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Short communication

SNAP and SIN-1 increase brain production of kynurenic acid

Piotr Luchowski [a], Ewa M. Urbanska [a,b,*]

[a] Department of Pharmacology, Skubiszewski Medical University, Jaczewskiego 8, 20-090 Lublin, Poland
[b] Department of Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, 20-950 Lublin, Poland

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Abstract

The influence of nitric oxide (NO) donors, *S*-nitroso-*N*-acetylpenicillamine (SNAP), and 3-morpholinosydnonimine (SIN-1), on the central production of an endogenous glutamate receptor antagonist, kynurenic acid, was evaluated *in vitro*. In cortical slices, SNAP and SIN-1 potently increased the extracellular concentration of kynurenic acid. A free radical scavenger, L-ascorbate reversed this effect. Neither SNAP nor SIN-1 altered the activity of kynurenic acid biosynthetic enzymes, kynurenine aminotransferases (KAT I and II). These data reveal a novel aspect of the brain response to studied herein NO donors and suggest that in the milieu containing NO-related free radicals the formation of kynurenic acid is enhanced.

Keywords: Kynurenic acid; Nitric oxide; Free radicals; Brain

1. Introduction

Kynurenic acid is an endogenous antagonist of presynaptic α7 nicotinic receptors and of ionotropic excitatory amino acid receptors. Kynurenic acid preferentially affects the glycine site of the *N*-methyl-D-aspartate (NMDA) receptor complex (Nemeth et al., 2005). Produced in situ kynurenic acid may affect glutamate-mediated neurotransmission and change neuronal function and viability (Nemeth et al., 2005). Brain synthesis of kynurenic acid can be regulated through e.g. the availability of its precursor, L-kynurenine, the activity of its biosynthetic enzymes, kynurenine aminotransferases I and II (KATs), or via status of mitochondrial oxidative phosphorylation (Luchowski et al., 2002; Luchowska et al., 2003). In addition, other endogenous factors capable of changing the production of kynurenic acid have been identified. The excitatory amino acid receptor agonists, such as L-cysteine-sulphinate, L-glutamate or L-aspartate, as well as ammonia, a neurotoxin implicated in the pathophysiological symptoms of hepatic encephalopathy were shown to inhibit the formation of kynurenic acid (Urbańska et al., 1997; Saran et al., 1998; Kocki et al., 2003). The opposite effects are exerted by adenosine, whereas D,L-homocysteine and *S*-adenosylhomocysteine act bimodally, either increasing or decreasing the production of kynurenic acid (Wu et al., 2004; Luchowska et al., 2005).

Nitric oxide (NO) is a gaseous intercellular signaling molecule and a modulator of neurotransmission which has been implicated in a broad spectrum of physiological brain functions (Contestabile et al., 2003; Guix et al., 2005). When excessively released, NO can contribute to neuropathological events, however, there are data suggesting that NO may also play a protective role (Contestabile et al., 2003; Guix et al., 2005; Figueroa et al., 2005; Khan et al., 2006). We have previously reported that nonselective NO synthase (NOS) inhibitors, *N*-G-nitro-L-arginine and its methyl ester inhibit the brain synthesis of kynurenic acid (Luchowski et al., 2001). Here, we report that two NO donors, *S*-nitroso-*N*-acetyl-penicillamine (SNAP) and 3-morpholinosydnonimine (SIN-1) enhance the production of kynurenic acid *in vitro*.

2. Materials and methods

2.1. Materials

Male Wistar rats (220–250 g) were experimental subjects. Animals were housed under standard laboratory conditions, at

* Corresponding author. Department of Pharmacology, Skubiszewski Medical University, Jaczewskiego 8, 20-090 Lublin, Poland. Tel.: +48 81 7405851.
E-mail address: emurbanska@poczta.onet.pl (E.M. Urbanska).

