



# Nicotinic $\alpha 7$ acetylcholine receptor-mediated currents are not modulated by the tryptophan metabolite kynurenic acid in adult hippocampal interneurons

Peter Dobelis<sup>1\*</sup>, Andrew L. Varnell<sup>1</sup>, Kevin J. Staley<sup>2</sup>, & Donald C. Cooper<sup>1</sup>

**The tryptophan metabolite, kynurenic acid (KYNA), is classically known to be an antagonist of ionotropic glutamate receptors. Within the last decade several reports have been published suggesting that KYNA also blocks nicotinic acetylcholine receptors (nAChRs) containing the  $\alpha 7$  subunit ( $\alpha 7^*$ ). Most of these reports involve either indirect measurements of KYNA effects on  $\alpha 7$  nAChR function, or are reports of KYNA effects in complicated in vivo systems. However, a recent report investigating KYNA interactions with  $\alpha 7$  nAChRs failed to detect an interaction using direct measurements of  $\alpha 7$  nAChRs function. Further, it showed that a KYNA blockade of  $\alpha 7$  nAChR stimulated GABA release (an indirect measure of  $\alpha 7$  nAChR function) was not due to KYNA blockade of the  $\alpha 7$  nAChRs. The current study measured the direct effects of KYNA on  $\alpha 7$ -containing nAChRs expressed on interneurons in the hilar and CA1 stratum radiatum regions of the mouse hippocampus and on interneurons in the CA1 region of the rat hippocampus. Here we show that KYNA does not block  $\alpha 7^*$  nAChRs using direct patch-clamp recording of  $\alpha 7$  currents in adult brain slices.**

Kynurenic acid (KYNA) is produced by the metabolism of tryptophan via the kynurenine pathway<sup>1,3</sup>. Classically, KYNA is known for its antagonist actions at ionotropic glutamate receptors, showing the greatest affinity for NMDA-mediated glutamatergic responses<sup>2</sup>. Altered levels of KYNA have been associated with several disease states; increased KYNA levels are seen with Alzheimer's disease, Down's syndrome, and schizophrenia while decreased KYNA levels are associated with end stage Parkinson's and Huntington's disease<sup>3</sup>. Additionally, animal studies indicate that increased brain levels of KYNA are neuroprotective and anti convulsant, while decreased KYNA levels are associated with an increased vulnerability to excitotoxic damage<sup>4</sup>.

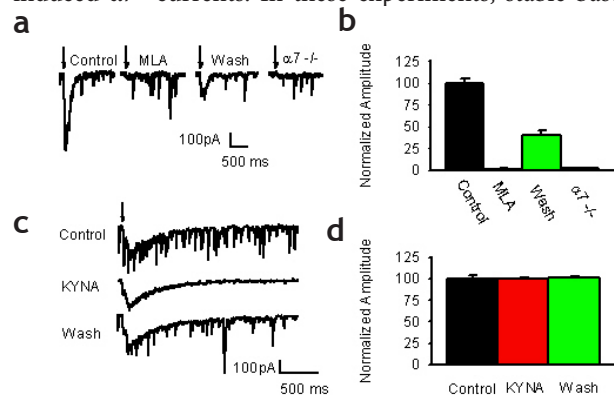
Another action attributed to KYNA is the antagonism of  $\alpha 7$  subunit-containing ( $\alpha 7^*$ ) nicotinic acetylcholine receptors (nAChRs). Several reports from Albuquerque and colleagues present data demonstrating that KYNA also blocks the activation of  $\alpha 7^*$  nAChRs<sup>4</sup>. However, a recent report from Mok and colleagues examining the effects of KYNA on several different ligand gated ion channels revealed that KYNA had no effect on  $\alpha 7^*$  nAChRs<sup>6</sup>. Here we present the results of our investigation of KYNA effects on  $\alpha 7^*$  nAChRs expressed on interneurons in the hilar and CA1 stratum radiatum (SR) regions of the mouse hippocampus, as well as  $\alpha 7^*$  nAChRs expressed on interneurons the rat CA1 SR.

