Genetic and Pharmacological Studies of GluR5 Modulation of Inhibitory Synaptic Transmission in the Anterior Cingulate Cortex of Adult Mice

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ABSTRACT: **In the anterior cingulate cortex (ACC), GluR5-containing kainate receptor mediated the small portion of excitatory postsynaptic current. However, little is known about its role in modulation of neurotransmitter release in this brain region. In the present study, we address this question by using selective GluR5 agonist and antagonist, as well as GluR5$^{-/-}$ mice. Our results showed that activation of GluR5 induced action potential-dependent GABA release, which is also required for the activation of voltage-dependent calcium channel and Ca^{2+} influx. The effect of GluR5 activation is selective to the GABAergic, but not glutamatergic synaptic transmission. Endogenous activation of GluR5 also enhanced GABA release to ACC pyramidal neurons and the corresponding postsynaptic tonic GABA current. Our results suggest the somatodendritic, but not presynaptic GluR5, in modulation of GABA release. The endogenous GluR5 activation and the subsequent tonic GABA current may play an inhibitory role in ACC-related brain functions.**
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Keywords: **kainate receptor; GluR5; GABAergic transmission; tonic GABA current; anterior cingulate cortex; mice**

INTRODUCTION

The anterior cingulate cortex (ACC) is involved in emotional and attentive responses to internal and external stimulation, such as intelligent behaviors, pain, and memory (Allman et al., 2001; Zhuo, 2004). Kainate receptor subunits, including GluR5, are expressed in the ACC (Huntley et al., 1993; Bahn et al., 1994). Recently, we have reported that both GluR5 and GluR6 contribute to a portion of excitatory synaptic currents in pyramidal neurons in the ACC (Wu et al., 2005b). However, whether and how GluR5 modulates GABAergic neurotransmission in the ACC remains unknown.

GluR5 is reported to modulate GABA release in hippocampus, basolateral amygdala, neocortex, and spinal dorsal horn. In hippocampal interneurons, GluR5 is combined with GluR6 to form functional heteromeric kainate receptors, which facilitate GABA release and increases tonic inhibition of pyramidal neurons (Cossart et al., 1998; Bureau et al., 1999; Mulle et al., 2000; Christensen et al., 2004). In the basolateral amygdala, presynaptic GluR5 is shown to bidirectionally modulate GABA release, possibly due to different types of GluR5-containing KA receptors with different agonist affinities (Braga et al., 2003). Presynaptic GluR5 is shown to depress GABA release in unitary connections between interneuron and pyramidal neurons in layer V of the motor cortex

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(Ali et al., 2001). GluR5 has also been located at presynaptic terminals of local interneurons in the dorsal horn of spinal cord, increasing both GABA and glycine release (Kerchner et al., 2001a, 2002). Although most studies agree that GluR5 activation increases spontaneous GABA release, whether the GluR5 is located at axon terminals remains controversial (Rodriguez-Moreno et al., 1997; Jiang et al., 2001; Braga et al., 2003). The reason for the discrepancy may reside in the different preparations, different regions, or even different types of synapses. Therefore, the most well-established results on GluR5 modulation of GABAergic transmission from hippocampus in young rats may not apply to the ACC in the adult mice.

Owing to the availability of selective GluR5 agonist and antagonist, as well as GluR5 knockout ($GluR5^{-/-}$) mice, we want to determine whether GluR5 modulates GABA release in the ACC. Our results demonstrate the presence of functional GluR5-containing kainate receptors on interneurons, which regulate the GABAergic neurotransmission to the pyramidal neurons in the ACC of adult mice.

METHODS

Animals

Adult C57BL/6 mice were purchased from Charles River (8–12 weeks old). $GluR5^{-/-}$ mice were obtained as gifts from Stephen F. Heinamann (Salk Institute, San Diego, CA) (Mulle et al., 1998; Sailer et al., 1999). $GluR5^{-/-}$ mice were maintained on a mixed 129Sv × C57BL/6 background, and wild-type littermates were used as controls. All mice were maintained on a 12-h light/dark cycle. Food and water were provided *ad libitum*. The Animal Care Committee at the University of Toronto approved the experimental protocols. All experiments were performed with the experimenter blinded to the genotype of the mice.

Whole-Cell Patch Clamp Recordings in Adult ACC Slices

Male mice (6–10 weeks old) were anesthetized with 1–2% halothane. Coronal slices (300 μm) containing the ACC were prepared using standard methods (Wu et al., 2005b). Slices were transferred to a room temperature submerged recovery chamber, with oxygenated (95% O_2 and 5% CO_2) solution containing (in m*M*): NaCl, 124; $NaHCO_3$, 25; KCl, 2.5; KH_2PO_4, 1; $CaCl_2$, 2; $MgSO_4$, 2; glucose, 10. In Ca^{2+}-free external solution, $CaCl_2$ was omitted and 2 m*M* EGTA was added. After 1-h recovery, slices were placed in a recording chamber on the stage of an Axioskop 2FS microscope (Zeiss) equipped with infrared DIC optics for visualized whole-cell patch clamp recordings. Pyramidal neurons or interneurons in Layer II-III in the ACC were recorded. Postsynaptic currents were recorded with an Axon 200B amplifier (Axon Instruments, CA). Local stimulations, about 100 μm away from the recording neurons in layer III, which activate local inhibitory circuitry and excitatory input from the thalamus (Wang and Shyu, 2004), were applied to induce evoked postsynaptic currents. Recording electrodes (2–5 MΩ) contained a pipette solution containing (in m*M*): Cs-$MeSO_3$, 120; NaCl, 5; $MgCl_2$ 1; EGTA, 0.5; Mg-ATP, 2; Na_3GTP, 0.1; HEPES, 10; pH 7.2; 280–300 mOsmol. When excitatory postsynaptic current and action potential of ACC neurons were recorded, Cs-$MeSO_3$ was replaced by K-gluconate. Access resistance was 15–30 MΩ and was monitored throughout the experiment. Data were discarded if access resistance changed more than 10% during an experiment. The membrane potential was held at −70 mV throughout the experiment. When recording $GABA_A$ receptor-mediated current, a holding potential of +10 mV was used as indicated.