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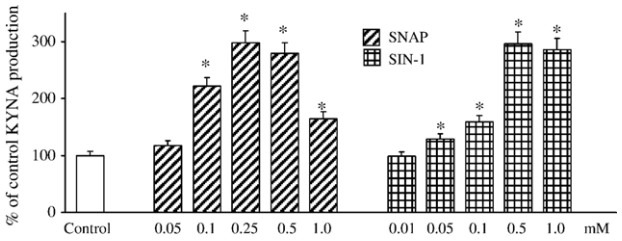

Fig. 1. The effect of SNAP and SIN-1 on the production of kynurenic acid in cerebral cortical slices. Data are mean values±S.D. of six determinations. *$P<0.05$ (ANOVA) vs. control (100%). Slices (1×1 mm base) were placed at culture wells (8 per well) containing 1 ml of oxygenated Krebs–Ringer buffer, pH 7.4. Following 10 min preincubation, the tissue was incubated (37 °C; 2 h) in the presence of 10 μM L-kynurenine and solutions of tested drugs.

20 °C environmental temperature, with food and water 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.

L-kynurenine (sulphate salt), kynurenic acid, L-ascorbate, L-pyruvate, pyridoxal-5′-phosphate, 2-mercaptoethanol, cellulose membrane dialysis tubing and plastic multiwell plates were obtained from Sigma (St. Louis, MO, USA). SNAP and SIN-1 were delivered by Tocris Bioscience (Ellisville, MI, USA), whereas all the high-performance liquid chromatography (HPLC) reagents were supplied by J.T. Baker Laboratory Chemicals (Phillipsburg, NJ, USA).

2.2. Methods

Kynurenic acid production *in vitro* was investigated using freshly obtained rat cortical slices, as previously described (Urbańska 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, pH 7.4. After the preincubation period (10 min), slices were incubated (37 °C; 2 h) in the presence of 10 μM L-kynurenine and various solutions of tested drugs, in a final volume of 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, media were rapidly separated from the tissue and acidified with 50% trichloroacetic acid (14 μl) and 1N HCl (100 μl). 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 three times.

Kynurenic acid was quantified fluorometrically (Varian HPLC system; ESA catecholamine HR-80, 3 μm, C18 reverse-phase column), as described before (Urbańska et al., 1997). The mean control production of kynurenic acid in the presence of 10 μM L-kynurenine was 8.21±1.35 pmol/h/well.

The activities of KAT I and KAT II were assayed in another set of animals, as described before (Kocki et al., 2003), in cortical brain tissue. Briefly, cortical brain tissue 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 and dialyzed overnight at 8 °C, against 41 of the Tris–acetate buffer composed as above. The obtained semi-purified enzyme preparation was incubated 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 tested drugs. The reaction was carried out at pH of 7.0 (KAT II) or 9.5 (KAT I). Six replicates were used for each concentration and each experiment was repeated twice. Glutamine (final concentration 2 mM), the inhibitor of KAT I, was added to samples assaying KAT II activity. Blanks contained the enzyme preparation that was heat-deactivated at 100 °C for 10 min. The incubation (37 °C, 2 h) was terminated by a rapid transfer of samples to an ice-cold water bath. Next, 14 μl of 50% trichloroacetic acid (wt:vol) and 100 μl of 1 N HCl were added to each sample. Further procedures were performed as indicated above.

The statistical evaluation of results was performed using one-way analysis of variance (ANOVA) with post-hoc comparisons according to the Bonferroni method.

3. Results

An exogenous NO donor, SNAP (0.1, 0.25, 0.5 and 1.0 mM) increased kynurenic acid synthesis to 222% ($P<0.001$), 298% ($P<0.001$), 279% ($P<0.001$) and 165% ($P<0.001$) of control, respectively (Fig. 1). Similarly, SIN-1 (0.05, 0.1, 0.5, and 1.0 mM) augmented kynurenic acid production in cortical slices to 129% ($P<0.05$), 159% ($P<0.001$), 296% ($P<0.001$), and 286% ($P<0.001$) of control, respectively (Fig. 1).

The use of antioxidant, L-ascorbate (3.0 mM) almost totally prevented the increase of kynurenic acid production evoked by either 0.5 mM SNAP (111% vs. 251% of control; $P<0.001$), or 0.5 mM SIN-1 (154% vs. 310% of control; $P<0.001$).