## RESULTS

### *Kynurenic acid effects on $\alpha 7^*$ nAChRs expressed on mouse hilar interneurons*

The results obtained from choline-induced  $\alpha 7^*$  currents in hilar interneurons are shown in Fig 1. We initially examined the prevalence of  $\alpha 7^*$  currents in hilar interneurons. Out of the 23 neurons studied, 20 displayed choline-induced and methyllycaconitine (MLA)

sensitive whole cell currents characteristic of  $\alpha 7^*$  nAChRs. Furthermore, in experiments using  $\alpha 7^*$  null mutant mice, no choline-induced currents were detected (Fig 1a and 1b). Next, we examined the effects of KYNA on the choline-induced  $\alpha 7^*$  currents. In these experiments, stable baseline



**Fig 1: a.** Representative traces for the characterization of  $\alpha 7^*$  currents in mouse hilar interneurons. The left most trace shows the control response to pressure applied choline. The next two traces show the response to choline in the presence of MLA (10 nM) and after 30 min washout, respectively. The last trace shows the lack of response to choline in  $\alpha 7$  null mutant mice. **b.** Methyllycaconitine (MLA) completely blocked the choline response ( $p = 0.0003$  control vs MLA,  $n = 8$ ), the MLA effect was reversed partially after a washout ( $p = 0.035$  washout vs. MLA,  $p = 0.005$  washout vs control,  $n = 8$ ), and the choline response for the  $\alpha 7^*$  null mutant mice differed significantly from wild type ( $p = 0.0003$ ,  $n = 8$ ). **c.** Representative traces for the effect of the bath applied KYNA on choline-evoked  $\alpha 7^*$  currents. The top trace shows the control response to pressure applied choline; note the overriding glutamatergic spontaneous EPSCs. The middle trace shows the choline response after a 30 min exposure to 1 mM KYNA; note the absence of the spontaneous EPSCs. The bottom trace shows the response to choline after a 20 min. washout of KYNA; note the reappearance of the spontaneous EPSCs. **d.** KYNA failed to produce any reduction in the choline response ( $p = 0.97$ ,  $n = 20$ ).

<sup>1</sup>Center for Neuroscience, Institute for Behavioral Genetics, Department of Neuroscience, University of Colorado at Boulder, Boulder Colorado 80303, USA

<sup>2</sup>Department of Neurology, Massachusetts General Hospital, 55 Fruit St, WAC 708-D, Boston, MA 02114, USA

\*Correspondence should be sent to peter.dobelis@colorado.edu

$\alpha 7^*$  currents were obtained, followed by bath application of KYNA (1 mM) for 30 min. Choline-evoked  $\alpha 7^*$  currents were then measured after a 20 min washout of KYNA (Fig 1c). The top trace shows a choline-evoked  $\alpha 7^*$  response with overriding spontaneous glutamatergic-mediated EPSCs. The middle trace shows the choline-evoked  $\alpha 7^*$  response after a 30 min bath application of 1 mM KYNA. The absence of the EPSCs indicates the presence of KYNA. The bottom trace shows the choline-evoked response after a 20 min washout of KYNA. Spontaneous EPSCs returned, indicating the removal of KYNA from the slice (Fig 1d). Out of the 20 hilar interneurons, KYNA failed to have any significant effect on the choline-evoked  $\alpha 7^*$ -mediated currents.

### KYNA effects on $\alpha 7^*$ nAChRs expressed on mouse and rat CA1 SR interneurons

To determine whether there are regional and/or species differences in  $\alpha 7^*$  nAChR sensitivity to KYNA, we tested the ability of KYNA to block  $\alpha 7^*$  nAChR currents in interneurons in the CA1 SR region in both mice and rats. The representative traces for choline-evoked  $\alpha 7^*$  currents in the mouse CA1 SR appear similar to the KYNA treated cells, indicating no change (Fig 2a). The top trace shows the control response to pressure applied choline (10 mM) and the bottom trace shows the choline response in the presence of 1 mM KYNA for 30 min. KYNA failed to significantly reduce choline-evoked  $\alpha 7^*$  currents in the five CA1 SR interneurons we tested (Fig 2b). Representative traces from  $\alpha 7^*$  currents after the pressure applied ACh (1 mM) and KYNA exposure in rat CA1 SR interneurons also showed no change (Fig 2c). The top trace shows the control response, the middle trace shows the response in the presence of 1 mM KYNA, and the bottom trace shows the response in the presence of the  $\alpha 7$ -selective antagonist MLA (10 mM). Exposure to a 20 min KYNA bath failed to inhibit  $\alpha 7^*$  currents, while subsequent exposure to MLA produced a greater than 80% blockade of  $\alpha 7^*$  currents (Fig 2d).