Pharmacological Inhibitors

AP-5 and GYKI 53655 were used to selectively block NMDA receptors and AMPA receptors, respectively. ATPA was used to selectively activate GluR5-containing KA receptors. All chemicals and drugs were obtained from Sigma (St. Louis, MO), except for ATPA, which was purchased from Tocris (Ellisville, MO). GYKI 53655 was a kind gift from Dr. John F. MacDonald (University of Toronto) and LY 293558 was a kind gift from Purdue Pharmaceutical Company (Purdue Pharma L.P.). Drugs were dissolved in ACSF and applied through perfusion.

Data Analysis

Data were expressed as mean (SEM). Statistical comparisons were performed with the Student *t*-test or one-way analysis of variance. Analysis of mIPSCs/sIPSCs was performed with cumulative probability plots and was compared using the Kolmogorov–Smirnov test for significant differences. In all cases, $p < 0.05$ was considered statistically significant. * indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$. These definitions are applied in all figures in the paper.

RESULTS

Activation of GluR5 Kainate Receptors Bidirectionally Modulates Evoked Inhibitory Synaptic Currents in the ACC

According to the morphological and electrophysiological properties, pyramidal neurons were selected from the layer II–III of ACC (Wu et al., 2005a). In the presence of AP5 (50 μM) and CNQX (20 μM), evoked inhibitory synaptic currents (eIPSCs) were

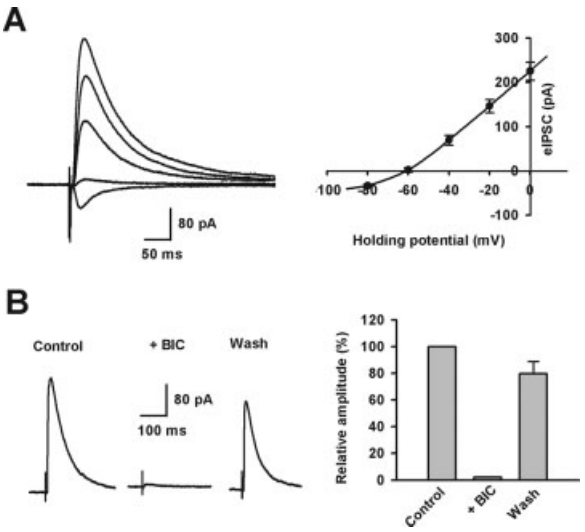

Figure 1 $GABA_A$ receptor-mediated eIPSCs in ACC pyramidal neurons. A. In the presence of AP5 (50 μM) and CNQX (20 μM), eIPSCs were recorded at holding potentials from −80 to 0 mV. The current–voltage plot showed linear property and the reversal potential of −65.2 ± 1.1 mV ($n = 7$). B. Bicuculline (BIC, 10 μM) could reversibly block eIPSCs in ACC pyramidal neurons ($n = 6$).

pharmacologically isolated by local stimulation. The reversal potential of eIPSCs, estimated from the *I-V* relationships, was −65.2 ± 1.1 mV ($n = 7$), which is close to the theoretical Cl^- equilibrium potential (−73.6 mV) calculated with the Nernst equation for the given extra- and intracellular Cl^- concentrations (130 and 7 m*M*, respectively) [Fig. 1(A)]. At holding potential of +10 mV, perfusion of bicuculline (10 μM) could reversibly block outward eIPSCs [$n = 6$, Fig. 1(B)], indicating that the current is mediated by $GABA_A$ receptors.

We have previously shown that both GluR5 and GluR6 are involved in mediating postsynaptic current in the ACC pyramidal neurons (Wu et al., 2005b). To study the role of GluR5 on GABAergic transmission, we first studied the effects of selective GluR5 agonist, ATPA, on eIPSCs in the ACC. In the presence of AP5 and GYKI53655 (100 μM), a selective AMPA antagonist, eIPSCs were induced and then ATPA was bath-applied. We found ATPA could biphasically regulate eIPSCs, depending on the concentration used. Our results showed that low concentration of ATPA had no effect on the amplitude of eIPSCs [0.1 μM ATPA, 96.3% ± 4.9%, $n = 6$, $p = 0.56$; 0.3 μM ATPA, 112.6% ± 7.4%, $n = 8$, $p = 0.18$, Fig. 2(C)]. At a high concentration (3 μM), ATPA significantly increased the amplitude of eIPSCs [191.2% ± 35.7%, $n = 12$, $p < 0.01$, Fig. 2(A–C)]. However, 10 μM ATPA significantly decreased eIPSCs [78.8% ± 9.0%, $n = 7$, $p < 0.05$, Fig. 2(C)]. The facilitatory or inhibitory effect triggered by ATPA application on eIPSCs was reversible. We then analyzed the effect of ATPA on the paired-pulse depression. We found that 3 μM ATPA decreased paired-pulse depression from 0.58 ± 0.06 to 0.75 ± 0.15 [$n = 7$, $p < 0.05$, Fig. 2(D)]. However, at concentrations of 0.1, 0.3, or 10 μM, ATPA had no significant effect on paired-pulse depression in the ACC pyramidal neurons.

Activation of GluR5 Kainate Receptors Regulates GABA Release

To further study the mechanism underlying modulation of GABAergic synaptic transmission by GluR5, we then tested the effect of ATPA on spontaneous inhibitory synaptic currents (sIPSCs) in ACC pyramidal neurons. We found that ATPA increased sIPSC frequency and amplitude in a concentration-dependent manner. The frequency of sIPSCs was significantly increased to 317.2% ± 54.4% ($n = 5$, $p < 0.05$) and 543.6% ± 115.1% ($n = 5$, $p < 0.05$) at 3 and 10 μM ATPA, respectively [Fig. 3(A,B)]. No significant effect was observed for 0.3 μM ATPA [131.5% ± 20.4%, $n = 5$, $p = 0.1$, Fig. 3(A,B)]. The mean amplitude of sIPSCs was also significantly increased at 10 μM ATPA (177.6% ± 34.0%, $n = 5$, $p < 0.05$) [Fig. 3(A,D)]. This facilitation was reversible after the washout of ATPA.

In the presence of non-NMDA receptor antagonist, CNQX (20 μM, $n = 6$), or selective GluR5 antagonist, LY293558 (30 μM, $n = 5$), the facilitatory effect of ATPA (3 μM) was completely abolished in the ACC neurons, indicating that the effect of ATPA is mediated through GluR5 [Fig. 3(C)]. Consistently, no effect of ATPA on sIPSC frequency or amplitude was found in $GluR5^{-/-}$ mice [$n = 5$, Fig. 3(C)]. These results indicate that GluR5 kainate receptor mediates ATPA-induced facilitation of GABA release.

