Regulation of GABAergic Inputs to CA1 Pyramidal Neurons by Nicotinic Receptors and Kynurenic Acid

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ABSTRACT

Impaired α7 nicotinic acetylcholine receptor (nAChR) function and GABAergic transmission in the hippocampus and elevated brain levels of kynurenic acid (KYNA), an astrocyte-derived metabolite of the kynurenine pathway, are key features of schizophrenia. KYNA acts as a noncompetitive antagonist with respect to agonists at both α7 nAChRs and N-methyl-D-aspartate receptors. Here, we tested the hypothesis that in hippocampal slices tonically active α7 nAChRs control GABAergic transmission to CA1 pyramidal neurons and are sensitive to inhibition by rising levels of KYNA. The α7 nAChR-selective antagonist α-bungarotoxin (α-BGT; 100 nM) and methyllycaconitine (MLA; 10 nM), an antagonist at α7 and other nAChRs, reduced by 51.3 ± 1.3 and 65.2 ± 1.5%, respectively, the frequency of GABAergic postsynaptic currents (PSCs) recorded from CA1 pyramidal neurons. MLA had no effect on miniature GABAergic PSCs. Thus, GABAergic synaptic activity in CA1 pyramidal neurons is maintained, in part, by tonically active α7 nAChRs located on the preterminal region of axons and/or the somatodendritic region of interneurons that synapse onto the neurons under study. L-Kynurenine (20 or 200 μM) or KYNA (20–200 μM) suppressed concentration-dependently the frequency of GABAergic PSCs; the inhibitory effect of 20 μM L-kynurenine had an onset time of approximately 35 min and could not be detected in the presence of 100 nM α-BGT. These results suggest that KYNA levels generated from 20 μM kynurenine inhibit tonically active α7 nAChR-dependent GABAergic transmission to the pyramidal neurons. Disruption of nAChR-dependent GABAergic transmission by mildly elevated levels of KYNA can be an important determinant of the cognitive deficits presented by patients with schizophrenia.

Introduction

Hippocampal dysfunction and a specific defect in hippocampal interneurons are consistent findings in the brains of patients that suffer from schizophrenia (Tammenga et al., 2010; Konradi et al., 2011), a disease that afflicts approximately 1% of the population worldwide. The contribution of hippocampal dysfunctions to the complex phenotype of the disease is underscored by numerous pharmacological studies. For instance, microinfusion of the GABA_A receptor antagonist picrotoxin in the ventral hippocampus of rats impairs prepulse inhibition of the startle reflex response, a measure of sensorimotor gating that is deficient in patients with schizophrenia (Bast et al., 2001). Intraventricular administration of the α7 nicotinic acetylcholine receptor (nAChR) antagonist α-bungarotoxin (α-BGT) to rats also impairs sensory gating as measured by the disruption of the attenuation of the amplitude of the second auditory-evoked potential in a pair of stimuli in the CA3 region of the hippocampus (Luntz-Leybman et al., 1992). Finally, systemic treatment of rats with NMDA receptor antagonists recapitulates major clinical features of schizophrenia that are ameliorated by treatment with nicotine and α7 nAChR-selective agonists (see references in Timofeeva and Levin, 2011). Among these features are deficits of working memory and decreased levels of glutamic acid decarboxylase 67, one of the enzyme isoforms that catalyzes the synthesis of GABA, and parvalbumin, a Ca^{2+}-binding protein expressed by interneurons, primarily in the stratum pyramidale.

Evidence also exists that a number of metabolic pathways are affected in the brains of patients with schizophrenia. The kynurenine pathway of the tryptophan metabolism is one of these pathways. Cerebral cortical levels of kynurenic acid (KYNA), an astrocyte-derived kynurenine metabolite, have been found to be higher in patients with schizophrenia than...
in age-matched control subjects (Schwarcz et al., 2001). KYNA is a neuroactive metabolite that interacts with a multitude of molecular targets in the brain. At concentrations ranging from tens to hundreds micromolar, KYNA acts as a competitive antagonist of glycine at NMDA receptors. The IC$_{50}$ values for KYNA to inhibit NMDA receptors are approximatively 15 μM in the absence of glycine and 230 μM in the presence of 10 μM glycine (Hilmas et al., 2001). With an IC$_{50}$ of approximately 7 μM, KYNA blocks noncompetitively α7 nAChRs (Hilmas et al., 2001; Lopes et al., 2007). Finally, with EC$_{50}$ values of 7 and 39 μM, KYNA activates the rat and the human orphan G-protein receptor 35, respectively (Wang et al., 2006).

