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β-adrenergic enhancement of brain kynurenic acid production mediated via cAMP-related protein kinase A signaling

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ABSTRACT

The central levels of endogenous tryptophan metabolite kynurenic acid (KYNA), an antagonist of *N*-methyl-D-aspartate (NMDA) and α7-nicotinic receptors, affect glutamatergic and dopaminergic neurotransmission. Here, we demonstrate that selective agonists of β_1-receptors (xamoterol and denopamine), β_2-receptors (formoterol and albuterol), α- and β-receptors (epinephrine), 8pCPT-cAMP and 8-Br-cAMP (analogues of cAMP) increase the production of KYNA in rat brain cortical slices and in mixed glial cultures. Neither betaxolol, β_1-adrenergic antagonist, nor timolol, a non-selective $\beta_{1,2}$-adrenergic antagonist has influenced synthesis of KYNA in both paradigms. In contrast, KT5720, a selective inhibitor of protein kinase A (PKA), strongly reduced KYNA formation in cortical slices (2–10 μM) and in glial cultures (100 nM). β-adrenergic antagonists and KT5720 prevented the β-adrenoceptor agonists-induced increases of KYNA synthesis. *In vivo*, β-adrenergic agonist clenbuterol (0.1–1.0 mg/kg) increased the cortical endogenous level of KYNA; the effect was blocked with propranolol (10 mg/kg). β-adrenoceptors agonists, cAMP analogues and KT5720 did not affect directly the activity of KAT I or KAT II measured in partially purified cortical homogenate. In contrast, the exposure of intact cultured glial cells to pCPT-cAMP, 8-Br-cAMP and formoterol has lead to an enhanced action of KATs. These findings demonstrate that β-adrenoceptor-mediated enhancement of KYNA production is a cAMP- and PKA-dependent event. PKA activity appears to be an essential signal affecting KYNA formation. Described here novel mechanism regulating KYNA availability may be of a potential importance, considering that various stimuli, among them clinically used drugs, activate cAMP/PKA pathway, and thus could counteract the central deficits of KYNA.

1. Introduction

Kynurenic acid (KYNA) is an endogenous tryptophan derivative, which multiple central actions have emerged within last years. KYNA blocks the glycine co-agonist site of *N*-methyl-D-aspartate (NMDA) receptor (Németh et al., 2005) and affects α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors — either facilitating or antagonizing their action, depending on the concentration (Prescott et al., 2006). At nanomolar levels, KYNA blocks also α7 cholinergic nicotinic receptors (Hilmas et al., 2001) and reduces the striatal content of dopamine, an effect that may locally modulate dopaminergic activity (Rassoulpour et al., 2005). In addition, KYNA regulates the release of glutamate via blockade of presynaptic NMDA autoreceptors (Carpenedo et al., 2001; Luccini et al., 2007).

Research data support the view that KYNA is an endogenous neuroprotective compound. Experimental inhibition of central KYNA synthesis leads to the excitotoxic neuronal loss (Urbanska et al., 1991; Eid et al., 1995), and to seizures in rodents (Turski et al., 1991), as opposed to the application of KYNA which prevents neurodegeneration and seizures (Németh et al., 2006). Altered central KYNA metabolism was associated with some human neuropathologies (Nemeth et al., 2006). It was shown that CSF and certain brain areas of schizophrenics contain abnormally high levels of KYNA (Miüller and Schwarz, 2007; Erhardt et al., 2007), whereas in depression the endogenous KYNA content is diminished (Myint et al., 2007; Miura et al., 2008).

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ANOVA, analysis of variance; BME, Basal Medium Eagle with Earle's salts; 8-Br-cAMP, 8-bromo-cyclic adenosine monophosphate; cAMP, cyclic adenosine monophosphate; 8pCPT-AMP, 8-(4-chlorophenyl-thio)-cyclic adenosine monophosphate; GFAP, glial fibrillary acidic protein; HPLC, high performance liquid chromatography; KAT, kynurenine aminotransferase; KRB, Krebs-Ringer buffer; KT 5720, (9R,10S,12S)-2,3,9,10,11,12-Hexahydro-10-hydroxy-9-meth yl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]py rrolo[3,4-i][1,6]benzodiazocine-10-carboxylic acid, hexyl ester, KYNA, kynurenic acid; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; PKA, cAMP-dependent protein kinase A.

