

The Tryptophan Metabolite Indole-3-Propionic Acid Raises Kynurenic Acid Levels in the Rat Brain In Vivo

International Journal of Tryptophan Research
Volume 17: 1–9
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/11786469241262876



Korrapati V Sathyaikumar^{1*}, Tonali Blanco-Ayala^{2*},
Yiran Zheng³, Lilly Schwieler³, Sophie Erhardt³,
Maximilian Tufvesson-Alm³, Burkhard Poeggeler⁴
and Robert Schwarcz¹

¹Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, USA. ²Neurobiochemistry and Behavior Laboratory, National Institute of Neurology and Neurosurgery “Manuel Velasco Suárez,” Mexico City, Mexico. ³Departments of Physiology and Pharmacology, Karolinska Institute, Stockholm, Sweden. ⁴Department of Physiology, Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Georg-August-Universität Göttingen, Germany.

ABSTRACT: Alterations in the composition of the gut microbiota may be causally associated with several brain diseases. Indole-3-propionic acid (IPrA) is a tryptophan-derived metabolite, which is produced by intestinal commensal microbes, rapidly enters the circulation, and crosses the blood-brain barrier. IPrA has neuroprotective properties, which have been attributed to its antioxidant and bioenergetic effects. Here, we evaluate an alternative and/or complementary mechanism, linking IPrA to kynurenic acid (KYNA), another neuroprotective tryptophan metabolite. Adult Sprague-Dawley rats received an oral dose of IPrA (200 mg/kg), and both IPrA and KYNA were measured in plasma and frontal cortex 90 minutes, 6 or 24 hours later. IPrA and KYNA levels increased after 90 minutes and 6 hours (brain IPrA: ~56- and ~7-fold; brain KYNA: ~4- and ~3-fold, respectively). In vivo microdialysis, performed in the medial prefrontal cortex and in the striatum, revealed increased KYNA levels (~2.5-fold) following the administration of IPrA (200 mg/kg, p.o), but IPrA failed to affect extracellular KYNA when applied locally. Finally, treatment with 100 or 350 mg IPrA, provided daily to the animals in the chow for a week, resulted in several-fold increases of IPrA and KYNA levels in both plasma and brain. These results suggest that exogenously supplied IPrA may provide a novel strategy to affect the function of KYNA in the mammalian brain.

KEYWORDS: Gut-brain axis, indoles, neuroprotection, tryptophan, kynurenic acid

RECEIVED: February 28, 2024. **ACCEPTED:** May 22, 2024.

TYPE: Original Research Article

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was funded by NIMH grant P50 MH103222 (Silvio O. Conte Center for Translational Mental Health Research) and The Swedish Research Council (2021-02251).

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: R.S. is co-founder of Kynexis BV, which develops kynurenic acid-related compounds for human use.

CORRESPONDING AUTHOR: Korrapati V Sathyaikumar, Maryland Psychiatric Research Center, Department of Psychiatry, University of Maryland School of Medicine, 55 Wade Avenue, Catonsville, MD 21228, USA. Email: saikumar@som.umaryland.edu

Introduction

The gut-brain axis is receiving increasing attention of neuroscientists since perturbations in the composition and function of the gut microbiota may be causally associated with several neuropsychiatric and neurological diseases.¹⁻⁴ Products of tryptophan degradation are often suggested to play a major role in this context.⁵⁻⁹ One of these metabolites, indole-3-propionic acid (IPrA), is produced by intestinal commensal microbes via the so-called indole pathway¹⁰⁻¹² and enters the circulation of the host. Notably, because of its lipophilic character, IPrA can readily cross the blood-brain barrier and is normally present in the cerebrospinal fluid of both humans and rodents.^{11,13-15}

IPrA possesses potent antioxidant properties¹⁶ and protects neurons against both β -amyloid-induced oxidative stress¹⁷ and ischemic damage.^{18,19} In view of its ability to enter the brain from the periphery, these neuroprotective effects suggested that IPrA may be of therapeutic use in Alzheimer's disease,²⁰ and IPrA is, in fact, currently under clinical investigation for reducing oxidative stress in patients

with Friedreich's Ataxia (ClinicalTrials.gov identifier NCT01898884). Moreover, recent studies revealed that IPrA can be used as a marker for the onset and development of metabolic disorders including type 2 diabetes and non-alcoholic fatty liver disease,²¹⁻²⁵ reduces the permeability of the intestinal barrier by pregnane X receptor activation,^{26,27} and attenuates septic injury and atopic dermatitis by aryl hydrocarbon receptor (AHR) activation.^{11,28-30}

Kynurenic acid (KYNA), an endogenous metabolite of the kynurenine pathway (KP) of tryptophan degradation, has biological qualities which are remarkably similar to those of IPrA.^{31,32} From a neurobiological perspective, KYNA is known mainly for its neuroprotective, anti-ischemic and anticonvulsant properties,³³⁻³⁶ and for its ability to bi-directionally influence cognitive processes.³⁷⁻⁴¹ These effects have been attributed primarily to KYNA's inhibition of excitatory amino acid receptors and to its actions as a free radical scavenger.⁴²⁻⁴⁴ Notably, however, KYNA also inhibits $\alpha 7$ nicotinic acetylcholine receptor function,⁴⁵ can serve as an agonist of the G-protein-coupled receptor GPR35⁴⁶ and of the AHR,⁴⁷ and acts as a substrate for the organic anion transporters OAT1 and OAT3.⁴⁸ KYNA is formed from its immediate bioprecursor kynurenine either

* Shared first authorship



through irreversible transamination by kynurenine aminotransferases (KAT I-IV)⁴⁹ or by non-enzymatic oxidation.⁵⁰ In the healthy mammalian brain, KAT II, which is almost exclusively localized in astrocytes,⁵¹ is the principal enzyme responsible for the neosynthesis of rapidly mobilizable KYNA.⁵²

In spite of the functional similarities of IPrA and KYNA and their putative biochemical relationship, the effect of IPrA on KYNA formation has not been explored so far. The present study was designed to examine this link in adult rats by either administering a single oral dose of IPrA or feeding animals IPrA for 7 days and then measuring treatment effects on both IPrA and KYNA levels in plasma and brain. In addition, we performed *in vivo* microdialysis experiments in the medial prefrontal cortex (mPFC) and in the striatum of freely moving rats to study the effects of both systemic administration and local IPrA perfusion on extracellular KYNA concentrations in the brain.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (300-350 g; Charles River Laboratories, Kingston, NY, USA) were used in all experiments. Two animals per cage were housed in a temperature-controlled animal facility (25°C; 40%-60% humidity) on a 12/12 hours-light/dark cycle (lights on at 06:00) with unlimited access to food and water.

Chemicals

IPrA and KYNA were purchased from Sigma-Aldrich (St. Louis, MO, USA). ³H-kynurenine was obtained from Amersham Corp. (Arlington Heights, IL, USA). All other chemicals were acquired from various suppliers and were of the highest commercially available purity.

In vivo studies

Systemic administration of IPrA. Rats received IPrA (50 or 200 mg/kg; dissolved in 0.9% saline) or an equal volume of 0.9% saline by oral gavage (p.o.). Ninety minutes, 6 or 24 hours later, the animals were euthanized in a CO₂ chamber and decapitated. Plasma samples and brain tissue (PFC) were rapidly collected from all animals, immediately frozen on dry ice and stored at -80°C until analysis.

