Amino Acids (2009) 37:637–641
DOI 10.1007/s00726-008-0183-x

ORIGINAL ARTICLE

High concentration of kynurenic acid in bile and pancreatic juice

Piotr Paluszkiewicz · Wojciech Zgrajka · Tomasz Saran · Janusz Schabowski · Jose L. Valverde Piedra · Olexandr Fedkiv · Sofia Rengman · Stefan G. Pierzynowski · Waldemar A. Turski

Received: 5 July 2008 / Accepted: 15 September 2008 / Published online: 5 October 2008

Abstract Kynurenic acid (KYNA) is an agonist of the G-protein-coupled receptor GPR35, which is predominantly expressed in gastrointestinal tissues. The aim of this study was to determine the content of KYNA in gastric juice, bile and pancreatic juice and intestinal content. KYNA was determined by means of high performance liquid chromatography. The mean concentrations of KYNA in human gastric juice is 9.91 ± 0.71 nM in contrast to human bile (832.5 ± 204.1 and 306.8 ± 35.2 nM) obtained from patients with cholecystolithiasis and obstructive jaundice, respectively. In pigs, the KYNA levels in bile and pancreatic juice are 1,113.3 ± 63.34 and 757.0 ± 394.4 nM, respectively. The KYNA concentration increases along the digestive system, reaching 1,638 nM in the colon content. We suggest that the liver and pancreas affect the content of kynurenic acid in the lumen of the digestive tract.

Keywords Kynurenic acid · Bile · Gastric juice · Pancreatic juice

P. Paluszkiewicz
Department of Surgery and Surgical Nursing, Medical University, Chodzki 6, 20-090 Lublin, Poland

W. Zgrajka · W. A. Turski
Department of Toxicology, Institute of Agricultural Medicine, Jaczewskiego 2, 20-950 Lublin, Poland

T. Saran · J. Schabowski
Department of Rehabilitation, Institute of Agricultural Medicine, Jaczewskiego 2, 20-950 Lublin, Poland

J. L. Valverde Piedra
Department of Animal Biochemistry and Physiology, University of Life Sciences in Lublin, Akademicka 12, 20-950 Lublin, Poland

O. Fedkiv · S. Rengman · S. G. Pierzynowski
Department of Cell and Organism Biology, Lund University, Helgonavägen 3b, Lund, Sweden

W. A. Turski (✉)
Department of Experimental and Clinical Pharmacology, Medical University, Jaczewskiego 8, 20-090 Lublin, Poland
e-mail: turskiwa@op.pl

Introduction

The 4-oxo-1,4-dihydroquinoline-2-carboxylic acid, an oxidative pathway metabolite of tryptophan, is known as kynurenic acid (KYNA). The role of KYNA was partially elucidated, especially as an endogenous neuroprotective agent in the central nervous system (Turski et al. 1988). The nanomolar concentrations of KYNA in the nervous system (Turski et al. 1988) are probably insufficient to block N-methyl-D-aspartate, alfa-7 nicotinic (Hilmas et al. 2001) or alpha-amino-3-hydroxy-5-methyl-4-isoxasole propionate (AMPA)/kainate receptors (Birch et al. 1988) localized within the brain. To date, the micromolar or millimolar concentrations sufficient to affect or block appropriate receptors (Birch et al. 1988; Hilmas et al. 2001) have not been identified in any mammal tissues. Unexpectedly, micromolar concentrations of KYNA were found in some physiological fluids, e.g. in human urine (Milart and Sikorski 1998) and liquid contents in rat intestine (Kuc et al. 2008). The role of KYNA outside the brain, especially in the gastrointestinal (GI) tract, is unknown. The action of KYNA may be dependent on specific activation of orphan G-protein-coupled receptor (GPR35) located predominately on enterocytes in intestinal crypts, as well as in various subpopulations of immune cells (Wang et al. 2006). The high KYNA concentrations in intestinal contents (up to 16 μM in distal part of small intestine (Kuc et al. 2008) suggest its

influence on GI immune response and enterocytes function. Recently we isolated the micromolar amount of KYNA in some flower-derived dietary compounds (Turski et al. 2008) and identified the production of KYNA by *E.coli* (Kuc et al. 2006). Despite these findings, the high-concentrations of KYNA in intestinal contents suggest the presence of other sources supplementing bacterial and dietary origin. Furthermore, the influence of enteral nutrition on mucosal integrity and GI inflammatory response has been widely accepted (Delegge 2008), whereas the role of KYNA and its mechanism of action in GI tract have not been presented to date. The aim of this study is to establish the additional sources of KYNA within GI tract, especially the role of bile as well as gastric and pancreatic juices.

