Study on reactive properties of voltage-dependent A-type K$^+$ channels to excitotoxicity in hippocampal neurons of rats

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흰쥐의 해마 신경세포에서 신경흥분독성에 대한

전압의존적 A-type 포타슘 채널의 반응 특성 연구

지도교수 : 정 성 철

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Study on reactive properties of voltage-dependent A-type K$^+$ channels to excitotoxicity in hippocampal neurons of rats

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(Supervised by Professor Sung-Cherl Jung)

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This thesis has been examined and approved.

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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 shape excitatory postsynaptic potentials, limit the back propagation of action potential, and prevent dendritic action potential initiation. I_A channels play roles to control the neuronal excitability in pyramidal neurons in hippocampi. We here tested if regulatory functions of I_A channels might be targeted by excitotoxic processings which are accompanied by the abnormal enhancement of synaptic inputs in dissociated hippocampal neurons (DIV 6~8). The application of high KCl in either recording solutions (10 mM, 2 min) or culture media (20 mM, 24 hours) to increase presynaptic glutamate release, significantly reduced the peak of somatic I_A with hyperpolarizing-shift of inactivation properties of channels. These changes of I_A channels are quietly different from those induced by exogenous glutamate application in either culture media (5 μM, 24 hours) or recording solution (5 μM, 5 min), which can activate both synaptic and extrasynaptic receptors. Glutamate application reduced the somatic I_A peak without any kinetic changes of I_A channels, indicating 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. These results suggest that synaptic inputs may determine the somatic membrane excitability by regulating both membrane expression and kinetic properties of I_A channels for more efficient correlations between neuronal inputs and outputs. It is also possible that neuronal processings by synaptic inputs are independent on the I_A regulations of excitotoxic effects induced by non-specific membrane excitability.

Keywords: A-type K^+ channel, glutamate, excitotoxicity, hippocampus, voltage-dependent channel

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Ⅰ. Introduction

Voltage-dependent A-type K^+ 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, A-type K^+ channels can also regulate the interspike interval and the duration of action potential (AP) in CNS neurons (Song *et al*., 1998; Kim *et al*., 2005; Khaliq and Bean., 2008). A-type K^+ channel is a transient outward K^+ current that activates rapidly upon suprathreshold, inactivates quickly and recovers fast from inactivation (Jerng *et al*., 2004). Electrophysiological studies have indicated that somatodendritic A-type currents (I_A) modulates subthreshold dendritic signal integration (Ramakers and Storm, 2002; Goldberg *et al*., 2003; Cai *et al*., 2004; Kim *et al*., 2005).

Within the *Shaker*-related Voltage-dependent K^+ (Kv) channels, four sub-families (Kv1, Kv2, Kv3 and Kv4) are present in the nervous system of diverse organisms in the animal kingdom (Salkoff *et al*., 1992; Gutman *et al*., 2003.). Although these Kv channels can produce delayed-rectifier and I_A, all members of the Kv4 subfamily (Kv4.1, Kv4.2 and Kv4.3) mediate I_A (Birnbaum *et al*., 2004; Jerng *et al*., 2004). Kv channels are composed of four α subunits that come together as either homotetrameric or heterotetrameric channels. The two transient K^+ channels Kv4.2 and Kv1.4 found in the hippocampal neurons by immunohistochemical techniques; Kv4.2 is primarily located in the soma and dendrites (Sheng *et al*., 1992; Serodio *et al*., 1994), whereas Kv1.4 is found mainly in axons and within or near the pre-synaptic terminal (Sheng *et al*., 1992, 1994; Rhodes *et al*., 1997; Cooper *et al*., 1998). Kv4.2 has also been found clustered on the postsynaptic membrane

