Strain-Specific Nicotinic Modulation of Glutamatergic Transmission in the CA1 Field of the Rat Hippocampus: August Copenhagen Irish Versus Sprague-Dawley

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Submitted 20 October 2006; accepted in final form 5 December 2006

Alkondon M, Pereira EF, Potter MC, Kauffman FC, Schwarzc R, Albuquerque EX. Strain-specific nicotinic modulation of glutamatergic transmission in the CA1 field of the rat hippocampus: August Copenhagen Irish versus Sprague-Dawley (SD) rats. In brain regions including the hippocampus, PPI is modulated by α7*-nicotinic receptors (nAChRs) and kynurenic acid (KYNA), a kynurenine metabolite that blocks α7 nAChRs. Here, KYNA levels and nAChR activities were measured in the hippocampi of 10- to 23-day-old ACI and SD rats of both sexes. Hippocampal KYNA levels were not different between ACI and SD rats. In hippocampal slices from both rat strains, choline (10 mM) evoked α7*-nAChR-mediated type IA currents in CA1 stratum radiatum (SR) interneurons. In the presence of α7 nAChR antagonists, acetylcholine (ACH, 1 mM) evoked αβ4*-nAChR-mediated type II currents. ACh also triggered excitatory postsynaptic currents (EPSCs) that resulted from α3β4*-nAChR activation in glutamatergic neurons/axons synapsing onto the interneurons. The magnitude of the nicotinic responses did not differ significantly between male and female rats. Only the magnitude of α3β4*-nAChR responses and the frequency of spontaneous EPSCs recorded from CA1 SR interneurons differed between the rat strains, being significantly larger in ACI than SD rats. These results indicate that the α3β4*-nAChR activity in glutamatergic neurons/axons and the number of glutamatergic terminals synapsing onto CA1 SR interneurons are larger in prepubertal ACI than SD rats. The differential sensitivity of these rats to PPI disruption by apomorphine may result from strain-specific levels of glutamatergic activity and its strain-specific modulation by α3β4*-nAChRs in the hippocampus.

INTRODUCTION

The August Copenhagen Irish (ACI) rat, an inbred strain, is well-known for its higher propensity to develop estrogen-dependent mammary and prostate cancers compared with the outbred Sprague-Dawley (SD) rats (Isaacs 1984; Sanchez et al. 2003; Shull et al. 1997; Spady et al. 1998). The brain of ACI rats is also highly sensitive to the actions of estradiol (Stakhiv et al. 2006), a sex hormone that appears to have a neuroprotective function in schizophrenia (Hafner et al. 1993; Seeman and Lang 1990) and to prevent disruption of prepulse inhibition (PPI) in laboratory animals and healthy women subjected to different treatments (Gogos et al. 2006; Van den Buuse and Eikels 2001). Notably, at prepubertal ages, ACI rats are more susceptible than SD rats to PPI disruption by the dopaminergic agonist apomorphine (Swerdlov et al. 2004).

PPI of the acoustic startle reflex, a phenomenon thought to reflect an individual’s ability to filter out a weak stimulus so that attention can be directed toward a startling stimulus (Kumari and Sharma 2002; Potter et al. 2006), is known to be impaired in patients with schizophrenia. Deficient PPI is currently thought of as a useful endophenotype of familial forms of schizophrenia (Braff and Light 2005), and there appears to be a close relationship between neuronal substrates that regulate PPI and the neuropathology of schizophrenia. One common biochemical change observed in individuals with schizophrenia is an elevation of brain and cerebrospinal fluid levels of kynurenic acid (KYNA) (Erhardt et al. 2001; Schwarzc et al. 2001), a glia-derived kynurenine metabolite that acts as a noncompetitive antagonist of both α7 nicotinic receptors (nAChRs) and N-methyl-D-aspartate (NMDA) receptors (Hilmas et al. 2001). Pharmacological manipulations that increase brain levels of KYNA cause PPI disruption in laboratory animals (Erhardt et al. 2004; Shepard et al. 2003). For >40 yr, schizophrenia has been referred to as a psychiatric disease resulting primarily from dopaminergic hyperactivity in the brain (Kienast and Heinz 2006). However, pharmacological, postmortem binding and noninvasive imaging studies have supported the concept that glutamatergic deficits and nicotinic cholinergic deficiencies in discrete areas of the brain, including the hippocampus and the striatum, contribute to the neuropathology of the disease (Tamminga 2006). NMDA receptor antagonists, including phencyclidine and dizocilpine, disrupt PPI in laboratory animals (Geyer et al. 2001). On the other hand, nicotine, a nonselective nAChR agonist, normalizes deficits in auditory sensory gating in patients with schizophrenia (Leonard et al. 1996) and reverses apomorphine-induced disruption of PPI in rats and mice (Suemaru et al. 2004). Initial genetic studies also suggested that PPI deficits observed in patients with schizophrenia are linked to...
the chromosome 15q13-14 region, which contains the gene coding for the α7 nAChR subunit (Freedman et al. 2001).

