Age Dependency of Inhibition of $\alpha 7$ Nicotinic Receptors and Tonically Active *N*-Methyl-D-aspartate Receptors by Endogenously Produced Kynurenic Acid in the Brain

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ABSTRACT

In the mouse hippocampus normal levels of kynurenic acid (KYNA), a neuroactive metabolite synthesized in astrocytes primarily by kynurenine aminotransferase II (KAT II)-catalyzed transamination of L-kynurenine, maintain a degree of tonic inhibition of $\alpha 7$ nicotinic acetylcholine receptors (nAChRs). The present *in vitro* study was designed to test the hypothesis that $\alpha 7$ nAChR activity decreases when endogenous production of KYNA increases. Incubation (2–7 h) of rat hippocampal slices with kynurenine (200 μ M) resulted in continuous *de novo* synthesis of KYNA. Kynurenine conversion to KYNA was significantly decreased by the KAT II inhibitor (S)-(–)-9-(4-aminopiperazine-1-yl)-8-fluoro-3-methyl-6-oxo-2,3,5,6-tetrahydro-4*H*-1-oxa-3a-azaphenylene-5-carboxylic acid (BFF122) (100 μ M) and was more effective in slices from postweaned than preweaned rats. Incubation of slices from postweaned rats with kynurenine inhibited $\alpha 7$ nAChRs and extrasynaptic *N*-methyl-D-aspartate receptors

(NMDARs) on CA1 stratum radiatum interneurons. These effects were attenuated by BFF122 and mimicked by exogenously applied KYNA (200 μ M). Exposure of human cerebral cortical slices to kynurenine also inhibited $\alpha 7$ nAChRs. The $\alpha 7$ nAChR sensitivity to KYNA is age-dependent, because neither endogenously produced nor exogenously applied KYNA inhibited $\alpha 7$ nAChRs in slices from preweaned rats. In these slices, kynurenine-derived KYNA also failed to inhibit extrasynaptic NMDARs, which could, however, be inhibited by exogenously applied KYNA. In slices from preweaned and postweaned rats, glutamatergic synaptic currents were not affected by endogenously produced KYNA, but were inhibited by exogenously applied KYNA. These results suggest that in the mature brain $\alpha 7$ nAChRs and extrasynaptic NMDARs are in close apposition to KYNA release sites and, thereby, readily accessible to inhibition by endogenously produced KYNA.

Introduction

The neuroprotective and neuroinhibitory effects of kynurenic acid (KYNA), a neuroactive metabolite of the kynurenine pathway of tryptophan degradation, have long been attributed to its action as an *N*-methyl-D-aspartate receptor (NMDAR) antagonist (Moroni, 1999). However, studies from numerous laborato-

ries have demonstrated that KYNA also inhibits $\alpha 7$ nicotinic acetylcholine receptors (nAChRs) (Hilmas et al., 2001; Alkondon et al., 2004; Lopes et al., 2007; Stone, 2007; Arnaiz-Cot et al., 2008). Identifying the molecular targets that are accessible to the actions of endogenously produced KYNA in the brain is key to understanding the pathophysiology of a number of catastrophic neurological disorders in which brain levels of KYNA are altered, including Alzheimer's disease, schizophrenia, and Huntington's disease (Beal et al., 1992; Baran et al., 1999; Erhardt et al., 2007).

Cerebral KYNA is formed enzymatically, primarily in astrocytes (Guillemin et al., 2001), by the irreversible transam-

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ABBREVIATIONS: KYNA, kynurenic acid; ACh, acetylcholine; nAChR, nicotinic ACh receptor; ACSF, artificial cerebrospinal fluid; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; APV, 2-amino-5-phosphonopivalic acid; α -BGT, α -bungarotoxin; PSC, postsynaptic current; EPSC, excitatory PSC; KAT, kynurenine aminotransferase; MLA, methyllycaconitine citrate; NMDAR, *N*-methyl-D-aspartate receptor; QX-314, *N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium; SRI, stratum radiatum interneuron; TFA, trifluoroacetate; ANOVA, analysis of variance; LK, L-kynurenine; BFF122, (S)-(–)-9-(4-aminopiperazine-1-yl)-8-fluoro-3-methyl-6-oxo-2,3,5,6-tetrahydro-4*H*-1-oxa-3a-azaphenylene-5-carboxylic acid.

ination of L-kynurenine, a tryptophan metabolite that readily crosses the blood-brain barrier (Fukui et al., 1991). In the rat brain, more than 70% of KYNA synthesis is catalyzed by kynurenine aminotransferase II (KAT II), one of four KATs present in the mammalian brain (Guidetti et al., 1997, 2007; Han et al., 2010). Newly synthesized KYNA is rapidly released into the extracellular milieu (Turski et al., 1989). Because of the absence of reuptake and degradation mechanisms, subsequent KYNA removal in vivo is accomplished by probenecid-sensitive brain efflux (Turski and Schwarcz, 1988).

Distinct experimental strategies have been used to identify and define the roles of endogenous KYNA in brain function and dysfunctions. One approach, pursued in our laboratories, relies on the use of mice with a null mutation in the gene that encodes KAT II [*mKat2*(-/-)] (Alkondon et al., 2004; Yu et al., 2004). These animals, which at early ages present with abnormally low levels of brain KYNA, have increased $\alpha 7$ nAChR activity in CA1 stratum radiatum interneurons (SRIs), but show no changes in glutamatergic synaptic activity (Alkondon et al., 2004). This finding constituted the first evidence that normal KYNA levels maintain a degree of tonic inhibition of $\alpha 7$ nAChR activity but are not sufficient to modulate the activity of synaptic NMDA receptors. Age-dependent adaptations resulting from germ-line elimination of KAT II (Alkondon et al., 2004; Yu et al., 2004), however, limit the usefulness of *mKat2*(-/-) mice for the study of KYNA neurobiology in the mature brain. Another approach relies on the use of pharmacological tools such as kynurenine and KAT II inhibitors to increase and decrease, respectively, KYNA production in the brain (Turski et al., 1989; Swartz et al., 1990; Wu et al., 2010). The pharmacological approach has a number of advantages. First, it circumvents potential confounds caused by chronic reductions of brain KYNA starting in utero in the *mKat2*(-/-) mice. Second, it can be used to address the impact of both decreasing and increasing brain levels of KYNA on neuronal functions. Third, it allows for acute and chronic manipulations of KYNA production in brain tissue either in vitro or in vivo.

The present study was designed to test the hypothesis that $\alpha 7$ nAChR activity in brain slices decreases as a result of an acute pharmacological intervention that increases KYNA production. To test this hypothesis, kynurenine was used to increase KYNA synthesis in hippocampal slices from preweaned (10–18 days old) and postweaned (23–35 days old) rats, because this age range shows an age-dependent increase in the amplitude of $\alpha 7$ nAChR currents (Alkondon et al., 2007). Experiments were also conducted in temporal lobe slices from two patients undergoing surgery to remedy intractable seizures. Evidence is provided that in the mature brain both $\alpha 7$ nAChRs and tonically active extrasynaptic NMDARs are inhibited by endogenously produced KYNA and that the $\alpha 7$ nAChR sensitivity to inhibition by KYNA is age-dependent.