***In vivo* microdialysis.** Rats were anesthetized in a chamber filled with 5% isoflurane using a vaporizer and were then mounted in a stereotaxic frame (David Kopf, Tujunga, CA, USA). Anesthesia was maintained during the entire surgery period using a nose mask which continuously delivered 2.0% to 3.0% isoflurane mixed with oxygen. A guide cannula (MAB 2.14.G, SciPro Inc., Sanborn, NY, USA) was then positioned over the mPFC (AP: 3.2 mm anterior to bregma, L: ±0.8 mm

from the midline, V: 2.0 mm below the dura) or the striatum (AP: 0.8 mm anterior to bregma, L: ±2.7 mm from the midline, V: 3.5 mm below the dura) and secured to the skull with anchor screws and acrylic dental cement. After surgery, the animals were allowed to recover and were housed individually in acrylic cages with full access to food and water.

On the next day, a microdialysis probe (MAB 9.14.2, membrane length: 2 mm; SciPro) was inserted through the guide cannula. The probe was then connected to a microinfusion pump, and the freely moving rats were perfused with Ringer solution (144 mM, NaCl; 4.8 mM, KCl; 1.7 mM, CaCl₂; 1.2 mM, MgSO₄; pH 6.7) at a speed of 1.1 µl/min. Microdialysates were collected every 30 minutes. After the collection of baseline samples (1.5-2.5 hours), animals received IPrA or vehicle orally, and sample collection continued for up to 8 hours. Dialysates were divided into two 15 µl samples for IPrA and KYNA measurements, respectively (see below). In separate rats, IPrA, dissolved in Ringer solution (pH 6.8), was applied to the mPFC by reverse dialysis. Microdialysates were again collected every 30 minutes. After obtaining baseline samples (2.5 hours), increasing concentrations of IPrA (10, 100, and 300 µM) were infused in succession (2 hours each). Subsequently, animals received a single dose of IPrA (50 mg/kg) by oral gavage, and sample collection continued for another 2 hours.

Subchronic administration of IPrA. Singly housed rats were fed chow containing IPrA or regular chow (controls) for 7 days. To this end, the compound was mixed into 20 g of chow to allow ingestion of 100 or 350 mg IPrA, respectively, per day. Chow was changed every 24 hours, and the animals had unlimited access to water. On Day 7 (~1 pm), rats were euthanized in a CO₂ chamber and decapitated. Plasma and brain tissue (PFC) were rapidly collected and stored as described above.

Analytical procedures

IPrA determination in plasma and brain. Plasma samples were diluted (1:10, v/v) in ultrapure water, and 100 µl of the sample were deproteinized by the addition of 50 µl of 6% perchloric acid. After thorough mixing, samples were centrifuged (16000 × g, 15 minutes). Twenty microliters of the supernatant were applied to a 3 µm C18 reverse phase column (BDS Hypersil; 100 mm × 4.6 mm; Thermo Fisher Scientific, Waltham, MA, USA), and IPrA was isocratically eluted using a mobile phase containing 10 mM sodium acetate, 25 µM EDTA, 0.01% triethylamine and 20% acetonitrile (pH 3.9) at a flow rate of 0.5 ml/min. In the eluate, IPrA was quantified by high-performance liquid chromatography (HPLC) with fluorimetric detection (excitation wavelength: 287 nm; emission wavelength: 340 nm, 2475 fluorescence detector; Waters, Milford, MA, USA). The retention time of IPrA was ~11 minutes.

Brain tissue was homogenized (1:5, w/v) by sonication (Branson Ultrasonics, Danbury, CT, USA) in ultrapure water. Fifty microliters of 6% perchloric acid were then added to 100 μ l of the homogenate, and the suspension was centrifuged (16 000 \times g, 15 minutes). IPrA was determined by HPLC in 30 μ l of the resulting supernatant as described above.

KYNA determination in brain. Twenty microliters of the supernatant used for the tissue determination of IPrA (see above) were applied to a 3- μ m ReproSil C18 column (100 mm \times 4 mm; Dr. Maisch GmbH, Ammerbuch, Germany) to quantify KYNA by HPLC with fluorimetric detection.⁵³ The retention time of KYNA was ~18 minutes.

Kynurenine and KYNA determination in plasma. Plasma samples were diluted (1:2, v/v, for kynurenine and 1:10, v/v, for KYNA) in ultrapure water, and 100 μ l of the sample were deproteinized by the addition of 25 μ l of 6% perchloric acid. After thorough mixing, samples were centrifuged (16 000 \times g, 15 minutes). Twenty microliters of the resulting supernatant were processed by HPLC as described above, and kynurenine and KYNA were quantified by fluorimetric detection.⁵³ The retention times of kynurenine and KYNA were ~6 and ~18 minutes, respectively.

IPrA and KYNA determination in microdialysate. For IPrA analysis, 15 μ l of the microdialysate were applied to a 3 μ m C18 reverse phase column (BDS Hypersil; 100 mm \times 4.6 mm; Thermo Fisher Scientific), and IPrA was determined in the eluate by the same method used for measurement of the compound in plasma and brain (see above).

For KYNA determination, 15 μ l of the dialysate were applied to a 3 mm C18 reverse phase column (BDS Hypersil; 100 mm \times 4.6 mm), and HPLC was performed using a mobile phase containing 250 mM zinc acetate, 50 mM sodium acetate, and 4.5% acetonitrile (pH 6.2) at a flow rate of 1 ml/min as previously described.⁵⁴ In the eluate, KYNA was detected fluorimetrically (excitation wavelength: 344 nm; emission wavelength: 398 nm; S200 fluorescence detector; Perkin-Elmer, Waltham, MA, USA). The retention time of KYNA was ~6 minutes.

Data were not corrected for recovery from the microdialysis probe.

In vitro studies

KAT II activity. Rats were euthanized, and PFC tissue was rapidly dissected out, frozen on dry ice, and stored at -80°C . On the day of the assay, the tissue was thawed and homogenized in ultrapure water (1:5, w/v; Branson Ultrasonics). After further dilution (1:1, v/v) in 5 mM Tris-acetate buffer (pH 8.0) containing 10 mM 2-mercaptoethanol and 50 μ M pyridoxal-5-phosphate, 80 μ l of the solution were incubated for 2 h at 37°C in a reaction mixture containing 150 mM Tris-acetate

buffer (pH 7.4), 2 μ M kynurenine, 0.79 μ M ^3H -kynurenine (23 nCi), 1 mM pyruvate, and 80 μ M pyridoxal-5-phosphate (total volume: 200 μ l). Where indicated, IPrA (final concentrations: 10, 30, 100, 300, or 1000 μ M) was added to the incubation mixture in 20 μ l aliquots (pH 7.0). Blanks contained aminoxyacetic acid (AOAA; 1 mM) in the incubation solution. The reaction was terminated by the addition of 20 μ l of 50% trichloroacetic acid and 1 ml of 0.1 M HCl, and the precipitated proteins were removed by centrifugation (16 000 \times g, 10 minutes). Newly produced ^3H -KYNA was then purified by cation exchange chromatography (Dowex 50W; H $^{+}$ -form) and quantitated by liquid scintillation spectrometry.⁵²