Eight α (α2-α7, α9, α10) and three β (β2-β4) nAChR subunits have been cloned from mammalian brain tissue (Lindstrom 2003). Although the exact subunit compositions of native nAChRs are still debated, it is generally accepted that the majority of nAChRs in the mammalian brain are heteromeric nAChRs are still debated, it is generally accepted that the majority of nAChRs in the mammalian brain are hetero-

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thetic nAChRs subunits have been made up of α7 subunits (Lindstrom 2003). Pharmacological studies using α4β2 and α7 nAChR agonists and antagonists have supported the involvement of α7 nAChRs in regulating PPI. Specifically, the ability of nicotine and α7 nAChR agonists to normalize sensory gating impaired by different experimental conditions can be blocked by α7 nAChR antagonists (Suemaru et al. 2004; Wishka et al. 2006).

Understanding how α3β4 nAChRs regulate specific aspects of cognitive functions has been complicated because these subunits are largely found in the peripheral nervous system. Recent findings that α3β4 (type III) nAChR activation modulates glutamatergic transmission in the rat hippocampus and striatum (Alkondon and Albuquerque 2006; Alkondon et al. 2003) provide an impetus to evaluate its function in the brain.

The present study was designed to investigate whether differences in hippocampal nicotinic cholinergic activity, glutamatergic activity, or KYN levels exist between prepubertal ACI and SD rats of both sexes. Evidence is provided herein that hippocampal levels of KYN and the activities of α7* and α4β2* nAChRs in CA1 SR interneurons are not significantly different between 10- to 23-day-old ACI rats and age-matched SD rats. However, glutamatergic synaptic transmission impinging onto CA1 SR interneurons and α3β4* nAChR activity regulating this transmission are significantly higher in ACI than SD rats. No sex differences were observed in the magnitude of nicotinic responses recorded from CA1 SR interneurons of ACI or SD rats. We conclude that strain-dependent differences in apomorphine-induced PPI disruption may be due to strain-specific levels of glutamatergic activity and its strain-specific modulation by α3β4* nAChRs in the hippocampus.

**Methods**

**Measurements of KYN levels in hippocampal tissue**

Male SD and ACI rats (18 day old) were anesthetized in a CO2 atmosphere and killed by decapitation. Their brains were rapidly dissected out, placed on liquid nitrogen, and stored at –80°C. On the day of the assay, the tissue was homogenized (1:10, wt/vol) in ultra-pure water. Aliquots (100 μl) of the homogenate were acidified with 25 μl of 6% perchloric acid. After centrifugation (10 min, 12,000 g), 30 μl of the supernatant was subjected to analysis by high-performance liquid chromatography (HPLC) using a mobile phase containing 250 mM zinc acetate and 4.5% acetonitrile (pH 6.2). KYN levels were determined by HPLC with fluorescence detection (excitation wavelength, 344 nm; emission wavelength, 398 nm) as described by Wu et al. (1992).

**Hippocampal slices**

Slices of 250-μm thickness were obtained from the hippocampi of 10- to 23-day-old rats according to the procedure described earlier.

According to the nomenclature for nAChRs and their subunits (Lukas et al. 1999), the asterisk next to nAChR subunits throughout text is meant to indicate that the exact subunit composition is not known.