Materials and Methods

Animals. Timed pregnant rats (Sprague-Dawley, gestation days 16–18) were purchased from Charles River Laboratories Inc. (Wilmington, MA) and housed individually in a temperature- and light-controlled animal care unit. Pregnant dams delivered their pups around gestation day 21. Some male pups were used for electrophys-

iological studies on postnatal days 10 to 11 (P10) or 16 to 18 (P17). Animals were weaned at 21 days of age. Male rats were then housed in groups of three to four per cage and used for experiments on postnatal days 23 to 25 (P24) or 29 to 35 (P30). Animals were handled according to the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care, in compliance with the standards of the Animal Welfare Act and in adherence to the principles of the 1996 National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Preparation of Rat Hippocampal Slices. Rats were euthanized by asphyxiation in a CO₂ atmosphere followed by decapitation using a guillotine. To reduce cell swelling, removal of the brains as well as dissection and slicing of the hippocampi were performed in an ice-cold solution consisting of a mixture of equal parts of regular artificial cerebrospinal fluid (ACSF) and sucrose-containing ACSF. Regular ACSF was composed of 125 mM NaCl, 26 mM NaHCO₃, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 2 mM CaCl₂, 1 mM MgCl₂, and 25 mM glucose. Sucrose-containing ACSF was composed of 230 mM sucrose, 2.5 mM KCl, 1.25 mM NaH₂PO₄, 26 mM NaHCO₃, 0.5 mM CaCl₂, 10 mM MgSO₄, and 10 mM glucose. The use of high glucose (25 mM) in the ACSF that supported hippocampal slice function in vitro (Tian and Baker, 2000; Youssef et al., 2009) did not interfere with the KYNA synthesis (see Fig. 1) or the outcome of the results. Hippocampal slices of 300- μ m thickness were cut using the vibratome Leica VT1000S (Leica Microsystems Inc., Bannockburn, IL) and transferred to an immersion chamber containing regular ACSF that was continuously bubbled with 95% O₂/5% CO₂. After 30-min incubation in regular ACSF, 12 hippocampal slices were transferred to a 50-ml chamber containing ACSF with test compounds that was continuously bubbled with 95% O₂/5% CO₂. The chambers were maintained in a water bath at 30°C. Time of incubation of the slices with the test compounds [kynurenine, (S)-(-)-9-(4-aminopiperazine-1-yl)-8-fluoro-3-methyl-6-oxo-2,3,5,6-tetrahydro-4H-1-oxa-3a-azaphenanthrene-5-carboxylic acid (BFF122), and KYNA] ranged from 2 to 7 h.

Preparation of Human Cerebral Cortical Slices. A specimen (7–8 mm wide) of human lateral neocortex was obtained from the temporal cortical lobes of a 25-year-old female and a 38-year-old

Fig. 1. Kynurenine increases the production of KYNA in rat hippocampal slices in vitro. Seven hippocampal slices were incubated in 3 ml of ACSF containing either LK (200 μ M) alone or the admixture of LK (200 μ M) with BFF122 (100 μ M). Concentrations of KYNA were measured in ACSF sampled at different times after the start of the incubation and expressed as femtomole of KYNA per milligram of protein. The total protein content of hippocampal slices was determined using the BCA protein assay. Data points and error bars represent mean and S.E.M., respectively, obtained from seven experiments using kynurenine-incubated slices from three P30 rats, 13 experiments using kynurenine-incubated slices from three P10 rats, and five experiments using kynurenine-plus-BFF122-incubated slices from two P30 rats. *, $p < 0.01$; **, $p < 0.001$; ***, $p < 0.0001$, all compared with kynurenine P30 rats by one-way ANOVA followed by Bonferroni comparison.

male undergoing surgery as treatment for intractable seizures. A combination of depth and surface recordings from implanted electrodes indicated that in these patients the focus of the seizures was in the temporal lobe. The tissue used here had neither histopathological abnormalities nor abnormal electrical activity and most likely did not include the epileptic focus. However, regardless of the location of the epileptic focus, there is still a remote possibility that the neuronal circuitry of the otherwise apparently normal neocortical tissue was altered in some subtle way because of the occurrence of persistent seizures. Consent was obtained from the patients regarding the use of the surgical brain sample. Within 2 min after removal from the brain, the tissue sample was placed in well oxygenated, ice-cold ACSF. Within 30 min after removal of the sample, 300- μ m-thick slices were cut using the vibratome according to the procedure described above for rat hippocampal slices. Slices were cut tangentially to the outer surface of the cortical specimen and, therefore, corresponded very closely to coronal or sagittal sections of the whole brain.

KYNA Neosynthesis in Rat Hippocampal Slices. Sets of seven hippocampal slices from rats of different ages (P10 or P30) were placed in immersion chambers containing 3 ml of ACSF that was bubbled with 95% O₂/5% CO₂ and maintained at 30°C in a water bath. Three sets of slices were incubated in ACSF containing no test compound. Three other sets were maintained in kynurenine (200 μ M)-containing ACSF, and three additional sets were kept in ACSF containing an admixture of kynurenine (200 μ M) + BFF122 (100 μ M). Immediately after the start of the incubation and various times thereafter, 50- μ l aliquots of ACSF were removed to determine KYNA concentrations in the extracellular milieu. Concentrations of KYNA were measured by high-performance liquid chromatography with fluorimetric detection as described previously (Hilmas et al., 2001). Appropriate volume corrections were made in the calculations. To correct for variations in tissue volume at different ages, final concentrations of KYNA were expressed as femtomole/milligram of protein of hippocampal tissue. The Pierce bicinchoninic acid protein assay (Thermo Fisher Scientific, Waltham, MA) was used to determine total protein content of hippocampal slices.

Electrophysiological Recordings. Whole-cell recordings were obtained from the soma of SRIs in rat hippocampal slices or the soma of neurons in human cortical slices according to the standard patch-clamp technique using an EPC9 amplifier (HEKA, Lambrecht, Germany). After incubation (2–7 h) in a 50-ml immersion chamber with ACSF containing no test compound (control), kynurenine (200 μ M), BFF122 (100 μ M), kynurenine (200 μ M) + BFF122 (100 μ M), or KYNA (2–200 μ M), slices were transferred to a 1-ml recording chamber where they were continuously superfused at 2 ml/min at room temperature. The test compounds and their respective concentrations present in the ACSF during the incubation in the immersion chamber were the same as those present in the ACSF used to superfuse the slices. Each slice was kept in the recording chamber for no longer than 30 min. On average, recordings were obtained from two neurons per rat.