Recombinant human KAT II. Recombinant human KAT II (hKAT II) was prepared as described⁵⁵ and stored at -80°C . On the day of the assay, the protein was thawed and diluted 400 times. Five microliters of the solution were then incubated at 37°C for 2 hours in 150 mM Tris-acetate buffer (pH 7.4) containing kynurenine (10 μ M), pyruvate (1 mM), and pyridoxal-5'-phosphate (80 μ M), in a total volume of 200 μ l. IPrA (final concentration: 1, 10, 100, or 1000 μ M) was added to the incubation mixture in 20 μ l aliquots (pH 7.0). Blanks were obtained by the addition of AOAA (1 mM). The reaction was terminated by the addition of 20 μ l of 50% trichloroacetic acid and 1 ml of 0.1 M HCl, and the precipitated protein was removed by centrifugation (16 000 \times g, 10 minutes). Twenty microliters of the supernatant were applied to a 3 μ m C18 reverse phase column (BDS Hypersil; 100 mm \times 4.6 mm), and KYNA was determined in the eluate by the method used for the measurement of KYNA in microdialysate (see above).

Data analysis

Data are expressed as the mean \pm SEM. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test for the effects of time and for subchronic treatment experiments. Two-way ANOVA with Bonferroni's multiple comparisons test for each timepoint between treatments was used for in vivo microdialysis experiments.

Results

Oral IPrA treatment rapidly and transiently raises both IPrA and KYNA levels in plasma and brain

Endogenous concentrations of IPrA were 6.4 ± 1.7 pmoles/ μ l in plasma and 0.6 ± 0.2 pmoles/mg tissue in brain. Based on preliminary data (not shown), a dose of 200 mg/kg was selected to assess the effects of IPrA on KYNA in vivo. Oral administration of IPrA raised the levels of the compound in the plasma approximately 51- and 32-fold and in the brain approximately 56- and 7-fold after 90 minutes and 6 hours, respectively. In both plasma and brain, IPrA levels were no longer significantly elevated after 24 hours ($P > .05$ each; Figure 1a and b).

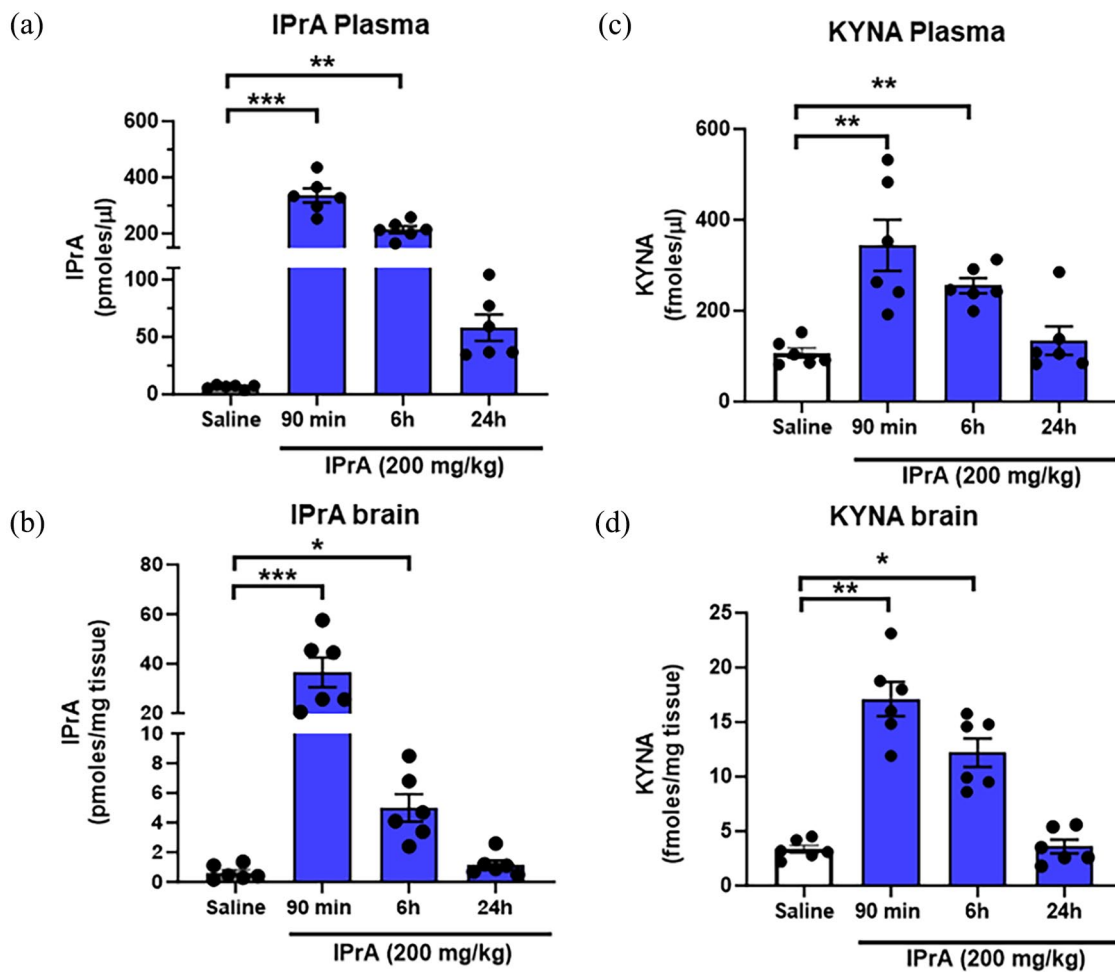


Figure 1. Time-dependent effects of a single administration of IPrA (200 mg/kg, p.o.) on IPrA and KYNA levels in plasma (a, c) and brain (PFC) (b, d). Controls received p.o. vehicle (0.9% saline; only examined after 90 minutes). Data are the mean \pm SEM ($n=6$ per group; individual data are shown as dots). *** $P < .001$, ** $P < .01$, * $P < .05$ versus saline (Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test).

Measured in the same samples as IPrA, endogenous levels of KYNA were 107.0 ± 11.3 fmoles/ μ l in plasma and 3.4 ± 0.4 fmoles/mg tissue in the brain. Oral IPrA administration raised the levels of KYNA in the plasma after 90 minutes and 6 hours approximately 3- and 2-fold, and in the brain approximately 4- and 3-fold, respectively, compared to basal levels. As in the case of IPrA (see above), plasma and brain levels of KYNA were no longer significantly elevated after 24 hours ($P > .05$ each) (Figure 1c and d).

Oral IPrA treatment does not alter kynurenine levels in plasma

Compared to basal levels (3.1 ± 0.3 pmoles/ μ l) and measured in the same samples as IPrA and KYNA (see above), plasma levels of kynurenine were unchanged at 90 minutes, 6 hours, and 24 hours after systemic administration of IPrA (data not shown).

