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Micromolar concentration of kynurenic acid in rat small intestine

Short Communication

D. Kuc[1], **W. Zgrajka**[2], **J. Parada-Turska**[3], **T. Urbanik-Sypniewska**[4], and **W. A. Turski**[1,2]

[1] Department of Experimental and Clinical Pharmacology, Medical University, Lublin, Poland
[2] Department of Toxicology, Institute of Agricultural Medicine, Lublin, Poland
[3] Department of Rheumatology and Connective Tissue Diseases, Medical University, Lublin, Poland
[4] Department of General Microbiology, Institute of Microbiology and Biotechnology, Maria Curie-Sklodowska University, Lublin, Poland

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Summary. Kynurenic acid is an antagonist of glutamate and alpha-7 nicotinic acetylcholine receptors and an agonist of the G-protein-coupled receptor GPR35, which is predominantly expressed in immune and gastrointestinal tissues. In this study, we report that kynurenic acid is present in the lumen of rat small intestine in micromolar concentration sufficient to affect the GPR35 receptor. Moreover, we show that kynurenic acid can be produced by *Escherichia coli*. We suggest that kynurenic acid may modulate gastrointestinal function and integrity.

Keywords: Kynurenic acid – Small intestine – Rat

Introduction

Kynurenic acid is an endogenous component of the mammalian body. Its presence was first demonstrated in urine by Liebig (1853). In 1988 it was identified in human brain tissue (Turski et al., 1988). Kynurenic acid acts as an antagonist at glutamate receptors (Birch et al., 1988) and the alpha-7-nicotinic acetylcholine receptor (Hilmas et al., 2001) and therefore its potential role as an endogenous neuroprotective agent has been suggested (Sas et al., 2007). However, this hypothesis has been questioned, because in the brain kynurenic acid reaches low nanomolar concentration (Moroni et al., 1988; Turski et al., 1988) while blockade of the glycine site of the N-methyl-D-aspartate receptor or inhibition of alpha-7 nicotinic receptors seems to require low micromolar concentration (Hilmas et al., 2001). Inhibition of the alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate receptors occurs at milimolar concentrations (Birch et al., 1988).

The importance of kynurenic acid outside the brain is unknown. Here, we report for the first time that kynurenic acid is present in the lumen of the small intestine of the rat, and that its concentration is sufficient to affect the glycine site of the NMDA receptor, the alpha-7 nicotinic receptor and G-protein-coupled receptor GPR35 (Birch et al., 1988; Hilmas et al., 2001; Wang et al., 2006).

Material and methods

Animals

Eight Wistar male rats (220–240 g) were housed in a temperature- and humidity-controlled animal unit at an ambient temperature of $21 \pm 2\,^{\circ}C$ and in a 12–12 h light–dark cycle. Before the experiment, the animals were deprived of food for 24 h. Water was available *ad libitum*. Rats were killed by decapitation and samples of intestinal fluid and tissue were collected. Samples were taken from the duodenum (between the pylorus and the Treitz ligament), jejunum (starting approximately 5 cm distal to the ligament of Treitz), proximal, middle and distal ileum (the ileo-caecal junction was the endpoint). The experiments were performed according to the rules of animal care and were approved by the Local Ethics Committees for the Use of Animals in Biomedical Experiments in Lublin, Poland.

Substances

Kynurenine sulphate and kynurenic acid were obtained from Sigma-Aldrich. All HPLC reagents used in the study were obtained from Baker (Germany) and were of the highest available purity.

KYNA determination

Samples were sonicated (1:5 wt/vol) in distilled water. The resulting homogenate was centrifuged. The supernatant was acidified with 14 µl of

50% trichloroacetic acid and centrifuged. Supernatant was applied on cation-exchange resin (Dowex 50 W+, Sigma). Eluted kynurenic acid was subjected to the HPLC (Hewlett Packard 1050 HPLC system: ESA catecholamine HR-30, 3 μm, C_{18} reverse-phase column) and quantified fluorometrically (Hewlett Packard 1046A fluorescence detector: excitation 344 nm, emission 398 nm).

