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Short communication

Endothelium-dependent production and liberation of kynurenic acid by rat aortic rings exposed to L-kynurenine

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Abstract

Rat aortic slices produced and liberated the endogenous antagonist of glutamate receptors, kynurenic acid, upon exposure to L-kynurenine. Endothelium-denuded slices did not synthesize any measurable amount of kynurenic acid, indicating its endothelial origin. Aortic kynurenic acid production was diminished by modification of the ionic milieu, hypoxia and hypoglycemia, as well as by L-glutamate and L-aspartate, endogenous glutamate receptor agonists, and aminooxyacetic acid, a non-selective inhibitor of aminotransferases and mitochondrial respiration. These data pave the way for future research aimed to clarify the role of kynurenic acid in the physiology and pathology of the endothelium and vasculature.
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Keywords: Aortic slice, rat; Kynurenic acid; Endothelium; Excitatory amino acid receptor; Glucose; Oxygen

1. Introduction

The endogenous excitatory amino acids, such as glutamate or aspartate, are major excitatory neurotransmitters interacting with specific iono- and metabotropic glutamate receptors in the mammalian central nervous system (CNS) (Meldrum, 1994). Increasing evidence suggests that excitatory amino acids might also play a modulatory role in peripheral tissues, including cardiovascular tissue (Lorenzo et al., 1994; Winter and Baker, 1995; Gill et al., 1998). The occurrence of ionotropic receptors of *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-izoxazole propionate (AMPA) type has been proposed in rat atria, whole heart tissue, cultured myocardial cells and portal vein by several groups (Lorenzo et al., 1994; Winter and Baker, 1995; Gill et al., 1998; Rossetti et al., 2000). Recent data suggest that NMDA receptors might be also present in aortic tissue (Crespi et al., 2000).

Kynurenic acid is the only known broad-spectrum, endogenous antagonist of glutamate receptors, displaying high affinity for the glycine site of the NMDA receptor complex (Stone, 2000). It has been implicated as a modulator of physiological glutamate neurotransmission, and its altered metabolism has been suggested as one of the factors contributing to the development of certain CNS disorders, possibly of excitotoxic nature (Schwarcz et al., 1984; Stone, 2000). The central production of kynurenic acid and mechanisms governing its regulation are relatively well described, but the peripheral role of kynurenic acid remains enigmatic. The presence of its biosynthetic enzymes was demonstrated in rat kidneys, liver and small intestine, and in human heart tissue (Noguchi et al., 1975; Baran et al., 1997). In contrast, there are no data until now on the synthesis of kynurenic acid by vascular tissue. Therefore, we aimed to evaluate and characterize the ability of rat aortic tissue to produce kynurenic acid under in vitro conditions.

Preliminary data have been presented in the form of an abstract (Stążka et al., 2001).

2. Materials and methods

The production of kynurenic acid by aortic slices was measured based on the method described by Turski et al.

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(1989). Male Wistar rats (220–250 g), housed under standard laboratory conditions (12-h light/dark cycle and free access to food and water), were used.

Animals were decapitated and perfused transcardially with 40 ml of ice-cold Krebs–Ringer buffer (KRB), pH 7.4 (composed as below). The thoracic portion of the aorta was rapidly removed and horizontal slices (width 0.75 mm; weight ~1.0 mg/slice) were prepared with a McIlwain tissue chopper. For the experiments with endothelium-denuded slices, the endothelial layer was gently removed by rubbing, and the accuracy of the procedure was checked at the end of experiments by light microscopy. Slices were placed in culture wells (five slices per well) containing 1 ml of standard oxygenated KRB (NaCl, 118.5 mM; KCl, 4.75 mM; $CaCl_2$, 1.77 mM; $MgSO_4$, 1.18 mM; NaH_2PO_4, 12.9 mM; Na_2HPO_4, 3 mM; glucose, 5 mM; pH 7.4). Based on preliminary studies in which different L-kynurenine concentrations and various incubation times were used, the experimental conditions were set as follows: concentration of substrate, 50 μM; duration of incubation, 1 h. Tested compounds (when used) were added to the incubation media 15 min before L-kynurenine.