directly opposed to the presynaptic terminal (Alonso and Widmer, 1997). This clustering is proposed to be attributable to interaction with post-synaptic density (PSD-95) via C-terminal valine-serine-alanine-leucine (VSAL) motif of Kv4.2 channel (Wong *et al.*, 2002). The Kv4.2 has six transmembrane domains (S1-S6) and N- and C- terminal cytoplasmic domains. The Kv4.2 N-terminus contains a T1 domain that mediates subfamily specification (Papazian, 1999), and also binds to auxiliary subunits (Gulbis *et al.*, 2000; Sewing et al., 1996). Kv4.2 C-terminal phosphorylation sites modulate the channel's trafficking and gating (Anderson *et al.*, 2000) and PKA phosphorylation is necessary for activity-dependent Kv4.2 internalization (Hammond *et al.*, 2008). Whole-cell A-type currents are selectively eliminated by Kv4 channels specific blocker heteropodatoxin-3 and by knockout of Kv4.2 gene (Ramakers and Storm, 2002; Chen *et al.*, 2006), demonstrating that Kv4.2 is most likely to encode somatodendritic I_A channels.

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 (Bliss and Collingridge, 1993). 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 (Zhang and Linden, 2003). 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 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 (Frick *et al.*, 2004). 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 (Charvez *et al.*, 1990). Increasing neuronal activity results in a redistribution of Kv4.2 channels out of spines (Kim *et al.*, 2007).

Glutamate acts as a major excitatory neurotransmitter in the CNS and plays a critical role in neuronal plasticity (Hardingham and Bading., 2003). Several studies have described the rapid redistribution of (±) α-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrate (AMPA) receptors (Lissin *et al*., 1999) and Kv2.1 channels (Misnou *et al*., 2004). 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 (Lei *et al*., 2008). However, there is no electrophysiological evidence to show the role of down-regulation of I_A channels in excitotoxic conditions of hippocampal neurons.

In the present study, I examined the down-regulation of Kv4.2 channels by both endo- and exogenous glutamate treatment using whole-cell patch clamp recordings of I_A. I observed that the activation of NMDA receptors contributes to the reduction of A-type K^+ current amplitude. Furthermore, selective activation of synaptic, but not extrasynaptic NMDA receptors caused the same effect as glutamate on A-type K^+ channels.

Ⅱ. Materials and Methods

1. Animals

Sprague-Dawley pregnant rats of 6~8 weeks were housed in a temperature (25 ± 3 ℃) 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.

2. 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$_2$·6H$_2$O, 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

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heads has been dissected, then triturated using 1 ml pipette. After triturating hippocampi, cells were resuspended in 5 ml pre-warmed (37 ℃) 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 ℃ 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.

3. Culture treatments

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

Overnight treatment protocol. 20 mM KCl and 5 μM glutamate were treated in culture medium and put into incubator for 24 hours. In some experiments, culture cells treat 20 mM KCl and 5 μM glutamate with DL-2-Amino-5-phosphonopentaonic acid (APV, 100 μM, 24 hours, Sigma-Aldrich) for block of NMDA receptor. And 20 mM KCl added in culture medium with (5S,10R)-(−)-5-Methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine hydrogen maleate (MK801, 25 μM, 24 hours, Tocris, Bristol, UK) for synaptic NMDA block,

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then 5 μM glutamate treats in culture medium for 24 hours.

Acute glutamate protocol. 5 μM glutamate was treated in 100 μM glycine contained recording solution (either Mg^{2+} or Mg^{2+}-free) for 5 min, then cells were washed with normal recording solution until resting membrane potential restoration. To blocked synaptic NMDA receptors, 25 μM MK801 (with 20 mM KCl) were treated in culture media for 24 hours before treated acute glutamate application.

4. 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).

5. 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 Microsoft 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.

Ⅲ. Results

1. **Short term activation of synaptic transmission by acute KCl application significantly reduces somatic I_A.**

Whole-cell patch-clamp recordings from cultured pyramidal neurons, in the presence of TTX to block voltage-dependent Na^+ channels, revealed a large outward current composed of a rapidly inactivating component along with sustained or slowly inactivating component. The A-type K^+ current 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 -20mV inactivated the A-type K^+ channels, leaving the sustained current alone. The averaged peak amplitude 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). All peak amplitude data are contained in Table 1.

