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ORIGINAL ARTICLE

Presence of kynurenic acid in food and honeybee products

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Abstract Kynurenic acid (KYNA) is an endogenous antagonist of ionotropic glutamate receptors and the α7 nicotinic acetylcholine receptor, showing anticonvulsant and neuroprotective activity. In this study, the presence of KYNA in food and honeybee products was investigated. KYNA was found in all 37 tested samples of food and honeybee products. The highest concentration of KYNA was obtained from honeybee products' samples, propolis (9.6 nmol/g), honey (1.0–4.8 nmol/g) and bee pollen (3.4 nmol/g). A high concentration was detected in fresh broccoli (2.2 nmol/g) and potato (0.7 nmol/g). Only traces of KYNA were found in some commercial baby products. KYNA administered intragastrically in rats was absorbed from the intestine into the blood stream and transported to the liver and to the kidney. In conclusion, we provide evidence that KYNA is a constituent of food and that it can be easily absorbed from the digestive system.

Keywords Kynurenic acid · Food · Honeybee product · Digestive system · Absorption

Introduction

Kynurenic acid (KYNA), a tryptophan metabolite, is the only known endogenous antagonist of ionotropic glutamate receptors (Perkins and Stone 1982; Turski et al. 1988, 1989). It has been also demonstrated that KYNA is a noncompetitive antagonist of the α7 nicotinic acetylcholine receptor (Hilmas et al. 2001). Its anticonvulsant and neuroprotective properties were experimentally proven. A modulatory effect of KYNA exerted on the neurotransmission in the brain has been suggested, based on electrophysiological studies (Hilmas et al. 2001; Scharfman et al. 2000).

In recent years, significant interest has been shown in identifying KYNA and its potential role outside the brain. The presence of KYNA was documented in urine, serum, amniotic fluid, and synovial fluid (Kazda et al. 1998; Milart et al. 2001; Parada-Turska et al. 2006). In vertebrates, KYNA is formed along the tryptophan–kynurenine pathway. The conversion from tryptophan to kynurenine is achieved by tryptophan-2,3-dioxygenase and indole-2,3-dioxygenase and KYNA is produced irreversibly from kynurenine by the activity of kynurenine aminotransferases (Nemeth et al. 2005). Recently, KYNA was found in human saliva (Kuc et al. 2006) and in rat's intestine fluid (Kuc et al. 2007). In this study, the presence of KYNA in food and honeybee products was investigated.

Materials and methods

Animals

Wistar male rats (220–240 g) were housed in a temperature- and humidity-controlled animal unit under an ambient temperature of 21 ± 2°C and in a 12–12 h light–dark cycle. Before the experiment animals were deprived of food for 24 h. Water was available ad libitum. KYNA was dissolved in saline and administered intragastrically at doses of 25 and 250 mg/kg. Rats were killed by

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decapitation 30, 60 and 120 min after KYNA administration and samples of blood, the liver and the kidney were collected. Each group consisted of six animals. Experimental procedures were approved by the I Local Ethical Committee in Lublin, Poland.

Food

BoboFrut—apple and carrot, Gerber—chicken and Gerber—vegetables with veal were produced by Alima-Gerber, Warsaw, Poland. BoboVita—vegetable soup was produced by Nutricia, Warsaw, Poland. Milk was collected from a woman in the third week of lactation and was stored in a sterile glass at a temperature of −20°C until further analysis. All other samples of food were of a commercial origin. They were used fresh. Additionally, carrot, cauliflower, broccoli and potato were separately boiled in tap water for 30 min.

Kynurenic acid determination

Kynurenic acid was isolated and determined according to the methods described previously (Shibata 1988; Turski et al. 1988, 1989). Blood samples were immediately centrifuged and supernatant was stored. The kidney tissue, the liver tissue and food samples were weighed, homogenized in distilled water (1:4, w/v) and centrifuged. Propolis was dissolved in dichlormethane and subsequently extracted in 5% sodium carbonate. Samples were acidified with 50% trichloroacetic acid. Denaturated proteins were removed by centrifugation (10 min, 6,000 rpm) and supernatant acidified with 1 N HCl was applied to columns containing cation-exchange resin (Dowex 50 W^+: 200–400 mesh) prewashed with 0.1 N HCl. Subsequently, columns were washed with 1 ml of 0.1 N HCl and 1 ml of water. The fraction containing KYNA was eluted with 4 ml of water. The eluate was subjected to the high performance liquid chromatography (HPLC) and KYNA was detected fluorometrically (Hewlett Packard 1050 HPLC system: ESA catecholamine HR-80, 3 μm, C_{18} reverse-phase column, mobile phase: 250 mM zinc acetate, 25 mM sodium acetate, 5% acetonitrile, pH 6.2, flow rate of 1.0 ml/min; fluorescence detector: excitation 344 nm, emission 398 nm). KYNA was obtained from Sigma–Aldrich (St Louis, USA). All HPLC reagents used in the study were obtained from Baker (Griesheim, Germany) and were of the highest available purity.