Studies of mice with a null mutation in the gene that encodes kynurenine aminotransferase II (KAT II), an enzyme responsible for more than 70% of the astrocytic synthesis of KYNA from kynurenine, have revealed the role of the metabolite in modulating hippocampal α7 nAChR activity (Alkondon et al., 2004). Decreased hippocampal KYNA levels in the mKat-2i(−/−) mice resulted in increased α7 nAChR activity in CA1 stratum radiatum interneurons and increased GABAergic transmission onto CA1 pyramidal neurons (Alkondon et al., 2004). Additional support for the ability of KYNA to regulate the activity of multiple neurotransmitter systems was obtained from recent studies in which KYNA was synthesized de novo in hippocampal slices, while synaptic transmission, action potentials, and nAChR activity were recorded from interneurons. Increasing de novo production of KYNA decreased agonist-induced activation of α7 nAChRs and inhibited tonically active NMDA receptors in stratum radiatum interneurons, in addition to reducing the rate of firing of these neurons (Alkondon et al., 2011a,b).

Tracing the consequences of increased production of KYNA on α7 nAChR activity and GABAergic synaptic transmission to CA1 pyramidal neurons, the major output of the hippocampus, is a significant step toward understanding the pathophysiology of schizophrenia. Therefore, the present study was designed to test the hypothesis that, under resting conditions, in a fully functional neurocircuitry, activation of α7 nAChRs by basal levels of choline and/or acetylcholine in hippocampal slices contributes to maintain GABAergic synaptic activity in CA1 pyramidal neurons and is reduced by increasing de novo synthesis of KYNA. To test this hypothesis, GABAergic postsynaptic currents (PSCs) were recorded from the somata of CA1 pyramidal neurons in slices that were subjected to incubation and/or superfusion with nAChR subtype-selective antagonists and different concentrations of kynurenine, the precursor of KYNA.

**Materials and Methods**

**Hippocampal Slices.** Male Sprague-Dawley rats at ages ranging between 30 and 35 days were used in this study. Animal care and handling were done strictly in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of the University of Maryland. Rats were euthanized by CO2 narcosis followed by decapitation. Their brains were removed in ice-cold artificial cerebrospinal fluid (ACSF), which was composed of 125 mM NaCl, 25 mM NaHCO$_3$, 2.5 mM KCl, 1.25 mM NaH$_2$PO$_4$, 2 mM CaCl$_2$, 1 mM MgCl$_2$, and 25 mM dextrose. The ACSF was bubbled with 95% O$_2$ and 5% CO$_2$ before recordings. Some of the slices were transferrered to a chamber containing ACSF with test compounds that was continuously bubbled with 95% O$_2$ and 5% CO$_2$. Slices were incubated with the test compounds for 2 to 5 h at room temperature, except 1-kynurenine-incubated slices were maintained at 30°C to maximize the activity of KAT II.

**Electrophysiological Recordings.** PSCs were recorded from the somata of CA1 pyramidal neurons at a holding potential of 0 mV according to the standard whole-cell mode of the patch-clamp technique by using an LM-EPC7 amplifier (List Electronics, Darmstadt, Germany). In the recording chamber, hippocampal slices were superfused with ACSF at 2 ml/min. All recordings were obtained in the presence of the muscarinic receptor antagonist atropine (0.5 μM), a concentration that does not affect α7 nAChR currents and does not modify KYNA-induced α7 nAChR inhibition (Lopes et al., 2007; Alkondon et al., 2011b). Test compounds were applied to the slices via bath perfusion. Signals were filtered at 3 kHz, digitized at 10 kHz through Digidata 1322A (Molecular Devices, Sunnyvale, CA), and recorded by using the Clampex module of pCLAMP9 software (Molecular Devices). The frequency of PSCs in control condition remained nearly the same even at a sampling rate of 20 kHz, so we sampled at the rate of 10 kHz throughout for ease of handling the data.

Patch pipettes were pulled from borosilicate glass capillaries (1.2 mm o.d.; World Precision Instruments, Inc., Sarasota, FL) with a P-97 Flaming-Brown puller (Sutter Instrument Company, Novato, CA). When filled with internal solution the patch pipettes had resistances between 4 and 6 MΩ. The leak current was generally between 50 and 150 pA, and when it exceeded 200 pA, the data were not included in the analysis. The access resistance was monitored during the course of the experiments and ranged between 15 and 20 MΩ. Data from any neuron were not considered for analysis when the access resistance increased more than 20% from the initial values. The internal pipette solution contained 0.5% biocytin in addition to 10 mM ethylene-glycol bis-(β-amino-ethyl ether)-N,N′-tetracetic acid, 10 mM HEPES, 130 mM Cs-methane sulfonate, 10 mM CaCl$_2$, 2 mM MgCl$_2$, and 5 mM N-(2,6-dimethylphenylcarbamoylmethyl) triethy lammonium bromide (QX-314), pH adjusted to 7.3 with CsOH. All recordings were done at room temperature (20–22°C). Only a single neuron was studied per slice. Therefore, the number of neurons represents the number of hippocampal slices analyzed. Biocytin staining was developed, and the morphology of biocytin-filled neurons identified them as pyramidal neurons.