Increase of GABA Release by GluR5 Activation Is Dependent on Action Potential and Voltage-Dependent Ca^{2+} Channels

Modulation of GABA release by kainate receptors has been studied for years, and different mechanisms were reported in different brain regions (Rodriguez-Moreno et al., 1997; Frerking et al., 1998; Frerking and Nicoll, 2000; Kerchner et al., 2001a, 2002; Semyanov and Kullmann, 2001; Braga et al., 2003; Lerma, 2003). To investigate whether presynaptic

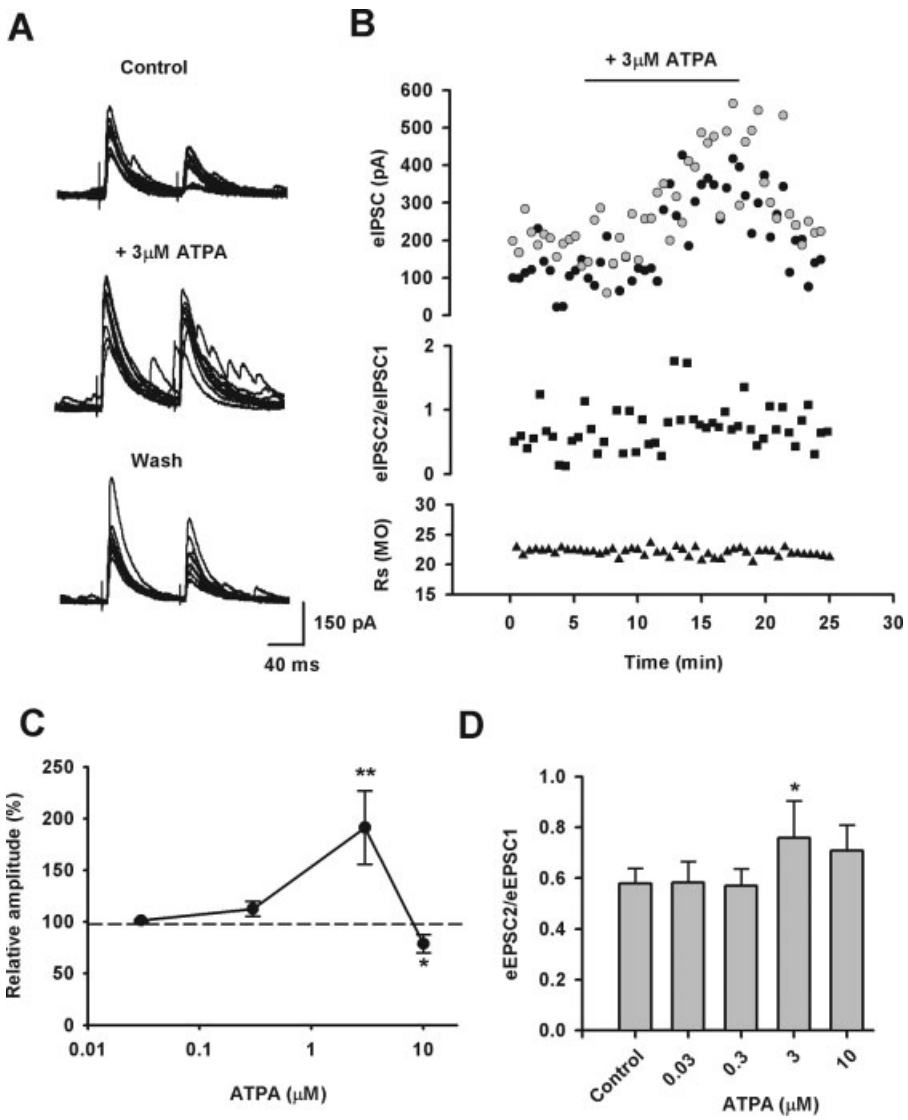

Figure 2 Biphasic modulation of ATPA on eIPSCs. A. Typical traces showed paired (100-ms interval) local stimulation-induced eIPSCs and paired-pulse depression, which are reversibly affected by ATPA (3 μM). B. Time course plot of eIPSC amplitude, pair-pulse depression (eIPSC2/eIPSC1), and input resistance in the neurons shown in (A). C. Concentration-dependent modulation of eIPSCs by ATPA. While 3 μM ATPA ($n = 12$) significantly enhanced eIPSCs, 10 μM ATPA ($n = 7$) decreased eIPSCs ($n = 7$). D. The effect of ATPA on the paired-pulse depression. Note that 3 μM ATPA significantly decreased the paired-pulse depression. * indicates a significant difference from control without treatment of ATPA.

GluR5 is involved in regulating GABA release in the ACC, we first studied the modulation of ATPA on miniature inhibitory synaptic currents (mIPSCs), which were recorded in the presence of tetrodotoxin (TTX, 1 μM) to block neuronal firing. Our results showed that ATPA (3 μM) has no effect on either frequency (98.4% $\pm$ 18.3% of control, $n = 7$, $p = 0.26$) or amplitude (101.3% $\pm$ 10.3% of control, $n = 7$, $p = 0.69$) of mIPSCs (Fig. 4). These results suggest that the facilitatory effect of ATPA on GABAergic transmission was not due to an enhancement of presynaptic GABA release or postsynaptic $GABA_A$ receptor function, but rather associated with triggering action potentials in interneurons.

