ORIGINAL ARTICLE

Thioperamide, antagonist of histamine H3 receptors, increases brain production of kynurenic acid

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Abstract: Brain histamine plays the role of neuromodulator and neurotransmitter in a variety of physiological and pathological processes. Kynurenic acid (KYNA) is an antagonist of the glycine site within the N-methyl-D-aspartate (NMDA) receptor complex, and of nicotinic α7 receptors in the brain of mammals. Here, we report that thioperamide, H_3 receptor antagonist/inverse agonist is capable of increasing KYNA synthesis *in vitro*. The effective concentrations were within the range of 0.25-1.0 mM in rat brain cortical slices, and 50-100 µM in mixed glial cultures. An inhibitor of protein kinase A, KT 5720 (0.5 µM) prevented the stimulatory effect of 0.5 mM thioperamide. Thioperamide also increased the activity of KYNA biosynthetic enzyme, KAT I, but not of KAT II. The above data suggest that the neuroprotective action of thioperamide may, at least in part, depend on an enhanced synthesis of KYNA.

Key words: kynurenic acid, *in vitro*, histamine, thioperamide, H3 receptors

INTRODUCTION

Brain histamine plays the role of neuromodulator and neurotransmitter in a variety of physiological and pathological processes, including e.g. immune response, memory formation, food intake, seizures or anxiety [1]. Histamine interacts with four distinct G-protein coupled receptors – H_1, H_2, H_3, H_4 – which are found in the brain and in the periphery [2]. Antagonists of H_1 and H_2 receptors are extensively used in the therapy of allergic disorders and peptic ulcer, respectively. Receptors of H_3 type control the release of histamine and other neurotransmitters, such as monoamines or amino acids [3]. Recently emerged data suggest the potential use of H_3 antagonists in neurodegeneration, obesity, or learning and memory impairment, but their mechanism of action has not been fully clarified [3].

Kynurenic acid (KYNA), a product of irreversible transamination of L-kynurenine, is an antagonist of glycine site within the N-methyl-D-aspartate (NMDA) receptor complex and of nicotinic α7 receptors in the brain of mammals [4]. Released locally, KYNA modifies the glutamate- and acetylcholine-mediated neurotransmission, exerts neuroprotective and antiepileptic properties and thus was implicated in the pathogenesis of neurodegeneration or seizures [4]. The central formation of KYNA can be modulated by intra- and extracellular processes, such as the activity of biosynthetic enzymes, kynurenine aminotransferases (KATs), the status of the mitochondrial oxidative phosphorylation, activity of protein kinase A, or the extracellular concentration of excitatory amino acids agonists [5-7]. Recently, we have

shown that some antiepileptic drugs, as well as memantine or β-adrenergic agonists, stimulate KYNA production [8-10]. Here, we report that thioperamide, H_3 receptor antagonist/inverse agonist is capable of increasing KYNA synthesis *in vitro*.

MATERIALS AND METHODS

Male Wistar rats, weighing 220–250 g, were used for *ex vivo* studies. Newborn Wistar rats (postnatal day 1–2) were used for the preparation of glial cultures. Animals were housed under standard laboratory conditions, at 20°C environmental temperature, with food and water freely available. The experimental procedures used were approved by the First Local Ethics Committee in Lublin and complied with the European Communities Council Directive on the use of animals in experimental studies. L-kynurenine sulphate salt, KYNA, thioperamide and KT 5720 were obtained from Sigma–Aldrich (St.Louis, USA). All the high pressure liquid chromatography (HPLC) reagents were purchased from J.T. Baker Laboratory Chemicals (Holland) and other reagents were obtained from POCH (Gliwice, Poland).

Synthesis of KYNA in rat cortical slices was carried out according to the method of Turski et al. [11]. Briefly, freshly obtained rat brain cortical slices (1x1 mm) were placed at random in culture wells (8/well), and incubated (37°C, 2 hrs) in a standard Krebs-Ringer buffer, pH 7.4 (118.5 mM NaCl, 4.75 mM KCl, 1.77 mM $CaCl_2$, 1.18 mM $MgSO_4$, 12.9 mM NaH_2PO_4, 3 mM Na_2HPO_4, 5 mM glucose), with 10 µM L-kynurenine and various concentrations of studied compounds. KYNA was quantified fluorimetrically (Varian HPLC system; ESA catecholamine HR-80, 3 µm, C18 reverse-phase column).