Effect of oral IPrA on extracellular IPrA and KYNA levels in the mPFC

Compared to basal levels (11.9 ± 2.8 fmoles/ μ l for IPrA and 3.5 ± 0.2 fmoles/ μ l for KYNA), the extracellular levels of IPrA

and KYNA in the mPFC were significantly elevated beginning at 90 minutes after IPrA administration (200 mg/kg, p.o.), and these increases were sustained up to 5 hours (Figure 2).

Effect of oral IPrA on extracellular KYNA levels in the striatum

Compared to basal levels (2.3 ± 0.2 fmoles/ μ l), oral administration of IPrA also rapidly raised extracellular KYNA levels in the striatum. Similar to the results in the mPFC, oral administration of 200 mg/kg IPrA maintained elevated KYNA levels in the striatum for 8 hours (Figure 3). Additionally, the oral administration of a relatively low dose of IPrA (50 mg/kg) significantly increased extracellular KYNA levels in the striatum, remaining elevated for up to 3.5 hours after administration (Figure 3).

Effect of local perfusion of IPrA on extracellular KYNA levels in the mPFC

Compared to basal levels (3.2 ± 0.4 fmoles/ μ l), successive local perfusion of IPrA (10, 100, and 300 μ M) did not affect extracellular KYNA levels in the mPFC (Figure 4). Subsequent systemic

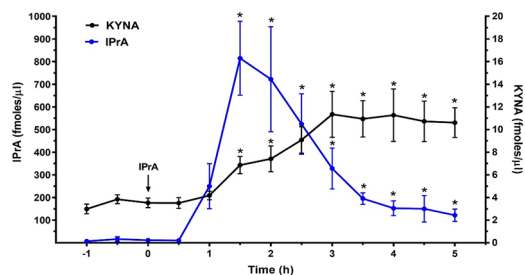


Figure 2. Effect of IPrA (200 mg/kg, p.o.) on extracellular IPrA and KYNA levels in the mPFC. Basal levels were collected for 1.5 hours before IPrA administration. Data are the mean \pm SEM (n=6). * $P < .05$ versus basal levels (two-way ANOVA followed by post hoc pairwise multiple comparisons using Bonferroni's test).

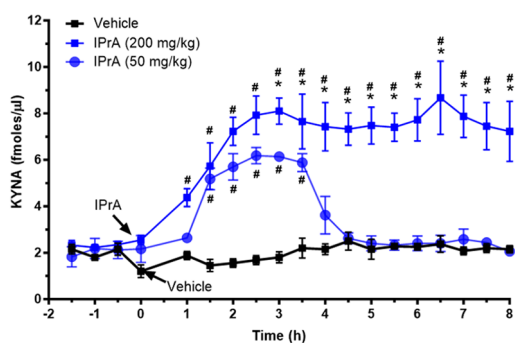


Figure 3. Effect of IPrA (50 or 200 mg/kg, p.o.) or vehicle (0.9% saline, p.o.) on extracellular KYNA levels in the striatum. Basal levels were collected for 2 hours before IPrA or vehicle administration (arrows). Data are the mean \pm SEM (n=5 per group). # $P < .05$ versus vehicle; * $P < .05$ versus 50 mg/kg p.o. (two-way ANOVA followed by post hoc pairwise multiple comparisons using Bonferroni's test).

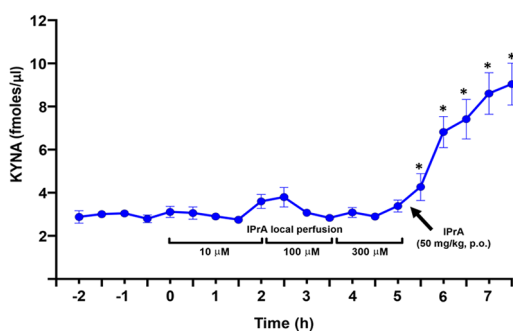


Figure 4. Effect of consecutive local perfusions with IPrA (10, 100, and 300 μ M) on extracellular KYNA levels in the mPFC. IPrA (50 mg/kg) was subsequently administered to the same animals by oral gavage (arrow). See text for experimental details. Data are the mean \pm SEM (n=4); * $P < .05$ versus basal levels (two-way ANOVA followed by post hoc pairwise multiple comparisons using Bonferroni's test).

administration of IPrA (50 mg/kg, p.o.) to the same animals promptly began to raise extracellular KYNA (Figure 4).

Effect of subchronic oral IPrA treatment

Endogenous concentrations of IPrA were 8.3 ± 1.4 pmoles/ μ l in plasma and 1.4 ± 0.5 pmoles/mg tissue in the PFC. Daily

feeding with 100 or 350 mg/day of IPrA for a week raised the levels of the compound approximately 19- and 27-fold in the plasma and approximately 2- and 3-fold in the PFC, respectively, compared to basal levels (Figure 5a and b).

Measured in the same samples as IPrA, endogenous levels of KYNA were 97.3 ± 7.2 fmoles/ μ l in plasma and 5.3 ± 0.7 fmoles/mg tissue in the PFC. Daily feeding with 100 or 350 mg/day of IPrA for a week raised KYNA levels 2- and 2-fold in the plasma and 2- and 3-fold in the PFC respectively, compared to basal levels (Figure 5c and d).

Effect of IPrA on KAT II activity in vitro

Basal values of KAT II activity in rat PFC tissue homogenate and pure hKAT II were 415.0 ± 41.7 fmoles/h/mg tissue and 0.30 ± 0.07 μ moles/h/mg protein, respectively. In both preparations, addition of IPrA failed to affect KAT II activity up to a concentration of 1 mM (all $P > .05$; triplicate measures in all cases; data not shown).

Discussion

The present study demonstrated that systemic IPrA administration causes a substantive increase in the brain levels of KYNA, a metabolite well known for its neuroinhibitory, neuroprotective, antioxidative, and free radical scavenging properties.^{33,42-44} Since IPrA is present endogenously in mammals and can also be detected in the serum of healthy humans (though with high individual variability^{10,13}), this finding may be of physiological significance since even relatively modest elevations of KYNA affect the function of $\alpha 7$ nicotinic acetylcholine and N-methyl-D-aspartate (NMDA) receptors, as well as other receptors including GPR35⁴⁶ and the AHR,⁴⁷ in the brain. In addition to its role in brain physiology and pathology, which includes regulation of the extracellular levels of classic neurotransmitters (dopamine, glutamate, GABA, and acetylcholine), endogenous KYNA also influences the function of peripheral ion channels and metabotropic receptors,^{36,56-61} and may thereby play significant roles in metabolic diseases such as diabetes and atherosclerosis.^{32,62,63} Of special note, physical exercise stimulates KYNA formation in skeletal muscle,⁶⁴ which in turn limits insulin resistance and adverse effects linked to inflammatory processes.⁶⁵

The biological role of tryptophan-derived indoles has recently gained increasing attention.^{8,11,66} The enzymatic chain involved in the synthesis and degradation of these compounds in the gut is present in many Gram-negative and Gram-positive bacteria, and there is consensus that IPrA is the terminal product of the catabolic cascade.⁶⁷ Thus, gut microbes that express aromatic amino acid aminotransferase catalyze the conversion of tryptophan to indole-3-pyruvic acid, which is subsequently converted to indole-3-lactic acid, further to indole-3-acrylic acid, and finally to IPrA.⁶⁸ Notably, IPrA modulates microbiota composition in the gut, inhibiting gut dysbiosis²³ and regulating gastrointestinal barrier function.²⁶ However, gut-derived IPrA also readily enters the circulation