Action potential-induced GABA release involves the external Ca^{2+} and voltage-dependent Ca^{2+} channels (VDCC). To confirm the idea, the effect of Cd^{2+} (100 μM), a general VDCC blocker, on ATPA-

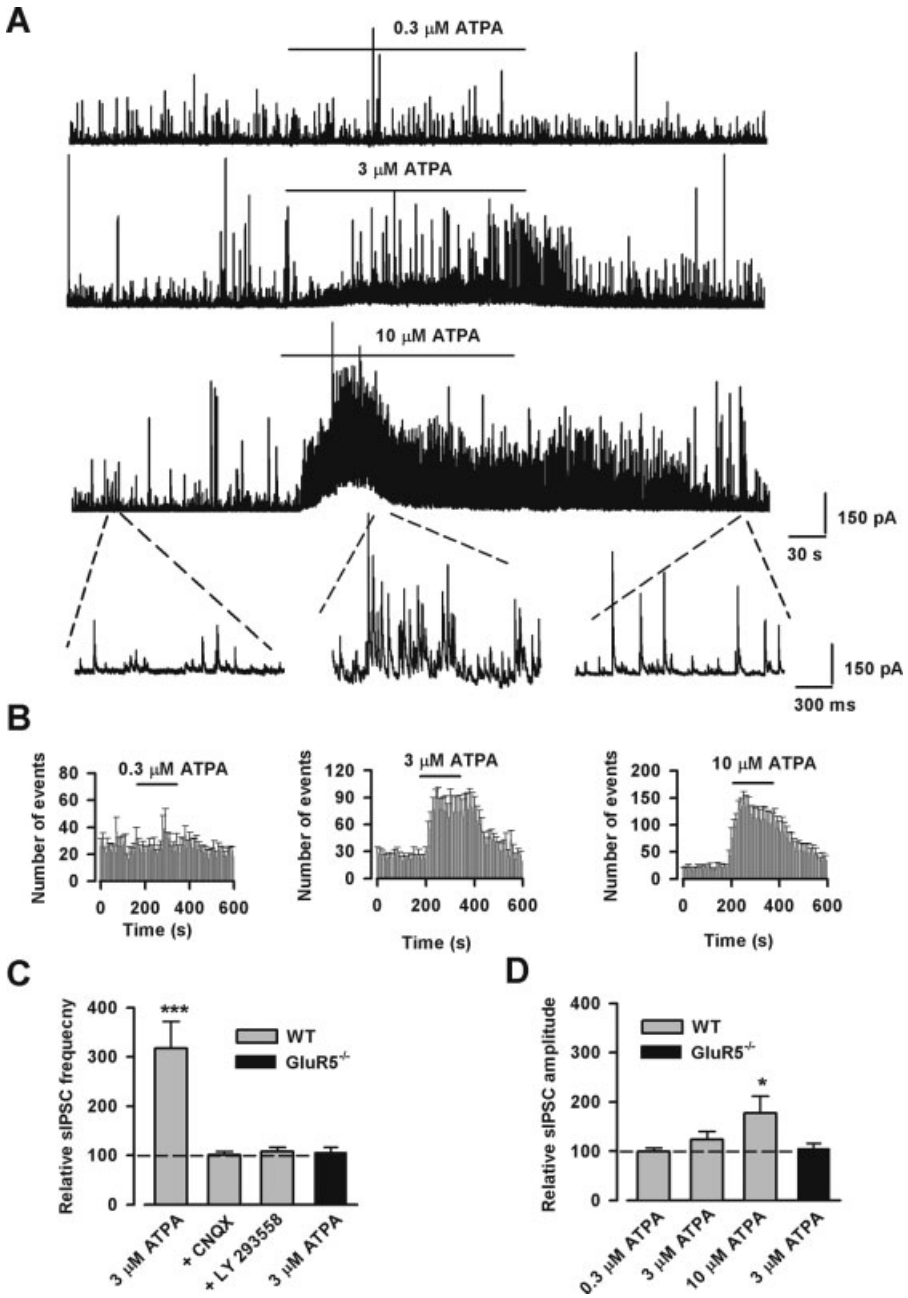

Figure 3 ATPA concentration-dependently and reversibly increased sIPSC frequency and amplitude. A. A representative example of ATPA (0.3 μM, 3 μM, 10 μM) modulation of sIPSCs in a pyramidal neuron. In the bottom, three separated traces are presented at an expanded scale. B. Time course for the ATPA-induced enhancement of sIPSC frequency at concentration of 0.3 μM ($n = 5$), 3 μM ($n = 5$), 10 μM ($n = 5$). Note the facilitatory effect of ATPA is reversible. C. The effect of ATPA (3 μM) was absent in GluR5$^{-/-}$ mice ($n = 5$), and could be blocked by CNQX (20 μM, $n = 6$) or LY 293558 (30 μM, $n = 5$), suggesting that the ATPA's action is mediated by GluR5. D. The effect of ATPA on sIPSC amplitude in wild-type and GluR5$^{-/-}$ mice.

induced facilitation of sIPSC frequency was tested. As shown in Figure 5(A,C), ATPA action on sIPSC was completely blocked in the presence of Cd^{2+} (frequency, 97.9% ± 2.3% of control, $n = 5$, $p = 0.17$; amplitude, 95.5% ± 1.3% of control, $n = 5$, $p = 0.07$). Finally, we examined the effect of Ca^{2+}-free external solution on ATPA-induced facilitation of sIPSC frequency. We found that the ATPA

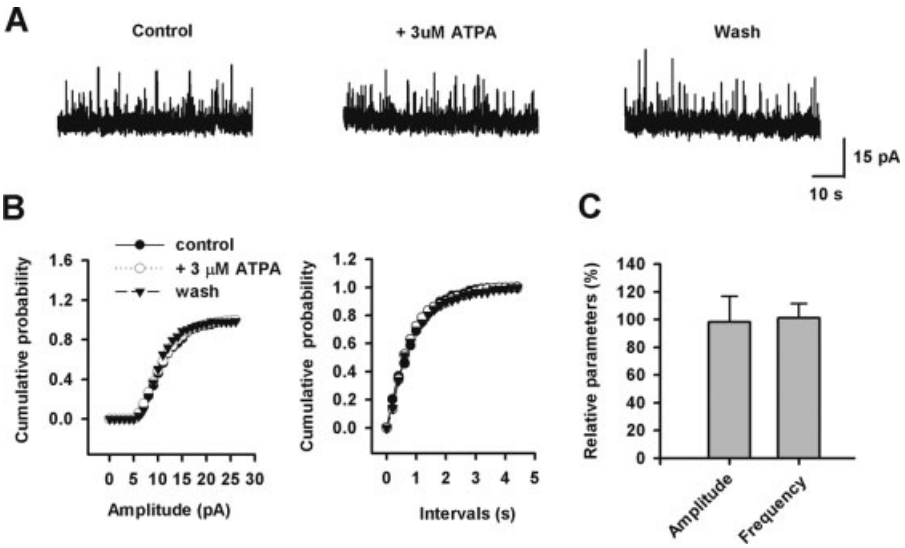

Figure 4 ATPA had little effect on mIPSCs. A. Typical examples showing the effect of ATPA (3 μM) on mIPSCs in the presence of TTX. B. Cumulative probability plot for mIPSC amplitude and inter-event intervals before, during, or after perfusion of ATPA in the neuron shown in (A). C. Statistical results showed no effect of ATPA on mIPSC amplitude or frequency ($n = 7$).

has no significant effect on either frequency or amplitude of sIPSC in the absence of Ca^{2+} in external ACSF [Fig. 5(B,D)]. Taken together, these results indicate that ATPA-induced facilitation of GABA release is dependent on action potentials and mediated by Ca^{2+} influx via VDCC activation.