**Electrophysiological recordings**

Excitatory postsynaptic currents (EPSCs) and agonist-evoked whole cell currents were recorded from the soma of various neurons according to the standard patch-clamp technique using an LM-EPCC amplifier (List Electronic, Darmstadt, Germany). Agonists were applied to the slices via a U-tube, and antagonists were applied via either bath perfusion or both U-tube and bath perfusion (Alkondon et al. 2003). Signals were filtered at 2 kHz and either recorded on a video tape recorder for later analysis or directly sampled by a microcomputer using the pCLAMP9 software (Axon Instruments, Foster City, CA). Neurons were superfused with ACSF at 2 ml/min. Atropine (0.5 μM) was added to the ACSF to block muscarinic receptors. Bicuculine (10 μM) was added to ACSF to block γ-aminobutyric acid A (GABA_A) receptor activity. Methyllycaconitine (MLA, 10 nM) was included in the ACSF to block type IA currents while studying non-type IA nAChR responses. Patch pipettes were pulled from a borosilicate glass capillary (1.2-mm OD) that, when filled with internal solution, had resistances between 3 and 5 MΩ. The series resistance ranged from 8 to 20 MΩ. At ~68 mV, 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 internal pipette solution contained 0.5% biocytin in addition to (in mM): ethyleneglycol bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 10; HEPES, 10; Cs-methane sulfonate, 130; CsCl, 10; MgCl2, 2; and lidocaine-N-ethyl bromide (QX-314), 5 (pH adjusted to 7.3 with CsOH; 340 mOsm). Membrane potentials were corrected for liquid junction potential. All experiments were carried out at room temperature (20–22°C).

**Data analysis**

Frequency, peak amplitude, 10–90% rise time and decay-time constant of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) EPSCs were analyzed using WinEDR V2.3 (Strathclyde Electrophysiology Software, Glasgow, Scotland). The peak amplitude of nicotinic currents and the net charge of NMDA receptor-mediated EPSCs and nicotinic currents were analyzed using the pCLAMP9 software (Axon Instruments, Foster City, CA). Typically, the net charge of agonist-evoked responses was calculated for the duration of the agonist pulse starting from the time when the solenoid valve was activated. Results are presented as means ± SE and compared for their statistical significance by Student’s t-test, Mann-Whitney U test, or Fisher’s exact test.

**Drugs used**

ACh chloride, (−)-bicuculline methiodide, choline chloride, QX-314 bromide, 5-aminophosphonovaleric acid (APV), α-bungarotoxin (α-BGT), and atropine sulfate were purchased from Sigma Chemical (St. Louis, MO). Stock solutions of all drugs were made in distilled water.
RESULTS

No significant strain-related differences in hippocampal tissue levels of KYNA and in characteristics and prevalence of nicotinic responses

In hippocampal tissue dissected from 18-day-old male SD rats and age-matched male ACI rats, levels of KYNA were 5.1 ± 0.6 and 4.4 ± 0.6 fmole/mg tissue (n = 4 animals/group), respectively. Statistical analysis (unpaired Student’s t-test) revealed that these levels are not significantly different.

In hippocampal slices from male or female ACI rats, U-tube-application of choline (10 mM, 12-s pulses) to CA1 SR interneurons induced inward currents at ~68 mV that decayed to the baseline level before the end of the agonist pulse (Fig. 1A). These currents were inhibited by bath application of MLA (10 nM, Fig. 1A) or α-BGT (100 nM; data not shown). The kinetics and pharmacological profile of these currents indicate that they correspond to the α7* nAChR-mediated type IA currents previously studied in CA1 SR interneurons of SD rats (Alkondon and Albuquerque 2004).

In the continuous presence of MLA (10 nM), CA1 SR interneurons in hippocampal slices from male or female ACI rats responded to ACh (0.1 mM, 12-s pulses) with slowly decaying inward currents at ~68 mV (see Fig. 1B). The peak amplitudes and net charge of these currents were decreased by >95% by bath application of 10 μM dihydro-β-erythroidine (see Fig. 1B). The kinetics and pharmacological properties of these currents suggested that they represent the α4β2* nAChR-mediated type II currents previously identified in CA1 SR interneurons of SD rats (Alkondon and Albuquerque 2004).