In all experiments, ACSF used to superfuse the slices contained the muscarinic antagonist atropine (0.5 μ M). At the concentration used, atropine had no significant effect on α 7 nAChR currents. In addition, there is evidence that atropine up to 1 μ M does not interfere with the interactions between KYNA and α 7 nAChRs (Lopes et al., 2007). In experiments that were not aimed at recording GABAergic postsynaptic currents (PSCs) ACSF also contained the GABA_A receptor antagonist bicuculline (final concentration: 10 μ M). Receptor agonists such as choline, acetylcholine (ACh), and NMDA were applied to individual neurons via a U-tube, whereas antagonists and other test compounds were applied via bath superfusion.

Signals were filtered at 3 kHz and either recorded on a videotape recorder for later analysis or directly sampled by a microcomputer using PULSE software (ALA Scientific Instruments, Inc., Westbury, NY). Patch pipettes were pulled from a borosilicate glass capillary (1.2 mm o.d.) that, when filled with internal solution, had resistances between 3 and 5 M Ω . The internal pipette solution contained 0.5%

biocytin in addition to 10 mM ethylene-glycol bis (β -amino-ethyl ether)-*N,N'*-tetraacetic acid, 10 mM HEPES, 130 mM Cs-methane sulfonate, 10 mM CsCl, 2 mM MgCl₂, and 5 mM *N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide (pH adjusted to 7.3 with CsOH; 340 mOsm). Membrane potentials were corrected for liquid junction potential. All recordings were carried out at room temperature (20–22°C).

Data Analysis. Peak amplitude and net charge of agonist-evoked currents were analyzed using pCLAMP9 software (Molecular Devices, Sunnyvale, CA). The frequency, peak amplitude, 10 to 90% rise time, and decay-time constants of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) excitatory PSCs (EPSCs) and GABAergic PSCs were analyzed using WinEDR V2.3 (Strathclyde Electrophysiology Software, Glasgow, Scotland). Baseline current standard deviation, which is a measure of tonic NMDA currents (Sah et al., 1989; Alkondon et al., 2003), was also analyzed using WinEDR based on 60-s segments of recordings obtained at –60 mV and sampled at 10 kHz. Any synaptic events present in the segments were manually removed before analysis. Results are presented as means $\pm$ S.E.M. Statistical significance was tested with StatsDirect software v 2.7.7 (StatsDirect Ltd., Cheshire, UK) using one-way ANOVA followed by an appropriate post hoc test or a paired or unpaired *t* test.

Test Compounds Used. (–)Bicuculline methochloride was purchased from Tocris Bioscience (Ellisville, MO). Acetylcholine chloride, atropine sulfate, choline chloride, L-kynurenine sulfate or free base (kynurenine), KYNA, *N*-(2,6-dimethylphenylcarbamoylmethyl)triethylammonium bromide (QX-314), trifluoroacetate (TFA), and 2-amino-5-phosphonovaleric acid (APV) were purchased from Sigma-Aldrich (St. Louis, MO). 6-Cyano-7-nitroquinoxaline-2,3-dione was purchased from Sigma/RBI (Natick, MA). α -Bungarotoxin (α -BGT) was purchased from Biotoxins Inc. (St. Cloud, FL). Methyllycaconitine citrate (MLA) was a gift from Professor M. H. Benn (University of Calgary, Alberta, Canada). BFF122 TFA salt was synthesized in the laboratories of Mitsubishi-Tanabe (Yokohama, Japan). Kynurenine-containing ACSF was always prepared on the day of the experiments. A stock solution of 1 M choline chloride was prepared in 150 mM NaCl solution and kept frozen. The working solution of 10 mM choline was prepared by diluting the stock solution in HEPES-buffered ACSF. A stock solution of 500 mM KYNA was made in 1 M NaOH and subsequently serially diluted in regular ACSF as needed. The pH of KYNA-containing and all other ACSF solutions, measured within 2 to 3 min after bubbling with 95% O₂/5% CO₂, was 7.38. Stock solutions of all other compounds were made in distilled water, kept frozen, and subsequently diluted in ACSF.

Results

Kynurenine Stimulates De Novo Synthesis of KYNA in Rat Hippocampal Slices. In accordance with previous studies (Turski et al., 1989; Gramsbergen et al., 1997), incubation of hippocampal slices from postweaned (P30) rats with 200 μ M kynurenine resulted in a time-dependent accumulation of KYNA in the extracellular milieu, reaching approximately 5.8 pmol/mg protein at 6 h (Fig. 1). The slope of the linear regression of KYNA concentration versus incubation time revealed that ACSF levels of the metabolite increased at a rate of 16 fmol/mg protein/min. This translated to a concentration increase of 4.8 nM/min in the 3-ml incubation chamber. Based on this finding, ACSF concentrations of KYNA at 2 and 6 h after the beginning of incubation in the 50-ml chamber were estimated to be 50 and 200 nM, respectively. Incubation of the slices with kynurenine (200 μ M) in the presence of the KAT II inhibitor BFF122 (100 μ M) significantly attenuated the synthesis of KYNA (Fig. 1).

Conversion of kynurenine (200 μ M) to KYNA was significantly less effective in hippocampal slices from preweaned

(P10) than postweaned (P30) rats. During incubation of hippocampal slices from P10 rats with 200 μ M kynurenine in the 3-ml chamber, ACSF levels of KYNA increased at a rate of 4 fmol/mg protein/min (Fig. 1) or 1.2 nM/min. This translated to ACSF KYNA concentrations of approximately 18 and 52 nM at 2 and 6 h, respectively, after the beginning of incubation of the slices in the 50-ml immersion chamber with kynurenine-containing ACSF. The relatively moderate de novo synthesis of KYNA in the developing rat brain was also noted in an earlier study (Gramsbergen et al., 1997) and is probably caused by low hippocampal KAT activity during the early postnatal period (Baran and Schwarcz, 1993).

Age-Dependent Effects of Kynurenine Incubation on $\alpha 7$ nAChR Currents in Rat Hippocampal Slices. In the presence of the GABA_A receptor antagonist bicuculline (10 μ M), rapidly decaying whole-cell currents were recorded in response to U-tube application of choline (10 mM) to CA1 SRIs voltage-clamped at -60 mV (Fig. 2A). As in previous studies of rat hippocampal slices (Albuquerque et al., 2009 and references therein), these currents were blocked by the $\alpha 7$ nAChR antagonists MLA (3–10 nM) and α -BGT (100 nM). The peak amplitude of choline-evoked currents (hereafter referred to as type IA currents) increased significantly with the age of the rats, being more than 2-fold larger in slices from postweaned (P30) than preweaned (P10) rats (Fig. 2, A and B).

