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**The optimal dose of kynurenic acid in improving memory and its
antidepressant-like effect in preclinical studies**

Summary of the Ph.D. Thesis

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Original publications directly related to the Ph.D. thesis:

- I. **Martos D.**, Tuka B., Tanaka M., Vécsei L. and Telegdy Gy. Memory enhancement with kynurenic acid and its mechanism in neurotransmission. MDPI Biomedicines 2022, 10, 849. Received in MDPI Biomedicines The Best Paper Award 2022. (original paper IF: 4.757, **Q1**)
- II. Tanaka M., Bohár Zs., **Martos D.**, Telegdy Gy., Vécsei L. Antidepressant-like effects of kynurenic acid in a modified forced swim test. Pharmacological Reports 2020, 72(2):449-455. (original paper IF: 3.024, **Q2**)

Cumulative classification of the publications directly to the thesis: **Q1 + Q2**

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Publications not directly related to the Ph.D. thesis:

- I. Dézsi L., Tuka B., **Martos D.** and Vécsei L. Alzheimer's disease, astrocytes and kynurenes. *Bentham Science Current Alzheimer Research* 2015, 12(5):462-480. (review IF: 3.040, **Q1**)
- II. Samavati R., Zador F., Szucs E., Tuka B., **Martos D.**, Veres G., Gaspa R., Mandity MI., Fülöp F., Vecsei L. et al. Kynurenic acid and its analogue can alter the opioid receptor G-protein signaling after acute treatment via NMDA receptor in rat cortex and striatum. *Journal of the Neurological Sciences* 2017, 376 pp. 63-70. (original paper IF: 2.448 **Q4**)
- III. Nánási N., Veres G., Cseh EK., Szentirmai M., **Martos D.**, Sümegei E., Hadady L., Klivényi P., Vécsei L., Zádori D. The detection of age-, gender-, and region-specific changes in mouse brain tocopherol levels via the application of different validated HPLC methods. *Neurochemical Research* 2018, 43:11 pp. 2081-2091. (original paper IF: 2.782 **Q2**)
- IV. Veres G., Tellér A., **Martos D.**, Szatmári I., Kiss L., Vécsei L., Zádori D. Determination of glutamate and GABA from rat central nervous system samples with HPLC utilizing fluorescent detection. *Proceeding of the 25th International Symposium on Analytical and Environmental Problems*. 2019, 464 pp. 427-431. Alapi T., Ilisz I. (eds). University of Szeged, Department of Inorganic and Analytical Chemistry, H-6720 Szeged, Dóm tér 7, Hungary. ISBN: [9789633067024](https://doi.org/10.1007/978-96-3306-702-4) (conference proceeding)
- V. Nánási N., Veres G., Cseh EK., **Martos D.**, Hadady L., Klivényi P., Vécsei L., Zádori D. The assessment of possible gender-related effect of endogenous striatal alpha tocopherol level on MPTP neurotoxicity in mice. *Heliyon* 2020, 6:e04425. (original paper, IF: 0.455, **Q1**)
- VI. **Martos D.**, Lőrinczi B., Szatmári I., Vécsei L. and Tanaka M. The Impact of C-3 Side Chain Modifications on Kynurenic Acid: A Behavioral Analysis of Its Analogs in the Motor Domain. *MDPI International Journal of Molecular Sciences* 2024, 25, 3394. (original paper, IF: 5.6, **Q1**)

- VII. Szabó Á., Galla Zs., Spekker E., Szűcs M., **Martos D.**, Takeda K., Ozaki K., Inoue H., Yamamoto S., Toldi J., Ono E., Vécsei L and Tanaka M. Oxidative and Excitatory Neurotoxic Stress in CRISPR/Cas9-Induced Kynurenine Aminotransferase Knockout Mice: A Novel Model for Despair-Based Depression and Post-Traumatic Stress Disorder. *Frontiers in Bioscience-Landmark* 2025, 30 (1):25706. (original paper, IF: 3.3 **Q2**)

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Poster not directly related to the Ph.D. thesis:

- I. **Martos D.**, Nyári A., Markó V., Tuka B., Tajti J., Vécsei L. *Effect of kynurenine analogues in mice behavioral test*. The 9th World Congress on Controversies Neurology (CONy)-E-poster presentations, 1st place on congress poster award. March 26-28, 2015, Budapest, Hungary.