In hippocampal slices from ACI rats of both sexes, CA1 SR interneurons that were voltage clamped at positive membrane potentials and continuously perfused with ACSF containing MLA (10 nM) and bicuculline (10 μM) responded to ACh (0.1 mM, 12-s pulses) with several overlapping EPSCs (see Fig. 1, C and D) that were sensitive to inhibition by the NMDA receptor antagonist APV (50 μM; Fig. 1D) and were, therefore, mediated primarily by NMDA receptors. Under this experimental condition, the contribution of AMPA receptors to the EPSCs was negligible. As previously observed in hippocampal slices from SD rats (Alkondon and Albuquerque 2004; 2006), ACh-evoked EPSCs recorded from interneurons of ACI rats were also sensitive to inhibition by mecamylamine (1 μM; Fig. 1C) or choline (100 and 300 μM; Fig. 2). These results support the concept that ACh-triggered EPSCs recorded from interneurons of ACI rats correspond to the type III responses originally identified in CA1 SR interneurons of SD rats. The pharmacological profile of these responses suggest that they result from activation of α3β4* nAChRs present in glutamatergic axons/neurons synapsing onto the interneurons under study.

The prevalence of all three types of nicotinic responses recorded from CA1 SR interneurons was not statistically different between the two rat strains according to Fisher’s exact test. Type I A, II, and III responses were recorded from 97% (36 of 37 neurons), 48% (16 of 33 neurons), and 96% (42 of 44

FIG. 1. Pharmacological characterization of nicotinic receptor (nAChR) responses in August Copenhagen Irish (ACI) rat neurons. Sample recordings of various nAChR responses from CA1 stratum radiatum (SR) interneurons of ACI rat hippocampal slices before (left) and 10 min after bath exposure to antagonists (right). Choline (10 mM)-evoked type IA current (A), acetylcholine (ACh, 0.1 mM)-evoked type II current (B), and ACh (0.1 mM)-evoked type III responses (C and D) were obtained from 4 different neurons. Type IA and type II currents were recorded at ~68 mV, whereas type III responses were recorded at ~40 mV. Artificial cerebrospinal fluid (ACSF) contained atropine (0.5 μM), bicuculline (10 μM), and methyllycaconitine (MLA, 10 nM). MLA was excluded while recording type IA currents. Agonists were applied to the neuron for 12 s (—) via a U-tube.

FIG. 2. Choline inhibits ACh-induced N-methyl-D-aspartate (NMDA) excitatory postsynaptic currents (EPSCs) in hippocampal slices from ACI rats. Sample recordings of ACh-induced NMDA EPSCs obtained from a CA1 SR interneuron under control conditions (top), 10 min after bath exposure to 300 μM choline (middle), and 10 min after washing of the slice with choline-free ACSF (bottom). Bar graph depicts the percent inhibition by choline of the net charge of ACh-induced NMDA EPSCs. Graph and error bars represent the means and SE, respectively, of results obtained from 4 (100 μM choline) or 5 (300 μM choline) neurons. Recording conditions were similar to those described in Fig. 1. The values were significantly different from control according to the paired Student’s t-test (**p < 0.01; ***p < 0.001).

ACI

Control

Choline (300 μM)

Wash

ACh, 0.1 mM

% Inhibition of ACh-induced NMDA EPSCs

0

100

20

30

40

50

60

70

80

90

100

100

Choline (μM)