Changes in the composition of standard KRB were made by selectively substituting buffer elements without changing the overall molarity of the solution. Thus, in the Na^+-free medium, sodium chloride was replaced by choline chloride and in Cl^--free medium, NaCl was replaced by sodium isethionate. Similarly, increases in the concentration of K^+ were compensated for by decreases in the concentration of NaCl. Tris–acetate buffer, pH 7.4, replaced phosphate buffer when a high concentration of Ca^{2+} (10 mM) was investigated. For the anoxic conditions, KRB was bubbled with helium, instead of oxygen, for 30 min.

Blank values were always evaluated and obtained by incubating the appropriate amount of L-kynurenine in KRB, composed as above, without the tissue slices, and under conditions specific for a given type of experiment.

Following the incubation period, culture wells were placed on ice and medium was rapidly separated from the tissue, mixed with 100 μl of 1 M HCl and 14 μl of 50%

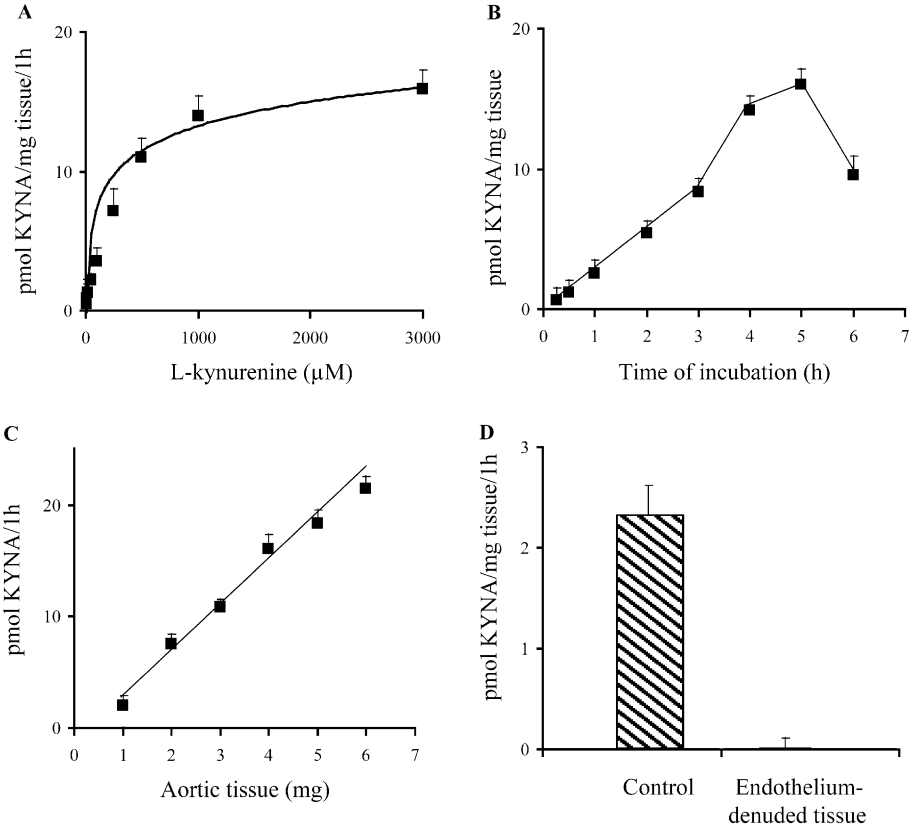

Fig. 1. (A) The effect of precursor concentration (L-kynurenine) on the production of kynurenic acid in rat aortic slices. Slices were placed in culture wells (five per well) containing 1 ml of oxygenated KRB, pH 7.4. Tissue was incubated in the presence of different concentrations of L-kynurenine for 1 h at 37 °C. (B) The effect of duration of incubation on kynurenic acid production in rat aortic slices. Slices were placed in culture wells (five per well) containing 1 ml of oxygenated KRB, pH 7.4. Tissue was incubated in the presence of 50 μM L-kynurenine for 0.25–6 h at 37 °C. (C) The effect of amount of tissue on kynurenic acid production in rat aortic slices. Slices were placed in culture wells (one to six per well) containing 