Kynurenine Aminotransferase II Inhibition by Glycyrrhetinic Acid: An *In Vitro* and *In Vivo* Study

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INTRODUCTION

Kynurenic acid (KYNA), a metabolite of the kynurenine pathway of tryptophan degradation, is thought to play an important role in the mechanism(s) underlying normal and abnormal cognitive processes, acting as an antagonist of α7 nicotinic and NMDA receptor function. Specifically, increased brain KYNA levels may have detrimental effects in schizophrenia (SZ) and other psychiatric diseases (Plitman et al., Schizophr. Bull., 43: 764-7, 2017; Sellgren et al., Transl. Psychiatry. 9: 37, 2019).

KYNA is synthesized from its immediate bioprecursor kynurenine - either by non-enzymatic oxidation or through irreversible enzymatic transamination by kynurenine aminotransferases. In the mammalian brain, kynurenine aminotransferase II (KAT II) is the principal enzyme responsible for the neosynthesis of rapidly mobilizable KYNA and therefore constitutes an attractive target for pro-cognitive interventions (Schwarcz et al., Nat. Rev. Neurosci., 13: 465-4, 2012).

Glycyrrhetinic acid (GA) is a bioactive constituent of the traditional Japanese medicine Yokukansan, which has been shown to alleviate clinical symptoms in people with SZ (Miyoaka et al., Phytopharmacology. 20: 654-8, 2013) and to improve cognitive function in people with senile dementia (Mizukami et al., Int. J. Neuropsychopharmacol., 12: 191-9, 2009).

Based on the discovery that GA selectively inhibits the activity of recombinant human and mouse KAT II (Yoshida et al., Sci Rep., 9: 10243, 2019), we now examined the effect of GA on KAT II activity in crude brain and liver tissue homogenates of mice, rats and humans *in vitro.* We then evaluated the effect of intraperitoneally (i.p.) administered GA (200 mg/kg) on KYNA neosynthesis by *in vivo* microdialysis in the medial prefrontal cortex (mPFC) of adult mice (C57Bl/6J, 2-4 months), both alone or 20 min prior to a challenge with systemically administered kynurenine (50 mg/kg, i.p.).

MATERIALS & METHODS

Animals: Adult male C57BL/6J (2-4 months; Jackson Laboratories, USA) were used for biochemical experiments. Animals were housed in a temperature-controlled, AAALAC-approved animal facility on a 12/12h-light/dark cycle with unlimited access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland School of Medicine and University of Ferrara, Ferrara, Italy.