0

50

100

150

200

250

300

ACh, 0.1 mM

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Table 1. Neurolucida-deduced dendritic length of CA1 SR interneurons and frequency, amplitudes, and kinetics of spontaneous or ACh-induced EPSCs recorded from these neurons in hippocampal slices of prepubertal ACI and SD rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ACI</th>
<th></th>
<th>SD</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Total Dendrite Length, μm</td>
<td>1073 ± 161</td>
<td>28</td>
<td>1058 ± 143</td>
<td>12</td>
</tr>
<tr>
<td>Frequency of Spontaneous AMPA EPSCs (Hz)</td>
<td>0.081 ± 0.013†**</td>
<td>27</td>
<td>0.039 ± 0.009</td>
<td>13</td>
</tr>
<tr>
<td>Frequency of ACh-induced AMPA EPSCs (Hz)</td>
<td>1.003 ± 0.196†***</td>
<td>14</td>
<td>0.362 ± 0.097</td>
<td>11</td>
</tr>
<tr>
<td>Peak Amplitude of Spontaneous AMPA EPSCs (pA)</td>
<td>15.9 ± 0.8</td>
<td>26</td>
<td>17.6 ± 1.8</td>
<td>10</td>
</tr>
<tr>
<td>Peak Amplitude of ACh-induced AMPA EPSCs (pA)</td>
<td>25.0 ± 2.1†***</td>
<td>13</td>
<td>26.4 ± 2.2†***</td>
<td>25</td>
</tr>
<tr>
<td>10–90% Rise Time of Spontaneous AMPA EPSCs (ms)</td>
<td>0.92 ± 0.06</td>
<td>23</td>
<td>0.80 ± 0.06</td>
<td>10</td>
</tr>
<tr>
<td>10–90% Rise Time of ACh-induced AMPA EPSCs (ms)</td>
<td>0.88 ± 0.06</td>
<td>12</td>
<td>1.03 ± 0.11</td>
<td>6</td>
</tr>
<tr>
<td>τ Decay of Spontaneous AMPA EPSCs (ms)</td>
<td>3.23 ± 0.21</td>
<td>24</td>
<td>3.39 ± 0.40</td>
<td>10</td>
</tr>
<tr>
<td>τ Decay of ACh-induced AMPA EPSCs (ms)</td>
<td>3.26 ± 0.28</td>
<td>10</td>
<td>3.49 ± 0.64</td>
<td>5</td>
</tr>
<tr>
<td>Net Charge Ratio of ACh-induced NMDA EPSCs (ACh 30 μM/ACh 300 μM)</td>
<td>0.454 ± 0.082</td>
<td>5</td>
<td>0.477 ± 0.089</td>
<td>3</td>
</tr>
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</table>

Values are means ± SD. ACI, August Copenhagen Irish; SR, stratum radiatum; ACh, acetylcholine; EPSC, excitatory postsynaptic current; SD, Sprague-Dawley; NMDA, N-methyl-D-aspartate. †Asterisks indicate that results are different from those obtained in the analysis of events recorded from SD rats: *P < 0.05, **P < 0.01 (unpaired Student’s t-test). ††Asterisks indicate that results obtained from the analysis of ACh-induced and spontaneously occurring EPSCs are significantly different: ***P < 0.001 (unpaired Student’s t-test).

Magnitude of type III nicotinic responses recorded from CA1 SR interneurons is significantly larger in ACI than SD rats

The peak amplitude or net charge of choline (10 mM)-evoked type IA currents recorded from CA1 SR interneurons did not differ between SD and ACI rats (Fig. 3, A and D). Likewise, no strain-related differences were detected in the net charge of ACh (0.1 mM)-induced type II currents recorded from the interneurons (Fig. 3, B and D). In contrast, interneurons from ACI rats exhibited significantly larger type III nAChR responses than interneurons from SD rats as judged from the net charge of ACh-evoked NMDA EPSCs (Fig. 3, C and D).

No sex-related differences were observed in the magnitude of type IA or type III responses recorded from CA1 SR interneurons in ACI or SD rats. For instance, the peak amplitude of type IA currents and the net charge of type III responses were 51 ± 14 pA and 698 ± 123 pC, respectively, in female ACI rats (n = 18–23 neurons) and 42 ± 11 pA and 492 ± 173 pC, respectively, in age- and litter matched male ACI rats (n = 14–15 neurons).