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 ± 1.96 mV; acute KCl V_h = - 68.07 ± 2.40 mV, $p = 0.90$, compared with control) and activation (control V_h = - 10.32 ± 3.03 mV; acute KCl V_h = - 17.85 ± 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. All kinetic properties data are contained in Table 2.

control

| without any application | recording |

|—————————————————|———————————|

5 min

acute KCl

| 10 mM KCl | wash out | recording |

2 min   2 min
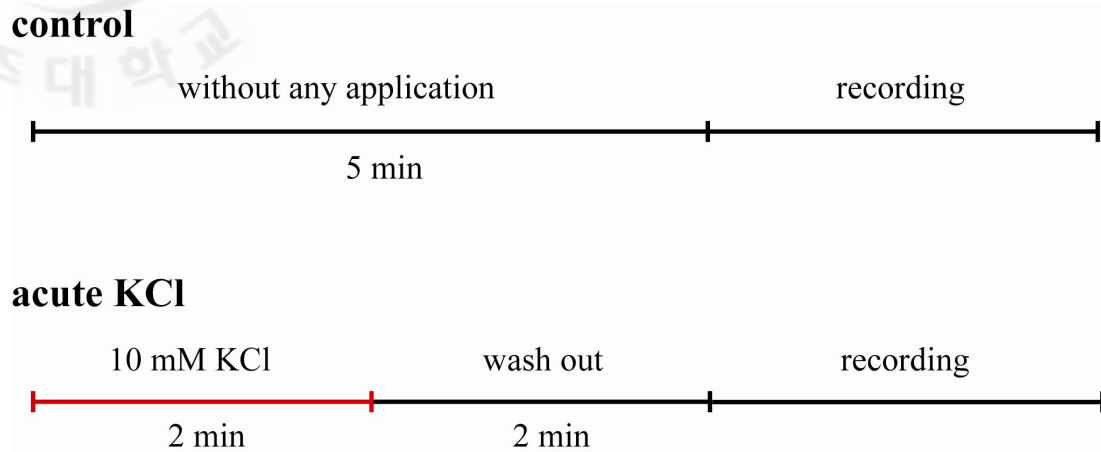
Figure 1. Experimental protocols of control and acute KCl groups.
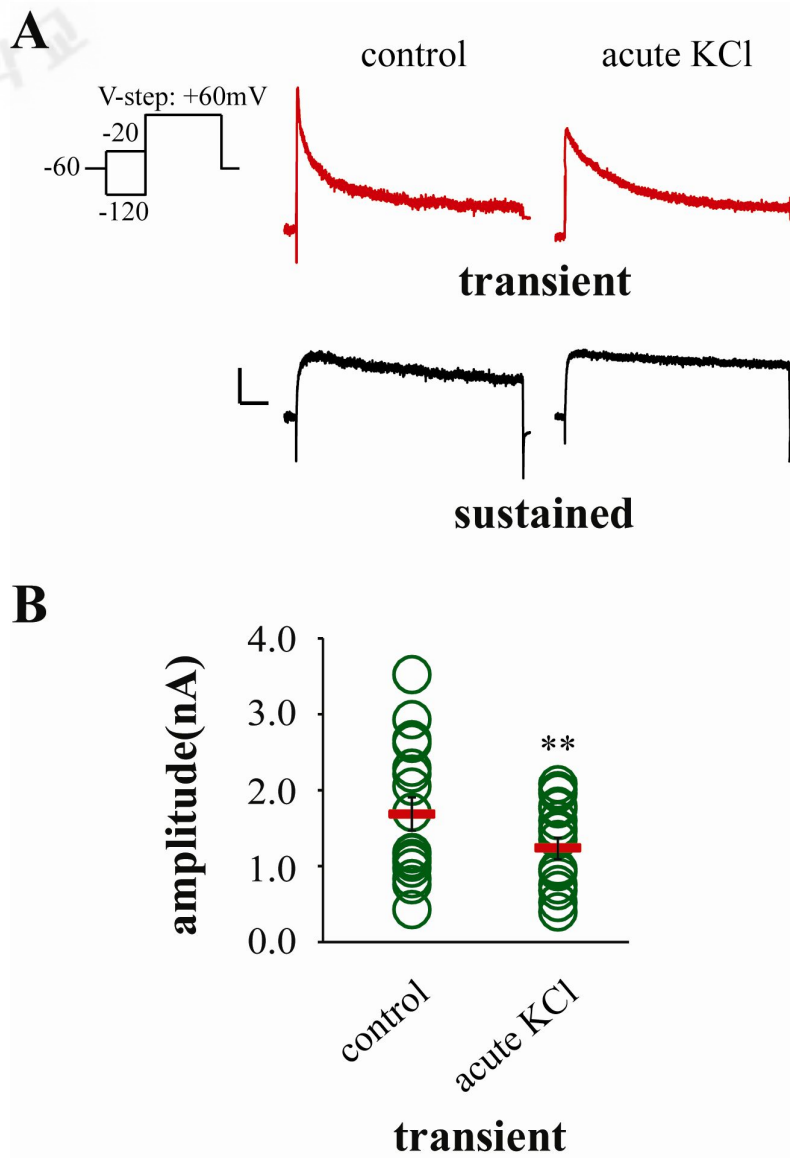
Figure 2. Acute KCl application significantly reduces somatic I$_A$ peak amplitude of hippocampal neurons.

