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Cerebrolysin lowers kynurenic acid formation — An *in vitro* study

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Abstract

The therapeutic effect of Cerebrolysin in the treatment of dementia and brain injury has been proposed because of neurotrophic properties of this compound. Since an increased kynurenine metabolism has been documented in several brain pathologies including dementia the aim of the present study was to investigate the biochemical properties of Cerebrolysin with respect to kynurenic acid (KYNA) formation in an *in vitro* study. KYNA is an endogenous metabolite of the kynurenine pathway of tryptophan degradation and is an antagonist of the glutamate ionotropic excitatory amino acid and of the nicotine cholinergic receptors. The activities of the KYNA synthesizing enzymes kynurenine aminotransferases I, II and III (KAT I, KAT II and KAT III) in rat liver, and rat and human brain homogenates were analysed in the presence of Cerebrolysin. KAT I, II and III activities were measured using a radio-enzymatic method in the presence of 1 mM pyruvate and 100 μM [H^3]L-kynurenine. Cerebrolysin, dose-dependently and significantly reduced KAT I, KAT II and KAT III activities of rat liver homogenate. Furthermore, Cerebrolysin exerted a dose-dependent inhibition of rat and human brain KAT I, KAT II and KAT III activities, too. The inhibitory effect of Cerebrolysin was more pronounced for KAT I than for KAT II and KAT III. The present study for the first time demonstrates the ability of Cerebrolysin to lower KYNA formation in rat liver as well as in rat and human brain homogenates. We propose Cerebrolysin as a

Abbreviations: KYNA, kynurenic acid; KAT, kynurenine aminotransferase; NMDA, N-methyl-D-aspartate; EAA, excitatory amino acid; CNS, central nervous system.

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compound susceptible of therapeutic exploitation in some disorders associated with elevated KYNA metabolism in the brain and/or other tissues. We suggest that the anti-dementia effect of Cerebrolysin observed in Alzheimer patients could be in part due to Cerebrolysin induced reduction of KYNA levels, thus modulating the cholinergic and glutamatergic neurotransmissions.

1. Introduction

Kynurenic acid (KYNA) is a well known endogenous antagonist of the glutamate ionotropic excitatory amino acid receptors N-methyl-D-aspartate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid and kainate (Birch et al., 1988; see Stone, 1993) and of the nicotine cholinergic subtype alpha-7 receptor (Hilmas et al., 2001) and has anticonvulsive and neuroprotective properties (Foster et al., 1984; see Stone, 1993). Furthermore, it has been shown in an *in vitro* study that KYNA increases the oxygen consumption of rat heart mitochondria (Baran et al., 2003) and this would suggest an essential role of KYNA in the cell function of myocardium and probably of other organs.

KYNA's concentration is regulated by many pyridoxal-5-phosphate-dependent enzymes: kynurenine aminotransferases, which catalyse the conversion of L-kynurenine to KYNA. In organs of mammalians several aminotransferases have been discovered (Kido, 1989). In peripheral tissues of rats, there are at least four types of proteins which are capable of catalysing the kynurenine-2-oxo acid transamination reaction to form KYNA (Kido, 1989; Ishikawa et al., 1989). In rat and human brain tissues also several kynurenine aminotransferases (KAT I–III) synthesize KYNA (Okuno et al., 1991; Schmidt et al., 1993; Baran et al., 1994; Yu et al., 2006; Guidetti et al., 2007a). KAT I and KAT II exert different catalytic characteristics (Okuno et al., 1991; Schmidt et al., 1993; Baran et al., 1994) which suggest that KAT II (pH = 7.4) substantially acts under physiological conditions, whereas KAT I (pH = 9.6) may have a particular importance in pathological conditions (Baran et al., 1999, 2000). There are also data suggesting that human KAT I is a multifunctional enzyme and might be an important player in KYNA synthesis under physiological conditions (Han et al., 2004). Furthermore, there are data indicating that human heart contains also three proteins KAT I (pH = 9.6), KAT II (pH = 7.4) and KAT III (pH = 8) showing comparable capacity to synthesize KYNA with those in human brain homogenate (Baran et al., 1997). Interestingly, in contrast to human brain KATs, human heart KAT I, KAT II and KAT III in the presence of tryptophan were significantly activated to synthesize KYNA, at least in an *in vitro* study (Baran et al., 1997).

An increase in brain KYNA and/or serum KYNA levels has been implicated in the symptoms associated with experimental dystonia (Richter et al., 1996), asphyxia (Baran et al., 2001), epilepsy (Baran et al., 1995) and ageing (Gramsbergen et al., 1992). The effect of ageing on NMDA receptor is associated with age-related declines in spatial memory and a coherency between lower densities of NMDA receptor binding and poor memory performance has been described (Magnusson, 1998). Interestingly, there is evidence that KYNA interferes with the working memory (Steele and Stewart, 1993) and an enhancement of endogenous KYNA levels induces spatial memory deficits (Chess et al., 2007). The assumption that an increase of KYNA levels in the human central nervous system (CNS) might be involved in cognitive decline is supported by the increased KYNA metabolism in Alzheimer's patients (Baran et al., 1999), in patients with DOWN syndrome (Baran et al., 1996), in patients with subcortical sclerotic encephalopathy (Kepplinger et al., 2001), in patients infected with HIV-1 virus (Heyes et al., 1992; Baran et al., 2000), in patients with Schizophrenia (Schwarcz et al., 2001) and in elder human subjects (Kepplinger et al., 2005).

Cerebrolysin is a peptidergic drug obtained by standardized enzymatic breakdown from purified porcine brain preparations, exhibiting neurotrophic and neuroprotective effects in experimental and clinical studies (Veinbergs et al., 2000; Ladurner et al., 2005; Riley et al., 2006). Cerebrolysin is also effective in preventing cognitive impairment in different experimental animal models (Masliah et al., 1999; Valousková and Gschane, 1999; Ren et al., 2007). Human studies indicated that Cerebrolysin improves dementia symptoms and cognitive performance in patients with Alzheimer's disease (AD) and in other types of senile dementia (Rüther et al., 1994; Ruether et al., 2001; Crook et al., 2005; Álvarez et al., 2006; Muresanu et al., 2008) and in elderly control subjects (Álvarez et al., 2000a).

The aim of the study was to investigate whether Cerebrolysin has an ability to influence KYNA formation in the rat and human tissues, in an *in vitro* study. A part of the data was published in abstract form (Baran and Kepplinger 2006a).

2. Experimental procedures

2.1. Compounds

L-kynurenine, KYNA and pyridoxal-5′-phosphate were purchased from Sigma. [^{3}H]L-kynurenine (specific activity, 41 Ci/mmol) was purchased from Amersham, England. The compound Cerebrolysin was obtained from EBEWE Pharma, Unterach, Austria. Cerebrolysin is produced by using a standardized controlled enzymatic breakdown of lipid-free porcine brain proteins and consists of free amino acids and peptides with molecular weights of less than 10 kD. In solution Cerebrolysin contains 40 mg dry substance per ml, with a nitrogen content of 5.3 mg. All other chemicals used were of the highest commercially available purity.

2.2. Animals

Male Sprague–Dawley 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