The different magnitude of type III nAChR response between ACI and SD rats could not be explained by variations in agonist affinity as the ratio of response magnitude evoked by low and high agonist concentration was not significantly different between the two strains (see Table 1). Experiments were then designed to determine whether the differences in magnitude of type III responses between the two rat strains could be solely accounted for by differences in the level of glutamatergic activity impinging onto the interneurons. To this end, EPSC recordings were obtained from neurons voltage clamped at −68 mV using Mg2+-containing ACSF. Under this experimental condition, NMDA receptors were blocked and EPSCs were mediated primarily by AMPA receptors. Due to their fast decay, AMPA EPSCs appeared as discrete events in the electric activity of the interneurons.
physiological recordings, making it easier to analyze both the amplitude and the frequency of events. The mean amplitude of spontaneous AMPA EPSCs recorded from CA1 SR interneurons was not significantly different between the two rat strains, whereas the frequency of these events was approximately twofold higher in interneurons of ACI than SD rats (Table 1). In hippocampal slices from ACI and SD rats, ACh (0.1 mM, 12-s pulses) increased the frequency and amplitude of AMPA-mediated EPSCs recorded from CA1 SR interneurons (Fig. 4, A and B; Table 1). Although no significant strain-related differences were detected in the mean amplitude of ACh-evoked AMPA EPSCs (Table 1), the frequency of ACh-induced AMPA EPSCs was significantly higher in ACI than in SD rats (Table 1). The rise- and decay-time constants of spontaneous and ACh-induced AMPA EPSCs were similar between the two rat strains (Table 1).

Analysis of Neurolucida drawings of biocytin-filled neurons (Fig. 5) revealed no significant differences in the dendrite length of CA1 SR interneurons between SD and ACI rats (Table 1). These findings ruled out the possibility that variations in dendrite length account for the strain-related differences in glutamatergic activity recorded from the CA1 SR interneurons.

A higher number of glutamatergic synapses per unit length of dendrite in the CA1 SR interneurons of ACI rats compared with SD rats (see Fig. 6A) could explain the higher spontaneous glutamatergic activity and the higher magnitude of type III nAChR responses recorded from interneurons of ACI rats. However, in recordings obtained from CA1 SR interneurons of SD rats, no significant correlation existed between the frequency of ACh-induced AMPA EPSCs and the frequency of spontaneous AMPA EPSCs (Fig. 6B). In recordings obtained from interneurons of ACI rats, the frequency of ACh-evoked AMPA EPSCs and the frequency of spontaneous AMPA EPSCs only correlated well in the range of 0.01 to 0.04 Hz (Fig. 6B). In addition, within a range of comparable spontaneous frequency (between 0.01 and 0.04 Hz) in the two rat strains, ACh always triggered more AMPA EPSCs in ACI interneurons than in SD interneurons (Fig. 6B).

**DISCUSSION**

The present study reveals that CA1 SR interneurons in the hippocampus of prepubertal ACI rats receive more glutama-
No significant differences were detected in hippocampal tissue was observed in the magnitude of nicotinic regulation of the hippocampus (Lapchak et al. 1990) and that the hippocampus of prepubertal ACI than SD rats. There are reports that sex ergic inputs than corresponding interneurons in the hippocampus of age-matched SD rats. It also demonstrates that the level of \( \alpha 3\beta 4 \) nAChR-modulated glutamate activity impinging onto CA1 SR interneurons is higher in the hippocampus of prepubertal ACI than SD rats. There are reports that sex hormones regulate nAChR expression/activity in the rat hippocampus (Lapchak et al. 1990) and that the hippocampus of prepubescent rats is sexually dimorphic (Davis et al. 1999; Diamond et al. 1983). However, no apparent sex dependency was observed in the magnitude of nicotinic regulation of glutamatergic activity in the hippocampus of ACI and SD rats. No significant differences were detected in hippocampal tissue concentrations of KYNA of age-matched ACI and SD rats. Likewise, levels of \( \alpha 7 \) or \( \alpha 4\beta 2 \) nAChR activities in CA1 SR interneurons were comparable between ACI and SD rats. As discussed in the following, higher levels of glutamatergic activity and its differential regulation by \( \alpha 3\beta 4 \) nAChRs in the hippocampus of ACI compared with SD rats could explain the strain-specific sensitivities to apomorphine-induced PPI disruption.