Table 1
The influence of NO donors on the cortical activity of KAT I and II

Substance (mM)	KAT I activity (% of control)	KAT II activity (% of control)
SNAP		
0.1	100.2±6.8	98.7±9.9
1.0	104.9±6.3	100.2±5.8
2.0	96.3±7.8	99.7±9.0
SIN-1		
0.1	104.2±6.2	96.2±8.7
1.0	100.3±9.3	98.1±3.5
2.0	93.2±10.1	95.7±7.5

Data are mean values±S.D. of six determinations (presented as % of control value). Tissue was incubated for 2 h, at 37 °C, in the presence of tested compound, 2 μM L-kynurenine, 1 mM pyruvic acid and 70 μM pyridoxal 5′-phosphate in 150 mM Tris–acetate buffer, at pH 7.0 (KAT II) or 9.5 (KAT I). Glutamine (final concentration 2 mM), the inhibitor of KAT I, was added to samples assaying KAT II activity. Blanks contained the enzyme preparation that was heat-deactivated at 100 °C for 10 min. Newly synthesized kynurenic acid was quantified fluorometrically, with HPLC.

The activity of KAT I was not changed in the presence of NO donors (both 0.5 and 1.0 mM), and reached respectively 104.9 ± 6.3 and 96.3 ± 7.8% of control (SIN-1), or 100.3 ± 9.3 and 93.2 ± 10.1% of control (SIN-1) (Table 1). The activity of KAT II was also not altered and reached respectively 100.2 ± 5.8 and 99.7 ± 9.0 (0.5 and 1.0 mM SNAP), or 98.1 ± 3.5 and 95.7 ± 7.5% of control (0.5 and 1.0 mM SIN-1) (Table 1).

4. Discussion

The above data demonstrate a novel aspect of the central actions induced by two NO donors, SNAP and SIN-1. Both compounds potently augmented the synthesis of kynurenic acid in cortical slices in the mechanism independent of KATs-mediated transamination of L-kynurenine. The activities of KAT I and KAT II remained unaltered even in the presence of milimolar levels of SNAP or SIN-1.

Production of kynurenic acid in cortical slices was enhanced at low, micromolar concentrations of both NO donors. Similar concentrations of SNAP and SIN-1 were demonstrated to modulate the function of central nervous system under various experimental paradigms (Moro et al., 1998; Polte et al., 1997; Figueroa et al., 2005). The mechanisms underlying central actions of NO are not fully understood, but direct *S*-nitrosylation of target proteins and activation of cyclic GMP-dependent protein phosphorylation cascades, are considered the key ones (Guix et al., 2005). Studied herein NO donors display various biochemical features. SNAP is the NO. generator and *S*-nitrosylating agent, whereas SIN-1 is the NO./peroxynitrite ($ONOO^-$) generator with a very weak *S*-nitrosylating activity (Contestabile et al., 2003; Guix et al., 2005). Considering the fact that both NO donors showed similar potency in our experimental paradigm, *S*-nitrosylation does not seem to contribute to the increase of kynurenic acid production.

NO donors generate substantial amounts of reactive oxygen species (Hogg et al., 1992; Beckman and Koppenol, 1996). Free radicals and their reactive reaction products may cause oxidative damage and thus play a role in neuronal loss. Paradoxically, it was shown that kynurenic acid, presumably a neuroprotectant, can be formed not only during KATs-mediated processes, but also in the reaction involving free oxygen radicals, without contribution of KATs (Zsizsik and Hardeland, 2002). Therefore, we hypothesized that the action of SNAP and SIN-1 is linked to the excessive release of reactive oxygen species. Indeed, the pretreatment of cortical slices with L-ascorbate, a free radical scavenger (Kirsch and de Groot, 2000), fully prevented the increase of kynurenic acid production evoked by NO donors. Thus, our data suggest that NO-related free radicals may enhance kynurenic acid synthesis in KATs-independent way. However, it cannot be excluded that cGMP-mediated events are also implicated in the observed effect, what will be the subject of further research.

The numerous experimental studies have clearly shown the dual role of NO in the mechanisms of neuroprotection and neurodegeneration (Contestabile et al., 2003; Guix et al., 2005). Studied herein NO donors, SNAP and SIN-1 were frequently demonstrated by others to induce as well as to prevent neuronal loss (Moro et al., 1998; Polte et al., 1997; Figueroa et al., 2005; Khan et al., 2006). Enhanced formation of kynurenic acid associated with the use of SIN-1 and SNAP may partially contribute to their neuroprotective effects. Such an increased synthesis of neuroinhibitory compound in the milieu containing excessive amounts of NO-related reactive species may represent a biological response attempted to limit the cellular damage.

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