Materials and methods

Patients

Gastric juice collection. 23 patients (15 women and 8 men, mean age 58 years, SEM 1.7), candidates for therapy with non-steroid anti-inflammatory drugs due to complaints of pain in the course of discopathia and arthrosis were included. Patients with signs of GI chronic disorders or who had proton pump inhibitors were excluded. All patients gave their informed written consent to participate in the study, which was approved by the local ethics committee (KE-0254/242/2002). Upper GI endoscopy was performed after 12 h fast. Gastric juice was aspirated via the suction channel. A 1 ml sample of gastric juice was immediately frozen to −78°C.

Bile collection

Patients with uncomplicated cholecystolithiasis. The group of 18 patients (12 women and 6 men, mean age 55 years, SEM 2.8) underwent elective cholecystectomy due to bile stones localized in the gallbladder with uneventful outcome. Samples of 5 ml of bile were obtained from the removed gallbladder extra-corporally.

Obstructive jaundice patients. The group of 11 patients (7 women and 4 men, mean age 52 years, SEM 5.4) who underwent elective surgery due to obstructive jaundice was included to the study. The causes of bile ducts obstruction were bile stones in eight cases and malignant tumours of the hepatico-pancreatico-biliary regions in another three cases. Open cholecystectomy and T-tube cholangiostomy were performed in five patients. In another five, who underwent cholecystectomy many years ago, the T-tube cholangiostomy supplemented extrahepatic bile duct revision. In one patient, cholangiostomy was reached by percutaneous transhepatic, sonography-guided puncture of the right hepatic duct. In all patients postoperative outcomes were uneventful. Samples of 10 ml of hepatic bile were obtained from cholangiostomy on the 6th post-operative day. No bacterial growth was observed in any bile samples. Sampled bile was frozen at −78°C for further analyses.

Animals

Pancreatic juice and gallbladder's bile collection. Three castrated male pigs crossbred [(Swedish Landrace × Yorkshire) × Hampshire] from Odarslöv research farm (Swedish University of Agricultural Sciences, Alnarp, Sweden) were used in the experiments. Pigs were surgically prepared for pancreatic juice collection according to the method described previously (Pierzynowski et al. 1988).

Surgical procedures. The pigs were fasted overnight and premedicated with azaperone (4.0 mg kg^{-1} i.m.) before transport and further handling occurred. At surgery the pigs were anaesthetised with 0.3% 2-bromo-2-1.1.1-triflouroethane mixed with air, and supplemented with oxygen using a closed-circuit system. A median laparotomy was performed and the accessory pancreatic duct was catheterised for collection of pancreatic juice and a T-shaped cannula was inserted into the duodenum for reintroduction of the pancreatic juice between the experimental phases. The catheters were exteriorized through the right side of the abdominal wall. The pigs were given one week of recovery before the experiments started. After overnight starvation pancreatic juice samples (1 ml) were collected and frozen at −78°C for further analyses.

Ligation of the pancreatic duct. Another set of ten pigs were used to study the effect of pancreatic duct ligation on KYNA concentration in the intestinal content and in the bile. For pancreatic duct ligation, six pigs at the age of 16 weeks were sedated and anesthetised as described above. A median laparotomy was performed and the accessory pancreatic duct was isolated and ligated. Two pigs from the same litter and two from another litter that did not undergo pancreatic duct ligation were kept as controls. Pigs were fed a standard diet twice daily and had free access to tap water and were kept in a 12/12 light/dark cycle. At the 26th week of age the pigs were euthanized and samples from the stomach, duodenum, jejunum, ileum and colon content and gallbladder bile were taken and frozen −78°C for further analysis. The experiments were approved by the Lund University Ethics Review Committee on Animal Experiments (M227-05).