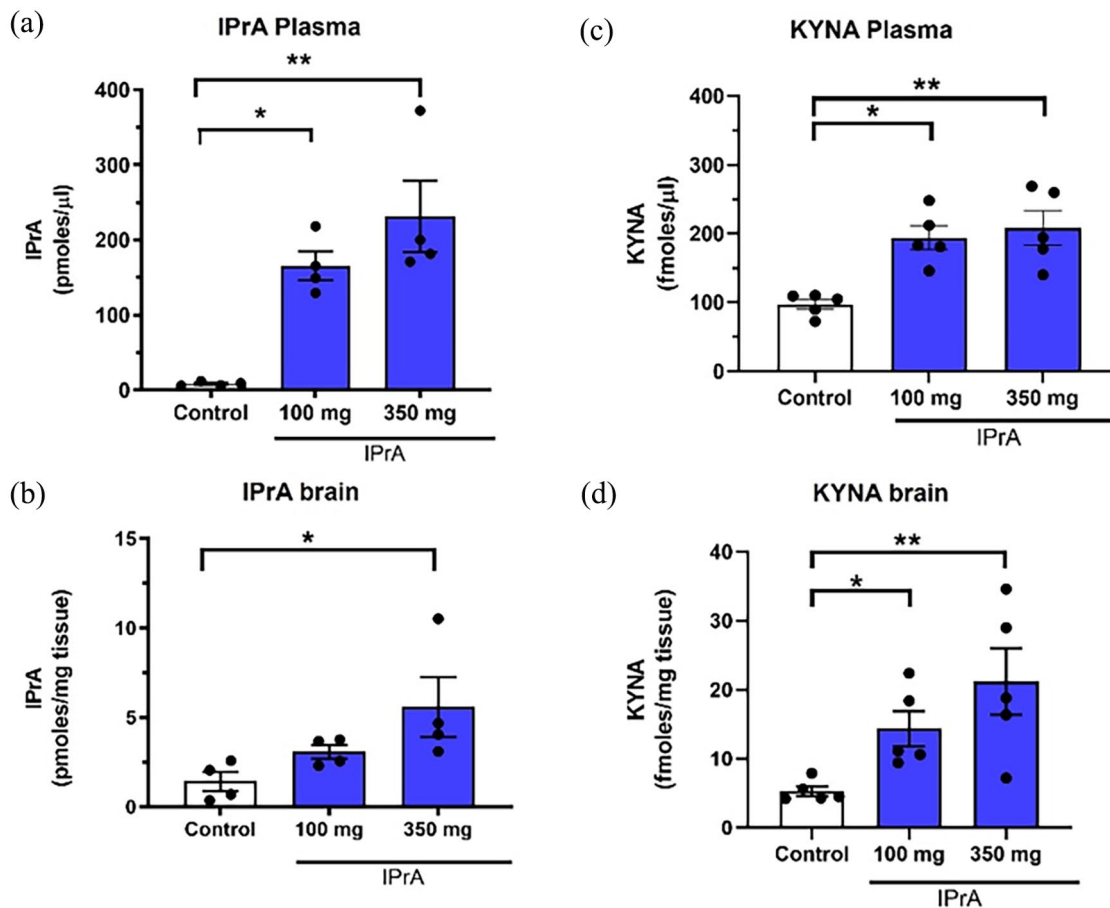


Figure 5. IPrA and KYNA levels in plasma (a, c) and brain (b, d) of rats fed orally with IPrA for 7 days (100 or 350 mg per day). Controls received regular chow. See text for experimental details. Data are the mean \pm SEM (n=4-5 per group). ** $P < .01$, * $P < .05$ versus the control group (Kruskal-Wallis test followed by Dunn's post hoc multiple comparison test).

and affects the function of several peripheral organs (see Jiang et al¹¹ for review) and, as a lipophilic compound, easily crosses the blood-brain barrier.¹³

Interest in the fate and function of IPrA is based on the remarkable, and remarkably complex, biological effects of the compound observed in pre-clinical and clinical studies. Thus, IPrA facilitates regeneration and functional recovery of sensory axons through an immune-mediated mechanism,^{69,70} promotes intestinal homeostasis,^{71,72} boosts muscle tissue development and reduces muscle cell inflammation,⁷³ modulates mitochondrial function in cardiomyocytes,⁷⁴ mediates abnormal synaptic pruning of hippocampal microglia, and affects susceptibility to autism spectrum disorders.⁷⁵ In the brain, IPrA can attenuate neuronal damage and oxidative stress,^{17,18,76} and modulates inflammation and immunity via AHR activation and its downstream effects.^{8,77} Of note, elevated IPrA levels are associated with cognitive impairment.⁷⁸ Taken together, IPrA impacts an array of immune, gastrointestinal, cardiovascular, and central nervous system functions in mammals. In several cases, these phenomena have been convincingly linked to the antioxidant properties of the metabolite and to its ability to inhibit the synthesis of proinflammatory cytokines (cf. Introduction).

A considerable number of biological effects of IPrA, both within and outside the gut, are qualitatively similar to those associated with elevated KYNA levels. Our present demonstration that either acute or subchronic oral IPrA application increases KYNA levels both in the plasma and in the brain therefore raises the question if and to what extent KYNA may play a substantive role in the beneficial and/or adverse consequences of endogenously produced or—as in the present study—exogenously administered IPrA. In other words, could (some of) the biological properties of IPrA be causally related to its ability to serve as a direct or indirect bioprecursor of KYNA?

Previously published data, as well as results from the present study, do not provide straightforward answers to this question. Both in vivo and in vitro studies in mammals have so far failed to show that IPrA, unlike its related metabolite indole-3-pyruvic acid, can be oxidized non-enzymatically to form KYNA⁸ (Poeggeler, unpublished observations). Moreover, we showed here that IPrA, when applied locally in vivo, had no impact on KYNA formation in the brain and does not affect KAT II activity, which accounts for ~75% of KYNA neosynthesis in the adult rat brain,⁵² in vitro. In fact,

IPrA inhibits 2 KP enzymes upstream from KYNA, namely tryptophan-2,3-dioxygenase⁷⁹ and KAT I,⁸⁰ and prevents the production of the pivotal KP metabolite kynurenine in human astrocytes.⁸¹ Also of note in this context, some central effects of IPrA, such as its ability to protect against hydrogen oxide-induced oxidative damage in vivo, are seen after either systemic or intracerebral application of the compound.¹⁶ Thus, although we cannot rule out that KAT III and/or KAT IV may play a role,^{49,51} mechanisms unrelated to KYNA neosynthesis from kynurenine can clearly account for some of IPrA's effects within the brain.⁸² Taken together, functionally significant connections between IPrA and KYNA ought to be explored further and may reveal, for example, the IPrA-induced peripheral formation of currently unknown, brain-penetrant compounds, which could secondarily raise KYNA within the brain. Future studies should also be designed to examine the possible effects of systemic IPrA administration on the levels of neuroactive KP metabolites other than KYNA, including 3-hydroxykynurenine, xanthurenic acid, cinnabarinic acid, picolinic acid, and quinolinic acid,⁸³ and to evaluate IPrA-induced KP changes in additional brain regions.