GluR5 Modulation of GABAergic But Not Glutamatergic Synaptic Transmission in the ACC

Modulation of glutamatergic neurotransmission by kainate receptors has been reported in the hippocampus (Vignes et al., 1998; Contractor et al., 2000; Schmitz et al., 2000) and spinal dorsal horn (Kerchner et al., 2001b, 2002; Youn and Randic, 2004). We want to know whether GluR5 also facilitates glutamate other than GABA release in the ACC. At holding potential of −70 mV, spontaneous excitatory synaptic currents (sEPSCs) were recorded in the presence of picrotoxin (100 μM), an antagonist of $GABA_A$ receptors. In the ACC pyramidal neurons, ATPA (3 μM) has little effect on the frequency of sEPSCs [100.6% ± 8.5% of control, $n = 6$, $p = 0.92$, Fig. 6(A,C)]. Likewise, ATPA showed no effect on sEPSC amplitude (95.1% ± 2.0% of control, $n = 6$, $p = 0.08$). We also studied the ATPA effect on the typical fast-spiking interneurons. Our results showed that ATPA did not affect either frequency or amplitude of sEPSCs in interneurons [Fig. 6(B,D)]. However, during the perfusion of ATPA, we found substantially larger sustained inward current in interneurons than in pyramidal neurons. Significant differences were found for the ATPA-induced current densities when comparing interneurons and pyramidal neurons (interneuron: 0.91 ± 0.09 pA/pF, $n = 5$; pyramidal neurons: 0.06 ± 0.02 pA/pF, $n = 6$; $p < 0.001$) [Fig. 6(E)]. These results indicate the selective modulation of GluR5 on GABA but not glutamate release. This selectivity may be due to the less expression of functional GluR5 in pyramidal neurons compared to interneurons.

Modulation of GABA Release and Tonic GABA Current by Endogenous Activation of GluR5

Although the aforementioned results directly show that GluR5 activation by exogenous agonist facilitates GABA release, it is still unknown whether endogenous activation of GluR5 has similar function in the ACC. To address this question, both selective GluR5 antagonist, LY293558, and $GluR5^{-/-}$ mice were used. First, we found that perfusion of LY293558 (30 μM) significantly reduced the frequency of sIPSCs in ACC pyramidal neurons (73.8% ± 4.5% of control, $n = 5$, $p < 0.05$, Fig. 7). However, the amplitude of sIPSCs was unaffected by LY293558 (107.9% ± 5.1% of control, $n = 5$, $p = 0.36$, Fig. 7). Moreover, LY293558 has no effect on either

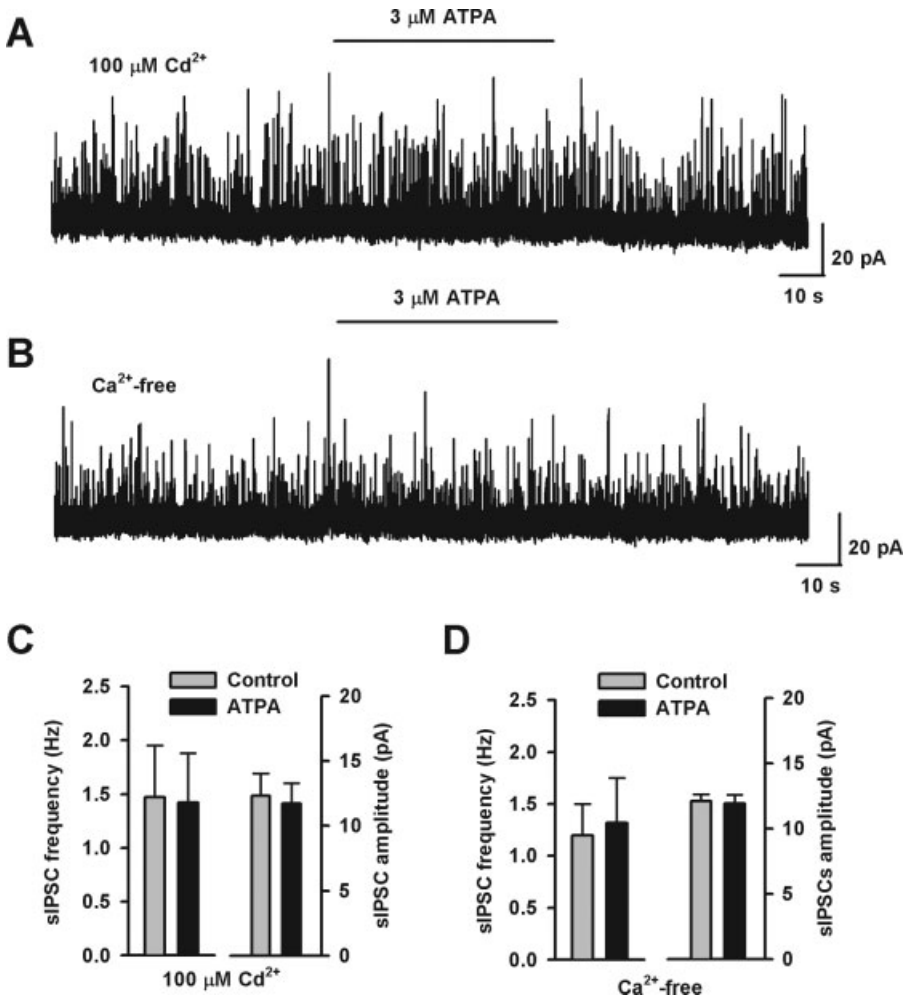

Figure 5 The ATPA-induced sIPSC facilitation is blocked by Cd^{2+} or Ca^{2+}-free external solution. A,B. Typical trace showing the effect of ATPA (3 μM) on sIPSCs in the presence of Cd^{2+}- (100 μM) or in a Ca^{2+}-free external solution. C,D. Statistical results showed that the effect of ATPA on sIPSC frequency or amplitude was abolished in 100 μM Cd^{2+} ($n = 5$) or in Ca^{2+}-free external solution ($n = 5$).