List of abbreviations

Ach	Acetylcholine
AHR	aryl hydrocarbon receptor
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
ATR	Atropine
BCL	Bicuculline
CPH	Cyproheptadine
DA	Dopamine
EAARs	Excitatory amino acid receptors
FST	Forced Swim Test
GABA	Gamma (γ)-aminobutyric acid
GABAA	GABA subunit A
GPR35	G-protein-coupled receptor 35
5-HT	5-hydroxytryptamine
HPD	Haloperidol
icv	Intracerebroventricular
ip	Intraperitoneal
KYN	Kynurenine
KYNA	Kynurenic acid
MDD	Major Depressive Disorder
NER	Norepinephrine
NMDA	N-methyl-D-aspartate
PHB	Phenoxybenzamine
PPL	Propranolol
SER	Serotonin
Trp	Tryptophan
YHB	Yohimbine

Introduction

Major depressive disorder (MDD) has been ranked as the third cause of the burden of disease worldwide in 2008 by WHO, which has projected that this disease will rank first by 2030. It has a lifetime prevalence of about 5 to 17 percent, with the average being 12 percent. The prevalence rate is nearly double in women than in men. The etiology of MDD is believed to be multifactorial, including biological, genetic, environmental, and psychosocial factors. MDD was earlier considered to be mainly due to abnormalities in neurotransmitters, especially serotonin (SER), norepinephrine (NER), acetylcholine (Ach) and dopamine (DA). By the cognitive theory the depression occurs as a result of cognitive skewness in persons who are susceptible to depression.

Worldwide, currently more than 55 million people have dementia, over 60% of whom live in low-and middle-income countries. There are nearly 10 million new cases in every year. Dementia is currently the seventh leading cause of death and it sizable a considerable physical, psychological, social and economic burden on for people with dementia, their family members and society. The cause of major neurocognitive disorders remains unknown, but it is considered to be caused by connection of multifactorial factors including genetic, environmental, infectious, and nutritional components, together with lifestyle, among others. Currently, there is no cure for neurodegenerative diseases, and available treatments mainly aim to alleviate symptoms and modify disease progression. Consequently, significant efforts have been dedicated to identifying pathomechanisms, discovering therapeutic targets, and developing novel pharmaceutical agents.

Kynurenic acid (KYNA) is a metabolite in the kynurenine (KYN) pathway from the essential amino acid tryptophan (Trp), which is also a precursor of SER and melatonin in the melatonin pathway. It is known, that KYNA is to possess a neuroprotective property. The neuroprotective activities are considered to be attributed to the antagonism of the excitatory amino acid receptors (EAARs) such as the N-methyl-D-aspartate (NMDA) receptor, the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor, and the kainic acid receptor. Moreover, KYNA acts as an agonist of the G-protein-coupled receptor 35 (GPR35) and the aryl hydrocarbon receptor (AHR). In addition, opioid receptors are presumed to be interacting partners with KYNA. For this reason, to better understand the mechanisms that may understand neuropsychological and neurocognitive diseases such as depression, anxiety and dementia. We investigated the effect of Janus-faced KYNA on the receptor pathways involved in the development of the above-mentioned diseases in mice.

Aims

The dissertation aims to investigate the antidepressant-like and cognitive-enhancing effects of KYNA by evaluating its impact on neurotransmitter receptor pathways in preclinical models. To determine the involvement of specific neurotransmitter systems, a series of receptor blockers (targeting SER-ergic, DA-ergic, adrenergic, and Gamma (γ)-aminobutyric acid (GABA)-ergic pathways) were co-administered with KYNA. Behavioral responses were measured, and statistical analyses were applied to evaluate dose-dependent effects. Ethical guidelines were strictly followed to ensure humane animal handling. The methodological framework was designed to elucidate the receptor-specific actions of KYNA, offering new insights into its potential therapeutic role in depression and cognitive disorders.

Materials and Methods

Animals

For the first study CD-1 male mice (body weight 28-35 g), for the second study CFLP mice of either sex (body weight 25–28 g) were used. Five animals per cage were housed under laboratory conditions with a 12 h dark/12 h light cycle (lights on at 6:00-18:00 h) in a temperature- and humidity-controlled room (24 ± 1 °C and 50 ± 10 %) in the Laboratory Animal House of the Department of Neurology in Szeged. Standard mouse chow and tap water were available ad libitum. Each animal was used only once in the experiments, at least 5 days of recovery post-surgery was allowed before the experiments. The suffering of the animals and the number of animals used were kept to a minimum.