Pharmacological approaches to up- or down-regulate KYNA function in the brain are increasingly considered as therapeutic options in major neurological and psychiatric diseases.⁸⁴⁻⁸⁷ In view of the fact that IPrA can be safely administered to humans even at high doses,⁸⁸ the translationally most relevant question generated by the present study is therefore whether systemically produced or exogenously applied IPrA, irrespective of its precise mechanism of action, predictably mimics—or is additive with—certain characteristics of KYNA function and dysfunction in the brain. In these cases, targeted genetic or pharmacological up- or down-regulation of IPrA levels in microbes or other cells could be expected to provide interesting, conceptually novel strategies for the treatment of a number of major brain disorders.

Acknowledgements

The authors thank Ms. Marian Thomas for outstanding technical assistance.

Author Contributions

Conceptualization, K.V.S., S.E., B.P., and R.S.; methodology, K.V.S., T.B.-A., and L.S.; software, Y.Z. and M.T.-A.; validation, K.V.S., L.S., and S.E.; formal analysis, K.V.S., L.S., and M.T.-A.; investigation, T.B.-A., Y.Z., and M.T.-A.; resources, S.E. and R.S.; data curation, K.V.S., T.B.-A., Y.Z., and M.T.-A.; writing: original draft preparation, K.V.S., T.B.-A., and Y.Z.; writing: review and editing, K.V.S., L.S., S.E., B.P., and R.S.; visualization, K.V.S., T.B.-A., Y.Z., and M.T.-A.; supervision, L.S., S.E., and R.S.; project administration, K.V.S. and T.B.-A.; funding acquisition, S.E. and R.S. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

Experimental protocols were approved by, and performed in accordance with, the guidelines of the Institutional Animal Care and Use Committee of the University of the Maryland School of Medicine (Baltimore). All efforts were made to minimize the number of animals used and to optimize their well-being.

ORCID iDs

Korrapati V Sathyasaikumar  <https://orcid.org/0000-0001-6646-9272>

Yiran Zheng  <https://orcid.org/0000-0002-2798-9037>

Data Availability Statement

Data are contained within the article.

REFERENCES

1. Nguyen TT, Kosciolk T, Eyler LT, Knight R, Jeste D V. Overview and systematic review of studies of microbiome in schizophrenia and bipolar disorder. *J Psychiatr Res.* 2018;99:50-61.
2. Zhuang Z, Yang R, Wang W, Qi L, Huang T. Associations between gut microbiota and Alzheimer's disease, major depressive disorder, and schizophrenia. *J Neuroinflammation.* 2020;17:288.
3. Generoso JS, Giridharan V V., Lee J, Macedo D, Barichello T. The role of the microbiota-gut-brain axis in neuropsychiatric disorders. *Braz J Psychiatry.* 2021;43:293-305.
4. Nikolova VL, Hall MRB, Hall LJ, Cleare AJ, Stone JM, Young AH. Perturbations in gut microbiota composition in psychiatric disorders: a review and meta-analysis. *JAMA Psychiatry.* 2021;78:1343-1354.
5. Moloney GM, O'Leary OF, Salvo-Romero E, et al. Microbial regulation of hippocampal miRNA expression: implications for transcription of kynurenine pathway enzymes. *Behav Brain Res.* 2017;334:50-54.
6. Agus A, Planchais J, Sokol H. Gut Microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe.* 2018;23:716-724.
7. Gheorghie CE, Martin JA, Manriquez FV, Dinan TG, Cryan JF, Clarke G. Focus on the essentials: tryptophan metabolism and the microbiome-gut-brain axis. *Curr Opin Pharmacol.* 2021;48:137-145.
8. Pappolla MA, Perry G, Fang X, Zagorski M, Sambamurti K, Poeggeler B. Indoles as essential mediators in the gut-brain axis. Their role in Alzheimer's disease. *Neurobiol Dis.* 2021;156:105403.
9. Wei GZ, Martin KA, Xing PY, et al. Tryptophan-metabolizing gut microbes regulate adult neurogenesis via the aryl hydrocarbon receptor. *Proc Natl Acad Sci USA.* 2021;118:e2021091118.
10. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci USA.* 2009;106: 3698-3703.
11. Jiang H, Chen C, Gao J. Extensive summary of the important roles of indole propionic acid, a gut microbial metabolite in host health and disease. *Nutrients.* 2023;15:151.
12. Tennoune N, Andriamihaja M, Blachier F. Production of indole and indole-related compounds by the intestinal microbiota and consequences for the host: the good, the bad, and the ugly. *Microorganisms.* 2022;10:930.
13. Young SN, Anderson GM, Gauthier S, Purdy WC. The Origin of indoleacetic acid and indolepropionic acid in rat and human cerebrospinal fluid. *J Neurochem.* 1980;34:1087-1092.
14. Morita I, Kawamoto M, Yoshida H. Difference in the concentration of tryptophan metabolites between maternal and umbilical foetal blood. *J Chromatogr B Biomed Sci Appl.* 1992;576:334-339.
15. Anderson GM. The quantitative determination of indolic microbial tryptophan metabolites in human and rodent samples: a systematic review. *J Chromatogr B.* 2021;1186:123008.
16. Poeggeler B, Pappolla MA, Hardeland R, et al. Indole-3-propionate: a potent hydroxyl radical scavenger in rat brain. *Brain Res.* 1999;815:382-388.
17. Chyan YJ, Poeggeler B, Omar RA, et al. Potent neuroprotective properties against the Alzheimer β -amyloid by an endogenous melatonin-related indole structure, indole-3-propionic acid. *J Biol Chem.* 1999;274:21937-21942.
18. Hwang IK, Yoo KY, Li H, et al. Indole-3-propionic acid attenuates neuronal damage and oxidative stress in the ischemic hippocampus. *J Neurosci Res.* 2009;87:2126-2137.

