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Short-term activation of synaptic transmission by acute KCl application significantly reduces somatic A-type K^+ current

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Abstract A-type K^+ (I_A) channels are transiently activated in the suprathreshold membrane potential and then rapidly inactivated. These channels play roles to control the neuronal excitability in pyramidal neurons in hippocampi. We here electrophysiologically tested if regulatory functions of I_A channels might be targeted by acute activation of glutamatergic synaptic transmission in cultured hippocampal neurons (DIV 6∼8). The application of high KCl in recording solutions (10 mM, 2 min) to increase presynaptic glutamate release, significantly reduced the peak of somatic I_A without changes of gating kinetics. This indicates that neuronal excitation induced by the enhancement of synaptic transmission may process with distinctive signaling cascades to affect voltage-dependent ion channels in hippocampal neurons. Therefore, it is possible that short-lasting enhancement of synaptic transmission is functionally restricted in local synapses without effects on intracellular signaling cascades affecting a whole neuron, efficiently and rapidly enhancing synaptic functions in hippocampal network.

Key words: A-type K^+ channel, Glutamate, Transient current, Hippocampus, Gating kinetics, Synaptic transmission

INTRODUCTION

Voltage-dependent A-type K^+ (I_A) channels mediate transient K^+ currents and play critical roles in regulating the excitability of neurons by preventing membrane depolarization and providing repolarization. Depending on the frequency of repetitive spikes firing, I_A channels can also regulate the interspike interval and the duration of action potential (AP) in CNS neurons[1,2]. I_A channel is a transient outward K^+ current that activates rapidly upon suprathreshold, inactivates quickly and recovers fast from inactivation[3]. Electrophysiological studies have indicated that somatodendritic I_A modulates subthreshold dendritic signal integration[2,4].

Activity-dependent changes in synaptic function such as long-term potentiation (LTP) and long-term depression (LTD) have been widely considered as mechanisms for learning and memory[5]. In addition to these synaptic changes, activity-dependent change in intrinsic excitability has been suggested to be the other side of the engram for learning and memory[6]. Activity-dependent plasticity of intrinsic excitability in postsynaptic neurons targets on modulation of Na^+, K^+ and Ca^{2+} channels. Recently, several studies have reported the activity-dependent modulation of A-type currents and one of subunits of voltage-dependent K^+ (Kv4.2) channels in hippocampal neurons. Induction of LTP causes a hyperpolarizing shift in the inactivation curve of I_A of hippocampal neurons from adult rat[7]. This shift has the effect of increasing local dendritic excitability, enhancing AP backpropagation. However, LTP also causes a decrease of AP firing, threshold and a global phenomenon[8]. Increasing neuronal activity results in a redistribution of Kv4.2 channels out of spines[9].

Glutamate acts as a major excitatory neurotransmitter in the CNS and plays a critical role in neuronal plasticity[10]. Several studies have described the rapid redistribution of α-Amino-3-hydroxy-5-methylisoxazole-4-pro-

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pionic acid hydrate (AMPA) receptors and Kv2.1 channels[11]. Brief glutamate exposure leads to reduction of total Kv4.2 levels and Kv4.2 clusters. In addition, the inactivation curve of I_A is shifted toward more hyperpolarized potentials following glutamate treatment[12]. However, there is no electrophysiological evidence to show the role of down-regulation of I_A channels in short-lasting activation of glutamatergic transmission in hippocampi.

In the present study, we electrophysiologically examined the down-regulation of I_A channels by endogenous glutamate which was acutely released by applying KCl. Results demonstrate that short-lasting enhancement of glutamatergic transmission directly reduced the amplitude of I_A while no significant changes in gating kinetics were observed. This indicates that shortly instant activation of synaptic transmission may rapidly potentiate local membrane excitability via down-regulating I_A channels

MATERIALS AND METHODS

Animals

Sprague-Dawley pregnant rats of 6∼8 weeks were housed in a temperature (25 ± 3°C) and humidity (50 ± 10%) controlled room on a 12 h light/dark cycle with pellet and water *ad libitum*. Experiments and all procedures with animals were performed in accordance with the Animal Care and Use Committee of Jeju National University.