**Data Analysis and Statistics.** PSCs were analyzed in 5-min recordings by using the Clampfit module of pCLAMP 9.0 software (Molecular Devices). Frequency, peak amplitude, rise time (10–90%) and decay-time constant ($t_d$) of synaptic events were measured. The threshold amplitude for detecting PSCs was set at 10 pA, and the PSCs detected by the software were visually inspected to minimize errors. Events that did not show a typical synaptic waveform were rejected manually. For kinetic analysis, only single events with a sharp rising phase and an exponential decay were chosen during visual inspection of the recordings. Double- and multiple-peak currents were excluded for determination of PSC properties but included for the calculation of frequency of PSCs. Rise times and $t_d$ were determined during the analysis of the averaged chosen single events aligned at half rise time. Data are expressed as mean ± S.E.M. of results obtained from various animals, and statistical significance was analyzed by using one-way ANOVA or $t$ test in SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA). Furthermore, the cumulative distributions of events in control versus treatment groups were compared by using the Kolmogorov-Smirnov (K-S) test. For this, events from different neurons in each group were pooled together and then subjected to the K-S test by using the Clampfit module of pCLAMP 9.0 software.
Drugs. (−)-Bicuculline methochloride, atropine sulfate, L-kynurenine sulfate (kynurenic acid), KYNA, QX-314, 2-amino-5-phosphonovaleric acid (APV), and tetrodotoxin (TTX) were purchased from Sigma (St. Louis, MO). 6-Cyano-7-nitroquinolin-2-3-dione (CNQX) was purchased from Sigma/RBI (Natick, MA). α-BGT was purchased from Biotoxins Inc. (St. Cloud, FL). Methyllycaconitine (MLA) was a gift from Professor M. H. Benn (University of Calgary, Calgary, Alberta, Canada). Kynurenic-containing ACSF was always prepared on the day of the experiments. 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 approximately 7.4. Stock solutions of all other compounds were made in distilled water, kept frozen, and subsequently diluted in ACSF.

Results

Spontaneous GABAergic Synaptic Activity in CA1 Pyramidal Neurons. Spontaneous synaptic currents recorded from CA1 pyramidal neurons at 0 mV appeared as outward events (see traces in Fig. 1, A and B). Under control conditions, the frequency of these events ranged from 1.05 to 1.27 Hz (mean ± S.E.M. = 1.18 ± 0.08; n = 94 neurons from 94 slices from 63 rats). These spontaneously occurring synaptic currents were completely blocked after 15-min superfusion of the hippocampal slices with ACSF containing 10 µM bicuculline (Fig. 1, A and C), indicating that they were mediated via GABA<sub>A</sub> receptors. The slow decay-time constant of the outward currents (approximately 35 ms; see Table 1) was consistent with the notion that they were GABAergic in nature. To examine the extent to which glutamatergic excitation of GABAergic interneurons that synapse onto the CA1 pyramidal neurons regulate the spontaneous GABAergic synaptic activity recorded from the latter, hippocampal slices were superfused with ACSF containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and NMDA receptor antagonists CNQX (10 µM) and APV (50 µM). The frequency of GABAergic PSCs recorded from the CA1 pyramidal neurons in the presence of both CNQX and APV was comparable with that recorded under control conditions (Fig. 1, B and C). These results suggest that the spontaneous GABAergic synaptic activity recorded from the pyramidal neurons is not regulated by the basal glutamatergic activity in the slices.

Suppression of Spontaneous GABAergic Synaptic Activity in CA1 Pyramidal Neurons Exposed to α7 nAChR Antagonists. To determine whether GABAergic transmission to CA1 pyramidal neurons is regulated by tonically active α7 nAChRs in interneurons, hippocampal slices were incubated for 2 h in ACSF containing the α7 nAChR antagonist MLA (10 nM) and subsequently continuously superfused in MLA-containing ACSF. The concentration of MLA used in this study was sufficient to produce complete block of α7 nAChR currents (Alkondon et al., 2009). The cumulative plot of interevent intervals recorded in the presence of MLA was displaced toward longer intervals compared with control (Fig. 2A). In addition, the mean frequency of events recorded in the presence of MLA was significantly lower than that recorded under control conditions (Fig. 2B). In contrast, MLA had no significant effect on the τ<sub>α</sub>, rise time, mean peak amplitude, or cumulative distribution of the peak amplitude of GABAergic PSCs (Table 1).

In another set of experiments, spontaneous GABAergic PSCs were recorded from pyramidal neurons before and dur-
of the effect was nearly the same regardless of whether the slices were incubated for 2 h or superfused for 15 min with MLA-containing ACSF (Fig. 2, B and C).