frequency (96.6% ± 6.4% control, $n = 6$, $p = 0.50$) or amplitude (97.1% ± 4.9% control, $n = 6$, $p = 0.51$) of sIPSCs in GluR5$^{-/-}$ mice. Second, we compared the sIPSC frequency in the wild-type and GluR5$^{-/-}$ mice. We found that the sIPSC frequency in the knock-out mice was significantly lower than that in wild-type mice [wild-type, 2.9 ± 0.2 Hz, $n = 15$; GluR5$^{-/-}$ 2.4 ± 0.1 Hz, $n = 12$, $p < 0.05$, Fig. 8(A,B)]. Therefore, these results indicate deletion of GluR5 or block GluR5 function decrease GABA release, suggesting that the endogenous activation of GluR5 may facilitate GABAergic transmission.

Presynaptic GABA release participates in the generation of tonic GABA current (Soltesz et al., 1995; Salin and Prince, 1996), a persistent form of GABAergic conductance that results from the activation of $GABA_A$ receptors by a low concentration of extracellular GABA (Mody, 2001; Farrant and Nusser, 2005). We examined whether GluR5 modulation on GABA release could affect tonic GABA current in the ACC. First, we compared tonic GABA currents in wild-type and GluR5$^{-/-}$ mice. At a holding potential of 10 mV, background tonic GABA current was revealed after the application of a selective competitive $GABA_A$ receptor antagonist, bicuculline (10 μM). As shown in Figure 8(A), bath application of bicuculline consistently caused an inward current by a shift in the holding current in wild-type mice (−15.1 ± 1.9 pA, $n = 11$). Conversely, tonic GABA currents were significantly decreased in GluR5$^{-/-}$ mice (−6.1 ± 1.1 pA, $n = 10$, $p < 0.01$). Second, we studied the tonic GABA current induced by ATPA,

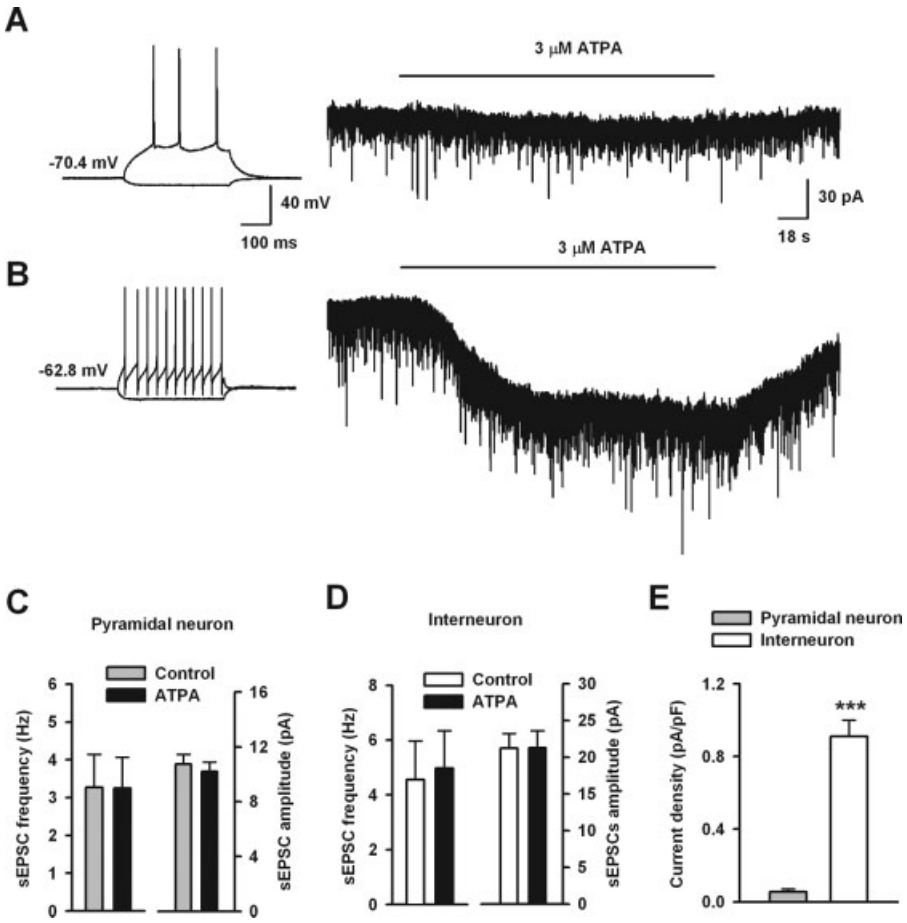

Figure 6 ATPA had no effect on sEPSCs in either pyramidal neurons or interneurons. A. Typical traces showing the firing properties of a pyramidal neuron in response to current steps that ranged from −50 to 100 pA within 400 ms (left) and the effect of ATPA (3 μM) on sEPSCs in the same neuron (right). B. Typical traces showing the fast-spiking properties of an interneuron in response to current steps that ranged from −50 to 50 pA within 400 ms (left) and the effect of ATPA (3 μM) on sEPSCs in the same neuron (right). C,D. Statistical results showed no effect of ATPA on sEPSC frequency or amplitude in pyramidal neurons ($n = 6$) or interneurons ($n = 5$). E. ATPA-induced current densities are significantly larger in interneurons ($n = 5$) than in pyramidal neurons ($n = 6$).

since ATPA could robustly increase synaptic GABA release. We found that ATPA (3 μM) induced an outward current by a shift in the holding current in wild-type mice (16.2 ± 3.1 pA, $n = 8$). However, no outward tonic GABA current was found in the GluR5$^{-/-}$ mice by ATPA (0.64 ± 1.1 pA, $n = 6$, $p < 0.01$). Taken together, these results indicate that the exogenous and endogenous activation of GluR5 could increase tonic GABA current in the ACC.