Primary cultures of hippocampal neurons

Hippocampal primary cultures were prepared from embryonic day 21 Sprague-Dawley rats. The embryonic rats were removed from deeply anesthetized rats, then transferred ice-cold normal tyrode solution containing the following (in mM) : 140 NaCl, 5.4 KCl, 2.3 $MgCl_2 \cdot 6H_2O$, 10 HEPES, 5 D(+)-glucose, pH 7.4 with NaOH. Embryos were removed from uterine membranes and placentae, dissected head, and then washout by normal tyrode. For hippocampal dissection, each head was transferred to the lid of a 100 mm-diameter dish containing ice-cold normal tyrode and positioned under microscope in culture room. The skin covering the skull was peeled away and the skull opened up using two pairs of fine-tipped forceps and scissors. Dissected hippocampi were transferred to ice-cold plating medium containing MEM (Sigma-Aldrich, St Louis, MO, USA) until the required number of heads has been dissected, then triturated using 1 mL pipette. After triturating hippocampi, cells were resuspended in 5 mL pre-warmed (37°C) plating medium containing MEM (Sigma-Aldrich) then quantified by obtaining and average of two tetragons from a hemocytometer. Then cells were seeded on 12 mm-diameter glass cover slips (Fisher Scientific, Pittsburgh, PA, USA) coated with poly-L-lysine (Sigma-Aldrich) at a density of 4.5×10^4 cells/well and put into a incubator (Nuaire, Plymouth, MN, USA) maintained at 37°C in 95% air and 5% CO_2. A day after seeding whole plating medium was changed to Neurobasal (Sigma-Aldrich) medium containing B-27 (Invitrogen, Carlsbad, CA, USA), and a half medium was changed once DIV5.

Culture treatments

Acute KCl protocol. 10 mM KCl treats pre-warmed (37°C) recording solution for 2 min. Then cells were washed with normal recording solution until resting membrane potential restoration.

Electrophysiology

For patch clamp recordings, coverslips containing DIV 6∼8 hippocampal primary culture neurons were transferred to a recording chamber with a continuous flow of recording solution containing the following (in mM) : 145 NaCl, 5 KCl, 2 $CaCl_2$, 1.3 $MgCl_2$, 10 HEPES, 10 glucose, pH 7.4 with NaOH, when bubbled with 95% O_2 and 5% CO_2. For recording acute glutamate groups, 100 μM glycine, 5 μM glutamate were included in the $MgCl_2$-free recording solution to treat neurons before recording. The patch pipettes (10 MΩ) were filled with an internal solution containing the following (in mM) : 20 KCl, 125 K D-gluconate, 4 NaCl, 10 HEPES, 0.5 EGTA, 4 ATP, 0.3 tris GTP, 10 phosphocreatin, pH 7.2 with KOH. The osmolarity was 290∼320 mOsm. Patch pipettes were pulled from borosilicate glass (Warner Instruments, Hamden, CT, USA) with the PP-830 electrode puller (Narishige, Tokyo, Japan). Whole-cell capacitances was 5∼14 pF. Series resistances (5∼30 MΩ) were continuously monitored by digital phosphor oscilloscope TDS3012 (Tectronix, Beaverton, OR, USA). Whole-cell currents were recorded in voltage-clamp mode from pyramidal neurons using Axopatch 200B amplifier and Digidata 1322A (Axon Instruments, Union City, CA, USA), low-pass filtered 5 kHz, and digitized at 10 kHz. Data were acquired and stored using pClamp 8 (Axon Instruments).

Data analysis

All patch clamp recordings were analyzed using clampfit 8.2 (Molecular Devices, Sunnyvale, CA, USA), Igor pro 6.0 (WaveMetrics, Lake Oswego, OR, USA) and Mi-

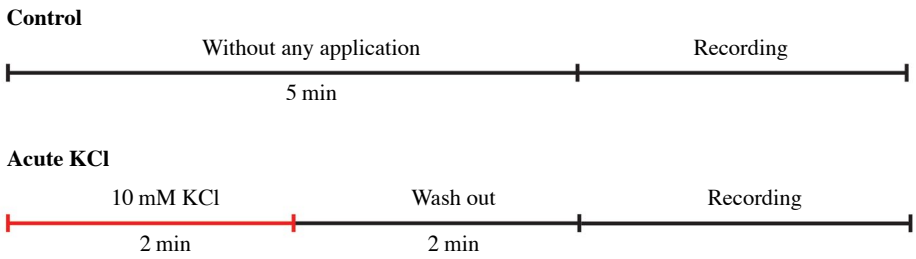

Figure 1. Experimental protocols of control and acute KCl groups.