The frequency of GABAergic PSCs was also significantly lower in slices that had been incubated for 1 h in ACSF containing a saturating concentration of the α7 nAChR antagonist α-BGT (100 nM) than in control slices. α-BGT caused a displacement of the cumulative distribution of interevent intervals toward longer intervals (Fig. 3A) and reduced the mean frequency of PSCs (Fig. 3B). α-BGT also decreased the frequency of PSCs in the presence of the glutamate receptor blockers CNQX (10 μM) and APV (50 μM) (Fig. 3B). The magnitude of the effect of combined bath application of MLA (10 nM) and α-BGT (100 nM) in slices

**Fig. 2.** Effect of MLA on frequency of GABAergic PSCs. A, cumulative probability plots of interevent intervals and peak amplitude (inset) of PSCs recorded from control and MLA-incubated slices. Plots represent data from five neurons from four rats for control and eight neurons from four rats for MLA incubation. MLA caused a significant rightward displacement of the cumulative distribution of interevent intervals (p < 0.01 according to K-S test). B, mean frequency of GABAergic PSCs recorded 1) under control conditions and 2) in the continuous presence of MLA after 2-h incubation with MLA. Graph and error bars represent mean and S.E.M., respectively, of data obtained from seven neurons from seven rats. ***p < 0.001 compared with control according to unpaired t test. C, mean frequency of GABAergic PSCs recorded under control condition followed by 15-min superfusion of the slices with MLA. Graph and error bars represent mean and S.E.M., respectively, of data obtained from seven neurons from seven rats. ***, p < 0.001 compared with control according to paired t test.

**Fig. 3.** Effect of α-BGT on frequency of GABAergic PSCs. A, cumulative probability plots of interevent intervals of PSCs recorded under control conditions and after 1-h incubation in ACSF containing 100 nM α-BGT. Plots represent data from four neurons from four rats in control and eight neurons from four rats in α-BGT. The cumulative distribution of interevent intervals obtained in the presence of α-BGT was significantly displaced to the right in comparison with control (p < 0.01 according to K-S test). B, quantification of the effects of α-BGT, CNQX + APV, and CNQX + APV + α-BGT. Results obtained from control slices were compared with those obtained from slices after 1-h incubation with CNQX + APV or 1-h incubation with CNQX + APV + α-BGT. Graph and error bars represent mean and S.E.M., respectively, of data obtained from five neurons from five rats in the control condition, eight neurons from five rats in α-BGT incubation, five neurons from three rats in CNQX + APV incubation, and six neurons from three rats in CNQX + APV + α-BGT. ***, p < 0.01 compared with control according to unpaired t test. ##, p < 0.01 compared with CNQX + APV by unpaired t test. C, frequency of PSCs recorded in the continuous presence of α-BGT (100 nM) was compared with that recorded in the continuous presence of α-BGT (100 nM) + MLA (10 nM). In these experiments, all slices were incubated for 1 h in α-BGT (100 nM)-containing ACSF and subsequently superfused with ACSF containing only α-BGT (100 nM) or both α-BGT (100 nM) and MLA (10 nM). Graph and error bars represent mean and S.E.M., respectively, of data obtained from seven neurons from four rats in α-BGT and seven neurons from four rats in α-BGT + MLA. *, p < 0.01 according to paired t test.
that had been preincubated with α-BGT was significantly larger than that of α-BGT alone (Fig. 3C).

**Suppression of GABAergic Synaptic Activity in CA1 Pyramidal Neurons Exposed to the Na⁺-Channel Blocker Tetrodotoxin.** To isolate action potential-independent from action potential-dependent GABAergic PSCs, hippocampal slices were first incubated for 1 h in TTX (200 nM)-containing ACSF and subsequently superfused with the same solution. The cumulative distribution of interevent intervals recorded in the presence of TTX was displaced toward longer intervals in comparison with control (Fig. 4A). TTX also reduced the mean frequency and the mean amplitude of GABAergic events by 68.3 ± 2.0 and 49.4 ± 1.3%, respectively (Fig. 4B; Table 2). Neither the rise time nor τₐ of the GABAergic PSCs were affected by TTX (Table 2). These results are consistent with TTX-induced block of the action potential-dependent GABAergic transmission; only miniature PSCs (mPSCs) remained in the presence of TTX.

In the continuous presence of TTX, MLA affected neither the cumulative distribution of interevent intervals (Fig. 4A) nor the mean frequency of mPSCs (Fig. 4B) recorded from CA1 pyramidal neurons. MLA was also devoid of any effect on the mean amplitude, rise time, and τₐ of mPSCs (Table 2).