Incubation of slices from preweaned rats with kynurenine (200 μ M) had no significant effect on the amplitudes of type IA currents recorded from SRIs. In contrast, in slices from postweaned rats incubation with kynurenine for 2 to 7 h significantly decreased the amplitude of choline-evoked type IA currents recorded from SRIs (Fig. 2B). Similar age-dependent results were obtained when the net charge of choline-evoked currents was analyzed. In slices of preweaned rats the net charge of choline-induced currents recorded under control conditions (289 ± 88 pC; $n = 16$ neurons) was not significantly different from that recorded in the presence of kynurenine (401 ± 82 pC; $n = 14$ neurons). On the other hand, in slices of postweaned rats the net charge of choline-induced currents decreased significantly from 795 ± 55 pC ($n = 88$ neurons) under control conditions to 354 ± 61 pC ($n = 30$ neurons) in the presence of kynurenine ($p < 0.0001$ according to ANOVA followed by Bonferroni comparison).

Brief superfusion (10 min) of the slices from postweaned rats with kynurenine (200 μ M) had no significant effect on the magnitude of $\alpha 7$ nAChR responses. The peak amplitudes of type IA currents recorded at 10 min after the beginning of superfusion with kynurenine-containing or kynurenine-free ACSF were $89.9 \pm 3.9\%$ ($n = 6$ neurons) and $95.5 \pm 1.4\%$ ($n = 9$ neurons) of the current amplitudes recorded before the start of superfusion; the difference between the two groups was not significant ($p = 0.134$ by unpaired Student's t test). Therefore, $\alpha 7$ nAChR inhibition in slices from postweaned rats could have resulted from kynurenine-derived KYNA.

Although KYNA levels in the ACSF surrounding slices from postweaned rats increased significantly between 2 and 6 h of incubation with kynurenine, the magnitude of reduction of $\alpha 7$ nAChR responses did not change significantly during the same time (Fig. 2C). This finding suggests that the concentration of KYNA at its release sites remained constant, but continuous diffusion into the surrounding ACSF resulted in a linear buildup of KYNA levels in the ACSF.

Fig. 2. Effects of kynurenine on $\alpha 7$ nAChR currents in rat hippocampal slices. **A**, sample recordings of whole-cell inward currents evoked by application of 10 mM choline to CA1 SRIs voltage-clamped at -60 mV. The agonist was applied via a U-tube for 12 s (indicated by bars at top of traces). Samples are representative of many such recordings in the two groups of slices at four age groups. Control represents slices incubated in LK-free ACSF. LK200 represents slices incubated in ACSF containing 200 μ M LK. Incubations lasted 2 to 7 h. In addition, 200 μ M LK was continuously present in the ACSF during the entire recording session in the LK group. Calibration in P30 applies to all traces. **B**, graph represents the peak amplitude of choline-evoked inward currents recorded from neurons in slices incubated in LK-free and LK-containing ACSF. The preweaned group consisted of animals from P10 and P18, and the postweaned group consisted of animals from P23 and P35. Graph and error bars represent mean and S.E.M., respectively, of results obtained from the numbers of neurons presented in parentheses. **, $p < 0.0001$, compared with preweaned controls; ***, $p < 0.0001$, compared with postweaned controls by one-way ANOVA followed by Bonferroni comparison. **C**, plot of the peak amplitude of choline-induced currents versus LK incubation time. Each symbol represents a neuron. The solid line represents the linear regression of the data points.

Age-Dependent Effects of Incubation with Kynurenine on GABAergic PSCs Evoked by $\alpha 7$ nAChR Activation in Rat Hippocampal Slices. In the absence of bicuculline, bursts of outward postsynaptic currents were recorded in

response to U-tube application of choline (10 mM, 12-s pulse) from CA1 SRIs voltage clamped at 0 mV in control and kynurenine-exposed slices (Fig. 3A). The GABAergic nature of these currents was confirmed by their blockade with bicuculline (10 μ M). EPSCs were absent at 0 mV, the reversal potential for cationic currents.

As in previous studies (Alkondon et al., 1999), choline-evoked GABAergic PSCs were sensitive to blockade by 10 μ M bicuculline and the $\alpha 7$ nAChR antagonists MLA (10 nM) and α -BGT (100 nM) and could not be detected in the presence of the Na⁺ channel blocker tetrodotoxin (100 nM). These results are consistent with the concept that choline-evoked GABAergic PSCs recorded from CA1 SRIs result from the activation of $\alpha 7$ nAChRs in interneurons that synapse onto the SRIs studied here.

When hippocampal slices from preweaned (P10) or postweaned (P30) rats were exposed to kynurenine (200 μ M), there was no significant change in the frequency, amplitude, 10 to 90% rise time, or decay-time constant of spontaneous GABAergic PSCs recorded from CA1 SRIs (Table 1). Incubation of slices from P10 rats with kynurenine (200 μ M) also had no significant effect on the net charge of choline-evoked GABAergic PSCs (Fig. 3B). However, incubation of slices from P30 rats with kynurenine

Fig. 3. Effects of kynurenine on $\alpha 7$ nAChR-dependent GABAergic PSCs in rat hippocampal slices. **A**, sample recordings of outward-going GABAergic PSCs obtained from SRIs during U-tube application of choline (10 mM) to slices from preweaned (P10) and postweaned (P30) rats. Holding potential = 0 mV. Control slices were incubated in ACSF, whereas LK slices were incubated in LK (200 μ M)-containing ACSF. Incubations lasted 2 to 7 h. **B**, graph represents the net charge of choline-induced GABAergic PSCs recorded from several neurons in the absence and presence of LK (200 μ M). Data are presented as mean and S.E.M. of results obtained from the number of neurons shown in parentheses. **, $p < 0.02$, compared with P30 controls, by one-way ANOVA followed by Bonferroni post hoc test.

TABLE 1

Characteristics of spontaneous GABAergic PSCs recorded in the presence and absence of kynurenine (200 μ M) from CA1 SRIs in hippocampal slices from preweaned (P10) and postweaned (P30) rats. Recordings were obtained from neurons in slices incubated for 2 to 7 h in kynurenine-free (control) and kynurenine (200 μ M)-containing ACSF. Data are presented as the mean $\pm$ S.E.M. of results obtained from the number of neurons presented in parentheses.

	P10		P30	
	Control (n = 8)	Kynurenine (n = 9)	Control (n = 6)	Kynurenine (n = 10)
Frequency (Hz)	0.69 $\pm$ 0.14	0.92 $\pm$ 0.18	1.42 $\pm$ 0.19	1.43 $\pm$ 0.21
Peak amplitude (pA)	22.7 $\pm$ 1.03	21.2 $\pm$ 1.50	32.6 $\pm$ 2.90	26.9 $\pm$ 1.50
Rise time (10–90%, ms)	2.60 $\pm$ 0.37	2.55 $\pm$ 0.20	1.63 $\pm$ 0.26	2.02 $\pm$ 0.30
Decay-time constant (ms)	33.7 $\pm$ 1.76	33.1 $\pm$ 1.91	23.3 $\pm$ 2.18	24.7 $\pm$ 1.80

significantly decreased the net charge of choline-induced GABAergic PSCs (Fig. 3B). These results are consistent with the age-dependent inhibition of $\alpha 7$ nAChRs by kynurenine-derived KYNA described above.