Statistics

Data were presented as a mean value and a standard error of the mean (SEM). Statistical analysis was accomplished using Student's *t* test or one-way ANOVA followed by post hoc Student–Newman–Keuls test. A p-value of less than 0.05 was considered significant.

Results

Kynurenic acid identification

A substance extracted from food was compared to authentic KYNA in the chromatographic system. A solution of authentic KYNA was added to samples of food, which were processed as described above. Retention times and shapes of obtained peaks were compared. In all cases, the shape and the retention time to peaks of isolated and authentic KYNA were identical. In food samples to which authentic KYNA was added only one peak was recorded on chromatograms (data not shown).

Kynurenic acid content in food

The presence of KYNA was determined in all 37 samples of food and honeybee products. The highest concentration of KYNA was obtained from honeybee products' samples, propolis (8.6 nmol/g), honey (1.0–4.6 nmol/g) and bee pollen (3.4 nmol/g). A high level was detected in fresh broccoli (2.2 nmol/g) and potato (0.7 nmol/g). The lowest content of KYNA was found in the commercial baby product, chicken from Gerber (5.1 pmol/g). Only traces of KYNA were found in red paprika (6.1 pmol/g) and fish (7.5 pmol/g) (Table 1).

Boiling for 30 min in tap water lowered KYNA concentration in carrot, cauliflower and broccoli to 63, 19 and 12%, respectively. Boiling did not affect the content of KYNA in potato (Fig. 1).

Kynurenic acid absorption

The absorption of intragastrically administered KYNA in rats was studied to evaluate its bioavailability in vivo.

The administration of KYNA at the dose of 25 mg/kg resulted in a fivefold elevation of its serum concentration in comparison to the control value. This effect lasted up to 120 min after drug administration (Table 2). Approximately twofold enhancement of KYNA concentration was detected in the liver and the kidney 30 min after drug administration (Table 2).

After KYNA administration at the dose of 250 mg/kg an approximately 100-fold increase of its concentration was recorded in serum and the kidney and approximately tenfold increase in the liver. The significant elevation of

Table 1 The content of kynurenic acid in food and honeybee products

Food and honeybee products	Kynurenic acid (pmol/g)
Gerber-chicken	5.1
Red paprika	6.1
Fish (roach)	7.5
BoboFrut—apple and carrot	10.3
Colza oil	10.9
Apple	12.1
Sunflower oil	14.3
Beef	16.6
Pork	19.4
Cucumber	20.0
Egg	23.9
BoboVita—vegetable soup	29.4
Rice	29.8
Tomato	31.1
Carrot	38.6
Fluor	44.3
Hard cheese	44.4
Pea	46.7
Pig liver	48.3
Barley	51.8
Kefir	56.8
Bread	78.0
Gerber—vegetables with veal	78.3
Corn	84.7
Yoghurt	90.1
Milk 1.5%	92.1
Onion	120.8
Human milk	136.0
Garlic	145.8
Cauliflower	250.1
Potato	688.3
Linden honey	947.7
Buckwheat honey	958.6
Broccoli	2,213.7
Bee pollen	3,415.2
Multiflorous honey	4,642.0
Propolis	8,572.8

Samples were determined in duplicates

KYNA concentration lasted at least up to 120 min after drug administration (Table 3).

Discussion

To the best of our knowledge, this is the first report on the presence of KYNA in food and honeybee products. Previously, KYNA was mentioned as a constituent of plants, *Gingko biloba* (Drieu 1986) and *Ephedra transitoria* (al-Khalil et al. 1998). However, it was neither unequivocally identified nor its content was measured. In this study, KYNA in food and honeybee products' samples was chromatographically identified. The highest amount of KYNA was found in honeybee products. Propolis, which is extensively used in food, beverages and as a folk medicine contained 8.6 nmol of KYNA/g. A very high level of KYNA was found in multiflorous honey and bee pollen, 4.6 and 3.4 nmol KYNA/g, respectively. Bee pollen is food for larvae and bees, considered to be a good complement to the human daily diet (Orzaez Villanueva et al. 2002). A high concentration of KYNA was measured in fresh broccoli, buckwheat, linden honey and potato. Unexpectedly, only a trace of KYNA was determined in the commercial baby product, chicken from Gerber. In other baby meals, apple + carrot from BoboFrut, vegetable soup from BoboVita and vegetables with veal from Gerber, KYNA was found in concentration distinctly lower than in human milk. We discovered that the boiling procedure lowered KYNA concentration in some vegetables such as carrot, cauliflower and broccoli by 37, 81 and 88%, respectively. On the other hand, boiling did not affect the content of KYNA in potato.