Ethics statement

All animal experiments complied with the principles of animal care outlined in the instructions of the Ethical Committee for the Protection of Animals in Research of the University of Szeged (Szeged, Hungary), which specifically approved the first study (XXIV/352/2012) and the second study (XI./240/2019). The protocols for animal care approved both by the Hungarian Health Committee (40/2013 (II.14.)) and by the European Communities Council Directive (2010/63/EU). The animals were kept and handled during the experiments in accordance with the guidelines of the 8th Edition of the Guide for the Care and Use of Laboratory Animals and the Use of Animals in Research of the International Association for the Study of Pain and the directive of the European Economic Community (86/609/ECC).

Surgery

The mice were anaesthetized for the first study with Isoflurane at 3-4% for induction and at 1-2% for maintenance, and for the second study was used 40% Euthazol (in a dose of 60 mg/kg administered ip). To allow intracerebroventricular (icv) administration, a polystyrene cannula was implanted into the right lateral brain ventricle of each mouse at the coordinates 0.2 mm posterior, 0.2 mm lateral to the bregma, and 2.0 mm deep from the dural surface [80]. The cannula was fixed with cyanoacrylate (Ferrobond) (Budapest, Hungary). The icv administration was performed 5 days after the surgery. The correct location of the cannula was controlled when dissecting the brain following the completion of the experiments. Only animals with the correct location of the cannula were used in the evaluation of the experiments. All experiments were performed during the morning period.

Materials

We used the following receptor blockers for the antidepressant-like effects study, 3.0 mg/bwkg CPH hydrochloride, 2 mg/bwkg PHB hydrochloride, 5 mg/bwkg YHB hydrochloride, 5 mg/bwkg PPL hydrochloride, 2 mg/bwkg ATR sulfate, 10 µg/bwkg HPD haloperidol and 2 mg/bwkg BCL methiodide. Physiological saline (0.9% NaCl) was used as a control. For the memory enhancement measurements the following receptor blockers were applied: 5 mg/kg CPH hydrochloride, 2 mg/kg PHB hydrochloride, 0.3 mg/kg naloxone, 10 µg/kg HPD, 2 mg/kg PPL hydrochloride, 2 mg/kg ATR sulfate. During both tests the KYNA was freshly dissolved in sterile pyrogen-free 0.9% aqueous saline solution and its pH was set to approximately 7.4 before use and administered icv via the cannula in a volume of 2 µL. The control animals received only 0.9% saline solution.

Experimental groups and treatments

The dose dependent examinations for the modified forced swim test (FST), 6 groups were examined (1 control and 5 for the different doses of KYNA applied). Animals for further studies were divided into 32 groups (8 control, 8 KYNA, 8 for the different receptor blockers, and 8 combined groups) and the treatments were carried out following the training behavioral test (pre-test day) on the second day. KYNA was administered through a polyethylene tube into the right lateral brain ventricle in a volume of 2 µL icv. The different receptor blockers were administered intraperitoneal (ip). The dose of KYNA was selected based on the results of the dose-effect study.

In the memory enhancement pilot study 4 groups of female mice were used (1 control and 3 different doses of KYNA). The dose range of KYNA was selected according to the results of the pilot study. The dose-effect examination for the memory enhancement study, 7 groups were examined (1 control and 6 for the different doses of KYNA applied). Animals for further studies were divided into 24 groups (6 control, 6 KYNA, 6 for the different receptor blockers, and 6 combined groups) and the treatments were carried out following the training behavioral test (post-trial) on the second day. KYNA was administered through a polyethylene tube into the right lateral brain ventricle in a volume of 2 μL icv. The different receptor blockers were administered ip. The dose of KYNA was selected based on the results of the dose-effect study; only the most effective dose was used during the different receptor blocker-testing experiments.

Behavioral tests

Modified forced swim test

The modified mouse FST was performed as reported previously [84]. The mice were placed individually in a glass cylinder of 12 cm in diameter and 30 cm in height. Water (25 ± 1 °C) was filled to a height of 20 cm. Fresh water was used for each mouse. A 15-min pretest was carried out 24 h before the 3-min test session. 30 min prior to the test session, KYNA was administered icv at a volume of 2 μL , at doses of 0.04 $\mu\text{g}/2$ μL , 0.2 $\mu\text{g}/2$ μL , 0.4 $\mu\text{g}/2$ μL , 0.6 $\mu\text{g}/2$ μL or 0.8 $\mu\text{g}/2$ μL . In the modified combination FST, 30 min prior to KYNA (0.8 $\mu\text{g}/2$ μL icv) administration, the following receptor blockers were administered ip. A time-sampling technique was conducted to count the duration of climbing, swimming, and immobility times.

