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Kynurenic acid has a dual action on AMPA receptor responses

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Abstract

Glutamate is the predominant excitatory neurotransmitter in the central nervous system. The receptors that bind glutamate, including *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subtypes, are strongly implicated in higher cognitive processes, especially learning and memory. Loss of glutamate receptor function impairs the ability to acquire and retain information in some patients subsequent to stroke or brain injury, and positive allosteric modulators of glutamate receptors can restore learning and memory in some of these patients. Here we demonstrate that kynurenic acid (KYNA), an endogenous tryptophan metabolite, acts upon heterologous AMPA receptors via two distinct mechanisms. Low (nanomolar to micromolar) concentrations of KYNA facilitate AMPA receptor responses, whereas high (millimolar) concentrations of KYNA competitively antagonize glutamate receptors. Low concentrations of KYNA appear to increase current responses through allosteric modulation of desensitization of AMPA receptors. These findings suggest the possibility that low concentrations of endogenous KYNA acting at AMPA receptors may be a positive modulator of excitatory synaptic transmission.
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KYNA is a neuroprotective endogenous tryptophan metabolite produced by astrocytes and neurons via the kynurenine pathway in both humans and rodents [6]. In human CSF, KYNA levels are in the low nanomolar range [4]; however, the KYNA levels that are measured in the CSF may not accurately reflect synaptic concentrations because the majority of neuronal KYNA is produced by astrocytes, many of which are directly associated with excitatory synapses and are capable of producing micromolar concentrations of KYNA [7,13]. KYNA levels are decreased in the frontal cortex in absence epilepsy, infantile spasms, and in the caudate nucleus in Huntington's disease, while KYNA levels are increased in Alzheimer's disease, Down's syndrome, Gilles de la Tourette syndrome, schizophrenia, some bacterial and viral infections, and old age [22,12]. Increasing evidence suggests that KYNA is an endogenous neuroprotective agent able to prevent neuronal loss following excitotoxic, ischemia-induced, and infectious neuronal injuries [20,15]. Specifically, KYNA's ability to act as a neuroprotectant in multiple epilepsy models has been well documented [3,27,19,25,16,9,26].

The molecular mechanisms of action of KYNA in the CNS have been studied with mixed results. At non-physiologic (millimolar) concentrations, KYNA has been reported to act somewhat non-selectively at several different receptor types, but at lower (low micromolar) concentrations, KYNA acts as a competitive antagonist at the glycine-binding site of the NMDA receptor and as a non-competitive antagonist at the α-7 nicotinic receptor [8]. Therefore, a significant component of KYNA's neuroprotective ability arises from its actions as an antagonist of glutamate receptors in brain regions with neuronal damage and concomitant NMDA receptor hyperexcitability. Rationally-designed structural derivatives of KYNA that are more selective for the glycine-binding site of the NMDA receptor are successfully making their way into clinical use [2]. In addition, KYNA is a lower-affinity competitive antagonist of AMPA receptors, competing for binding at the glutamate-binding pocket in the extracellular ligand-binding domain.

In the course of performing concentration-response experiments using very low concentrations of KYNA to reduce glutamatergic activation of AMPA receptors, we made an unexpected observation that suggested to us in some cases KYNA might facilitate AMPA synaptic transmission. We hypothesized, based on these data that KYNA might act both as a competitive antagonist and a positive allosteric modulator of AMPA receptor

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activity. Positive allosteric modulators of AMPA receptors, such as cyclothiazide or aniracetam, modulate receptor desensitization and thereby increase AMPA-mediated current responses. Co-crystallization of the ligand-binding core of GluR2 with cyclothiazide [23] or aniracetam [11] identified a positive modulatory binding site at the interface between two adjacent subunit dimers. KYNA has never been reported to interact with this site, however. The goal of the present work was to determine if low concentrations of KYNA facilitate responses of cloned, heterologously expressed rat AMPA receptors, and if so to explore the molecular mechanism of the facilitation.

Adult (4–6 weeks old) male Sprague–Dawley rats were anesthetized with 100 mg/kg pentobarbital and decapitated. The brain was rapidly dissected out and placed into ice-cold high sucrose solution containing (in mM) 0.5 $CaCl_2$, 7 $MgCl_2$, 2.5 KCl, 25 $NaHCO_3$, 87 NaCl, 1.25 NaH_2PO_4, 25 glucose, and 75 sucrose. Then, 400 μm-thick coronal hemibrain slices were cut using a Leica VT-1000E vibratome (Leica, Nussloch, Germany) and kept for at least 90 min in an interface chamber containing 50% high sucrose solution and 50% ACSF, containing (in mM) 2 $CaCl_2$, 2 $MgCl_2$, 2.5 KCl, 26 $NaHCO_3$, 126 NaCl, 1.25 NaH_2PO_4, and 10 glucose (pH 7.4). Slices were then transferred to a recording chamber, where they were bathed in a modified ACSF solution containing (in mM) 1.3 $CaCl_2$, 0.9 $MgCl_2$, 3.3 KCl, 26 $NaHCO_3$, 126 NaCl, 1.25 NaH_2PO_4, and 10 glucose (pH 7.4). All solutions were saturated with 95% O_2/5% CO_2 and both interface and recording chambers were maintained at 35 °C. Experiments were approved by the University of Colorado Health Science Center Institutional Animal Care and Use Committee.