Substances. Kynurenic acid (KYNA) was 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 (1:2 vol/vol or 1:5 wt/vol) in distilled water. The resulting

homogenate was centrifuged. The 1 ml of supernatant was acidified with 28 µl of 50% trichloroacetic acid and centrifuged. Supernatant was applied on cation-exchange resin (Dowex 50 W+, Sigma). Eluted KYNA 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).

Statistical analysis. Data are presented as the mean ± standard error median (SEM). Statistical analysis was accomplished using Student *t* test; *P* values <0.05 were considered significant.

Results

KYNA content in human gastric juice

The presence of KYNA was determined in all samples of gastric juice. In samples to which authentic KYNA was added, only one peak was recorded on the chromatogram at the retention time of KYNA (data not shown). The mean KYNA content was 9.91 ± 0.71 nM (Fig. 1). The level of KYNA was not gender- or age-dependent.

KYNA content in human bile

The presence of KYNA was determined in all samples of gallbladder and hepatic bile. In samples to which authentic KYNA was added, only one peak was recorded on the chromatogram at the retention time of KYNA (data not shown). Mean concentrations of KYNA in bile obtained from patients with uncomplicated cholecystolithiasis and obstructive jaundice were 832.5 ± 204.1 and 306.8 ± 35.2 nM, respectively (Fig. 2).

KYNA concentration in pig pancreatic juice and bile

The concentration of KYNA in the pancreatic juice and gallbladder bile of pigs was 757.0 ± 394.4 and 1,113.3 ± 63.3 nM, respectively.

KYNA content in pig GI tract

In the GI tract of pigs the mean content of KYNA in the stomach, duodenum, jejunum, ileum and colon was 165.5 ± 57.3, 220.5 ± 16.0, 257.0 ± 15.0, 950.0 ± 168.0 and 1,637.5 ± 265.7 pmol/g, respectively (Fig. 3).

In the GI tract of pigs with ligated pancreatic duct the mean content of KYNA in the stomach, duodenum, jejunum, ileum and colon was 133.0 ± 8.8, 116.0 ± 11.0, 100.7 ± 16.7, 596.0 ± 118.9 and 1,621.7 ± 346.3 pmol/g, respectively (Fig. 3).

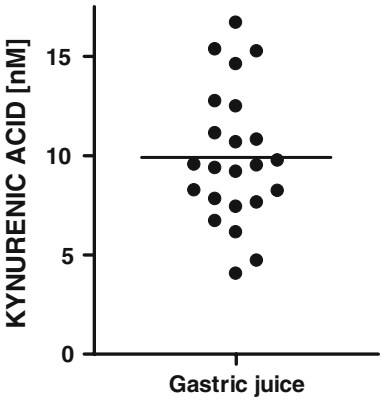

Fig. 1 Scatter plot of kynurenic acid (KYNA) concentration in human gastric juice obtained from 23 patients. *Horizontal bar* represents the mean value

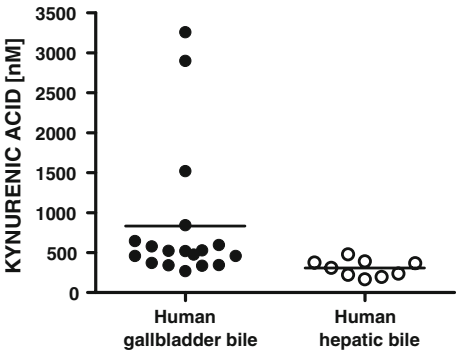

Fig. 2 Scatter plots of kynurenic acid concentration in human gallbladder and hepatic bile obtained from 18 and 11 patients, respectively. *Horizontal bar* represents the mean value