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KYNA is produced from its bioprecursor, L-kynurenine, in enzymatic reaction catalyzed by at least three kynurenine aminotransferases (KATs) (Guidetti et al., 2007). KAT II is assumed to synthesize approximately 75% of KYNA in the brain (Guidetti et al., 1997). The central levels of KYNA are affected by various factors including the availability of substrate for KATs, the ionic composition of extracellular milieu or the status of oxidative phopshorylation (Urbanska et al., 1997; Luchowski et al., 2002; Kocki et al., 2003; Luchowska et al., 2005; Chmiel-Perzyńska et al., 2007). Notably, the clinical activity of some antiepileptics and memantine may be in part related to an increase of brain KYNA synthesis (Kocki et al., 2006; Kloc et al., 2008). However, much less is known about intracellular regulation of KYNA production.

β-adrenergic neurotransmission is an important factor regulating brain activity such as neuronal and glial survival, plasticity, membrane transport or cellular metabolism (Marien et al., 2004; Hertz et al., 2004). Intracellular β-adrenergic signaling, *via* stimulatory G protein (G_s), activates two major down-stream effectors i.e. adenylate cyclase and cAMP-dependent protein kinase A (PKA) (Marien et al., 2004; Hertz et al., 2004). Both neurons and astrocytes express functional β_1- and β_2-adrenergic receptors on their surface and remain under strong influence of adrenergic impulsation (Hertz et al., 2004). Agonists of β-adrenoceptors were shown to regulate glial activation, to promote the release of cytokines and homocysteic acid or to modify L-arginine uptake (Maimone et al., 1993; Feinstein and Rozelman, 1997; Do et al., 1997).

Recently, we have shown that clenbuterol, β_2-adrenergic agonist enhances the central production of KYNA in brain cortical slices and in glial cultures (Luchowska et al., 2008). The aim of this study was to evaluate the effects of various β_1 and β_2-adrenergic agonists and antagonists on the central production of KYNA, under *in vitro* and *in vivo* conditions, and to investigate the contribution of the cAMP pathway to the endogenous formation of KYNA.

2. Materials and methods

2.1. Animals

Male Wistar rats (220–250 g) were experimental subjects. Animals were housed under standard laboratory conditions, at 20 °C environmental temperature, with food and water available *ad libitum*. Experimental procedures have been approved by the Local Ethical Committee in Lublin and are in agreement with the European Communities Council Directive on the use of animals in experimental studies.

2.2. Substances and materials

L-kynurenine (sulfate salt), KYNA, L-pyruvate, pyridoxal-5′-phosphate, 2-mercaptoethanol, timolol maleate, albuterol hemisulfate salt, denopamine, epinephrine HCl, noradrenaline HCl, 8-(4-chlorophenyl-thio)-cyclic adenosine monophosphate (8pCPT-AMP), 8-bromo-cyclic adenosine monophosphate (8-Br-cAMP), poly-L-lysine hydrobromide, cellulose membrane dialysis tubing, cell culture T-Flasks and plastic multiwell plates were obtained from Sigma (St. Louis, MO, USA). Xamoterol hemifumarate, betaxolol HCl, (9R,10S,12S)-2,3,9,10,11,12-Hexahydro-10-hydroxy-9-meth yl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3',2',1'-kl]pyrrolo[3,4-i][1,6] benzodiazocine-10-carboxylic acid, hexyl ester (KT 5720), formoterol hemifumarate were delivered by Tocris Bioscience (Ellisville, MI, USA). All the high-performance liquid chromatography (HPLC) reagents were supplied by J.T. Baker Laboratory Chemicals (Phillipsburg, NJ, USA). Hanks' salt solution, Basal Medium Eagle with Earle's salts (BME), penicillin/streptomycin, L-glutamine, trypsin and trypsin/EDTA solution were obtained from Biochrom AG (Berlin, Germany), whereas phosphate-buffered saline (PBS) and foetal bovine serum were delivered by GIBCO/Invitrogen (Carlsbad, CA, USA). Deoxyribonuclease was supplied by Worthington Biochemical Corporation (Lakewood, NJ, USA).