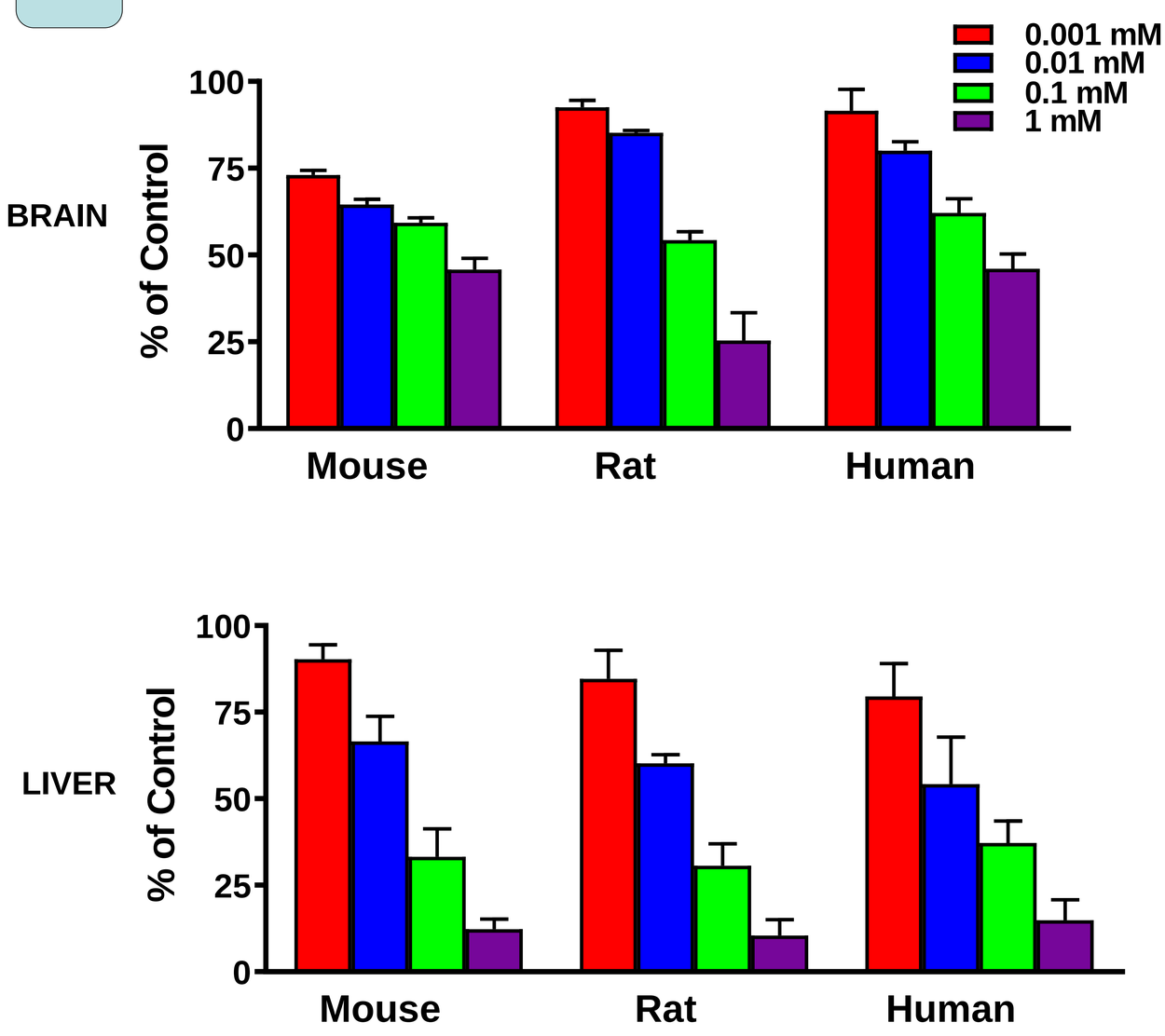
KAT II assay: Tissue samples (mouse, rat, and human) were homogenized (brain: 1:5 w/v; liver: 1:10, w/v) by sonication (Branson Ultrasonics Corp., Danbury, CT, USA) in ultrapure water. Tissues were further diluted [1:2 (w/v) for brain, 1:30 (w/v) for liver] as previously described (Giorgini et al., J. Biol.Chem., 288: 36554-66, 2013).To analyze the effect of GA on KAT II activity, 20 µL of GA (final concentrations: 1 µM, 10 µM, 100 µM and 1000 µM) were added to the incubation mixture. Blanks were obtained by adding 20 µL of AOAA (final concentration 1 mM) to the incubation solution. The reaction was terminated by the addition of 20 µL of 50% (w/v) trichloroacetic acid and 1 mL of 0.1 M HCl. In the eluate, KYNA was measured by HPLC with fluorimetric detection (Shibata et al., J. Chromatogr., 430: 376-80, 1988).

Microdialysis: Microdialysis in the mPFC was performed as previously described (Beggiato et al., Front Psychiatry.13: 996406, 2022) to assess extracellular KYNA levels. The coordinates used were AP: 1.8, L: ± 0.4; V: -3.0 below the dura (Paxinos and Franklin, 5th Edition, 2019).This methodology was used to examine the effect of GA on the neosynthesis of KYNA from peripherally administered kynurenine (50 mg/kg, ip) *in vivo*.