1 ml of oxygenated KRB, pH 7.4. Tissue was incubated in the presence of 50 μM L-kynurenine for 1 h at 37 °C. (D) Endothelium dependence of kynurenic acid production in rat aortic slices. Control slices (endothelium-preserved) and endothelium-denuded slices were placed in culture wells (five per well) containing 1 ml of oxygenated KRB, pH 7.4. Tissue was incubated in the presence of 50 μM L-kynurenine for 1 h at 37 °C. All data are mean values±S.D. of six determinations. Kynurenic acid was detected fluorimetrically using HPLC.

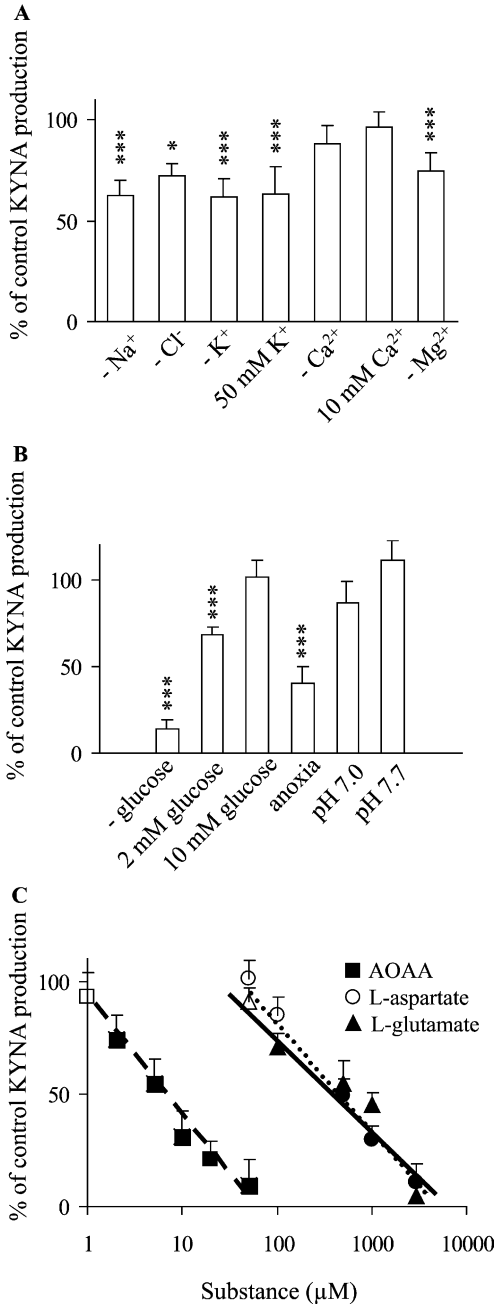

Fig. 2. (A) The effect of ionic changes on the production of kynurenic acid in rat aortic slices. (B) The effect of pH, glucose concentration and hypoxic conditions on the production of kynurenic acid in rat aortic slices. Slices were placed in culture wells (five per well) containing 1 ml of control or modified as needed, oxygenated or non-oxygenated KRB of appropriate pH. Tissue was incubated in the presence of 50 μM L-kynurenine for 1 h at 37 °C. *P<0.05, ***P<0.001 vs. control; Student's t-test. (C) The effect of aminooxyacetic acid (AOAA), L-glutamate and L-aspartate on kynurenic acid production in rat aortic slices. Slices were placed in culture wells (five per well) containing 1 ml of oxygenated KRB, pH 7.4. Tissue was incubated in the presence of 50 μM L-kynurenine and solutions of tested drug for 1 h at 37 °C. Filled symbols represent data points that reached statistical significance (P<0.05; ANOVA test) vs. control (100%). AOAA: y=−21.975x+89.43, r=−0.99; L-aspartate: y=−22.103x+186.05, r=−0.99; L-glutamate: y=−18.558x+163.29, r=−0.97. All data are mean values ±S.D. of six determinations. X-axis shows log concentrations. Kynurenic acid was detected fluorimetrically using HPLC.