Mixed glial cell cultures were prepared from the brains of newborn Wistar rats, as described previously [10]. The

experiments were performed on cultures held *in vitro* for 20–22 days. The cells were exposed to studied substances during 24 hrs incubation time (22 hrs in tissue culture medium and 2 hrs in standard Krebs-Ringer buffer). Quantification of produced KYNA was performed as indicated above.

The activities of KAT I and KAT II were assayed as described previously [5]. Briefly, freshly obtained cortical brain tissue from naïve rats was homogenized (1:9; wt:vol) in 5 mM Tris–acetate buffer, pH 8.0, containing 50 µM pyridoxal-5′-phosphate and 10 mM 2-mercaptoethanol. The resulting homogenate was centrifuged (12,000 rpm for 10 min), the supernatant was placed in cellulose membrane dialysis tubing (Sigma) and dialyzed overnight at 8°C, against 4 L of Tris–acetate buffer, composed as above. The obtained semi-purified enzyme preparation was incubated (37°C, 2 hr) in the reaction mixture containing 2 µM L-kynurenine, 1 mM pyruvate, 70 µM pyridoxal-5′-phosphate, 150 mM Tris–acetate buffer, and solutions of the tested compound. KAT II was analyzed at pH 7.0, whereas KAT I at pH 9.5 and in the presence of KAT II inhibitor (2 mM glutamine). Further procedure was carried out as described above.

Data are presented as a percentage of control values ± SD. The statistical analyses were performed using one way analysis of variance (ANOVA), with the adjustment of P value by the Bonferroni method.

RESULTS

Thioperamide increased the synthesis of KYNA *ex vivo* in rat brain cortical slices at concentrations 0.25-1.0 mM (Figure 1A). An inhibitor of protein kinase A, KT 5720 (0.5 µM) prevented the stimulatory effect of 0.5 mM thioperamide (Figure 1B). Thioperamide also enhanced the production of KYNA in mixed primary glial cultures, but with higher potency. The effective doses were within the range of 50-100 µM (Figure 1C). Enzymatic studies revealed that thioperamide increases the activity of KAT I, but not of KAT II, in cortical semi-purified homogenate (Table 1).

Table 1 The effect of thioperamide on the activity of kynurenine aminotransferase (KAT) I and II.

Compound (mM)		KAT I (% of control activity)	KAT II (% of control activity)
Thioperamide	0.02	98 ± 5	99 ± 9
	0.1	111 ± 8*	101 ± 7
	0.25	120 ± 9*	98 ± 8
	0.5	127 ± 7*	100 ± 9

The data are expressed as % of control value ± SEM. *P<0.05 vs control (ANOVA with Bonferroni adjustment of P value).