KYNA production

Escherichia coli ATCC 25922 were grown in Luria-Bertani broth (LB, Lab.Conda, Madrid, Spain) at 37 °C to the early exponential phase (OD_{600} 0.1–0.2). On the day of the experiment, the medium was replaced by Hank's balanced salt solution (HBSS, Sigma) enriched with 0.2% glucose. To the cell suspension of final density of $2.5 \pm 0.5 \times 10^2$ cfu/ml (colony forming units per ml) 10 μM of L-kynurenine (Sigma) was added and bacteria were incubated with shaking (120 rpm) at 37 °C for 6 h. At the end of incubation time supernatants were collected and treated with 14 μl of 50% trichloroacetic acid. Precipitated proteins were removed by centrifugation. Supernatants were analyzed for KYNA content as previously described. The number of *E. coli* colony forming-units was estimated by plating of serial decimal dilutions of the aliquots of incubation media and by count of the number of colonies grown on agar plates after overnight incubation at 37 °C.

Statistical analysis

Data are presented as the mean ± S.E.M. Statistical analysis was accomplished using one-way ANOVA with post hoc Student-Newman-Keuls test. $P < 0.05$ were considered significant.

Results

Kynurenic acid content in small intestine

The presence of kynurenic acid was determined in all samples of intestinal fluid. In samples to which authentic kynurenic acid was added, only one peak was recorded on the chromatogram (data not shown). Mean concentration of kynurenic acid in the jejunum, proximal, middle and distal ileum was 1.49 ± 0.37, 3.30 ± 0.67, 8.08 ± 3.23 and 16.10 ± 5.56 μM, respectively (Fig. 1).

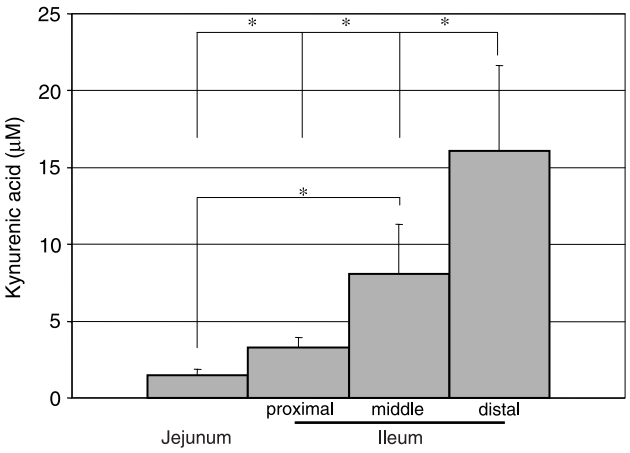

Fig. 1. Kynurenic acid content in rat intestinal fluid. Data are presented as a mean ± S.E.M.; one-way ANOVA with post hoc Student-Newman-Keuls test; $^*P < 0.05$

The content of kynurenic acid in the wall of duodenum, jejunum and ileum was 0.29 ± 0.04, 0.21 ± 0.08 and 0.28 ± 0.12 nmol/g wet tissue, respectively.

Kynurenic acid production

In the presence of L-kynurenine, *E. coli* produced kynurenic acid and liberated it into incubation medium in a dose- and time-dependent manner (data not shown). The mean production of kynurenic acid in the presence of 10 μM L-kynurenine was 6.07 ± 1.14 pmol/$3.0 \pm 0.5 \times 10^4$ cfu/6 h.