2.3. Production of KYNA — brain cortical slices

KYNA production *in vitro* was investigated using freshly obtained rat cortical slices, as previously described (Urbanska et al., 1997). Briefly, animals were killed by decapitation. Their brains were rapidly removed from the skull and cortical slices (1×1 mm base) were immediately prepared with McIlwain tissue chopper. Slices ($n = 8$) were randomly transferred into the incubation wells containing oxygenated Krebs–Ringer buffer (KRB), pH 7.4. After the preincubation period (10 min), slices were incubated (37 °C; 2 h) in the presence of 10 μM L-kynurenine (KYNA substrate) and various solutions of tested drugs (final volume 1 ml). Blanks contained all of the incubation buffer components except for the brain tissue. Following the incubation period, the wells were transferred into an ice-cold water bath, and media were rapidly separated from the tissue. After addition of 50% trichloroacetic acid (14 μl) and 1 N HCl (100 μl), the denaturated protein was removed by centrifugation. Obtained supernatant was stored (−72 °C) until the day of analysis. At least 6 wells were used for each studied concentration and the experiments were repeated at least twice.

KYNA was separated from the supernatant using Dowex 50W$^+$ ionic exchanger and quantified fluorometrically (Varian HPLC system; ESA catecholamine HR-80, 3 μm, C18 reverse-phase column), as described before (Urbanska et al., 1997). The mean control production of KYNA in the presence of 10 μM L -kynurenine was 7.95 ± 1.41 pmol/h/well.

2.4. Production of KYNA — mixed glial cultures

Mixed glial cell cultures were prepared from the brains of newborn Wistar rats, as described previously (Luchowska et al., 2008). Briefly, brain tissue of newborn rats was seeded at a density of one brain/75 cm^2 culture flasks (Falcon, Switzerland) containing 10 ml of Basal Medium Eagle with Earle's salts (BME, Biochrom AG, Berlin), supplemented with 2 mM L-glutamine, penicillin–streptomycin (500 IU/ml–500 UG/ml; Gibco-BRL) and containing 10% heat-inactivated fetal bovine serum (Gibco, InVitrogen). The medium was replaced twice a week. Cultures were maintained at 37 °C in a 5% CO_2/95% air atmosphere. After 14–15 days, the cells were replated on poly-L-lysine-coated 24-microwell plates (Nunc), and the medium was replaced every three days. After one week, a confluent astrocytic monolayer developed with scattered oligodendrocytes and microglia on top. Approximately 75–80% of the cells were glial fibrillary acidic protein (GFAP) — positive (as revealed by immunostaining). The production of KYNA was assessed in tissue cultures after 20–22 days *in vitro*.

The influence of tested compounds on the synthesis of KYNA was evaluated using different exposure times. Standard procedure involved 2 or 24 h incubation. During 2 h incubation, the medium was replaced with the freshly prepared KRB (composed as above). Substances were added to cultures 15 min prior to the addition of L-kynurenine (final concentration 10 μM). The incubation with KYNA substrate lasted always for 2 h. During longer exposure times, the medium was initially changed into the fresh one, and the compounds were added to cultures (start point determining the beginning of incubation). Prior to the addition of L-kynurenine, i.e. 2 h before the end of incubation, the medium was replaced again with freshly prepared KRB. The tested compounds were added once more to the incubation media. This procedure ensured that cells were exposed to studied substances throughout the entire incubation time. Blanks contained all of the incubation buffer components, except for the cultured cells. Controls received an appropriate amount of saline. Further analyses were performed as indicated above.