**Differential glutamatergic synaptic transmission and its regulation by \( \alpha 3\beta 4 \) nAChRs in prepubertal SD and ACI rats**

Electrophysiological recordings obtained from CA1 SR interneurons revealed that the frequency of EPSCs mediated by AMPA receptors in hippocampal slices from prepubertal ACI was higher than that observed in age-matched SD rats. This difference could be accounted for by a larger number of glutamatergic synapses impinging onto the neurons under study in ACI rats compared with SD rats. The finding that the average amplitudes of AMPA-mediated EPSCs were comparable between the two rat strains is consistent with the conclusion that there are no significant differences in the number of postsynaptic AMPA receptors or quantal size of glutamate released at the neurons under study in hippocampal slices from ACI and SD rats. Given that the dendritic length of the interneurons was not significantly different between the two rat strains, it is conceivable that the number of glutamatergic terminals per dendritic length is higher in the interneurons of ACI than SD rats.

In ACI rats, the frequency of AMPA EPSCs triggered by ACh-induced activation of \( \alpha 3\beta 4 \) nAChRs in glutamatergic neurons/axons synapsing onto the interneurons only correlated with the frequency of spontaneous AMPA EPSCs when the latter ranged between 0.01 and 0.04 Hz. In neurons showing frequency of spontaneous EPSCs \( > 0.04 \) Hz, there was no proportional increase in the frequency of ACh-induced EPSCs. These findings suggested that in the hippocampus of ACI rats at least two subsets of glutamate neurons/axons synapse onto CA1 SR interneurons. One subset carries \( \alpha 3\beta 4 \) nAChRs and, under resting conditions, is less active than the other subset that is devoid of nAChRs. In fact, glutamatergic axons from several brain regions have been shown to make synapses in the CA1 SR region of the rat hippocampus (Somogyi and Klawe 2005).

The strain-related differences in ACh-triggered EPSCs could not be accounted for exclusively by the larger degree of glutamatergic synaptic activity impinging onto the neurons of ACI compared with SD rats. The finding that within the low range of frequency of spontaneous AMPA EPSCs ACh triggered more AMPA EPSCs in CA1 SR interneurons of ACI than SD rats supports the hypothesis that the activity/density of \( \alpha 3\beta 4 \) nAChRs is higher in glutamatergic neurons/axons synapsing onto the interneurons of prepubertal ACI rats compared with age-matched SD rats. There are reports that chronic exposure to nicotine of rats during the second postnatal week significantly disrupts the development of glutamatergic synapses in the auditory cortex (Aramakis et al. 2000; Hsieh et al. 2002). The present demonstration that a high \( \alpha 3\beta 4 \) nAChR activity/expression leads to a high level of glutamatergic synaptic activity in the hippocampus of prepubertal rats suggests that \( \alpha 3\beta 4 \) nAChRs may mediate some of the disruptive effects of nicotine exposure.

**FIG. 6.** Diagrammatic representation of nAChR-modulated glutamate transmission in CA1 SR interneurons of ACI and SD rats based on the correlation between the frequency of spontaneous AMPA EPSCs and ACh-induced EPSCs recorded from interneurons. A: scheme representing varying numbers of glutamatergic synapses made onto the dendrites of SR interneurons and varying numbers of type III nAChRs present on the glutamatergic axons. B: correlation between the frequencies of spontaneous and ACh-induced AMPA EPSCs. Each data point represents a single interneuron from either ACI (●) or SD (○) rats. ---, linear regression of data points \( \pm 0.04 \) Hz and \( \pm 0.04 \) Hz. The regression coefficient, \( r^2 \), was 0.97 and 0.04 for results obtained from ACI neurons showing spontaneous EPSC frequencies \( \pm 0.04 \) and \( > 0.04 \) Hz, respectively. Likewise, \( r^2 \) was 0.06 and 0.28 for results obtained from SD neurons showing spontaneous EPSC frequencies \( \pm 0.04 \) and \( > 0.04 \) Hz, respectively.
effects of nicotine on glutamatergic transmission during brain development.

**Inter-strain differences in brain nAChR expression and responsiveness to nicotinic agonists and antagonists in rats and mice**

Strain-dependent variations in nAChR density have been reported in the brains of mice and rats. Ultimately, differential levels of expression of specific nAChR subtypes in discrete brain regions contribute to the behavioral responses of the animals to nicotine and other nicotinic agonists (Gahring et al. 2004, 2005).