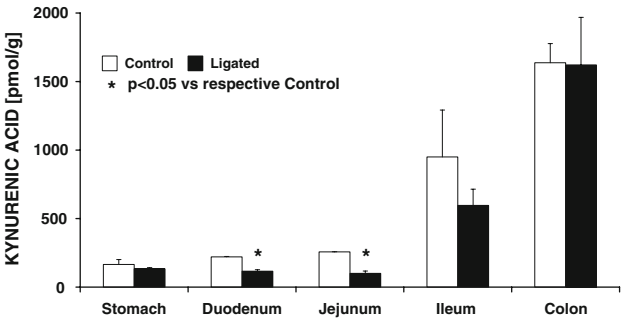

Fig. 3 Kynurenic acid (KYNA) content in gastrointestinal tract of sham-operated pigs (control, $N = 4$) and pigs with ligated pancreatic duct (ligated, $N = 6$). Data are presented as a mean ± SEM; $P < 0.05$ versus respective control, Student's *t* test

Discussion

In this study, we report that KYNA is present in the human bile and gastric juice. The concentration of KYNA in thickened bile obtained from gallbladder was 832 nM, and

was higher than in gastric juice (~10 nM) and bile obtained from a T-tube placed in the extrahepatic bile ducts (307 nM). Similarly, a high concentration of KYNA (1,113 nM) was found in bile obtained from pigs. The lower KYNA concentration in bile obtained from the extrahepatic bile ducts may be due to its direct hepatic flow and the lack of thickening in the gallbladder. Previously, the presence of KYNA in a concentration of 3.4 nM was reported in human saliva (Kuc et al. 2006). More recently, we found that KYNA is a constituent of rat intestinal fluid and that its level increases along the small intestine, reaching a concentration of 16 μM in its distal part (Kuc et al. 2008). In this study we confirmed these findings. In pig, in the intestinal content KYNA concentration increased from 166 pmol/g (~0.17 μM) in the stomach to 1,638 pmol/g (~1.64 μM) in the colon. Moreover, we found that its concentration in the pancreatic juice of pigs was 0.76 μM. It must be emphasized that pancreatic duct ligation lowered KYNA concentration in small intestinal but not in colonic contents. These results suggest that bile and pancreatic juice are important sources of KYNA in the lumen of the small intestine. Moreover, they further support our previous hypothesis of the principal role of bacterial production of KYNA in maintaining its content in the distal GI tract, especially in the colon (Kuc et al. 2008).

KYNA is a metabolite of tryptophan formed along the oxidative pathway via kynurenine. The secretion of KYNA to bile was probably the consequence of kynurenine aminotransferases activity found in hepatic tissue (Costa et al. 1999). The source of high concentration of KYNA in pancreatic juice is unknown. The distribution and activity of kynurenine transaminases in pancreatic tissue have not been described to date, thus the mechanism of KYNA formation in pancreas, especially in exocrine acinar cells, should be elucidated.

Both the absorption of KYNA in the intestine presented recently (Turski et al. 2008) and its presence in bile and pancreatic juice reported here suggest the existence of hepatic-pancreatic-intestinal secretion–absorption functional cycle creating high concentration of KYNA in intestinal fluid.

It can be expected that in normally nourished subjects the final concentration of KYNA in intestinal contents can be even higher than that observed in the presented study. Previously, the presence of this compound in dietary components was reported, and the highest concentration was found in honeybee products (Turski et al. 2008). Interestingly, the administration of KYNA-rich products such as honey or propolis, reduced bacterial translocation and atrophy of intestinal mucosa in experimental obstructive jaundice (Sabuncuoglu et al. 2007), and diminished ultra-structure injuries of liver observed after bile duct ligation (Kilicoglu et al. 2008). The potential role of KYNA in these conditions needs to be justified.

KYNA in micromolar concentration found in the digestive system may interact with glycine site of N-methyl-D-aspartate (NMDA) glutamate receptor, α-7 nicotinic receptor and GPR35 receptor (Birch et al. 1988; Hilmas et al. 2001; Wang et al. 2006). Indeed, NMDA and α-7 nicotinic receptors were found in submucosal and myenteric nervous plexi (Liu et al. 1997). Moreover, it was found that KYNA infused intravenously attenuated intestinal motility in experimental colonic obstruction in dogs (Kaszaki et al. 2008). However, the concentration of KYNA reported in the wall of rat intestine does not exceed 0.3 nmol per gram of wet tissue (~0.3 μM) (Kuc et al. 2008). This finding suggests that trans-mucosal access of KYNA from the lumen of the digestive system to the enteric nervous system is limited, and such an interaction of KYNA seems to be less probable.