DISCUSSION

In the present study, we investigated GluR5 kainate receptor modulation on synaptic transmission in the ACC from adult mice, using pharmacological tools as well as GluR5$^{-/-}$ mice. Our results showed that exogenous or endogenous activation of GluR5 facilitates GABA release and tonic GABA current in ACC pyramidal neurons. To our knowledge, this is the first study to directly show that endogenous GluR5 activation contributes to tonic GABA current. Second, somatodendritic GluR5 mediated action potential-dependent GABA release in the ACC is different from previous studies in spinal cord, where presynaptic GluR5 is involved (Kerchner et al., 2001a, 2002), indicating region-specific modulation of GABAergic transmission by GluR5. Third, we observed the preferential modulation of GABAergic, but not glutamatergic transmission by GluR5 activation, suggesting

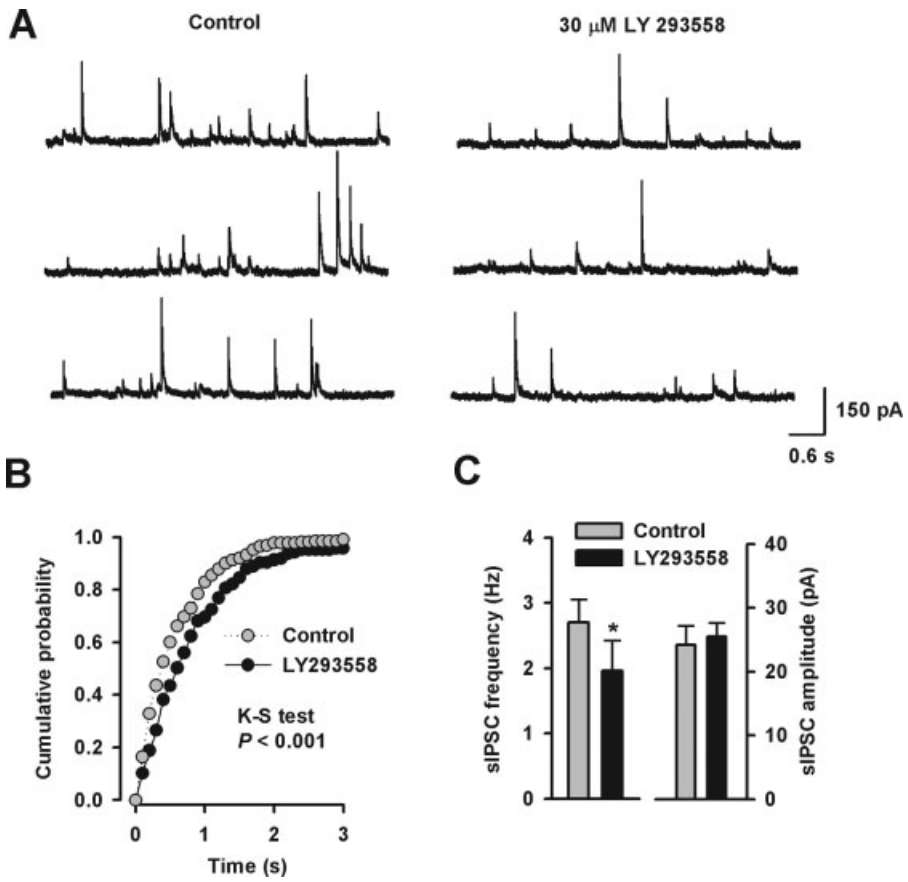

Figure 7 GluR5 antagonist inhibited the frequency of sIPSCs. A. Typical samples showing sIPSCs before and during perfusion of LY293558 (30 μM). B. Cumulative probability plot for sIPSC frequency before or during perfusion of LY293558 in the neuron shown in (A). C. Statistical results showed that LY293558 inhibited frequency but not amplitude of sIPSCs ($n = 5$).

the selective action of GluR5 on different synapses in the ACC from adult mice.

Mechanisms of Modulation of GABA Release by GluR5 in the ACC

GluR5 has been shown to be involved in the GABA release in hippocampus (Cossart et al., 1998; Bureau et al., 1999; Mulle et al., 2000), basolateral amygdala (Braga et al., 2003), motor cortex (Ali et al., 2001), and spinal dorsal horn (Kerchner et al., 2001a, 2002). However, the mechanism of GluR5 modulation of GABA release is not consistent. In the present study, we investigated whether or not GluR5-containing kainate receptors modulate presynaptic GABA release in the ACC.

To address whether GluR5 is located on presynaptic terminals or somatodendrites, we studied three aspects of modulation on GABAergic transmission by GluR5 activation in the ACC. First, we tested the effect of ATPA on the sIPSCs. Our results showed that ATPA facilitated sIPSC frequency in a concentration-dependent manner. Second, we tested the effect of ATPA on the mIPSCs. Our results showed that ATPA had little effect on either frequency or amplitude of mIPSCs. Third, we tested ATPA-induced facilitation of GABA release in the presence of Cd^{2+}- or Ca^{2+}-free external solution. Our results showed that the effect of ATPA was blocked in both conditions. Based on these findings, we proposed that GluR5 may be located somatodendritcically rather than on presynaptic terminals in interneurons in the ACC. Therefore, the activation of GluR5 is predicted to induce depolarization and action potential generation, and the subsequent activation of VDCCs in interneurons, triggering the robust GABA release. Consistent with this idea, activation of GluR5 by ATPA evoked substantial inward currents in fast-spiking interneurons.