Field excitatory postsynaptic potentials (fEPSPs) were recorded using glass electrodes (resistance 5–6 MΩ) filled with 150 mM NaCl placed in the stratum radiatum of the CA1 region or the CA3 pyramidal cell layer. A bipolar platinum–iridium stimulating electrode was placed approximately 0.5 mm lateral to the recording electrode in the CA1 or 0.5 mm medial to the recording electrode in the CA3. The strength of the electrical stimulus was modified to obtain the maximal fEPSP amplitude without evoking a population spike. Each individual stimulus was 20 μs in duration and the interstimulus interval was 10 s. Recordings were performed with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) and digitized at 5 kHz using a PCI-DAS 1602/16 (Computer Boards, Middleboro, MA). The change in response was determined as the average fEPSP slope or amplitude during drug application (20 min) normalized to the average baseline (20 min) fEPSP slope or amplitude, respectively.

Recordings were collected using programs written in Visual Basic 6.0 by Dr. K.J. Staley. All data are presented as the mean of the normalized responses, with standard errors shown.

Wild type and mutant GluR2 cDNAs served as a template for in vitro transcription using T7 polymerase, and oocytes were prepared from anesthetized *Xenopus laevis* as previously described [14]. Forty-eight hours after injection, responses to varying concentrations of glutamate, with or without KYNA, were recorded. An electronic valve control system and electronic valves were used to exchange solutions. A Macintosh G4 computer with

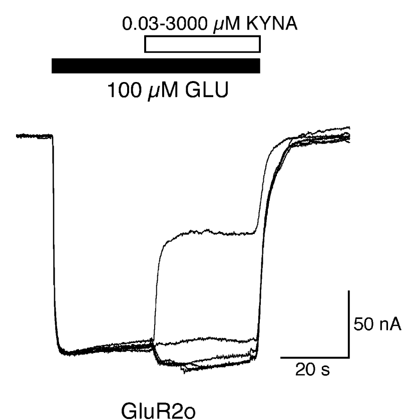

Fig. 1. KYNA has a dual effect on GluR2 glutamate-evoked currents. Overlay of six responses measured with two-electrode voltage clamp (TEVC) from an oocyte expressing GluR2o. After a 30 s exposure 100 μM glutamate, each oocyte was exposed to 30 s of a solution containing 100 μM glutamate and increasing concentrations of KYNA (0.03, 0.3, 3.0, 30, 300 and 3000 μM). Responses are normalized to the glutamate current immediately preceding application of KYNA, to negate the effects of receptor rundown over the 15–20 min needed to perform the entire experiment. $N = 6–21$ oocytes. Lower concentrations resulted in a modest potentiation of the glutamate-evoked currents, whereas concentrations of 300 and 3000 μM inhibited these currents.

an A/D interface running the program Synapse (Synergistic Research Systems, Silver Springs, MD) was used to acquire the current response. Oocytes were perfused with a solution of 100 μM glutamate for 30 s, followed by 30 s with 100 μM glutamate plus different concentrations of KYNA. Oocyte responses were analyzed by measuring the average amplitude of the control response and the average amplitude of the response in the presence of KYNA. Potentiation was measured as $I_{Glu+KYNA}/I_{Glu}$. Differences were measured using an ANOVA test.

All salts and drugs were obtained from Sigma (St. Louis, MO). Drugs were bath applied, unless otherwise stated.

We used a heterologous expression system in order to test the hypothesis that low concentrations of KYNA have positive allosteric effects on isolated AMPA receptors. We first examined the effects of KYNA on recombinant rat GluR2 responses. We found that concentration-dependent effects of KYNA were evident in the glutamate response of GluR2 receptors heterologously expressed in *Xenopus* oocytes, as measured by two-electode voltage clamp recording (TEVC) (Figs. 1 and 3). Low concentrations (0.03–30 μM) of KYNA potentiated steady-state responses to 100 μM glutamate of GluR2o by up to 110 ± 3% (3 μM KYNA), whereas high concentrations of KYNA inhibited responses to 40 ± 4% (3000 μM). Application of KYNA in the absence of agonist did not alter the holding current of the oocyte membrane. There was no significant difference in the ability of KYNA to potentiate the flop versus flip isoform (data not shown).

We reasoned that KYNA might be modulating receptor desensitization, as do other positive allosteric modulators. To determine if KYNA modulates desensitization, we tested a "non-desensitizing" GluR2o receptor mutant, $L_{483}Y$ [21]. Consistent with this hypothesis, KYNA was not able to potentiate responses of GluR2o $L_{483}Y$ (Figs. 2 and 3), but was still able to inhibit responses at the higher concentrations. We also tested a GluR2o

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