On the other hand, KYNA in micromolar concentration found in the digestive system may interact with the GPR35 receptor which is located mainly on enterocytes (Wang et al. 2006). Interestingly, the expression of GPR35 was predominantly localized in intestinal crypts and disappeared in the apex of villi (Wang et al. 2006). The high-mitotic activity of crypt cells and their migration to villi-apex is usually classified as aberrant crypt formation (Pacha 2000). The hypothesis has been formulated that the direct contact of intestinal content and mucosal surface is the route of antiapoptotic signal transduction in mucosal epithelial cells (Pacha 2000). Taken together, these findings may suggest a potential role of KYNA in the regulation of mitotic activity or differentiation of GI epithelium. This hypothesis can be indirectly supported by the reports showing that the obstructive jaundice aggravates intestinal oxidative stress (Assimakopoulos et al. 2006), induces apoptosis in intestinal epithelium cells, and increases intestinal wall permeability for GI flora (Scopa et al. 2000). Moreover, it was reported that the gastric mucosal susceptibility to injury depends on normal bile flow in duodenal lumen (Cingi et al. 2002). Interestingly, it was found that KYNA significantly blocks restraint-cold stress ulcers, ethanol ulcers and basal, non-stimulated gastric acid secretion in normal rats and in rats subjected to excitatory amino acid agonist—domoic acid (Glavin and Pinsky 1989). It protects also against gastric and duodenal ulcers, duodenal hyperemia and peritoneal ascites resulted from administration of an extract prepared from poisonous Atlantic mussels in mice (Glavin et al. 1989, 1990). The protective effect was particularly evident when KYNA was given 60 or 75 min after intoxication (Glavin et al. 1989, 1990). Thus, the role of KYNA in complex duodenal mucosa protection system can be not excluded.

It was also shown that GPR35 receptors are located on various subpopulations of the immune cells (Wang et al. 2006); therefore, it may be suggested that KYNA may contribute to GI immune response via interaction with these receptors. Further, the inhibition exerted by KYNA on the tumor necrosis factor α secretion in peripheral blood monocytes (Wang et al. 2006), xanthine oxidase activity (Kaszaki et al. 2008) and high mobility box 1 protein, as well as nitric oxide release (Moroni et al. 2007), suggest its anti-inflammatory effect. Taking into consideration these properties the finding that the experimental cholestasis protects the liver from ischemic injury and inflammation by blockade of the activation of NFkappaB, attenuation of the synthesis of tumour necrosis factor α and reduction of the neutrofil infiltration (Georgiev et al. 2007) may be speculatively linked, at least in part, to the action of KYNA.

In conclusion, we report that the high concentration of KYNA in bile and pancreatic juice provide evidences for the contribution of these sources to the maintenance of high KYNA content in GI tract. Moreover, we suggest a possible role of luminal KYNA in the modulation of enterocytes apoptosis and mitogenic activities that take place during the gut epithelium remodeling process.

Acknowledgments Technical assistance from Franciszek Wysocki, MD, Department of Surgery, Regional Hospital, Lublin, Poland, is gratefully acknowledged.

References

Assimakopoulos SF, Thomopoulos KC, Patsoukis N, Georgiou CD, Scopa CD, Nikolopoulou VN et al (2006) Evidence for intestinal oxidative stress in patients with obstructive jaundice. Eur J Clin Invest 36:181–187. doi:10.1111/j.1365-2362.2006.01616.x

Birch PJ, Grossman CJ, Hayes AG (1988) Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. Eur J Pharmacol 154:85–87. doi:10.1016/0014-2999(88)90367-6

Cingi A, Ahiskali R, Oktar BK, Gülpinar MA, Yegen C, Yegen BC (2002) Biliary decompression reduces the susceptibility to ethanol-induced ulcer in jaundiced rats. Physiol Res 51:619–627

Costa CV, Ragazzi E, Caparrotta L, Bertazzo A, Biasiolo M, Allegri G (1999) Liver and kidney kynurenine aminotransferase activity in different strains of rats. Adv Exp Med Biol 467:629–635

Delegge MH (2008) Enteral feeding. Curr Opin Gastroenterol 24:184–189. doi:10.1097/MOG.0b013e3282f4dbab