A. Example traces of A-type 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).
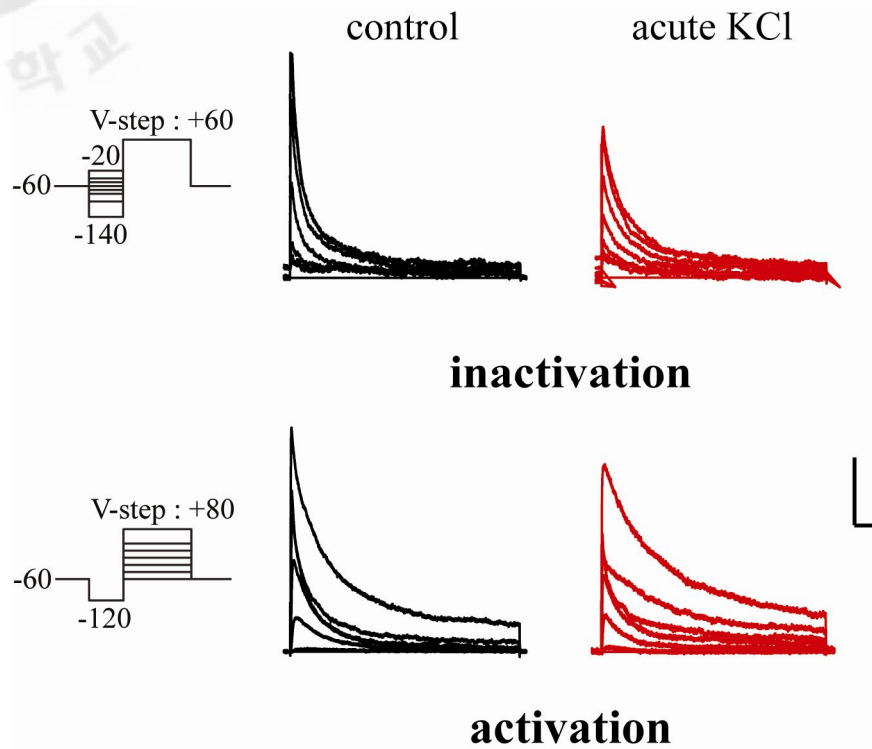
Figure 3. Example traces of inactivation and activation properties.