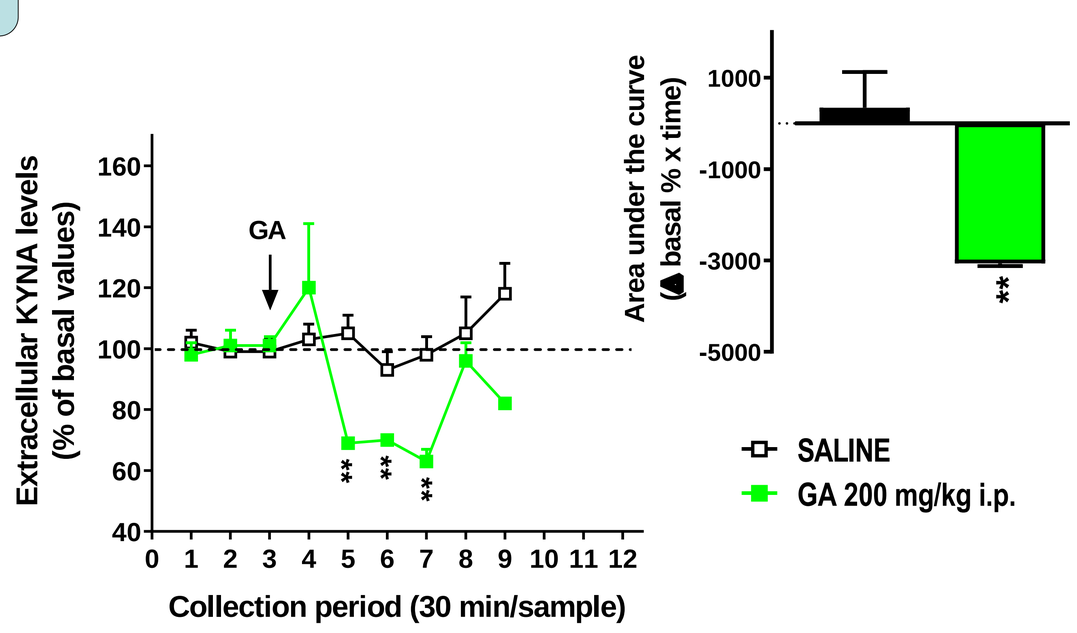
Statistical analysis: Statistical analysis was performed using Two-way ANOVA followed by Bonferroni or student's t-test, when appropriate* p<0.05, ** p<0.01.

RESULTS

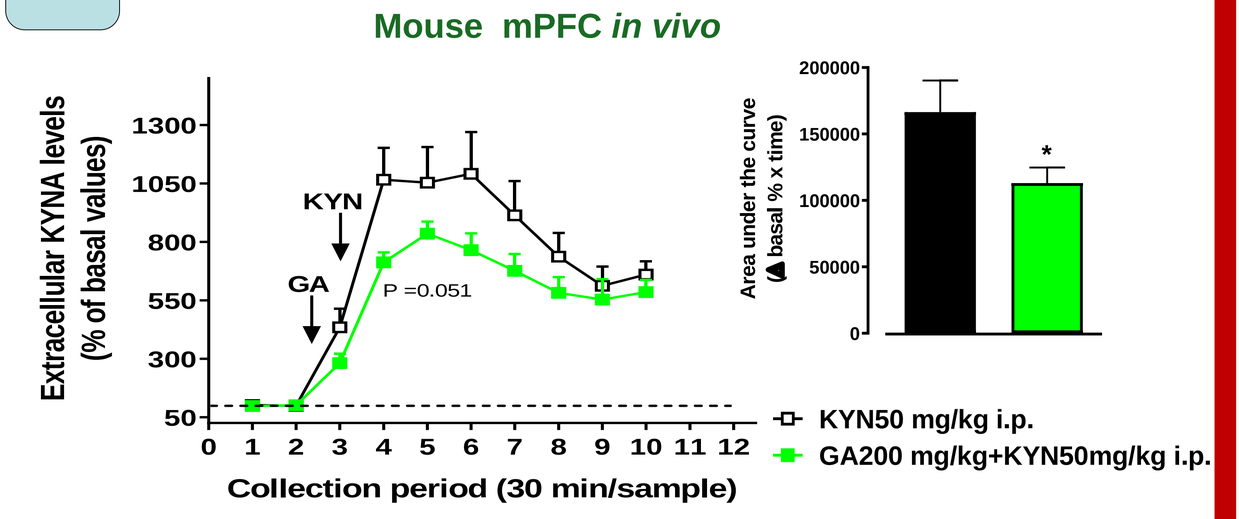
1 GA Inhibits Rodent and Human KAT II *in vitro*