DISCUSSION

Using brain cortical slices and primary mixed glial cultures we have shown that thioperamide increases the synthesis of KYNA. The concentrations of thioperamide effective in our paradigm were within a similar range described by others in tissue culture systems [12, 13]. The action of thioperamide was reversed by PKA inhibitor, KT 5720, indicating that the observed effect is mediated, at least partially, *via* PKA-related pathway. Moreover, thioperamide directly enhanced the
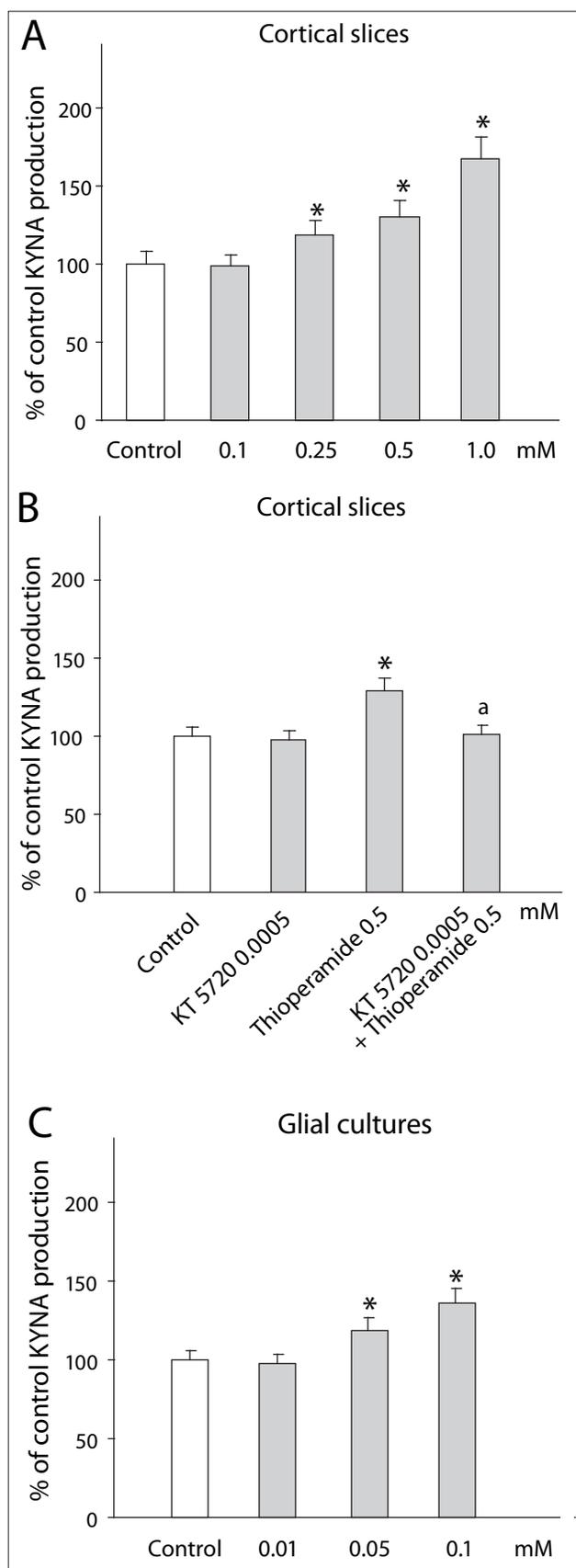

Figure 1 The influence of thioperamide on the production of kynurenic acid (KYNA) in rat brain cortical slices (A, B) and in primary mixed astroglial cultures (C). Panel (C) demonstrates the influence of protein kinase A inhibitor, KT 5720, on the stimulatory effect of thioperamide. The data are expressed as % of control value ± SEM. *P<0.05 vs respective control, ᵃP<0.05 *vs* thioperamide alone (ANOVA with Bonferroni adjustment of P value).

activity of KYNA biosynthetic enzyme, KAT II, as measured in brain homogenate.

Previous studies have shown that thioperamide displays a neuroprotective effect in various experimental models. In organotypic cultures treated with kainic acid, the protective action of thioperamide was associated mainly with the stimulation of histamine release [13]. Others have shown that thioperamide attenuates NMDA-induced excitotoxicity, possibly due to an increased release of γ-amino-butyric acid (GABA) evoked by NMDA stimulation [12].

Thioperamide is an antagonist/reverse agonist at H_3 receptors, thus influencing not only histamine synthesis and release, but also the release of other neurotransmitters, such as glutamate, acetylcholine, dopamine or GABA [14]. H_3 receptors present an interesting feature i.e. apart from an agonist-induced signaling, they are also constitutively active and capable of signal transduction independently of agonist presence [15,16]. Stimulation of H_3 receptors may lead to various intracellular changes, including e.g. inhibition of adenylate cyclase, activation of phopsholipase A2 or phosphorylation of the serine/threonine protein kinase B (Akt)/glycogen synthase 3β (GSK-3β) [16,17]. Initially, the H_3 receptors were demonstrated only on neurons, but recent data indicate their location also on glial cells [18], which are the major source of KYNA in the brain.

We have previously demonstrated that activation of the cAMP/protein kinase A (PKA) pathway enhances production of KYNA [9,10]. Bearing in mind that high constitutive activity of H_3 receptors inhibits cAMP/PKA-dependent pathway [16], thioperamide would reduce the constitutive H_3-mediated inhibition of cAMP/PKA-related events. This should be reflected by an increase of KYNA synthesis, which indeed was the case. Furthermore, the inhibitor of PKA prevented this effect, indicating that the action of thioperamide, apart from stimulation of KAT activity, is associated with PKA-mediated signaling.

Higher brain levels of KYNA may inhibit neuronal loss, which coincides with the described experimental profile of thioperamide. In view of presented data, its neuroprotective action may, at least in part, depend on an enhanced synthesis of KYNA.

ACKNOWLEDGEMENTS

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