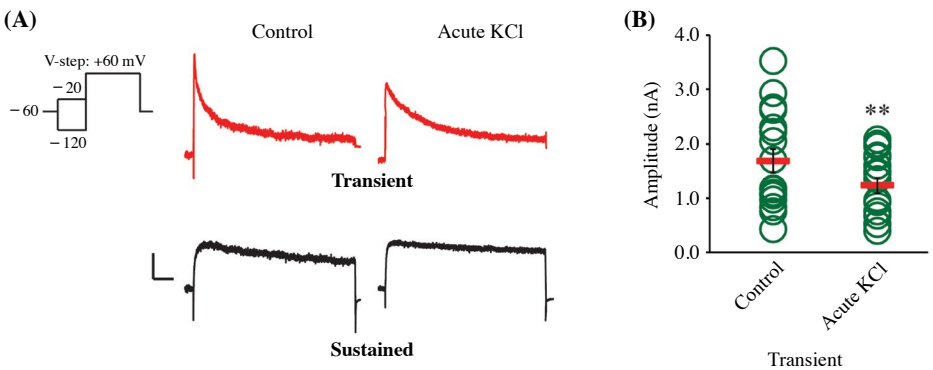

Figure 2. Acute KCl application significantly reduces somatic I_A peak amplitude of hippocampal neurons. A. Example traces of I_A currents recorded in hippocampal neurons with and without acute KCl application. Left traces are input pulses for recording transient currents. Scale bars: 0.5 nA, 50 ms. B. The averaged values of I_A peak with individual values with and without acute KCl application. $^{**}p<0.01$ by using student's t-test (unpaired).

crosoft Exel 2007 (Microsoft, Redmond, WA, USA). Results were expressed as the mean ± SEM from at least ten independent biological samples. Statistical significance was evaluated using Student's t test (unpaired). *P* Values were represented with 0.05 or 0.01.

RESULTS

Whole-cell patch-clamp recordings from cultured pyramidal neurons, in the presence of tetrodotoxin to block voltage-dependent Na^+ channels, revealed a large outward current composed of a rapidly inactivating component along with sustained or slowly inactivating component. I_A was routinely isolated from the sustained current by the voltage protocol. To examine down-regulation of I_A channel by short-term endogenous glutamate in culture condition, 10 mM KCl was added to recording solution for 2 min to induce depolarization of presynaptic neurons, and then high K^+ ions was washed during resting membrane potential (RMP) restoration (Fig. 1).

The voltage dependence of I_A peak amplitude was studied by holding the membrane potential of neurons from −120 mV to 60 mV (Fig. 2A, left trace). And a 200 ms prepulse to −20 mV inactivated the I_A channels, leaving the sustained current alone. The averaged peak amplitude

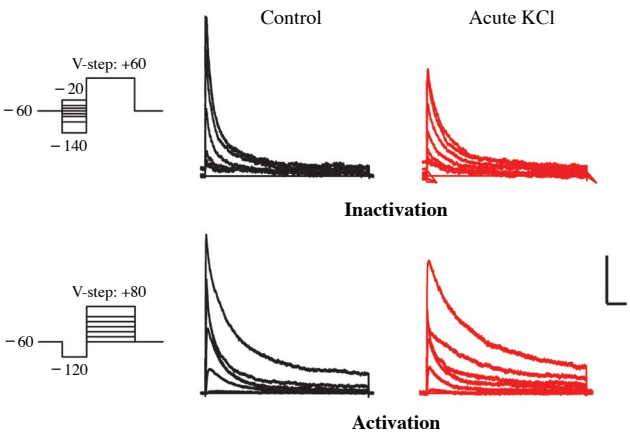

Figure 3. Example traces of inactivation and activation properties. Left traces are input pulses for recording kinetic properties. Acute KCl application reduces the amplitude of I_A by releasing endogenous glutamate. Scale bars: 0.5 nA, 50 ms.