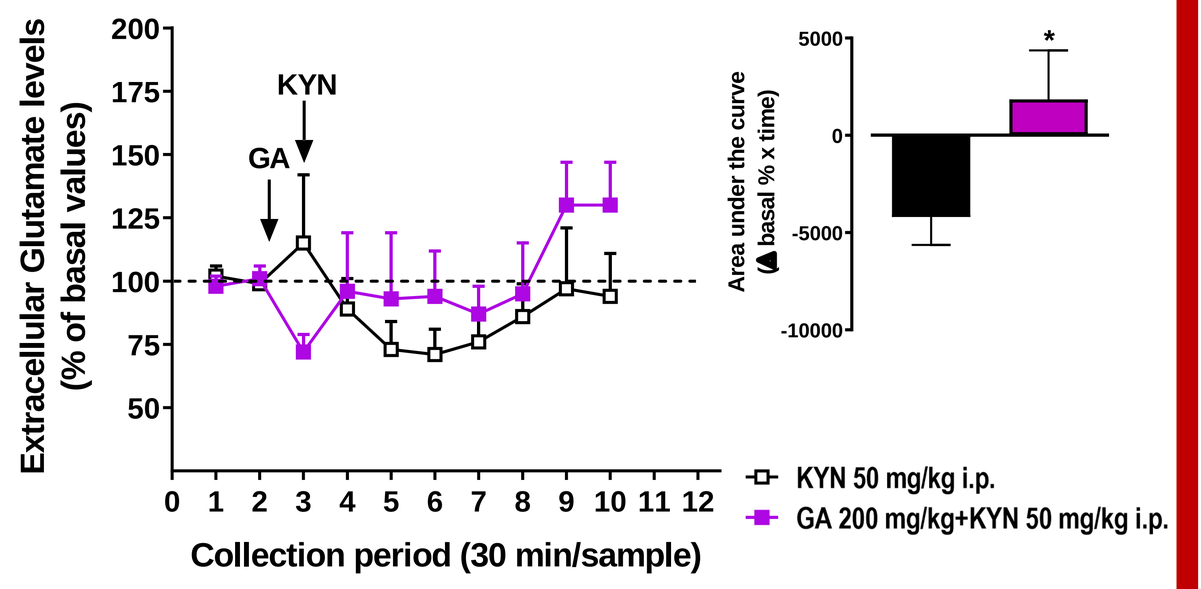
2 Systemic Administration of GA Reduces Endogenous Extracellular KYNA Levels in the Mouse mPFC *in vivo*



3 Systemic Administration of GA Attenuates Kynurenine-induced *de novo* Synthesis of KYNA in the Mouse mPFC *in vivo*



4 Systemic Administration of GA Attenuates the Kynurenine-induced Reduction in Extracellular Glutamate Levels in the Mouse mPFC *in vivo*



SUMMARY & CONCLUSIONS

- In mice, rats, and humans, GA inhibits KAT II activity in brain and liver homogenates *in vitro* with IC_{50} values in the range of ~50 µM to ~500 µM.
- Systemic administration of GA reduces extracellular KYNA levels in the mouse mPFC.
- Systemic administration of GA attenuates the kynurenine-induced reduction in extracellular glutamate levels in the mouse mPFC.
- Taken together, these results indicate that GA may exert its neurobiological effects at least in part by reducing cerebral KYNA levels via KAT II inhibition. GA can therefore be envisioned to be used as a tool to modulate newly produced KYNA in rodents and humans.

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