**GABAergic Synaptic Activity Recorded from CA1 Pyramidal Neurons Decreased in the Presence of Increasing Concentrations of L-Kynurenine.** Studies from our laboratory and others have shown that incubation of rat hippocampal slices with kynurenine increases the production of KYNA in situ (Scharfman et al., 1999; Alkondon et al., 2011b). To examine the effects of newly synthesized KYNA on GABAergic transmission in CA1 pyramidal neurons, hippocampal slices were first incubated for 2 to 5 h in ACSF containing 2, 20, or 200 μM kynurenine. At the end of the incubation time, slices were transferred to the recording chamber, where they were continuously superfused with kynurenine-containing ACSF while GABAergic PSCs were recorded from CA1 pyramidal neurons. Control slices were incubated and maintained in kynurenine-free ACSF.

Kynurenine caused a concentration-dependent reduction of the mean frequency of GABAergic PSCs (Fig. 5, A and B). At 20 and 200 μM kynurenine also caused a significant displacement of the cumulative distribution of interevent intervals toward longer intervals (Fig. 5A). The mean peak amplitude, rise time, and τₐ of PSCs were not affected by the test concentrations of kynurenine (Table 3).

In the absence of preincubation, 15-min superfusion of hippocampal slices with ACSF containing kynurenine (200 μM) had no significant effect on the frequency of GABAergic PSCs (Fig. 5B). The slow onset of the action of kynurenine on GABAergic transmission is in agreement with the concept that this action is the result of the metabolic conversion of kynurenine into a neuroactive metabolite. The onset of inhibition was further assessed in experiments in which recordings were obtained from neurons before and during their superfusion with ACSF containing 20 μM kynurenine. As illustrated in Fig. 5C, a significant reduction in the frequency of GABAergic PSCs was detected after 35 min of bath application of kynurenine. The percentage of inhibition increased from approximately 13% at 35 min to nearly 22% at 45 min, the maximum recording time within which there was no noticeable change in access resistance. The effect of 20 μM kynurenine had not fully developed by 45 min, because the frequency of GABAergic PSCs recorded from CA1 pyramidal neurons in slices incubated for 2 to 5 h with 20 μM kynurenine was approximately 32% lower than that recorded under control conditions (Fig. 5, B and C). Furthermore, in the kynurenine (20 μM)-incubated slices, the frequency of GABAergic PSCs was 0.76 ± 0.02 Hz (n = 5 neurons) at 2 to

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**TABLE 2**

Characteristics of GABAergic PSCs recorded from CA1 pyramidal neurons in hippocampal slices incubated with TTX (200 nM) alone or together with MLA (10 nM). Data are presented as mean ± S.E.M. of results obtained from five neurons from five rats in control, 10 neurons from six rats in TTX, and six neurons from four rats in TTX plus MLA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amplitude</th>
<th>Rise Time 10 to 90%</th>
<th>τₐ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSF</td>
<td>0.69 ± 0.26</td>
<td>2.19 ± 0.18</td>
<td>34.8 ± 1.59</td>
</tr>
<tr>
<td>200 nM TTX</td>
<td>1.34 ± 0.52**</td>
<td>1.87 ± 0.15</td>
<td>38.6 ± 2.98</td>
</tr>
<tr>
<td>200 nM TTX plus 10</td>
<td>1.41 ± 0.84**</td>
<td>1.81 ± 0.24</td>
<td>39.9 ± 3.04</td>
</tr>
<tr>
<td>nM MLA</td>
<td>1.84**</td>
<td>1.87 ± 0.15</td>
<td>38.6 ± 2.98</td>
</tr>
</tbody>
</table>

**P < 0.01 compared with control according to one-way ANOVA followed by Dunnett post hoc test.**
3 h and 0.74 ± 0.02 Hz at 3 to 5 h (n = 5 neurons), suggesting that maximum inhibition was achieved by 2- to 3-h incubation with the drug.

Exogenously Applied KYNA Reduced GABAergic Synaptic Activity in CA1 Pyramidal Neurons. To analyze the effects of exogenously applied KYNA on GABAergic transmission to CA1 pyramidal neurons, hippocampal slices were first incubated for 2 to 5 h in ACSF containing a test concentration of KYNA (100 nM-200 μM) and subsequently superfused with KYNA-containing ACSF. The mean frequency of GABA PSCs recorded in the continuous presence of 200 nM KYNA was lower than that recorded under control conditions (Fig. 6A). The magnitude of the effect increased with increasing concentrations of KYNA (Fig. 6A) and was comparable with that of equimolar concentrations of kynurenine, particularly at the highest concentrations. In the presence of 200 nM TTX, incubation of the slices with KYNA (20 μM) had no significant effect on the frequency of mIPSCs (Fig. 6B).

To determine the onset time for KYNA to suppress GABAergic PSCs, the frequency of events recorded from neurons before and during superfusion with ACSF containing 200 μM KYNA was analyzed. In this set of experiments, slices were first exposed to ACSF containing CNQX (10 μM) and APV (50 μM) followed by the addition of KYNA (200 μM). As shown in Fig. 6C, the frequency of GABAergic PSCs was significantly reduced at 15 min of bath application of KYNA, and the magnitude of the effect increased further with time.