19. Ganesh BP, Blasco MP, Hamamy A El, et al. Restoring a balanced pool of host-derived and microbiota-derived ligands of the aryl hydrocarbon receptor is beneficial after stroke. Published online 2023. doi:10.21203/rs.3.rs-3143015/v1
20. Bendheim PE, Poeggeler B, Neria E, Ziv V, Pappolla MA, Chain DG. Development of indole-3-propionic acid (OXIGON™) for Alzheimer's disease. *J Mol Neurosci*. 2002;19:213-217.
21. Abildgaard A, Elfving B, Hokland M, Wegener G, Lund S. The microbial metabolite indole-3-propionic acid improves glucose metabolism in rats, but does not affect behavior. *Arch Physiol Biochem*. 2017;124:306-312.
22. Tuomainen M, Lindström J, Lehtonen M, et al. Associations of serum indole-propionic acid, a gut microbiota metabolite, with type 2 diabetes and low-grade inflammation in high-risk individuals. *Nutr Diabetes*. 2018;8:35.
23. Zhao ZH, Xin FZ, Xue Y, et al. Indole-3-propionic acid inhibits gut dysbiosis and endotoxin leakage to attenuate steatohepatitis in rats. *Exp Mol Med*. 2019;51:1-4.
24. Sehgal R, de Mello VD, Männistö V, et al. Indolepropionic acid, a gut bacteria-produced tryptophan metabolite and the risk of type 2 diabetes and non-alcoholic fatty liver disease. *Nutrients*. 2022;14:4695.
25. Zhang B, Jiang M, Zhao J, Song Y, Du W, Shi J. The mechanism underlying the influence of indole-3-propionic acid: a relevance to metabolic disorders. *Front Endocrinol (Lausanne)*. 2022;13:841703.
26. Venkatesh M, Mukherjee S, Wang H, et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and toll-like receptor 4. *Immunity*. 2014;41:296-310.
27. Ismael S, Rodrigues C, Santos GM, et al. IPA and its precursors differently modulate the proliferation, differentiation, and integrity of intestinal epithelial cells. *Nutr Res Pract*. 2023;17:616-630.
28. Huang ZB, Hu Z, Lu CX, et al. Gut microbiota-derived indole 3-propionic acid partially activates aryl hydrocarbon receptor to promote macrophage phagocytosis and attenuate septic injury. *Front Cell Infect Microbiol*. 2022;12:1015386.
29. Fang Z, Pan T, Wang H, et al. *Limosilactobacillus reuteri* attenuates atopic dermatitis via changes in gut bacteria and indole derivatives from tryptophan metabolism. *Int J Mol Sci*. 2022;23:7735.
30. Konopelski P, Konop M, Gawrys-Kopczynska M, Podosadni P, Szczepanska A, Ufnal M. Indole-3-propionic acid, a tryptophan-derived bacterial metabolite, reduces weight gain in rats. *Nutrients*. 2019;11:591.
31. Turska M, Paluszkiwicz P, Turski WA, Parada-Turska J. A review of the health benefits of food enriched with kynurenic acid. *Nutrients*. 2022;14:4182.
32. Zhen D, Liu J, Zhang XD, Song Z. Kynurenic acid acts as a signaling molecule regulating energy expenditure and is closely associated with metabolic diseases. *Front Endocrinol (Lausanne)*. 2022;13:847611.
33. Foster AC, Vezzani A, French ED, Schwarcz R. Kynurenic acid blocks neurotoxicity and seizures induced in rats by the related brain metabolite quinolinic acid. *Neurosci Lett*. 1984;48:273-278.
34. Schneiderman JH, MacDonald JF. Excitatory amino acid blockers differentially affect bursting of In vitro hippocampal neurons in two pharmacological models of epilepsy. *Neuroscience*. 1989;31:593-603.
35. Chiamulera C, Costa S, Reggiani A. Effect of NMDA- and strychnine-insensitive glycine site antagonists on NMDA-mediated convulsions and learning. *Psychopharmacology*. 1990;102:551-552.
36. Wyant GA, Yu W, Doulamis IiP, et al. Mitochondrial remodeling and ischemic protection by G protein-coupled receptor 35 agonists. *Science (1979)*. 2022;377:621-629.
37. Shepard PD, Joy B, Clerkin L, Schwarcz R. Micromolar brain levels of kynurenic acid are associated with a disruption of auditory sensory gating in the rat. *Neuropsychopharmacology*. 2003;28:1454-1462.
38. Erhardt S, Schwieler L, Emanuelsson C, Geyer M. Endogenous kynurenic acid disrupts prepulse inhibition. *Biol Psychiatry*. 2004;56:255-260.
39. Chess AC, Simoni MK, Alling TE, Bucci DJ. Elevations of endogenous kynurenic acid produce spatial working memory deficits. *Schizophr Bull*. 2007;33:797-804.
40. Potter MC, Elmer GI, Bergeron R, et al. Reduction of endogenous kynurenic acid formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior. *Neuropsychopharmacology*. 2010;35:1734-1742.
41. Wu HQ, Okuyama M, Kajii Y, Pocivavsek A, Bruno JP, Schwarcz R. Targeting kynurenine aminotransferase II in psychiatric diseases: promising effects of an orally active enzyme inhibitor. *Schizophr Bull*. 2014;40 Suppl 2:S152-S158.
42. Perkins MN, Stone TW. An iontophoretic investigation of the actions of convulsant kynurenines and their interaction with the endogenous excitant quinolinic acid. *Brain Res*. 1982;247:184-187.
43. Goda K, Hamane Y, Kishimoto R, Ogishi Y. Radical scavenging properties of tryptophan metabolites. Estimation of their radical reactivity. *Adv Exp Med Biol*. 1999;467:397-402.
44. Lugo-Huitrón R, Blanco-Ayala T, Ugalde-Muñiz P, et al. On the antioxidant properties of kynurenic acid: free radical scavenging activity and inhibition of oxidative stress. *Neurotoxicol Teratol*. 2011;33:538-547.
45. Albuquerque EX, Schwarcz R. Kynurenic acid as an antagonist of $\alpha 7$ nicotinic acetylcholine receptors in the brain: facts and challenges. *Biochem Pharmacol*. 2013;85:1027.
46. Wang J, Simonavicius N, Wu X, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. *J Biol Chem*. 2006;281:22021-22028.
47. DiNatale BC, Murray IA, Schroeder JC, et al. Kynurenic acid is a potent endogenous aryl hydrocarbon receptor ligand that synergistically induces interleukin-6 in the presence of inflammatory signaling. *Toxicol Sci*. 2010;115:89-97.
48. Tang J, Shen H, Zhao X, et al. Endogenous plasma kynurenic acid in human: a newly discovered biomarker for drug-drug interactions involving organic anion transporter 1 and 3 inhibition. *Drug Metab Dispos*. 2021;49:1063-1069.
49. Han Q, Cai T, Tagle DA, Li J. Structure, expression, and function of ky aminotransferases in human and rodent brains. *Cell Mol Life Sci*. 2010;67:353-368.
50. Ramos-Chávez LA, Lugo Huitrón R, González Esquivel D, et al. Relevance of alternative routes of kynurenic acid production in the brain. *Oxid Med Cell Longev*. 2018;2018:5272741.
51. Guidetti P, Amori L, Sapko MT, Okuno E, Schwarcz R. Mitochondrial aspartate aminotransferase: a third kynurenate-producing enzyme in the mammalian brain. *J Neurochem*. 2007;102:103-111.
52. Guidetti P, Okuno E, Schwarcz R. Characterization of rat brain kynurenine aminotransferases I and II. *J Neurosci Res*. 1997;50:457-465.
53. Pocivavsek A, Baratta AM, Mong JA, Viechweg SS. Acute kynurenine challenge disrupts sleep-wake architecture and impairs contextual memory in adult rats. *Sleep*. 2017;40:zsx141.
54. Shibata K. Fluorimetric micro-determination of kynurenic acid, an endogenous blocker of neurotoxicity, by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl*. 1988;430:376-380.
55. Blanco-Ayala T, Sathyasaikumar K V, Uys JD, Pérez-de-la-Cruz V, Pidugu LS, Schwarcz R. N-acetylcysteine inhibits kynurenine aminotransferase II. *Neuroscience*. 2020;444:160-169.
56. Carpenedo R, Pittaluga A, Cozzi A, et al. Presynaptic kynurenate-sensitive receptors inhibit glutamate release. *Eur J Neurosci*. 2001;13:2141-2147.
57. Wu HQ, Rassoulpour A, Schwarcz R. Kynurenic acid leads, dopamine follows: a new case of volume transmission in the brain? *J Neural Transm*. 2007;114:33-41.
58. Zmarowski A, Wu HQ, Brooks JM, et al. Astrocyte-derived kynurenic acid modulates basal and evoked cortical acetylcholine release. *Eur J Neurosci*. 2009;29:529-538.
59. Pocivavsek A, Wu HQ, Potter MC, Elmer GI, Pellicciari R, Schwarcz R. Fluctuations in endogenous kynurenic acid control hippocampal glutamate and memory. *Neuropsychopharmacology*. 2011;36:2357-2367.
60. Beggato S, Tanganelli S, Fuxe K, Antonelli T, Schwarcz R, Ferraro L. Endogenous kynurenic acid regulates extracellular GABA levels in the rat prefrontal cortex. *Neuropharmacology*. 2014;82:11-18.
61. Curto M, Lionetto L, Negro A, et al. Altered kynurenine pathway metabolites in serum of chronic migraine patients. *J Headache Pain*. 2015;17:47.
62. Moroni F, Cozzi A, Sili M, Mannaioni G. Kynurenic acid: a metabolite with multiple actions and multiple targets in brain and periphery. *J Neural Transm*. 2012;119:133-139.
63. Koziel K, Urbanska EM. Kynurenine pathway in diabetes mellitus—novel pharmacological target? *Cells*. 2023;12:460.
64. Agudelo LZ, Femenía T, Orhan F, et al. Skeletal muscle PGC-1 α 1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. *Cell*. 2014;159:33-45.
65. Agudelo LZ, Ferreira DMS, Cervenka I, et al. Kynurenic acid and Gpr35 regulate adipose tissue energy homeostasis and inflammation. *Cell Metab*. 2018;27:378-392.e5.
66. Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. *Nat Commun*. 2018;9:3294.
67. Gao K, Mu CL, Farzi A, Zhu WY. Tryptophan metabolism: a link between the gut microbiota and brain. *Adv Nutr*. 2020;11:709-723.
68. Roth W, Zadeh K, Vekariya R, Ge Y, Mohamadzadeh M. Tryptophan metabolism and gut-brain homeostasis. *Int J Mol Sci*. 2021;22:2973.
69. Serger E, Luengo-Gutierrez L, Chadwick JS, et al. The gut metabolite indole-3-propionate promotes nerve regeneration and repair. *Nature*. 2022;607:585-592.
70. Kim CS, Jung S, Hwang GS, Shin DM. Gut microbiota indole-3-propionic acid mediates neuroprotective effect of probiotic consumption in healthy elderly: a randomized, double-blind, placebo-controlled, multicenter trial and in vitro study. *Clin Nutr*. 2023;42:1025-1033.
71. Alexeev EE, Lanis JM, Kao DJ, et al. Microbiota-derived indole metabolites promote human and murine intestinal homeostasis through regulation of Interleukin-10 receptor. *Am J Pathol*. 2018;188:1183-1194.
72. Cao B, Zhao RY, Li HH, et al. Oral administration of asparagine and 3-indolepropionic acid prolongs survival time of rats with traumatic colon injury. *Mil Med Res*. 2022;9:37.