Kynurenic acid administered intragastrically in rats was absorbed from the intestine into the blood stream and transported to the liver and to the kidney. This effect was time- and dose-dependent. The administration of KYNA at the dose of 25 mg/kg resulted in an increase of KYNA content in serum lasting for 2 h and a transient increase of this compound in the liver tissue and in the kidney tissue recorded 30 min after drug application. The administration of KYNA at the dose of 250 mg/kg resulted in a steady-state increase of KYNA content in serum, the liver and the kidney lasting for at least 2 h. It should be noted that KYNA concentration achieved after its administration at the dose of 250 mg/kg reached micromolar level, which is sufficient to interact with G-protein-coupled (GPR35), *N*-methyl-D-aspartate (NMDA) and α7 nicotinic acetylcholine receptors (Birch et al. 1988; Hilmas et al. 2001; Wang et al. 2006).

Kynurenic acid is a broad-spectrum antagonist of all subtypes of ionotropic, glutamate receptors, acting preferentially as a competitive blocker of the strychnine-insensitive glycine co-agonist site of NMDA, and as a non-competitive antagonist of the α7 nicotinic acetylcholine receptor (Birch et al. 1988; Hilmas et al. 2001). It is known that KYNA possesses anticonvulsant and neuroprotective properties in the brain. However, its permeability through the blood–brain barrier is poor (Fukui et al. 1991).

Glutamatergic and α7 nicotinic receptors are also present outside the brain. Glutamatergic neurons were found in the digestive system. Most of glutamate-containing axons

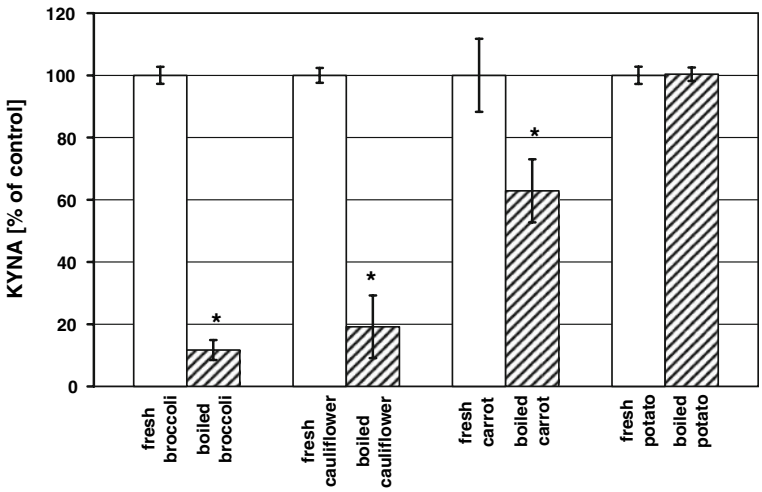

Fig. 1 KYNA content in fresh and boiled vegetables. Results are expressed as a percentage of fresh vegetable (control); mean ± SEM; $n = 4$ independent experiments; * $P < 0.05$ versus respective control (100%), Student's t test

Table 2 The content of kynurenic acid in serum, the liver and the kidney after the intragastric administration of kynurenic acid at the dose of 25 mg/kg in rats

Time (min)	Serum (pmol/ml)	Liver (pmol/g wet tissue)	Kidney (pmol/g wet tissue)
0	29.6 ± 2.7	227.8 ± 19.8	396.2 ± 20.4
30	166.0 ± 22.2*	409.2 ± 38.7*	878.1 ± 72.0*
60	162.0 ± 21.0*	281.8 ± 24.8	332.8 ± 58.3
120	149.9 ± 12.9*	271.2 ± 28.1	315.4 ± 58.5

* $P < 0.05$ versus respective control (0 min), ANOVA followed by post hoc Student–Newman–Keuls test

Table 3 The content of kynurenic acid in serum, the liver and the kidney after the intragastric administration of kynurenic acid at the dose of 250 mg/kg in rats