Effects of Kynurenine on $\alpha 7$ nAChRs in Human Cerebral Cortical Neurons. As described under *Materials and Methods*, cerebral cortical slices were obtained from specimens sampled from two patients who underwent surgery for intractable seizures. Reconstruction of the images of the biocytin-filled neurons studied in these slices confirmed that they were interneurons (Fig. 4A). Electrophysiological data were obtained from three such neurons.

Fig. 4. Kynurenine suppresses $\alpha 7$ nAChR-mediated responses in human cerebral cortical slices. **A**, neuroLucida drawing of a biocytin-filled interneuron in a human lateral cortical slice. **B**, sample recording of a whole-cell current recorded at -60 mV during U-tube application of choline (10 mM) to the interneuron shown in **A**. **C** and **D**, sample recordings of choline (10 mM)-induced GABA PSCs obtained from interneurons voltage-clamped at 0 mV in cortical slices maintained in LK-free ACSF (**C**) or LK (200 μ M)-containing ACSF (**D**). **E** and **F**, sample recordings of ACh (0.1 mM)-induced GABA PSCs obtained from neurons voltage-clamped at 0 mV in cortical slices maintained in LK-free ACSF (**E**) or LK (200 μ M)-containing ACSF (**F**). In **C** to **F**, incubations lasted 3 h.

U-tube application (12-s pulses) of choline (10 mM) to the interneurons voltage-clamped at -60 mV induced inward whole-cell currents that decayed to the baseline despite the presence of the agonist (Fig. 4B). These currents were type IA, because choline is a selective $\alpha 7$ nAChR agonist and choline-induced currents in human cerebral cortical neurons have been shown to be blocked by the $\alpha 7$ nAChR antagonist MLA (Albuquerque et al., 2009).

In the absence of bicuculline, U-tube application of choline (10 mM) to cerebral cortical interneurons voltage-clamped at 0 mV increased the frequency of GABAergic PSCs (Fig. 4C), an effect attributed to the activation of $\alpha 7$ nAChRs on interneurons that synapse onto the neurons under study (Alkondon et al., 2000). The net charge of choline-evoked GABAergic PSCs recorded from a neuron in a kynurenine-treated cortical slice was lower (9.3 versus 29 pC) than that recorded from a neuron in a control slice (Fig. 4, C and D). In contrast, the net charge of ACh-induced GABAergic PSCs, a measure of $\alpha 4\beta 2$ nAChR activation (Alkondon et al., 2000), was not decreased after incubation with kynurenine (Fig. 4, E and F). The limited availability of human brain samples precluded a quantitative analysis of the effects of kynurenine on choline-induced $\alpha 7$ nAChR activation. However, these qualitative findings were in line with the quantitative results obtained from hippocampal slices from postweaned rats.

The KAT II Inhibitor BFF122 Prevents the Effects of Kynurenine on $\alpha 7$ nAChR Currents in CA1 SRIs from Postweaned Rats. Incubation of P30 rat hippocampal slices with the KAT II inhibitor BFF122 (100 μ M) for 2 to 7 h had no significant effect on the peak amplitude of choline (10 mM)-evoked currents recorded from SRIs (Fig. 5A). The amplitude of choline-evoked currents recorded in the presence of the admixture of BFF122 and kynurenine was significantly larger than those recorded in the presence of kynurenine alone (Fig. 5). At the concentration tested, BFF122 did not completely abolish the effect of kynurenine. The amplitudes of choline-evoked currents recorded in the presence of the admixture of BFF122 and kynurenine were still somewhat smaller than those recorded under control conditions (Fig. 5A).

The salt of BFF122, TFA, is a selective inhibitor of the glial tricarboxylic acid cycle and, as such, is gliotoxic (Clarke, 1991). Therefore, the effect of BFF122 could have been confounded by TFA-induced loss of astrocyte-producing KYNA. Incubation (2–7 h) with TFA (100 μ M), however, had no significant effect on the amplitude of type IA currents (Fig. 5A). In addition, type IA currents recorded from SRIs after 2 to 7 h incubation with an admixture of kynurenine and TFA had amplitudes comparable with those recorded from neurons that had been exposed to kynurenine alone (Fig. 5). These findings confirmed that the ability of BFF122 to attenuate the kynurenine-induced inhibition of $\alpha 7$ nAChRs was not caused by a nonspecific gliotoxic effect of TFA.

Externally Applied KYNA Mimics the Actions of Kynurenine on $\alpha 7$ nAChRs in CA1 SRIs of Hippocampal Slices. The results described in the previous sections suggested that kynurenine suppresses $\alpha 7$ nAChR activity via newly synthesized KYNA. However, even an extended incubation of slices with 200 μ M kynurenine yielded no more than approximately 200 nM KYNA in the 50-ml immersion chamber (see above). This estimate reflects dilution of newly formed and released KYNA in the surrounding ACSF so that

Fig. 5. The KAT II inhibitor BFF122 (BFF) attenuates the inhibitory effect of kynurenine on $\alpha 7$ nAChR currents. A, graph shows the peak amplitude of choline (10 mM)-evoked type IA currents recorded under various experimental conditions. Graph and error bars represent mean and S.E.M., respectively, of data obtained from the numbers of neurons shown in parentheses. Hippocampal slices were incubated for 2 to 7 h in control ACSF or ACSF containing different test compounds. All data are from postweaned animals. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ according to one-way ANOVA followed by Bonferroni post hoc test. B, normalized peak amplitude of choline (10 mM)-evoked currents in three of the experimental groups depicted in A. Results obtained in the presence of LK were normalized to those obtained under control conditions. Results obtained in the presence of BFF122 + kynurenine were normalized to those obtained in the presence of BFF122 alone, and results obtained in the presence of TFA + LK were normalized to those obtained in the presence of TFA alone. ***, $p < 0.0001$ compared with LK by one-way ANOVA followed by Bonferroni comparison.

the actual concentration of KYNA could be far higher in the immediate vicinity of the astrocytic release site (see also Scharfman et al., 1999). To obtain further insight into the pharmacologically active concentrations of KYNA at the release sites, we next measured the magnitude of $\alpha 7$ nAChR inhibition by known concentrations of exogenously supplied KYNA.

Hippocampal slices from postweaned (P24) rats were incubated for 2 to 7 h in ACSF containing KYNA (2, 100, or 200 μ M). As illustrated in Fig. 6A, KYNA caused a concentration-dependent suppression of the peak amplitude of choline (10 mM)-evoked currents. KYNA at 2 μ M, a concentration that is nearly 10-fold higher than that measured in the ACSF surrounding slices incubated with 200 μ M kynurenine, had no significant effect on the amplitude of type IA currents (Fig.