## DISCUSSION

These results clearly indicate that KYNA has no effect on  $\alpha 7^*$  nAChRs in the hilar and CA1 regions of adult mouse and rat hippocampal interneurons. These results are consistent with those reported by Mok and colleagues<sup>6</sup>. Others have concluded that KYNA does interact with the  $\alpha 7^*$  nAChRs using indirect measures of  $\alpha 7^*$  function<sup>4</sup>. KYNA has recently been shown to activate the orphan G-protein receptor GPR-35<sup>1</sup>. GPR-35 is coupled to the  $G_{i/o}$  pathway and is expressed throughout the rodent brain<sup>1</sup>. This, in addition to the interactions that KYNA has with other ligand-gated receptors<sup>4</sup> suggests that prior direct  $\alpha 7^*$  mediated physiological effects attributed to KYNA must be made with caution. Further studies are needed to resolve the apparent effects of KYNA on  $\alpha 7^*$  nAChRs. Until then the most direct evidence indicates no role for KYNA on  $\alpha 7^*$  nAChR function.

## METHODS

### Slice preparation and recordings

Hippocampal slices were prepared as described by Proctor and colleagues<sup>7</sup>. Whole-cell recordings were made using a

standard potassium gluconate based internal solution and a standard ACSF solution. KYNA and MLA were both bath applied to the tissue slices. Both choline and acetylcholine were pressure applied to the slices using established protocols. Expanded methods and recordings can be found at

<http://www.neuro-cloud.net/nature-precedings/dobelis/>

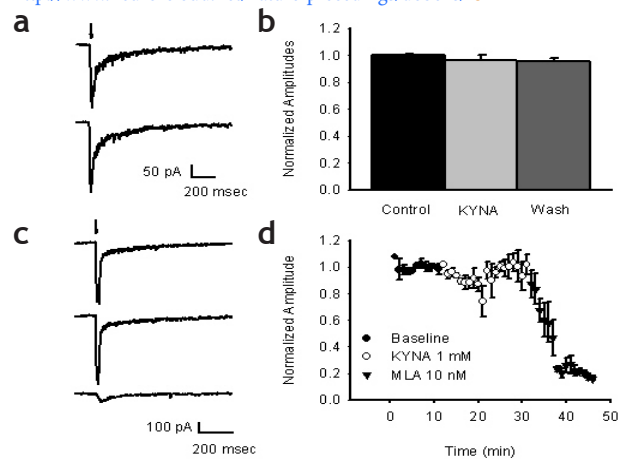


Fig 2. **a.** Representative traces for choline-evoked  $\alpha 7^*$  currents in mouse *stratum radiatum* (SR) interneurons. The top trace is the control response to pressure applied choline (10 mM, 50 ms). The bottom trace shows the choline response after a 30 min. bath exposure to 1 mM KYNA. **b.** KYNA failed to reduce the choline response ( $p = 0.44$ ,  $n = 5$ ). **c.** Representative traces for pressure applied ACh-evoked  $\alpha 7^*$  currents in rat CA1 SR interneurons. The top trace shows the control response to 1 mM ACh. The middle trace shows the ACh response after the 30 min. bath exposure to 1 mM KYNA. The bottom trace shows the ACh response after a 20 min. bath exposure to 10 nM MLA. **d.** The last five traces were averaged for each condition: control, KYNA, and MLA. KYNA failed to block the ACh-induced  $\alpha 7^*$  currents ( $p = 0.71$ ) while MLA significantly blocked the response ( $p = 0.0001$ ,  $n = 5$ ).

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## PROGRESS AND COLLABORATIONS

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## AUTHOR CONTRIBUTIONS

P.D., D.C.C. & K.J.S. designed the experiments. P.D. recorded and analyzed the data. P.D., A.L.V. & D.C.C. wrote and prepared the manuscript.

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