Time (min)	Serum (pmol/ml)	Liver (pmol/g wet tissue)	Kidney (pmol/g wet tissue)
0	27.4 ± 4.5	352.7 ± 32.2	403.2 ± 37.8
30	3,437.4 ± 700.8*	3,502.6 ± 212.4*	46,537.4 ± 5,162.8*
60	3,286.8 ± 675.2*	3,563.3 ± 422.0*	44,787.7 ± 4,767.2*
120	3,285.1 ± 620.5*	3,398.7 ± 407.7*	37,381.6 ± 3,864.5*

* $P < 0.05$ versus respective control (0 min), ANOVA followed by post hoc Student–Newman–Keuls test

in the intestine and the stomach wall originate from cell bodies within the myenteric and the submucous plexus. Some of the enteric glutamatergic neurons are likely to be sensory neurons and glutamate may be involved in transferring sensory information from the mucosa to enteric plexuses (Tsai 2005). L-Glutamate shows a powerful, stimulating effect on the majority of smooth muscle layers in the stomach via NMDA and kainate receptors (Jankovic et al. 1999). Moreover, glutamate agonists inhibit the histamine- and oxotremorine-stimulated gastric acid secretion. This effect is diminished by antagonists of the NMDA receptor (Tsai 2005). Interestingly, several groups have reported an increase in food intake after systemic treatment with MK-801, an NMDA receptor antagonist (Tsai 2005). It seems that such a result may be obtained by acceleration in gastric emptying and the subsequent increase in daily food intake (Covasa et al. 2000).

The α7 subtype of nicotinic acetylcholine receptor is expressed in submucosal and myenteric plexuses (Obaid et al. 2005). However, its function is not known.

The regulation of food intake occurs also in the brain. NMDA receptors in the lateral hypothalamus are believed to be involved in the stimulation of feeding behavior. Furthermore, 7Cl-kynurenate, an analog of KYNA and NMDA antagonist, administered into the lateral hypothalamus decreases food intake in rats (Lee and Stanley 2005). It was found out that the level of KYNA in cerebrospinal fluid is significantly reduced in underweight anorectics in comparison to the control. However, there were no differences when considering KYNA levels in bulimics and the control (Demitrack et al. 1995). It is known that nicotine reduces appetite resulting in the body weight loss. There is evidence that nicotine administration reduces appetite and alters feeding patterns via α7 nicotinic acetylcholine receptors in

the lateral hypothalamus (Jo et al. 2005). Thus, KYNA regulative role in the brain in terms of food intake seems to be complex. In the hypothalamus KYNA interacting with NMDA receptors should decrease, whilst KYNA interacting with α7 nicotinic receptors should increase food intake. However, KYNA does not cross the blood–brain barrier easily (Fukui et al. 1991). Therefore, it can be assumed that its peripheral action may be predominant. Due to the fact that KYNA is an antagonist of NMDA and non-NMDA receptors, it can be expected that KYNA acting peripherally increase food intake. In contrast to this expectation it has been recently reported that memantine, a non-competitive NMDA antagonist, administered orally, reduced appetite and led to the marked body weight reduction within a few days in obese young women (Hermanussen and Tresguerres 2005). In the light of contradictory results and hypothesis it seems essential to discover the effect of dietary KYNA on food intake control.

Recently, KYNA has been reported as a ligand for GPR35 receptor (Wang et al. 2006). It was found out that KYNA is an agonist eliciting calcium mobilization and inositol phosphate production in a GPR35-dependent manner (Wang et al. 2006). Interestingly, expression analysis revealed that both human and mouse GPR35 receptors were predominantly expressed in immune and gastrointestinal tissues, with limited expression in other tissues. Therefore, it was suggested that KYNA acting on GPR35 receptors may contribute to the modulation of the gastrointestinal immune response and to the process of regeneration of the gastrointestinal epithelium (Wang et al. 2006). Moreover, it was found out that KYNA inhibits intestinal hypermotility and the xanthine oxidase activity during the experimental colon obstruction in dogs (Kaszaki et al. 2008).

Until now, KYNA has been regarded as a mammalian body constituent of a potential biological activity, which is produced from kynurenine by an enzymatic reaction catalyzed by kynurenine aminotransferases. In our recent study a high micromolar concentration of KYNA was found in the lumen of the rat small intestine and its production by intestinal microflora was suggested (Kuc et al. 2007). Here, we have shown that human food contains KYNA and that it can be easily absorbed from the intestinal lumen. Moreover, we found out that the enteric route of KYNA administration is efficient enough to obtain a long-lasting elevation of the peripheral tissue content of KYNA. Accumulated data pointed to the conclusion that KYNA warrants further investigation so as to ascertain its potential role both in the digestive system pathology and food intake.

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