trichloroacetic acid, and centrifuged for 5 min at 12 000 rpm. The resulting supernatants were applied to cation-exchange columns (Dowex 50 W, H^+). Kynurenic acid was eluted with H_2O and quantified fluorimetrically using a high-performance liquid chromatography (HPLC) system (Varian, USA) with a fluorescence detector (excitation 246 nm, emission 404 nm) as previously described (Urbańska et al., 1997). The mean control production of kynurenic acid in the presence of 50 μM L-kynurenine was 1.98±0.38 pmol/mg tissue/1 h.

IC_{50} values, i.e. the concentrations of compounds necessary to induce 50% inhibition of kynurenic acid synthesis (with 95% confidence limits), were calculated using computerized linear regression analysis of quantal log dose-probit functions. Statistical analyses of the data were performed using ANOVA test with the adjustment of P value by the method of Bonferroni.

3. Results

In the initial studies, we evaluated the ability of aortic slices to produce kynurenic acid. The synthesis of kynurenic acid was linear with the concentration of its precursor, L-kynurenine, up to 0.25 mM, and reached a plateau at 0.5 mM (Fig. 1A). Synthesis proceeded linearly with the incubation time, up to 5 h, and later on decreased (Fig. 1B). It was proportional to the amount of aortic tissue used for the incubation (studied up to 6 mg) (Fig. 1C).

The production of kynurenic acid did not occur following the removal of endothelium from aortic rings (Fig. 1D).

The synthesis of kynurenic acid decreased in the absence of Na^+, K^+, Cl^- and Mg^{2+} to 62.4% (P<0.001), 62.0% (P<0.001), 72.1% (P<0.05) and 74.3% (P<0.001) of control, respectively (Fig. 2A). High K^+ (50 mM) inhibited the production of kynurenic acid to 63.0% (P<0.001) of control, respectively (Fig. 2A). In contrast, the synthesis of kynurenic acid was not altered by the removal of Ca^{2+} or high Ca^{2+} (10 mM) (Fig. 2A). Low glucose, removal of glucose and absence of oxygen suppressed kynurenic acid formation to 68.3% (P<0.001), 14.2% (P<0.001) and 40.7% (P<0.001) of control, respectively (Fig. 2B). No changes in the production of kynurenic acid were observed at pH 7.0 or 7.7 (Fig. 2B).

L-Glutamate, L-aspartate and aminooxyacetic acid diminished the production of kynurenic acid with IC_{50} values of 403.3 (312.1–521.3), 461.3 (346.5–514.1) and 5.5 (4.4–6.9) μM, respectively (Fig. 2C).

4. Discussion

The presented data indicate the ability of rat aortic slices to produce and liberate kynurenic acid, an endogenous antagonist of ionotropic excitatory amino acid receptors. The synthesis of kynurenic acid by rat aortic slices pro-

ceeded linearly with the concentration of its precursor, L-kynurenine, with the incubation time and with the amount of tissue used. Moreover, the production of kynurenic acid seems to be entirely endothelium-dependent, as the endothelium-denuded aortic rings did not produce any measurable amount of this compound. Preliminary studies with endothelial cells in culture showed that the endothelium indeed intensively synthesizes kynurenic acid (unpublished data).