Fig. 6. Age and concentration dependencies of KYNA-induced inhibition of $\alpha 7$ nAChR currents in rat hippocampal slices. A, graph of the peak amplitude of choline (10 mM)-evoked inward currents in control slices and slices incubated with KYNA (2–200 μ M) for 2 to 7 h. Slices were obtained from preweaned (P10) and postweaned (P24) rats. B, the peak amplitude of choline (10 mM)-evoked currents recorded in the presence of KYNA or LK was normalized to that recorded in the absence of the two test compounds. The mean peak amplitude recorded from neurons in slices incubated with control ACSF was taken as 100% for each age group and used to normalize the peak amplitude of currents recorded after 2- to 7-h incubation of the slices with KYNA or LK. Graph and error bars represent mean and S.E.M., respectively, of results obtained from the numbers of neurons in parentheses. Results are significantly different from control according to one-way ANOVA followed by Dunnett's post hoc test: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

6A). In contrast, both 100 and 200 μ M KYNA produced significant inhibition of choline-evoked currents (Fig. 6). The degree of $\alpha 7$ nAChR inhibition induced by 200 μ M KYNA was comparable with the 64% inhibition seen in the presence of 200 μ M kynurenine (Fig. 6B).

Incubation of hippocampal slices from preweaned (P10) rats with 200 μ M KYNA had no significant effect on the amplitude of type IA currents (Fig. 6) or on the net charge of GABAergic PSCs triggered by activation of $\alpha 7$ nAChRs. Thus, the net charge of choline-induced GABAergic PSCs recorded in the absence and presence of KYNA (200 μ M) was 269 ± 64 and 365 ± 95 pC, respectively ($n = 5$ neurons in each group from three rats; $p = 0.45$ according to one-way ANOVA). These results demonstrate that $\alpha 7$ nAChRs in im-

mature hippocampal neurons are insensitive to inhibition by KYNA.

Differential Effects of Endogenously Produced and Exogenously Applied KYNA on Spontaneous AMPA EPSCs, Type III Nicotinic Currents, and NMDA Currents Recorded from CA1 SRIs. Spontaneous EPSCs recorded in the presence of bicuculline (10 μ M) from CA1 SRIs voltage-clamped at -60 mV were mediated primarily by AMPA receptors, because they were inhibited by the AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (10 μ M) and could not be blocked by the NMDAR antagonist APV (50 μ M). As illustrated in Fig. 7A, the frequency, amplitude, and kinetics (10–90% rise time and decay-time constant) of spontaneous AMPA EPSCs were not significantly altered after

Fig. 7. Comparison of the effects of kynurenine and exogenously applied KYNA on AMPA EPSCs recorded from CA1 SRIs in hippocampal slices from postweaned rats. Slices were incubated with ACSF alone or in the presence of various test compounds for 2 to 7 h and superfused with the same chemicals during the recordings. A, graphs represent the frequency, peak amplitude, 10 to 90% rise time, and decay-time constant of spontaneously occurring AMPA EPSCs recorded in the absence or in the presence of LK (200 μ M), BFF122 (100 μ M), or LK + BFF122. B, graphs represent the frequency, peak amplitude, 10 to 90% rise time, and decay-time constant of spontaneously occurring AMPA EPSCs recorded in the absence or presence of KYNA (100 or 200 μ M) or APV (50 μ M). Graph and error bars are mean and S.E.M., respectively, of the results obtained from the numbers of neurons in parentheses. *, $p < 0.05$; **, $p < 0.01$ compared with respective control by one-way ANOVA followed by Bonferroni post hoc test.

incubation of slices from postweaned (P30) rats with kynurenine (200 μ M), BFF122 (100 μ M), or a combination of kynurenine (200 μ M) and BFF122 (100 μ M). In sharp contrast, incubation of the slices with KYNA (100 or 200 μ M) caused a concentration-dependent reduction of the frequency and decreased the amplitude of spontaneous AMPA EPSCs (Fig. 7B).

Superfusion of the slices with the NMDAR antagonist APV (50 μ M) had no effect on the frequency and amplitude of AMPA EPSCs. In addition, 10-min bath application of the $\alpha 7$ nAChR antagonist MLA (10 nM) had no effect on the amplitude and decreased the frequency of AMPA EPSCs to only $90.7 \pm 4.9\%$ ($n = 4$) of control, whereas 100 and 200 μ M KYNA decreased the AMPA EPSC frequency to 46.6 and 16.3% of control, respectively (Fig. 7B). These results ruled out the contribution of NMDARs and $\alpha 7$ nAChRs to the effects of exogenously applied KYNA on AMPA EPSCs.

In slices from postweaned rats, incubation with kynurenine (200 μ M) had no significant effect on the magnitude of ACh-induced NMDA EPSCs recorded in the presence of bicuculline from SRIs voltage-clamped at +40 mV (Fig. 8). As reported previously, this ACh-evoked response is a measure of type III ($\alpha 3\beta 4\beta 2$) nAChR activity in glutamatergic neurons that synapse onto CA1 SRIs (Alkondon et al., 2003). The net charge of NMDA-evoked currents recorded at +40 mV from CA1 SRIs in the continuous presence of bicuculline (10 μ M) was also unaffected by incubation with 200 μ M kynurenine (Fig. 8). Similar results were obtained from slices using preweaned rats (Fig. 8). In contrast, 10-min bath application of KYNA (200 μ M) caused significant suppression of both responses in slices from preweaned or postweaned rats (Fig. 8).

The use of nominally Mg^{2+} -free ACSF reveals the existence of a tonic NMDA current in CA1 SRIs (Alkondon et al., 2003). As shown in Fig. 9A, the baseline current noise recorded from CA1 SRIs at -60 mV was larger in the nominal

Fig. 8. Effects of kynurenine and KYNA on NMDA currents and ACh-evoked EPSCs in hippocampal slices from rats at different ages. ACh (0.1 mM)-induced NMDA EPSCs (type III nAChR response) or NMDA (50 μ M)-induced currents were recorded from CA1 SRIs at +40 mV. A 12-s pulse of ACh or a 30-s pulse of NMDA (no added glycine) was used. Hippocampal slices were incubated with ACSF containing no test compound or LK (200 μ M) for 2 to 7 h. In experiments with KYNA, control responses recorded from any given neuron were compared with responses recorded from the same neuron at 10 min after the start of bath application of KYNA (200 μ M). The net charge was calculated for the duration of the pulse. Graph and error bars represent mean and S.E.M., respectively, of results obtained from the numbers of neurons in parentheses. *, $p < 0.05$; **, $p < 0.01$ compared with control by paired t test.

