study was to examine the mechanism of D-cycloserine action, whether D-cycloserine has an ability to influence KYNA synthesis in liver and brain tissues, in an in vitro study.

2. Experimental procedures

2.1. Compounds

D-Cycloserine, L-kynurenine, KYNA and pyridoxal-5′-phosphate were purchased from Sigma. All other chemicals used were of the highest commercially available purity.

2.2. Animals

Male Wistar rats (Forschungsinstitut für Versuchstierzucht, Himberg, Austria) of 250–280 g body weight were used. The animals were housed in groups of four to five per cage, in a room with controlled light/dark cycle (12 h light/12 h dark), and were given free access to laboratory chow and tap water. Rats were sacrificed in the morning, the liver and brain was immediately removed, the homogenates were prepared and frozen at −60 °C until analysis. The number of rats used was $N=7$.

2.3. Subjects

Post mortem human samples of frontal cortices of normal subjects, aged between 52 and 77 years were received from the Institute of Neurology, Medical University Vienna, Austria and the tissue was stored at −60 °C until analysis. Macroscopic examination of the brain revealed no changes. Post mortem time was 12.4±2.9 (h), mean of age was 65±2.96 (7) and sex was 4 M and 3 F. The number of tissue samples was $N=7$.

2.4. Assay of KAT I, KAT II and KAT III activities

Preparation of homogenate: The tissue samples were homogenised in an ice bath in 5 volumes (wt/vol) of 5 mM tris-acetate buffer pH 8.0 containing 50 μM pyridoxal-5′-phosphate and 10 mM mercaptoethanol and the homogenates obtained were used for KAT I, II and III activities determination.

KAT assay

KAT I, KAT II and KAT III activities in the homogenate were measured using an enzymatic assay described by Baran and Kepplinger (2009). In brief, the reaction mixture contained homogenate, 100 μM L-kynurenine, 1 mM pyruvate, 70 μM pyridoxal-5′-phosphate and 150 mM 2-amino-2-methyl-l-propranol buffer pH 9.6 for KAT I or 150 mM tris-acetate buffer pH 7.4 for KAT II or 150 mM tris-acetate buffer pH 8.0 for KAT III, in a total volume of 200 μl. After incubation for 2 h at 37 °C the reaction was stopped by adding 14 μl of 50% trichloroacetic acid and 1 ml of 0.1 M HCl. Denatured proteins were removed by centrifugation and the synthesised KYNA was purified on Dowex 50W cation-exchange column and quantitated by high performance liquid chromatography (HPLC) method. Blanks were prepared by boiling samples of homogenate for 15 min before adding the reaction mixture.

HPLC method for KYNA detection

Measurement of KYNA was performed as described by Baran and Kepplinger (2009). The HPLC system consisted of the following: Merck Hitachi Elite LaChrom Pump L-2130, Autosampler L-2200, Fluorescence Detector L-2485 and a data processor Windows^{R} XP Professional HP. The mobile phase consisted of 50 mM sodium acetate, 250 mM zinc acetate, an 4% acetonitril, pH 6.15, and was pumped through a 10 cm × 0.4 cm column (HR-80, C-18, Particle size 3 μM, InChrom, Austria) at flow rate of 0.9 ml/min. The fluorescence detector was set at an excitation wavelength of 340 nm and an emission wavelength of 398 nm. The injection volume was 50 μl. The retention time of KYNA was approximately 6 min, with a sensitivity of 25 fmol per injection (signal: noise ratio=5).

2.5. Effect of D-cycloserine on rat liver KAT I, KAT II and KAT III activities

To verify the D-cycloserine effect on rat liver KAT I, KAT II and KAT III activities, the homogenate of rat liver (1:100 wt/vol) was incubated in the present of different concentrations of D-cycloserine (0.673, 6.73 and 67.3 μM) under standard assay condition and the amount of KYNA formed was determined.

2.6. Effect of D-cycloserine on rat brain KAT I, KAT II and KAT III activities

To verify the D-cycloserine effect on rat brain KAT I, KAT II and KAT III activities, the homogenate of rat brain (1:10 wt/vol) was incubated in the present of different concentrations of D-cycloserine (0.673, 6.73 and 67.3 μM) under standard assay condition and the amount of KYNA formed was determined. Seven independent experiments were performed.

2.7. Effect of D-cycloserine on human brain KAT I, KAT II and KAT III activities

To verify the D-cycloserine effect on human brain KAT I, KAT II and KAT III activities, the homogenate of human frontal cortex (1:20 wt/vol) was incubated with different concentrations of D-cycloserine