Georgiev P, Navarini AA, Eloranta JJ, Lang KS, Kullak-Ublick GA, Nocito A et al (2007) Cholestasis protects the liver from ischaemic injury and post-ischaemic inflammation in the mouse. Gut 56:121–128. doi:10.1136/gut.2006.097170

Glavin GB, Bose R, Pinsky C (1989) Kynurenic acid protects against gastroduodenal ulceration in mice injected with extracts from poisonous Atlantic shellfish. Prog Neuropsychopharmacol Biol Psychiatry 13:569–572. doi:10.1016/0278-5846(89)90148-6

Glavin GB, Pinsky C (1989) Kynurenic acid attenuates experimental ulcer formation and basal gastric acid secretion in rats. Res Commun Chem Pathol Pharmacol 64:111–119

Glavin GB, Pinsky C, Bose R (1990) Gastrointestinal effects of contaminated mussels and putative antidotes thereof. Can Dis Wkly Rep 16(Suppl 1E):111–115

Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX (2001) The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J Neurosci 21:7463–7473

Kaszaki J, Palasthy Z, Erczes D, Racz A, Torday C, Varga G et al (2008) Kynurenic acid inhibits intestinal hypermotility and xanthine oxidase activity during experimental colon obstruction in dogs. Neurogastroenterol Motil 20:53–62

Kilicoglu B, Gencay C, Kismet K, Serin KS, Erguder I, Erel S et al (2008) The ultrastructural research of liver in experimental obstructive jaundice and effect of honey. Am J Surg 195:249–256. doi:10.1016/j.amjsurg.2007.04.011

Kuc D, Rahnama M, Tomaszewski T, Rzeski W, Wejksza K, Urbanik-Sypniewska T et al (2006) Kynurenic acid in human saliva: does it influence oral microflora? Pharmacol Rep 58:393–398

Kuc D, Zgrajka W, Parada-Turska J, Urbanik-Sypniewska T, Turski WA (2008) Micromolar concentration of kynurenic acid in rat small intestine. Amino Acids 35:503–505. doi:10.1007/s00726-007-0631-z

Liu MT, Rothstein JD, Gershon MD, Kirchgessner AL (1997) Glutamatergic enteric neurons. J Neurosci 17:4764–4784

Milart P, Sikorski R (1998) Kynurenic acid concentration in blood and urine during normal pregnancy. Ginekol Pol 69:968–973

Moroni F, Fossati S, Chiarugi A, Cozzi A (2007) Kynurenic acid actions in brain and periphery. Int Congr Ser 1304:305–313

Pacha J (2000) Development of intestinal transport function in mammals. Physiol Rev 80:1633–1667

Pierzynowski SG, Weström BW, Karlsson BW, Svendsen J, Nilsson B (1988) Pancreatic cannulation of young pigs for long-term study of exocrine pancreatic function. Can J Anim Sci 68:953–959

Sabuncuoglu MZ, Kismet K, Kilicoglu SS, Kilicoglu B, Erel S, Muratoglu S et al (2007) Propolis reduces bacterial translocation and intestinal villus atrophy in experimental obstructive jaundice. World J Gastroenterol 13:5226–5231

Scopa CD, Koureleas S, Tsamandas AC, Spiliopoulou I, Alexandrides T, Filos KS et al (2000) Beneficial effects of growth hormone and insulin-like growth factor I on intestinal bacterial translocation, endotoxemia, and apoptosis in experimentally jaundiced rats. J Am Coll Surg 190:423–431. doi:10.1016/S1072-7515(99)00285-9

Turski MP, Turska M, Zgrajka W, Kuc D, Turski WA (2008) Presence of kynurenic acid in food and honeybee products. Amino Acids. doi:10.1007/s00726-008-0031-z

Turski WA, Nakamura M, Todd WP, Carpenter BK, Whetsell WO Jr, Schwarcz R (1988) Identification and quantification of kynurenic acid in human brain tissue. Brain Res 454:164–169. doi:10.1016/0006-8993(88)90815-3

Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H et al (2006) Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem 281:22021–22028. doi:10.1074/jbc.M603503200