Effects of the Admixture of Kynurenine and the a7 nAChR Antagonist α-BGT on GABAergic Synaptic Activity in CA1 Pyramidal Neurons. To determine whether α7 nAChRs contribute to the effects of kynurenine on GABAergic transmission, hippocampal slices were first incubated for 1 h in ACSF containing a saturating concentration of the α7 nAChR antagonist α-BGT (100 nM) and for an additional 2 to 5 h in ACSF containing both α-BGT (100 nM) and kynurenine (20 or 200 μM).

The mean frequency of GABAergic PSCs recorded in the presence of α-BGT alone was not statistically different from that recorded in the presence of α-BGT plus 20 μM kynurenine (Fig. 7A). α-BGT (100 nM) alone decreased the frequency of PSCs by 51.3 ± 1.3% (Fig. 7B). After incubation of the slices with kynurenine (20 μM) alone, the mean frequency of events was 30.7 ± 1.5% lower than that recorded under control conditions (Fig. 7B). The magnitude of the effect of the admixture of α-BGT (100 nM) and kynurenine (20 μM) on the frequency of GABAergic PSCs was comparable with that of α-BGT alone (Fig. 7, A and B), indicating that

### Table 3

Characteristics of GABAergic PSCs recorded from CA1 pyramidal neurons in hippocampal slices incubated with kynurenine (200 μM) alone or together with 10 nM MLA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Amplitude (μA)</th>
<th>Rise Time 10 to 90% (ms)</th>
<th>τd (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSF</td>
<td>24.9 ± 0.83</td>
<td>2.25 ± 0.21</td>
<td>39.3 ± 2.14</td>
</tr>
<tr>
<td>200 μM Kynurenine</td>
<td>19.9 ± 0.96</td>
<td>2.07 ± 0.12</td>
<td>43.5 ± 2.25</td>
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<tr>
<td>200 μM Kynurenine plus</td>
<td>15.3 ± 0.44*</td>
<td>1.73 ± 0.18</td>
<td>52.4 ± 2.98</td>
</tr>
<tr>
<td>10 nM MLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 μM KYNA</td>
<td>20.2 ± 0.89</td>
<td>2.02 ± 0.24</td>
<td>40.8 ± 2.61</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with control according to one-way ANOVA followed by Dunnett post hoc test.
there is no additive inhibitory effect for the combined treatment.

After 2- to 5-h incubation in ACSF containing 200 μM kynurenine, the frequency of GABAergic PSCs was 49.2 ± 1.9% lower than that recorded under control conditions (Fig. 7B). The effect of the admixture of 100 nM α-BGT and 200 μM kynurenine on the mean PSC frequency was larger than that of either chemical alone (Fig. 7). Likewise, the magnitude of the effect of the admixture of 10 nM MLA plus 200 μM kynurenine on the frequency of GABAergic PSCs was larger of experiments, ACSF contained 10 μM CNQX + 50 μM APV during and 10 min before application of KYNA. The onset time for the effect KYNA was approximately 15 min. **, p < 0.01 according to one-way ANOVA followed by Tukey post hoc test.
than that seen with either 10 nM MLA or 200 μM kynurenine (Fig. 8). Both rightward displacement of the cumulative plot of interevent intervals and reduction of the mean frequency of PSCs were more pronounced in the presence of both 10 nM MLA and 200 μM kynurenine than in the presence of either chemical alone (Fig. 8). In addition, the admixture of 10 nM MLA and 200 μM kynurenine caused a significant reduction of the amplitude of PSCs (Table 3).

**Discussion**

The results presented in the present study demonstrate that 1) under resting conditions, α7 nAChRs located on interneurons are active in hippocampal slices and contribute to maintaining GABAergic synaptic input to CA1 pyramidal neurons, and 2) incubation of hippocampal slices with low micromolar concentrations of kynurenine, the precursor of KYNA, leads to the de novo synthesis of levels of KYNA that are sufficient to inhibit basal activation of α7 nAChRs, and, thereby, suppress α7 nAChR-dependent GABAergic synaptic transmission to the pyramidal neurons. The physiological and clinical relevance of these findings is discussed herein.

The Use of nAChR Antagonists Reveals that There Is a Basal Level of α7 nAChR Activity in Hippocampal Slices that Controls GABAergic Synaptic Inputs to CA1 Pyramidal Neurons. Glutamatergic activity provides major excitatory input to many neuron types. Yet, in the present study, blockade of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and NMDA receptors in the slices had no significant effect on the frequency of GABAergic PSCs recorded from the pyramidal neurons (see Fig. 1). These results strongly suggest that GABAergic synaptic activity recorded from CA1 pyramidal neurons is controlled primarily by factors other than the prototypical glutamate inputs.