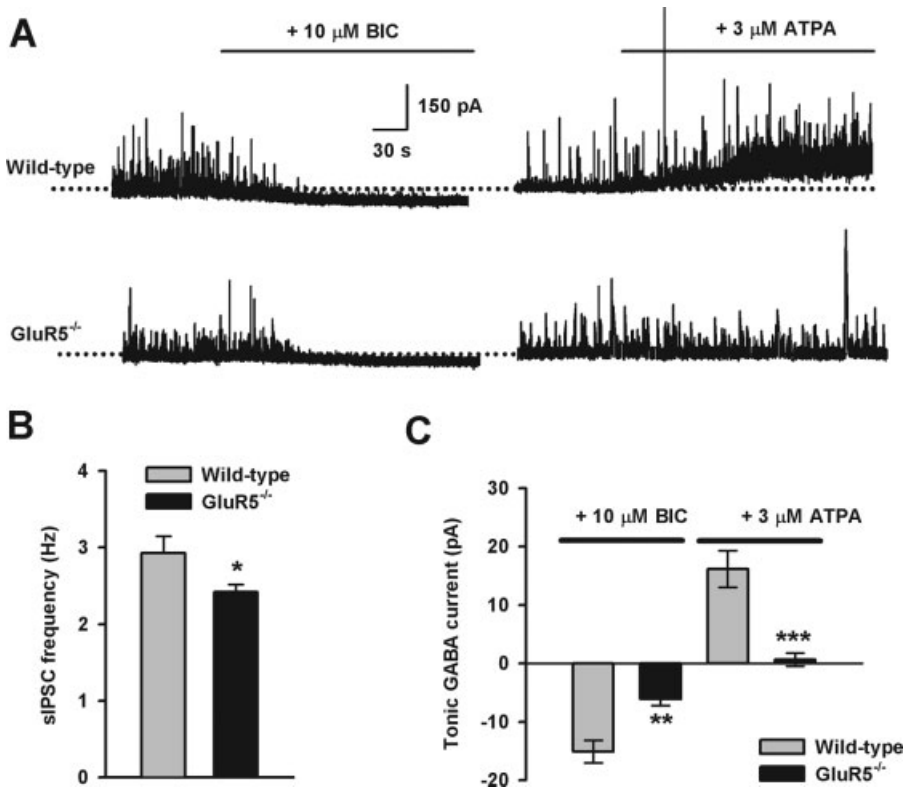

Figure 8 Modulation of tonic GABA currents by GluR5. A. Typical samples showing tonic GABA current revealed by adding bicuculline (BIC, 10 μM) in the wild-type and GluR5$^{-/-}$ mice (left). The opposite shift in tonic current was induced by perfusion of ATPA (3 μM) (right). B. Compared with that in wild-type mice ($n = 15$), frequency of sIPSCs was reduced in ACC pyramidal neurons from GluR5$^{-/-}$ mice ($n = 12$). C. Statistical results showed that tonic GABA current was reduced in GluR5$^{-/-}$ mice ($n = 10$) than in wild-type mice ($n = 11$). The ATPA induced the increase of tonic current in wild-type mice ($n = 8$), but the current was abolished in the knock-out mice ($n = 6$).

Our findings suggest that GluR5 activation increases efficacy of GABAergic synapses, which may also explain the biphasic effect of ATPA on the eIPSCs. We showed that 3 μM ATPA increased eIPSC amplitude but decreased the paired-pulse depression: two seemingly controversial results. This may be due to the decreased firing threshold in interneurons in the presence of ATPA. Therefore, the same intensity of stimulation would induce more interneuron firing, and thus the increased eIPSC amplitude. The second response to paired-pulse stimulations would also recruit new interneuron firing during activation of GluR5, thereby decreasing paired-pulse depression. At 10 μM ATPA, the robust release of GABA activated postsynaptic $GABA_A$ receptors, which would shunt pyramidal neurons or cause use-dependent depression of eIPSCs. A similar mechanism has been proposed in the kainate receptor modulation of GABA release in the hippocampus (Frerking et al., 1999). However, we cannot exclude the possibility that the regulatory mechanism may involve G-proteins, which are reported to decrease the GABA release with kainate receptor activation (Rodriguez-Moreno et al., 1997).

The action potential- and Ca^{2+}-dependent modulation of GluR5 on GABA release in the ACC pyramidal neurons is similar to those reported in the hippocampal CA1 pyramidal neurons (Cossart et al., 1998; Frerking et al., 1998; Frerking et al., 1999; Mulle et al., 2000; Cossart et al., 2001; Jiang et al., 2001), but different from those reported in the spinal cord dorsal horn (Kerchner et al., 2001a, 2002) or basolateral amygdala (Braga et al., 2003), where action potential-independent presynaptic GluR5 causes direct depolarization of presynaptic terminals and the release of GABA. In hippocampus interneuron-interneuron synapses, presynaptic GluR6 may increase action potential-independent GABA release (Mulle

et al., 2000; Cossart et al., 2001). The different results of GluR5 modulation on GABA release could be attributed to the different brain regions, or different species or animal ages.

Functional Significance of GluR5 Modulation on Synaptic Transmission in the ACC

Our previous studies have shown that GluR5 is involved in mediating postsynaptic current in the ACC pyramidal neurons (Wu et al., 2005b). In the present study, we further showed that more of functional GluR5 is expressed in interneurons than that in pyramidal neurons. The result may explain the selective action of GluR5 on GABAergic but not glutamatergic neurotransmission. Moreover, the selective effect of GluR5 on GABA release suggests the overall inhibitory role of GluR5 on ACC activity (Cossart et al., 1998).

Tonic GABA current is mediated by extrasynaptic $GABA_A$ receptors, which sense extracellular GABA and is important in modulation of neuronal excitability and synaptic efficacy (Semyanov et al., 2004). It has been proposed that tonic GABA current plays a role in pathological conditions such as seizure and anxiety (Overstreet and Westbrook, 2001; Stell et al., 2003) or physiological functions such as learning and memory (Caraiscos et al., 2004). In the present study, we found that activation of GluR5 increased tonic GABA current, whereas $GluR5^{-/-}$ mice showed reduced tonic GABA current in the ACC. Considering the robust facilitation of GABA release by both exogenous and endogenous GluR5 activation, we believed that the modulation is critical for the GABA tonic current and the subsequent inhibitory drive in ACC pyramidal neurons.

GluR5 kainate receptor has been implicated in etiology of epilepsy (Ben-Ari and Cossart, 2000), chronic pain (Li et al., 1999; Ben-Ari and Cossart, 2000; Ko et al., 2005a), and anxiety (Wu et al., unpublished data). It is conceivable that inhibitory mechanisms involved GluR5-induced facilitation of GABA neurotransmission in the ACC may partially mediate the pathophysiological behavioral phenotypes, particularly chronic pain and fear memory (Calejesan et al., 2000; Wei and Zhuo, 2001; Ko et al., 2005b; Tang et al., 2005). Thus, the present study provides useful information on the synaptic basis for GluR5 in ACC-related functions.

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