73. Du L, Qi R, Wang J, Liu Z, Wu Z. Indole-3-propionic acid, a functional metabolite of clostridium sporogenes, promotes muscle tissue development and reduces muscle cell inflammation. *Int J Mol Sci.* 2021;22:12435.
74. Gesper M, Nonnast ABH, Kumowski N, et al. Gut-derived metabolite indole-3-propionic acid modulates mitochondrial function in cardiomyocytes and alters cardiac function. *Front Med (Lausanne).* 2021;8:648259.
75. Wang T, Chen B, Luo M, et al. Microbiota-indole 3-propionic acid-brain axis mediates abnormal synaptic pruning of hippocampal microglia and susceptibility to ASD in IUGR offspring. *Microbiome.* 2023;11:245.
76. Owumi SE, Adedara IA, Oyelere AK. Indole-3-propionic acid mitigates chlorpyrifos-mediated neurotoxicity by modulating cholinergic and redox-regulatory systems, inflammatory stress, apoptotic responses and DNA damage in rats. *Environ Toxicol Pharmacol.* 2022;89:103786.
77. Rothhammer V, Mascanfroni ID, Bunse L, et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med.* 2016;22:586-597.
78. Huang YL, Lin CH, Tsai TH, et al. Discovery of a metabolic signature predisposing high risk patients with mild cognitive impairment to converting to alzheimer's disease. *Int J Mol Sci.* 2021;22:10903.
79. Eguchi N, Watanabe Y, Kawanishi K, Hashimoto Y, Hayaishi O. Inhibition of indoleamine 2,3-dioxygenase and tryptophan 2,3-dioxygenase by β -carboline and indole derivatives. *Arch Biochem Biophys.* 1984;232:602-609.
80. Han Q, Robinson H, Cai T, Tagle DA, Li J. Structural insight into the inhibition of human kynurenine aminotransferase I/Glutamine transaminase K. *J Med Chem.* 2009;52:2786-2793.
81. Garcez ML, Tan VX, Heng B, Guillemin GJ. Sodium butyrate and indole-3-propionic acid prevent the increase of cytokines and kynurenine levels in LPS-induced human primary astrocytes. *Int J Tryptophan Re.* 2020;13:1178646920978404.
82. Mimori S, Kawada K, Saito R, et al. Indole-3-propionic acid has chemical chaperone activity and suppresses endoplasmic reticulum stress-induced neuronal cell death. *Biochem Biophys Res Commun.* 2019;517:623-628.
83. Bohár Z, Toldi J, Fülöp F, Vécsei L. Changing the face of kynurenines and neurotoxicity: therapeutic considerations. *Int J Mol Sci.* 2015;16:9772-9793.
84. Schwarcz R. Manipulation of brain kynurenines: glial targets, neuronal effects, and clinical opportunities. *J Pharmacol Exp Ther.* 2002;303:1-10.
85. Erhardt S, Olsson SK, Engberg G. Pharmacological manipulation of kynurenine acid: potential in the treatment of psychiatric disorders. *CNS Drugs.* 2009;23:91-101.
86. Bai MY, Lovejoy DB, Guillemin GJ, Kozak R, Stone TW, Koola MM. Galantamine-memantine combination and kynurenine pathway enzyme inhibitors in the treatment of neuropsychiatric disorders. *Complex Psychiatry.* 2021;7:19-33.
87. Mithaiwala MN, Santana-Coelho D, Porter GA, O'Connor JC. Neuroinflammation and the kynurenine pathway in CNS disease: molecular mechanisms and therapeutic implications. *Cells.* 2021;10:1548.
88. Rodríguez LR, Lapeña T, Calap-quintana P, Moltó MD, Gonzalez-cabo P, Langa JAN. Antioxidant therapies and oxidative stress in friedreich's ataxia: the right path or just a diversion? *Antioxidants.* 2020;9:664.