Numbers of α-BGT- and cytosine-binding sites, which represent primarily α7* and α4β2* nAChRs, respectively, have been found to be significantly higher in specific regions of the brain of Wistar normotensive rats compared with spontaneously hypertensive rats (Gattu et al. 1997). It has been suggested that the poorer cognitive performance of spontaneously hypertensive compared with Wistar normotensive rats relates to their differential expression of nAChRs in the brain (Gattu et al. 1997). Our study is the first to report differences in α3β4* nAChR activity/expression in the hippocampus of ACI and SD rats.

**Role of nAChRs in auditory gating**

Several lines of evidence support the role of α7* nAChRs in sensorimotor gating. First, intracerebroventricular administration of the α7 nAChR antagonist α-BGT to rats induces auditory sensory gating deficits (Luntz-Leybman et al. 1992). Second, systemic administration of the α7 nAChR antagonist MLA to rats antagonizes nicotine-mediated reversal of apomorphine-induced PPI deficits (Suemaru et al. 2004). In contrast, α7 nAChR agonists reverse auditory gating and PPI deficits observed in isolation-reared rats (Cilia et al. 2005; O’Neill et al. 2003). Third, auditory gating deficits have been observed in inbred mouse strains, and the severity of these deficits correlates well with the degree of decreased expression of α7* nAChRs in the hippocampus (Stevens et al. 1996). In addition, pharmacological manipulations that increase the brain levels of KYNA, a glia-derived metabolite that acts as an endogenous regulator of α7* nAChR activity (Alkondon et al. 2004), have been shown to impair PPI in rats (Erhardt et al. 2004; Shepard et al. 2003). The findings of the present study suggest that neither α7* nAChRs nor KYNA is involved in the differential sensitivity of prepubertal ACI and SD rats to apomorphine-induced disruption of PPI. These observations are also consistent with the earlier reports that mice with a null mutation in the gene coding for α7 nAChR subunits exhibit normal sensorimotor gating (Paylor et al. 1998) and that specific nAChR subtypes are differentially involved in PPI modulation in distinct rodent species and strains (Schreiber et al. 2002).

Because GABAergic interneurons are critical for gating sensory information within the corticollimbic system (Benes and Beretta 2001) and contain various nAChR subtypes that control their excitability (Alkondon et al. 2003), different nAChRs can potentially play a role in sensorimotor gating. As demonstrated here, at an age when strain-specific sensitivities to apomorphine-induced disruption of PPI are evident (Swerdlov et al. 2004), ACI compared with SD rats have a significantly higher level of α3β4* nAChR activity on glutamatergic neurons/axons that synapse onto CA1 SR interneurons. It has been proposed that regulation of dopaminergic activity by a direct glutamatergic hippocampal projection to the nucleus accumbens represents a potential mechanism by which the hippocampus modulates PPI in rats (Kelley and Domesick 1982). Thus it can be hypothesized that ACI rats are more sensitive than SD rats to apomorphine-induced PPI disruption because the former display a higher degree of excitation of SR interneurons via an increased level of expression/activity of α3β4* nAChRs in glutamatergic axons/neurons. In the ACI rats, increased excitation of the CA1 SR interneurons may contribute to the inhibition of CA1 pyramidal neurons and, consequently, decrease the glutamatergic hippocampal stimulation of the nucleus accumbens.

Based on the results presented herein and considering the previous reports that ACI rats are exquisitely responsive to estradiol, prepubertal ACI rats emerge as a potential animal model in which to identify the role of α3β4* nAChRs and the potential interplay between the nicotinic cholinergic system and estradiol in the regulation of PPI. Sorting of this phenotype into pharmacologically and genetically meaningful groups can provide invaluable clues regarding the pathophysiology of schizophrenia and lead to the development of new drugs to treat the disease.

**Acknowledgments**

We thank M. Zelle for technical assistance and for editing the manuscript. We are also grateful to B. Alkondon for excellent technical assistance in the preparation of hippocampal slices, biocytin processing and neuronal drawings. MLA hydrochloride was a gift from Professor M. H. Benn, Department of Chemistry, University of Calgary, Alberta, Canada.

**Grants**

This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-41671 and NS-25296.

**References**


The distribution of the projection from the forebrain.

Hafner H, Maurer K, Loffler W, Riecher-Rossler A.