Fig. 9. Kynurenine and KYNA suppress tonic NMDA currents in rat hippocampal slices. A, sample recordings obtained from CA1 SRIs under the whole-cell patch configuration at -60 mV illustrating baseline current fluctuations under various conditions. B, graphs represent the percentage change in the baseline current S.D. recorded from SRIs in the nominal absence of Mg^{2+} under control condition and in the presence of LK (200 μ M) or KYNA (100 or 200 μ M). Baseline current S.D. in nominally Mg^{2+} -free ACSF is expressed as percentage of that in Mg^{2+} -containing ACSF in all groups. Whole-cell currents were recorded from CA1 SRI of hippocampal slices from preweaned (P10) or postweaned (P24) rats. Slices were incubated for 2 to 7 h with ACSF containing LK (200 μ M) or superfused for 10 min with KYNA (100 or 200 μ M)-containing ACSF. Graph and error bars represent mean and S.E.M., respectively, of results obtained from the numbers of neurons in parentheses. *, $p < 0.05$; ***, $p < 0.001$ compared with control, by one-way ANOVA followed by Bonferroni comparison.

absence than in the presence of Mg^{2+} (Fig. 9A). This is caused by the release of the Mg^{2+} block of extrasynaptic NMDARs (Sah et al., 1989). As reported previously (Alkondon et al., 2003), tonic NMDAR activity recorded from CA1 SRIs was decreased by the NMDAR antagonist APV (50 μ M) and unaffected by the $\alpha 7$ nAChR antagonists MLA (10 nM) and α -BGT (100 nM).

In all subsequent experiments, the baseline current noise was measured in CA1 SRIs of kynurenine-incubated or KYNA-exposed slices during a 5-min superfusion with nominally Mg^{2+} -free ACSF followed by a 5-min superfusion with Mg^{2+} -containing ACSF. It should be noted that synaptic NMDAR activity also increased with the use of nominally Mg^{2+} -free ACSF. However, synaptic events present in the segments were manually removed before analysis of baseline noise. The ratio of the baseline current amplitudes recorded

in the presence and absence of Mg^{2+} provided a measure of the magnitude of the Mg^{2+} -sensitive tonic NMDAR activity that contributes to the baseline current under the different experimental conditions. In slices from postweaned rats, tonic NMDAR activity was significantly reduced by 200 μM kynurenine (Fig. 9) and exogenously applied KYNA (Fig. 9B). In slices from preweaned rats, tonically active NMDARs were unaffected by incubation with kynurenine (200 μM), but were significantly inhibited by exogenously applied KYNA (200 μM) (Fig. 9B). Taken together, the results shown in Figs. 8 and 9 suggest that, in contrast to postsynaptic NMDARs, tonically active (extrasynaptic) NMDARs are in close proximity to the sites where kynurenine-derived KYNA is released in the mature hippocampus.

Discussion

This study demonstrates for the first time that in brain slices increased production of KYNA from its precursor kynurenine inhibits $\alpha 7$ nAChRs and extrasynaptic NMDARs. Evidence is also provided that the $\alpha 7$ nAChR sensitivity to inhibition by KYNA is age-dependent. The significance of these results is discussed below.

Kynurenine Enhances De Novo Synthesis of KYNA to Biologically Effective Levels that Selectively Inhibit $\alpha 7$ nAChRs and Extrasynaptic NMDARs. Consistent with previous reports that acute in vitro exposure of brain slices to kynurenine enhances KYNA synthesis (Turski et al., 1989; Gramsbergen et al., 1997; Scharfman et al., 1999), incubation of rat hippocampal slices with 200 μM kynurenine led to a continuous de novo synthesis of endogenous KYNA. Conversion of kynurenine to KYNA was catalyzed primarily by KAT II, because it was significantly reduced, although not totally abolished, by the KAT II inhibitor BFF122. The more efficient conversion of kynurenine to KYNA in hippocampal slices from postweaned compared with preweaned rat is likely to be related to the age-dependent increase in hippocampal KAT activity (Baran and Schwarcz, 1993).

Incubation of hippocampal slices from postweaned rats and human cerebral cortical slices with kynurenine significantly decreased $\alpha 7$ nAChR activity. Inhibition of $\alpha 7$ nAChRs was mediated by kynurenine-derived KYNA because it was attenuated by the KAT II inhibitor BFF122. The observation that BFF122 did not fully prevent the inhibitory effect of kynurenine (Fig. 5B) is consistent with the data that some KYNA was still produced by the tissue slices during incubation with both kynurenine and BFF122 (see above). In the absence of added kynurenine, production of KYNA was negligible. As a result, BFF122 alone had no significant effect on $\alpha 7$ nAChRs. These results support the concept that the tonic inhibition of $\alpha 7$ nAChR by physiological levels of KYNA seen in vivo (Konradsson-Geuken et al., 2010; Potter et al., 2010) apparently cannot be reproduced in hippocampal slices.

In hippocampal slices from postweaned rats, increases in the endogenous production of KYNA not only reduced $\alpha 7$ nAChR activity but also inhibited tonically active NMDARs in CA1 SRIs. $\alpha 3\beta 4\beta 2$ nAChRs, postsynaptic glutamatergic (NMDA and AMPA) receptors, or GABA_A receptors, on the other hand, were not significantly affected. Because incubation with kynurenine did not increase NMDAR activity, it is quite unlikely that tissue incubation with kynurenine led to

the production of the NMDAR agonist quinolinic acid in a competing branch of the kynurenine pathway (Chen and Guillemain, 2009). However, quinolinic acid levels were not measured in the present study.

We were surprised to find that the potency of exogenously applied KYNA to inhibit $\alpha 7$ nAChRs was similar to that of kynurenine in slices from postweaned rats. Thus, incubation with either 200 μM KYNA or 200 μM kynurenine decreased the activity of $\alpha 7$ nAChRs by approximately 60%, and a similar inhibition was seen using 100 μM KYNA. On the other hand, no significant effect was seen with 2 μM KYNA (Fig. 6). Consequently, the nanomolar concentrations of KYNA measured in the medium surrounding the slices incubated with kynurenine (Fig. 1) were apparently not sufficient to inhibit $\alpha 7$ nAChRs, suggesting that much higher concentrations of KYNA were generated and accumulated at the release sites. In fact, a hippocampal slice, assumed to resemble a rectangular box of 4000- μm length, 1800- μm width, and 300- μm height, occupies a volume of $\sim 2.16 \mu l$. Considering that seven hippocampal slices were used in the immersion chamber, this results in a ~ 200 -fold dilution of KYNA in 3 ml of ACSF. Correcting for this dilution factor using the estimated rate of release of 4.8 nM/min (see *Results*), as much as 958 nM ($\sim 1 \mu M$) KYNA may be released per minute from a single slice incubated with 200 μM kynurenine. Because KYNA is produced and released primarily by astrocytes (Guillemin et al., 2001; Guidetti et al., 2007) and astrocytic processes occupy only approximately 5% of the CA1 SR neuropil (Ventura and Harris, 1999), as much as 20 μM KYNA/min may become available at the release sites during incubation with 200 μM kynurenine. This assumes that the astrocytes release KYNA continuously. If the release process is intermittent, even higher concentrations may be available at the release sites.