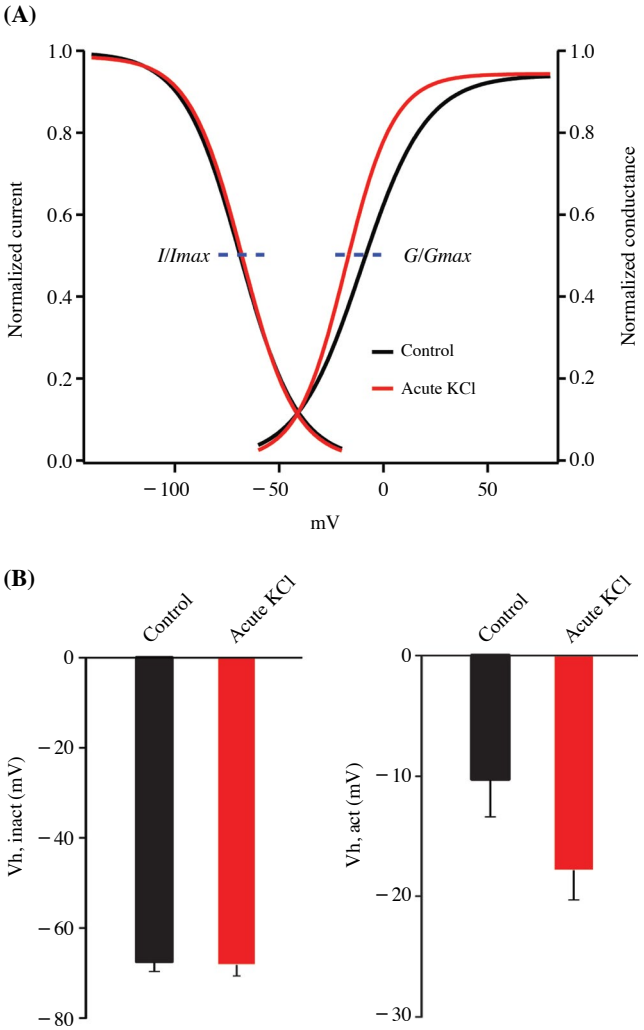

Figure 4. Kinetic properties of I_A channels are unaltered by acute KCl in hippocampal neurons. A. Inactivation and activation curves of I_A channels. This figure shows the effect of acute KCl to induce the hyperpolarizing shift of activation properties. B. The averaged V_h values of inactivation and activation curves of I_A channels with and without acute KCl.

of I_A before conditioning stimulation (control) is 1.82 ± 0.13 nA (Fig. 2A, middle, Fig. 2B). Applying acute KCl application (10 mM, 2 min), endogenous glutamate significantly reduced peak amplitude of I_A (Fig. 2A, right). The I_A was decreased 30% in acute KCl group, compared with control group. The peak amplitude of I_A is 1.24 ± 0.14 nA in this group (Fig. 2B, $p = 0.002$, compared with control group).

Figure 3 shows the kinetic change of I_A by endogenous glutamate in hippocampal neurons. Both inactivation and activation properties of I_A using different voltage protocols were measured in control group and in acute KCl group using whole-cell patch-clamp. First, the voltage dependence of inactivation was assessed by measuring amplitudes of currents evoked by a +60 mV test pulse (400 ms), after a 200 ms prepulse followed by main pulses between −140 and −20 mV with 10~40 mV steps (Fig. 3, upper left trace). Second, the voltage dependence of activation was assessed by measuring amplitudes of currents evoked by pulses (400 ms) between −60~+80 mV with 10~20 steps after a 200 ms prepulse followed by a main pulse of −120 mV (Fig. 3, lower left trace).

The I_A voltage range of inactivation curve was unaltered by acute KCl (Fig. 4A). In contrast to inactivation curve, the I_A voltage range of activation curve is shifted approximately 7 mV to the hyperpolarized direction after the short-term release of endogenous glutamate by acute KCl (Fig. 4A), but there is no significance ($p = 0.06$, compared with control). Figure 4B shows half I_A voltage values (V_h) of inactivation (control $V_h = -67.69 \pm 1.96$ mV; acute KCl $V_h = -68.07 \pm 2.40$ mV, $p = 0.90$, compared with control) and activation (control $V_h = -10.32 \pm 3.03$ mV; acute KCl $V_h = -17.85 \pm 2.45$ mV) curves.

This finding suggests that the short-term release of endogenous glutamate by high K^+ concentration in recording solution altered I_A expression in hippocampal neurons. However, short-term induction of endogenous glutamate is not altered inactivation and activation properties.

DISCUSSION

Voltage-dependent I_A channels in hippocampal neurons are primary regulators of membrane excitability[13]. Modulation of these channels dynamically and selectively control signals propagation through dendrites. Somatodendritic I_A play important roles in regulating suprathreshold excitability of neurons, such as the back-propagation of dendritic APs, Ca^{2+} plateau potential, AP initiation, half-width of APs and frequency-dependent AP broadening[1,4,14].

Glutamate is a principle excitatory neurotransmitter in the CNS, acting via NMDA receptors, non-NMDA (AMPA and kainite) receptors, and metabotropic glutamate receptors[15]. It has been proposed that the activation of post-synaptic NMDA receptors is required for both LTP and LTD and that Ca^{2+} influx through the activated NMDA receptors triggers a series of intracellular cascades that lead to persistent changes in the numbers and properties of post-synaptic AMPA receptors[16]. Further-

more, a recent study has found that a brief glutamate application leads to reduction of Kv4.2 channels expression levels and Kv4.2 clusters in hippocampal neurons of rat. In addition, the steady state inactivation of I_A currents is shifted toward more hyperpolarized potentials following glutamate treatment[12].

In the present study, the down-regulation of I_A channels in hippocampal neurons by activation of glutamate transmission is studied. Short-lasting activation of synaptic transmission reduced the amplitude of I_A, indicating that the expression level of somatic I_A channels may be targeted by synaptic activities of neurons for higher efficiency of excitatory postsynaptic potential (EPSP)-AP coupling. However, gating kinetics of I_A channels, inactivation and activation properties, was not affected by acute activation of synaptic glutamate release. This means that short-lasting enhancement of synaptic transmission may be functionally restricted in local synapses without any effects on intracellular signaling cascades affecting a whole neuron. Consequently, these results indicate that shortly instant activation of synaptic transmission may rapidly potentiate local membrane excitability via down-regulating I_A channels, efficiently enhancing synaptic functions in hippocampal network.

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