Passive avoidance test

The passive avoidance test was performed as previously described in Palotai et al. 2016 [85-88]. On the first day of testing, the mice were placed on an illuminated platform and were allowed to enter the dark compartment for 2 min. Since mice prefer the dark to the light, they normally entered within 5 second (sec). This session was repeated 3 times with all animals, and an additional trial was performed on the following day. However, during this second trial, when the mice entered the dark part of the box, an unavoidable but not harmful mild electric footshock (0.75 mA, 2 sec) was given through the grid floor. The gate between the light and dark compartments was closed and the animal could not escape. This learning trial was not repeated, but the mice were immediately removed from the apparatus and treated with

receptor blockers or saline one minute, with KYNA 30 minutes after the footshock. The consolidation of passive avoidance behavior was tested 24 h later. Each animal was placed on the light platform and the latency to enter the dark compartment was measured up to a maximum of 300 sec per test.

Statistical analysis

Following the analyses of normality and variance, parametric tests were used in all cases of the receptor blocker measurements, but a nonparametric test was carried out in the KYNA dose–response investigation. The one-way analysis of variance (ANOVA) test was followed by Tukey's post hoc test for multiple comparisons with unequal cell size. Kruskal–Wallis rank sum test was followed by pairwise comparisons using Tukey's and Kramer (Nemenyi) test with Tukey's-Dist approximation for independent samples. For the modified FST the analysis of variance (two-way ANOVA) test was followed by Tukey's test for multiple comparisons with unequal cell size. Probability values (p) of less than 0.05 were considered significant. The data in the plots are presented as means \pm SEM.

Results

The modified forced swim test

Dose dependent studies

During the analysis of dose effect examination we have been found, that compared to control group, the dose of 0.4 $\mu\text{g}/2 \mu\text{L}$ KYNA significantly decreased immobility time. 0.6 $\mu\text{g}/2 \mu\text{L}$ significantly decreased immobility time and significantly increased swimming time. The dose of 0.8 $\mu\text{g}/2 \mu\text{L}$ significantly decreased immobility time, significantly increased climbing time and significantly increased swimming time. The results suggest that KYNA induces antidepressant-like effects with doses of 0.4 $\mu\text{g}/2 \mu\text{L}$, 0.6 $\mu\text{g}/2 \mu\text{L}$ and 0.8 $\mu\text{g}/2 \mu\text{L}$.

Examination of different receptor blockers

Pretreatment with CPH significantly increased immobility time and significantly decreased swimming time compared to KYNA. It suggests the possible involvement of the SER receptor in KYNA-induced antidepressant-like effects. Pretreatment with PHB did not reverse immobility, climbing, or swimming times compared to KYNA, thus the NER receptor may not be involved in KYNA-induced antidepressant-like effects. Likewise, pretreatment with YHB did not reverse immobility, climbing, and swimming times compared to KYNA, so the α_2 -ADR receptor also may not be involved in KYNA-induced

antidepressant-like effects nor did pretreatment with PPL reverse climbing, swimming, and immobility times compared to KYNA, so the β -ADR is not involved in KYNA-induced antidepressant-like effects. Pretreatment with HPD did not change the immobility or swimming times but did decrease climbing time compared to KYNA. It suggests a minimal involvement of the D2, D3, D4 DA receptor in KYNA-induced antidepressant-like effects. Pretreatment with ATR did not affect immobility, climbing, or swimming times compared to KYNA, so the muscarinic Ach receptor may not be involved in KYNA-induced antidepressant-like effects. Pretreatment with BCL increased immobility time, decreased climbing time, and significantly decreased swimming time compared to KYNA, suggesting a possible involvement of the GABA subunit A (GABAA) receptor in KYNA-induced antidepressant-like effects. The above results revealed the presence of antidepressant-like effects of KYNA in a modified mouse FST, and the antidepressant-like actions of KYNA strongly interacted with 5-HT₂ SER-ergic receptors, weakly interacted with D2, D3, D4 DA-ergic receptors, and moderately interacted with GABAA receptors.

The memory enhancement

Pilot study

In order to determine the most preferable, effective dose of KYNA in the cognitive processes, 10, 20 and 40 μ g KYNA in 2 μ L saline were applied icv route. Female mice were used (n=5/group) to identify the differences between the treated groups. It was observed that the 40 μ g KYNA could greatly decrease the avoidance latency, while the lower doses did not influence significantly this parameter compared to the control animals. These results suggested that the positive impact of KYNA would be expected in lower doses than 10 μ g in the aspect of cognition.