Age-Dependent Inhibition of $\alpha 7$ nAChRs and Tonically Active NMDARs by Endogenously Produced KYNA. Although incubation with kynurenine resulted in significant inhibition of both $\alpha 7$ nAChRs and extrasynaptic NMDARs in CA1 SRIs from postweaned (P23–P35) rats, it had no significant effect on these receptors in neurons from preweaned (P10–P18) animals.

The lack of effect of kynurenine on extrasynaptic NMDARs in the hippocampus of preweaned rats is probably caused by low levels of KYNA produced from kynurenine in the developing brain (Baran and Schwarcz, 1993). This contention is supported by the findings that: 1) KYNA-induced inhibition of tonically active NMDA receptors is concentration-dependent, being negligible at 100 μM (Fig. 9), and 2) KYNA production from kynurenine is approximately 3-fold lower in slices from preweaned animals than in slices from postweaned animals (Fig. 1). On the other hand, the lack of effect of kynurenine on $\alpha 7$ nAChRs in the immature hippocampus (P10–P18) seems to be the result of an age-dependent change in $\alpha 7$ nAChR sensitivity to inhibition by KYNA. In fact, 200 μM KYNA had no significant effect on $\alpha 7$ nAChRs in slices of preweaned rats and blocked approximately 60% of the $\alpha 7$ nAChR activity in slices of postweaned (P23–P35) rats. The wide range of ages of animals used in different laboratories may account for variabilities in the degree of $\alpha 7$ nAChR inhibition by KYNA reported in the literature (Hilmas et al., 2001; Lopes et al., 2007; Stone, 2007; Arnaiz-Cot et al., 2008; Mok et al., 2009).

The exact mechanism by which endogenously produced KYNA suppresses $\alpha 7$ nAChR responses remains to be determined. One possibility includes allosteric inhibition of $\alpha 7$ nAChRs by KYNA binding to specific receptor sites (Hilmas et al., 2001; Lopes et al., 2007; Stone, 2007; Arnaiz-Cot et al., 2008), and accessibility and affinity to these sites can change because of age-dependent posttranslational modifications. In fact, posttranslational modifications of $\alpha 7$ nAChRs regulate the formation of ligand-binding sites on these receptors (Rakhilin et al., 1999; Alexander et al., 2010a). Another possibility includes KYNA-induced changes in $\alpha 7$ nAChR expression/turnover. Alterations in receptor expression/turnover may depend on proteins that are essential for proper folding and surface expression of functional $\alpha 7$ nAChRs. One such protein is Ric-3, whose expression increases upon cellular differentiation (Halevi et al., 2003; Castelán et al., 2008; Alexander et al., 2010b). The absence of such mechanisms in oocytes and other expression systems may also explain why $\alpha 7$ nAChRs ectopically expressed in these systems are insensitive to inhibition by KYNA (Mok et al., 2009).

Physiological and Clinical Relevance of the Selective Actions of Endogenously Produced Versus Exogenously Applied KYNA. Only $\alpha 7$ nAChRs and tonically active NMDA receptors were inhibited by endogenously produced KYNA. Although glutamatergic transmission was insensitive to kynurenine-derived KYNA, it was suppressed by exogenously applied KYNA. As proposed earlier (Scharfman et al., 1999), differential actions of endogenously produced versus exogenously applied KYNA would be measurable only if the receptors sensitive to KYNA are located in close proximity to astrocytic processes that release KYNA (see Fig. 10). The current findings are therefore in line with the concept of tripartite synapses in the brain, whereby synaptic activity is

tuned by astrocyte-derived regulatory signals (Volterra and Meldolesi, 2005; Albuquerque et al., 2009).

Fluctuations in brain KYNA levels occur in various catastrophic neurological and psychiatric diseases, including schizophrenia, amyotrophic lateral sclerosis, and Alzheimer's disease (Baran et al., 1999; Erhardt et al., 2007; Chen et al., 2009). It is conceivable that KYNA-induced $\alpha 7$ nAChR inhibition contributes to the cognitive deficits seen in schizophrenia and Alzheimer's disease (Wonodi and Schwarcz, 2010). In contrast, KYNA levels are significantly lower than normal in the brain of patients with Huntington's disease (Beal et al., 1992). Here, the progressive neurodegeneration characteristic of this disease may result from continued disinhibition of tonically active, extrasynaptic NMDA receptors by chronically low levels of KYNA. In fact, striatal neurons are more susceptible to excitotoxicity in *mKat2*($-/-$) than in age-matched control mice (Sapko et al., 2006). In the developed brain, extrasynaptic NMDAR activation can trigger a shutoff cAMP response element-binding signaling pathway that increases neuronal vulnerability to glutamate-induced excitotoxicity (Hardingham and Bading, 2002).

The demonstration that both $\alpha 7$ nAChRs and extrasynaptic NMDARs in the mature brain are selectively inhibited by endogenously produced KYNA supports the contention that the kynurenine pathway is a potential target for drug development to treat pathological conditions that are related to fluctuations in brain KYNA levels, $\alpha 7$ nAChR dysfunction, and excitotoxicity.

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Authorship Contributions

Participated in research design: Alkondon, Pereira, Schwarcz, and Albuquerque.

Conducted experiments: Alkondon.

Contributed new reagents or analytic tools: Eisenberg and Kajii.

Performed data analysis: Alkondon.

Wrote or contributed to the writing of the manuscript: Alkondon, Pereira, Eisenberg, Schwarcz, and Albuquerque.

Other: Schwarcz and Albuquerque acquired funding for the research.

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Fig. 10. Schematic representation of the relative locations of KYNA-sensitive targets. This simplified scheme illustrates the role of astrocyte-derived KYNA in selectively modulating the activity of $\alpha 7$ nAChRs and tonically active NMDARs in a CA1 SRI. An axon from a glutamatergic neuron bearing $\alpha 3\beta 4\beta 2$ nAChRs and an axon from a cholinergic neuron are shown synapsing onto the CA1 SRI. Surrounding these synaptic contacts are astrocytes, where KAT II catalyzes the transamination of kynurenine into KYNA. Results presented in this study support the contention that exposure to kynurenine increases the astrocytic neosynthesis of KYNA, which is, in turn, released at sites in close apposition to $\alpha 7$ nAChRs and tonically active (extrasynaptic) NMDARs. The distance between the KYNA-release sites and synaptically located NMDARs and AMPA receptors (AMPA) spares these receptors from inhibition by endogenously produced KYNA. In such tripartite synapses made up of astrocytes in addition to presynaptic and postsynaptic neurons, neuronal activity is ultimately modulated by astrocyte-derived KYNA.

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