Discussion

In this study, we report that kynurenic acid is present in the intestinal fluid and that the concentration of this compound increases along the small intestine, reaching mean concentration of 16 μM in its distal part. The origin of kynurenic acid in the small intestine is unknown. Since the standard animal food contains kynurenic acid in the mean amount of 623 pmol/g (unpublished data), the rats were deprived of food for 24 h, so that the digestive system was free from food particles. Therefore, it can be concluded that food was not a source of kynurenic acid. The content of kynurenic acid in the wall of non-perfused small intestine varied from 0.21 to 0.29 nmol/g (~0.2–0.3 μM), which is much lower than in the intestinal fluid. Thus, it seems unlikely that the high amount of kynurenic acid is produced in the wall of the small intestine and secreted to the intestinal fluid. It is possible that kynurenic acid is a product of intestinal microflora. It was demonstrated that *E. coli* possesses an enzyme with high kynurenine aminotransferase and glutamine transaminase K activities, and that the enzyme responsible for these activities is AspAT (Han et al., 2001). Both enzymes catalyze the production of kynurenic acid from kynurenine (Cooper, 2004). An accumulation of kynurenic acid was also observed in the reaction mixtures containing cell-free extracts of *E. coli* supplemented with L-kynurenine and pyruvate (Han et al., 2001). Furthermore, we found that in the presence of L-kynurenine, whole bacteria produce kynurenic acid and liberate it to the incubation medium. In our previous study, we reported the bactericidal effect exerted by kynurenic acid in milimolar concentrations and suggested its contribution to the control of oral microflora (Kuc et al., 2006).

Recently, kynurenic acid was reported to act as a ligand for the orphan G-protein-coupled receptor GPR35 (Wang et al., 2006). It was found that kynurenic acid is an agonist eliciting calcium mobilization and inositol phosphate pro-

duction in a GPR35-dependent manner (Wang et al., 2006). The EC_{50} concentration of kynurenic acid for human, rat and mouse GPR35 was 39.2, 7.4, and 10.7 μM, respectively (Wang et al., 2006).

It should be emphasized that the concentration of kynurenic acid found in our study increases along with microflora concentration in the jejunum and ileum and reaches the highest value in the distal part of rat small intestine (16 μM) that is sufficiently high to affect the GPR35 receptor. Interestingly, expression analysis by quantitative reverse transcriptase mediated PCR revealed that both human and mouse GPR35 were predominantly expressed in immune and gastrointestinal tissues, with limited expression in other tissues (Wang et al., 2006). In humans and mice, high levels of GPR35 expression were detected in the gastrointestinal tract. Accordingly, specific GPR35-positive signals were recorded in the duodenum, jejunum, ileum, cecum, colon, and rectum (Wang et al., 2006). In various regions of the intestine, GPR35 was primarily expressed in the epithelial cells located in the crypts of Lieberkühn, with lower expression in the intestinal villi. No significant signals were detected in lamina propria, muscularis propria, and enteric neurons (Wang et al., 2006). Among the various subpopulations of immune cells, GPR35 was detected in CD14 monocytes, T cells, neutrophils, and dendritic cells, with lower expression in B cells, eosinophils, basophils, and platelets (Wang et al., 2006).

Therefore, these findings raise the question regarding the biological role of kynurenic acid in the digestive system.

Expression analysis indicating that GPR35 receptors are predominantly located in immune cells and the gastrointestinal tract may suggest that they contribute to the modulation of gastrointestinal immune response and the process of regeneration of gastrointestinal epithelium (Hauck et al., 2005; Wang et al., 2006). The finding that kynurenic acid inhibits LPS-induced TNFα secretion in peripheral blood monocytes (Wang et al., 2006) suggests an anti-inflammatory effect and further supports this hypothesis.

On the other hand, kynurenic acid is an antagonist of glutamate receptors and the alpha-7 nicotinic receptor (Birch et al., 1988; Hilmas et al., 2001). In fact, glutamate receptors were found in the enteric nervous system on neurons in both submucosal and myenteric plexuses (Liu et al., 1997). Similarly, alpha-7 immunoreactivity was found on both nerve fibers and enteric cell bodies (Kirchgessner and Liu, 1998). The role of glutamate and alpha-7 nicotinic receptors in the gastrointestinal tract has to be elucidated.

Taking into account that the concentration of kynurenic acid in rat small intestine is sufficient to interact with GPR35, NMDA and alpha-7 nicotinic receptors, it can be suggested that kynurenic acid may modulate the gastrointestinal function and integrity.

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Authors' address: Waldemar A. Turski, Department of Experimental and Clinical Pharmacology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland,
Fax: +48-81-7408744, E-mail: turskiwa@op.pl