In numerous studies, the use of nicotinic agonists and antagonists demonstrated that hippocampal interneurons express a number of pharmacologically distinct nAChR subtypes, the most prevalent being the α-BGT-sensitive α7 nAChRs (Alkondon et al., 1997; Jones and Yakel, 1997; Frazier et al., 1998; McQuiston and Madison, 1999; Ji and Dani, 2000). Electrical stimulation of specific hippocampal pathways have led to the identification of α7 nAChR-mediated synaptic transmission in small subsets of CA1 stratum radiatum and stratum pyramidale interneurons and in CA1 and CA3 pyramidal neurons (Alkondon et al., 1998; Frazier et al., 1998; Heft et al., 1999; Stone, 2007; Albuquerque et al., 2009; Grybko et al., 2011). The low probability of finding synaptic transmission mediated by α7 nAChRs in the hippocampus has been accounted for by the predominant non-synaptic localization of these receptors (Umbrico et al., 1995; Aznavour et al., 2005). Here, the use of nAChR subtype-selective antagonists led to the identification of hippocampal neurocircuitries in which basal levels of cholinergic transmitter activate α7 nAChRs to control GABAergic synaptic activity in the pyramidal neurons.

Two α7 nAChR antagonists were used in this study: α-BGT and MLA. The finding that α-BGT reduced the frequency without altering the amplitude of GABAergic PSCs recorded from CA1 pyramidal neurons demonstrated that in the hippocampal slices synaptic release of GABA onto the pyramidal neurons is maintained, in part, by α7 nAChRs that are activated by basal levels of acetylcholine and/or choline. MLA also reduced the frequency of PSCs recorded from the pyramidal neurons, although to a greater extent than did α-BGT. The larger effect of MLA compared with that of α-BGT (see Figs. 2 and 3) may be caused by the ability of MLA to block some heteromeric α7 or non-α7 nAChRs. The α7 nAChR currents in hippocampal neurons that are sensitive to both α-BGT and MLA (Albuquerque et al., 2009) arise from single channels with conductance in the range of 73 to 91 pS (Castro and Albuquerque, 1993; Mike et al., 2000). In chick sympathetic neurons, however, Yu and Role (1998) described the existence of both homomeric and heteromeric α7 nAChRs with single-channel conductance of 18 pS being sensitive to α-BGT and 35 pS channels being sensitive to MLA. Because hippocampal interneurons coexpress β2 and α5 subunits in abundance with α7 subunits (Sudweeks and Yakel, 2000; Son and Winzer-Serhan, 2008), it is conceivable that MLA-sensitive/α-BGT-insensitive α7 nAChRs that contain β2 and/or α5...
subunits contribute to regulation of the activity of GABAergic inputs to CA1 pyramidal neurons seen here. Alternatively, some of the MLA effects may arise from blockade of non-α7 heteromers such as α4α6ε5β2 or α3α4ε5β2 nAChRs, as proposed by Klink et al. (2001) for some GABA neurons in the substantia nigra.

The frequency of GABAergic mPSCs recorded from the CA1 pyramidal neurons in the presence of TTX was not affected by MLA. Therefore, the α7 and non-α7 nAChRs that are sensitive to MLA and modulate GABAergic transmission to CA1 pyramidal neurons are not located on the presynaptic terminals of interneurons. Instead, these receptors are located on the somatodendritic and/or preterminal axon region of interneurons that synapse onto the pyramidal neurons. These receptors may also be located on the somatodendritic, preterminal, or axon regions of glutamatergic neurons that synapse onto interneurons that in turn synapse onto the pyramidal neurons from which recordings are obtained. The contribution of nAChRs present on glutamate neurons is limited because glutamate receptor blockers CNQX and APV had no significant effect on the frequency of spontaneous GABAergic PSCs (see Fig. 1) and did not prevent the inhibitory effect of α-BGT (Fig. 3B). However, α7 nAChRs present on glutamate neurons play an important role in regulating glutamate input activity to CA1 pyramidal neurons, and such activity is suppressed by concentrations of KYNA as low as 1 μM (Banerjee et al., 2012). In situ hybridization studies have demonstrated that the majority of the hippocampal interneurons express mRNA encoding the α7 nAChR, whereas subsets of stratum oriens interneurons express mRNA encoding the α2 nAChR subunit, and subsets of stratum radiatum and stratum lacunosum moleculare interneurons express mRNAs that encode the α5 nAChR subunit (Winzer-Serhan and Leslie, 2005; Son and Winzer-Serhan, 2008). Low levels of mRNA encoding the α3 nAChR subunit have also been detected in the hippocampus, but not in interneurons (Son and Winzer-Serhan, 2008). Functional nAChRs that have the pharmacological properties of α3-containing nAChRs have been found in glutamatergic neurons/axons that synapse onto stratum radiatum interneurons (Alkondon et al., 2004).