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.
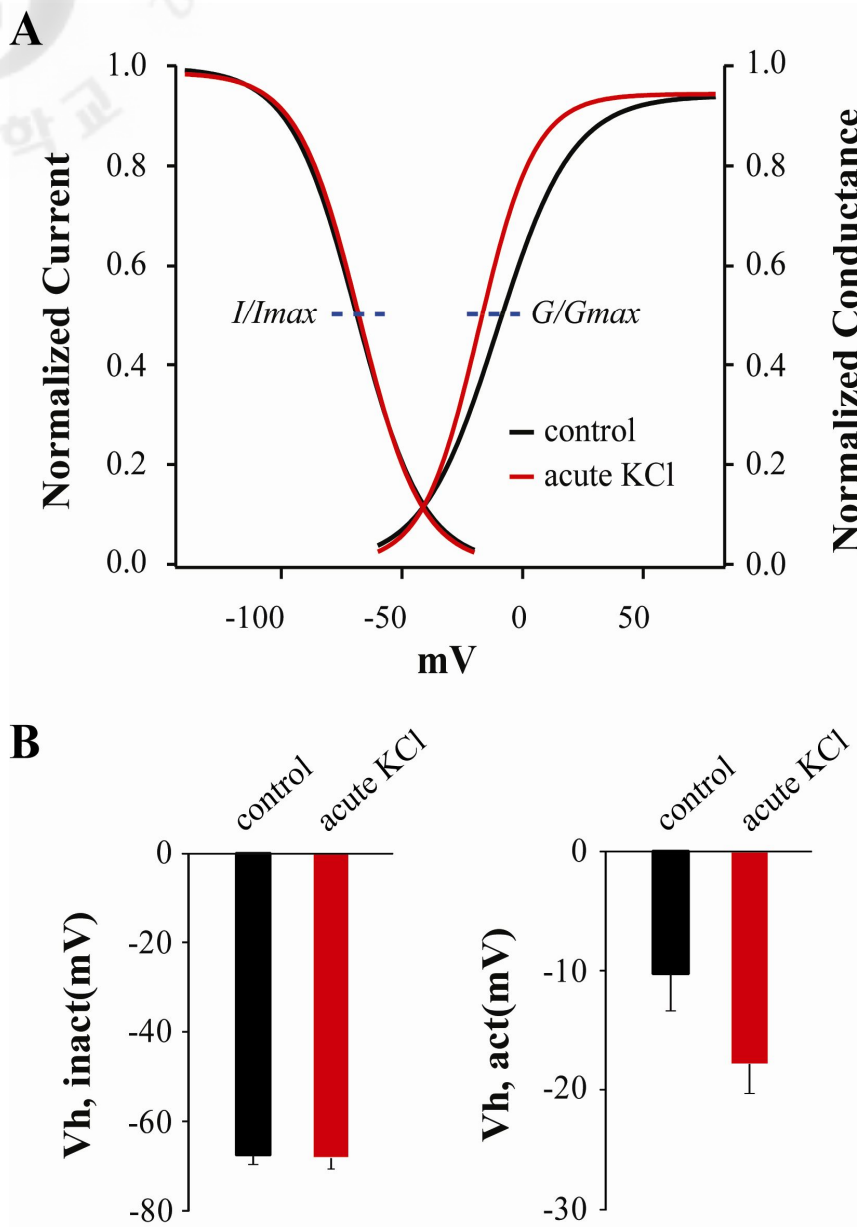
Figure 4. Kinetic properties of I_A channels are unaltered by acute KCl in hippocampal neurons.

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.

2. Long-lasting synaptic release of endogenous glutamate induces the reduction of I_A as well as hyperpolarizing shift of inactivation kinetics of I_A channels through NMDA receptors.

Hyperpolarizing-shift of inactivation curve increase neuronal excitability in hippocampal neurons (Frick *et al*, 2004). Short-term induction of endogenous glutamate simulation is not enough to induce the hyperpolarizing-shift of inactivation curve. To examine peak amplitude reduction and hyperpolarizing-shift of inactivation curve of I_A channel by long-term endogenous glutamate under a culture condition, 20 mM KCl was added in culture media for 24 hours to induces endogenous glutamate release from presynaptic neurons (Fig 5).

In applying KCl Ovnt application (20 mM, 24 hours), long-term release of endogenous glutamate significantly reduces the peak amplitude of I_A (Fig 6A, middle). The I_A of pyramidal cells were decreased up to 43% in KCl Ovnt group (I_A = 1.03 ± 0.12 nA, Fig 6B, *p* < 0.00, compared with control group). Applying 100 μM non-specific NMDARs blocker (APV) to culture media in the presence of 20 mM KCl abolished the reduction of I_A peak (I_A = 1.47 ± 0.16 nA, *p* = 0.04, compared with KCl Ovnt group). All peak amplitude data are contained in Table 1.

Figure 7 shows kinetic change of I_A by long-term endogenous glutamate release in hippocampal neurons of rats. Both inactivation and activation properties of I_A were measured by using different voltage protocols in control group, KCl Ovnt, and KCl + APV Ovnt group using whole-cell patch-clamp. KCl Ovnt application reduced peak amplitude of inactivation by long-term release of endogenous glutamate. KCl Ovnt application also reduced the peak amplitude of activation. The reduction of both inactivation and activation peak amplitude

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was abolished by 100 μM APV.