Dose dependent studies

Male mice were used (n = 10–27/group) to determine the dose of KYNA that could significantly increase the avoidance latency. We investigated the effect of KYNA in doses of 0.25, 0.5, 1, 2, 4, and 8 μ g in 2 μ L saline. The 0.5 μ g of KYNA prominently elevated the time until the animals entered the shock-associated dark part of the box, as compared with the control group (p < 0.044). We concluded that KYNA in a dose of 0.5 μ g improved memory consolidation; therefore, this dose was used for further testing. Higher doses of KYNA were associated with significantly shorter avoidance latency as compared with the 0.5 μ g KYNA-treated group (2 μ g KYNA vs. 0.5 μ g KYNA, p < 0.013; 4 μ g KYNA vs. 0.5 μ g KYNA, p < 0.001). Other doses did not significantly influence the avoidance behavior of mice.

Examination of different receptor blockers

In all cases, the 0.5 µg/2 µL dose of KYNA significantly increased the avoidance latency of mice as compared with the healthy control group in the passive avoidance behavioral test. All groups of the tested receptor blockers were associated with significantly shorter avoidance latency as compared with the 0.5 µg KYNA-treated group. Furthermore, the groups receiving combined treatments (KYNA plus different receptor blocker compounds) were associated with significantly diminished time spent in the light part of the box, as compared with the group treated with 0.5 µg of KYNA alone, except for the one receiving ATR. Compared to the control group, the applied receptor blockers did not influence remarkably the avoidance latency, whereas the latency values observed in the combination groups did not differ significantly from those observed in the groups treated with the respective receptor blocker alone.

Discussion

Future research must focus on improving KYNA delivery to the brain, potentially through nanoparticle-based drug formulations or prodrug approaches. Moreover, developing receptor-selective KYNA analogs could help fine-tune its effects, minimizing unwanted side effects while maximizing therapeutic benefits. Advanced neuroimaging and electrophysiological techniques may further clarify KYNA's receptor interactions and dose-dependent actions, helping to design more targeted interventions for mood and cognitive disorders.

This study builds on prior work suggesting a link between KYN pathway metabolites and neuropsychiatric disorders. While KYNA has been widely recognized for its neuroprotective and NMDA receptor antagonist properties, this study expands its relevance by demonstrating its antidepressant-like effects and cognitive-enhancing potential in a preclinical setting. By identifying specific receptor pathways involved in these effects, our findings bridge the gap between theoretical neurobiology and practical pharmacological applications, offering a new avenue for mood disorder and cognitive impairment treatments.

This series of research underscores the potential therapeutic relevance of KYNA in depression and cognitive dysfunction, highlighting its ability to modulate multiple neurotransmitter systems. Theoretically, these findings contribute to a deeper understanding of the KYN pathway's role in mood regulation and memory processes. Practically, low-dose KYNA or its analogs could serve as novel treatment strategies for patients with mood disorders or neurodegenerative diseases. However, further studies are required to assess long-term safety, optimal dosing strategies, and delivery mechanisms to enhance clinical applicability. Future

research should explore KYNA's role in human models, assess its interactions with existing antidepressants, and develop optimized derivatives with improved pharmacokinetics. By addressing these areas, KYNA-based interventions could hold promise for personalized medicine approaches in neuropsychiatric treatment.

Conclusion

This research provides compelling evidence for the role of kynurenic acid (KYNA) in modulating mood and cognitive processes, reinforcing the significance of kynurenine pathway metabolites in major depressive disorder (MDD). For the first time, KYNA's antidepressant-like effects were demonstrated in a modified forced swim test (FST), highlighting its strong interaction with 5-hydroxytryptamine (5-HT) 2 SER-ergic receptors, weaker association with dopaminergic (D2, D3, D4) receptors, and moderate involvement of GABAA receptors. These findings contribute to a growing body of research suggesting KYNA as a potential target for antidepressant development. Furthermore, this study confirmed that low-dose KYNA enhances learning and memory consolidation, offering insights into its cognitive-enhancing properties. While these results establish KYNA's neuromodulatory potential, further investigations are needed to elucidate its dose-dependent effects, optimize its pharmacokinetics, and assess its long-term impact on cognition and mood regulation. Future studies should explore KYNA's translational relevance in neurodegenerative diseases and cognitive disorders, particularly through chronic administration and alternative delivery strategies. By deepening our understanding of KYNA's neurobiological mechanisms, this research paves the way for novel therapeutic interventions targeting both mood disorders and cognitive impairments.

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