The production of kynurenic acid by aortic rings is influenced by the ionic composition of the incubation medium. The amount of synthesized kynurenic acid was significantly decreased by the absence of K^+, Cl^- and Mg^{2+}, as well as by a high concentration of K^+. The inhibitory effect of high K^+ seems surprising since depolarizing concentrations of K^+ usually increase the release of most neurotransmitters and hormones from cells. However, data similar to ours were obtained with cortical slices subjected to high K^+ (Turski et al., 1989). In the brain, kynurenic acid is produced mainly within glial cells. There is no evidence of its vesicular storage and it seems to be produced de novo and liberated, not released, from these cells (Turski et al., 1989). Possibly, such a mechanism could also apply to the cells of aortic rings.

As in the brain (Turski et al., 1989), the aortic production of kynurenic acid was not affected by the lack of or by high levels of Ca^{2+} in the medium. In contrast to brain tissue though, where depletion of Na^+ increases the synthesis of kynurenic acid (Turski et al., 1989), aortic slices exposed to medium low in the Na^+ ions produced less kynurenic acid.

Both L-glutamate, a neurotransmitter itself and an important metabolite, and L-aspartate reduced the aortic production of kynurenic acid. The range of inhibitory potency in aortic slices corresponded with the inhibitory activity revealed in brain cortical tissue (Turski et al., 1989; Urbańska et al., 1997). The inhibitory activity of L-glutamate and L-aspartate might result from their interference with the enzyme(s) synthesizing kynurenic acid. Indeed, L-glutamate diminishes the activity of kynurenine aminotransferases in the brain (Guidetti et al., 1997). Bearing this in mind, one could hypothesize that the action of high K^+ discussed above might be due to the release of inhibitory compound(s), such as L-glutamate, which could in turn diminish the activity of kynurenic acid biosynthetic enzyme(s). This will be the subject of further studies.

The vascular production of kynurenic acid is affected by ischemia-like conditions in vitro. A hypoxic and hypoglycemic milieu and addition of aminooxyacetic acid strongly reduced the synthesis of kynurenic acid by aortic tissue. Aminooxyacetic acid, a non-selective inhibitor of pyridoxal phosphate-dependent enzymes, including kynurenine aminotransferases (Turski et al., 1989), impairs oxidative phosphorylation via blockade of the malate-apartate shuttle (Kauppinen et al., 1987). In this study, aminooxyacetic acid effectively blocked the synthesis of kynurenic acid in a very low, micromolar range. These data could suggest that the enzymatic activity and/or status of mitochondrial respiration contribute to the aortic production of kynurenic acid.

We demonstrated that aortic rings synthesize kynurenic acid. Is the amount of vasculature-derived kynurenic acid sufficient to play physiological role? Calculations based on the amount of L-kynurenine found in serum (~2 μM; Morita et al., 1990), the production of kynurenic acid by human arterial slices at this L-kynurenine concentration (16 fmol/1 mg tissue/1 h, unpublished data), the total weight of human endothelium (1 kg; Cines et al., 1998) and the average serum volume (3.5 l) give a concentration of kynurenic acid of approximately 4.5 pmol/100 μl. The concentration of kynurenic acid in human serum reaches 1.9 pmol/100 μl (Milart et al., 1999), which is less than the above estimated value. However, kynurenic acid is efficiently excreted in urine (Rubaltelli et al., 1974), so its concentration in serum must be lower than the calculated concentration. Thus, the vascular endothelium seems to be an important source of circulating kynurenic acid.

The endothelium is equipped with various receptors for proteins, metabolites, hormones, etc., and is currently viewed as a dynamic organ possessing vital secretory, metabolic and other functions (Cines et al., 1998). The role of possibly another endothelial factor, kynurenic acid, in the function of the endothelium, and thus in the physiology and pathology of the circulatory system, requires close assessment. Produced in relatively high amounts kynurenic acid might be an inert metabolite or an active product modulating certain endothelial/vascular functions.

The results of our study pave the way for the future research necessary to clarify the potential role of kynurenic acid in the physiology and pathology of the endothelium and vasculature.

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