Typically, CA1 pyramidal neurons receive GABAergic inputs from multiple interneuron types located in various layers of the CA1 region (Miles et al., 1996). Interneurons located in the stratum radiatum and lacunosum moleculare innervate distal regions of the apical dendrites. As a result, GABAergic inputs from these interneurons are of very low amplitude and slow rise times and are rarely detected in voltage-clamp recordings obtained from the soma of pyramidal neurons (Ouardouz and Lacaille, 1997; Buhler and Dündidde, 2002). Two more lines of evidence rule out the contribution of GABAergic PSCs from stratum radiatum interneurons. First, APV, although able to suppress the excitability of stratum radiatum interneurons by 70% (Alkondon et al., 2011a), had no significant effect on the frequency of GABAergic PSCs (see Fig. 1). Second, MLA, which is least effective in suppressing the spontaneous action potential frequency in the stratum radiatum interneurons (Alkondon et al., 2011a), was found to be highly effective in suppressing the frequency of GABAergic PSCs recorded from CA1 pyramidal neurons (see Fig. 2). It is therefore conceivable that most of the GABAergic PSCs recorded from the pyramidal neurons in the present study arose from interneurons in the stratum pyramidale and the stratum oriens. Interneurons in the stratum pyramidale, classified in other studies as basket cells, provide multiple innervation sites around the pyramidal cell soma and axon initial segment (Cope et al., 2002). Because nicotinic synaptic potentials sensitive to MLA and α-BGT have been detected in basket cells (Stone, 2007), it is likely that the activation of α7 nAChRs by basal levels of acetylcholine and/or choline in the slices causes excitation of these basket cells, and, thereby, contributes to most of the nicotinic regulation of GABAergic synaptic activity in the CA1 pyramidal neurons.

**KYNA Generated by Low Micromolar Concentrations of Kynurenine Disrupts GABAergic Synaptic Transmission in CA1 Pyramidal Neurons via Inhibition of α7 nAChR Activity: Clinical Relevance.** Astrocyte processes intermingle with neuronal structures, thereby providing an ideal environment for multiple types of neuron–glia interaction (Black and Waxman, 1988; Butt et al., 1994; Theodosius and Poulan, 1999). The KAT II enzyme present primarily in astrocytes is actively involved in the conversion of the substrate kynurenine into KYNA (Guidetti et al., 2007). Previous studies have demonstrated that in vitro kynurenine can be converted into KYNA in hippocampal slices (Scharfman et al., 1999; Alkon et al., 2011b). In the present study, incubation of hippocampal slices with kynurenine (20 or 200 μM) resulted in a concentration-dependent suppression of the frequency of GABAergic PSCs in the CA1 pyramidal neurons. The slow onset of the effect of kynurenine (see Fig. 5C) suggested that the effect was mediated by kynurenine-derived KYNA.

After inhibition of the slices with α-BGT, 20 μM kynurenine had no inhibitory effect on GABAergic synaptic transmission. This finding supported the contention that suppression of GABAergic transmission by levels of KYNA generated in slices incubated with low micromolar concentrations of kynurenine was the result of KYNA-induced inhibition of α7 nAChRs. On the other hand, the admixture of 200 μM kynurenine with 100 nM α-BGT or 10 nM MLA produced more suppression of GABAergic transmission than each chemical alone. Therefore, levels of KYNA generated by exposure of the slices to high concentrations of kynurenine affect additional targets that contribute to the regulation of GABAergic synaptic activity in the pyramidal neurons. Indeed, it has been shown that concentrations of KYNA generated by exposure of hippocampal slices to 200 μM kynurenine are sufficient to inhibit extrasynaptic NMDA receptors in CA1 interneurons (Alkondon et al., 2011b). Inhibition of these receptors can decrease the excitability of the interneurons, and thereby, suppress GABAergic transmission to the pyramidal neurons. In addition, the potential contribution of metabolites other than KYNA to the effects of high concentrations of kynurenine on GABAergic transmission cannot be ruled out.

The present demonstration that GABAergic transmission to CA1 pyramidal neurons is suppressed as a result of inhibition of α7 nAChRs by mild increases of de novo synthesis of KYNA has significant clinical relevance. Brain tissue levels of KYNA have been found to be higher in patients with schizophrenia as opposed to those detected in age-matched controls (Schwarz et al., 2001). Disruption of hippocampal GABAergic inhibition, particularly originating from parval-
bumin-positive interneurons, i.e., interneurons in the stratum pyramidale of the hippocampus, is a hallmark of the disease and seems to be a major determinant of the cognitive deficits that these patients present (Freedman et al., 2000; Freedman and Goldowitz, 2010; Konradi et al., 2011; Timofe-eva and Levin, 2011). Thus, it is tempting to speculate that suppression of the activity of GABAergic synaptic inputs to CA1 pyramidal neurons caused by KYNA-induced inhibition of α7 nAChRs, particularly in parvalbumin-positive interneurons, contributes to this pathophysiology of this catastrophic disorder.

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