In inactivation curve, it was found that the I_A voltage range of inactivation curve was shifted approximately 7 mV to the hyperpolarized voltage direction by the long-term release of endogenous glutamate (Fig 8A, $p = 0.02$, compared with control). However, in activation curve, the I_A voltage range of activation curve was unaltered by KCl Ovnt application (Fig 8A). In addition, Figure 8B shows half I_A voltage values (V_h) of inactivation (control $V_h = -67.69 \pm 1.96$ mV; KCl Ovnt $V_h = -74.66 \pm 2.08$ mV, $p = 0.02$, compared with control) and activation (control $V_h = -10.32 \pm 3.03$ mV; KCl Ovnt $V_h = -11.95 \pm 2.15$ mV, $p = 0.66$, compared with control) curves. The hyperpolarizing-shift of inactivation curve was abolished by 100 μM APV (inactivation $V_h = -68.68 \pm 3.19$ mV, $p = 0.80$, compared with control group, $p = 0.15$, compared with KCl Ovnt group; activation $V_h = -10.45 \pm 2.08$ mV, $p = 0.97$, compared with control group, $p = 0.62$, compared with KCl Ovnt group).

This finding suggests that the long-term release of endogenous glutamate by high K^+ alters both I_A expression and inactivation property in hippocampal neurons, but not activation property. All kinetic properties data are contained in Table 2.

control

without any application	recording

5 min

KCl Ovnt

20 mM KCl	wash out	recording

24 hours   2 min

KCl, APV Ovnt

20 mM KCl, 100 μM APV	wash out	recording

24 hours   2 min
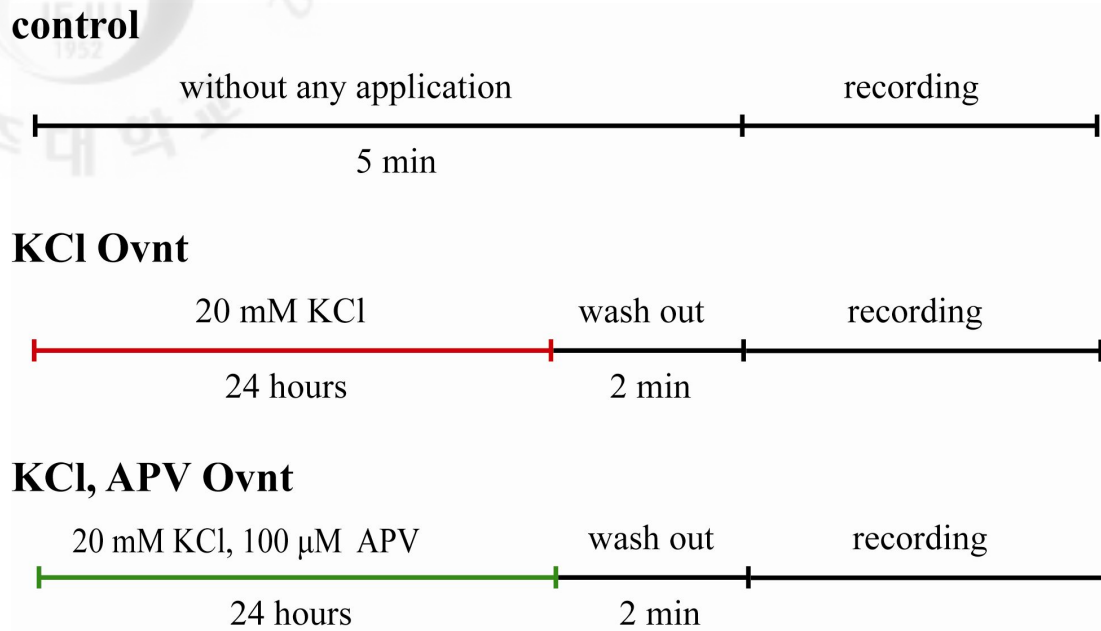
Figure 5. Experimental protocols of control, KCl Ovnt and KCl APV Ovnt groups.
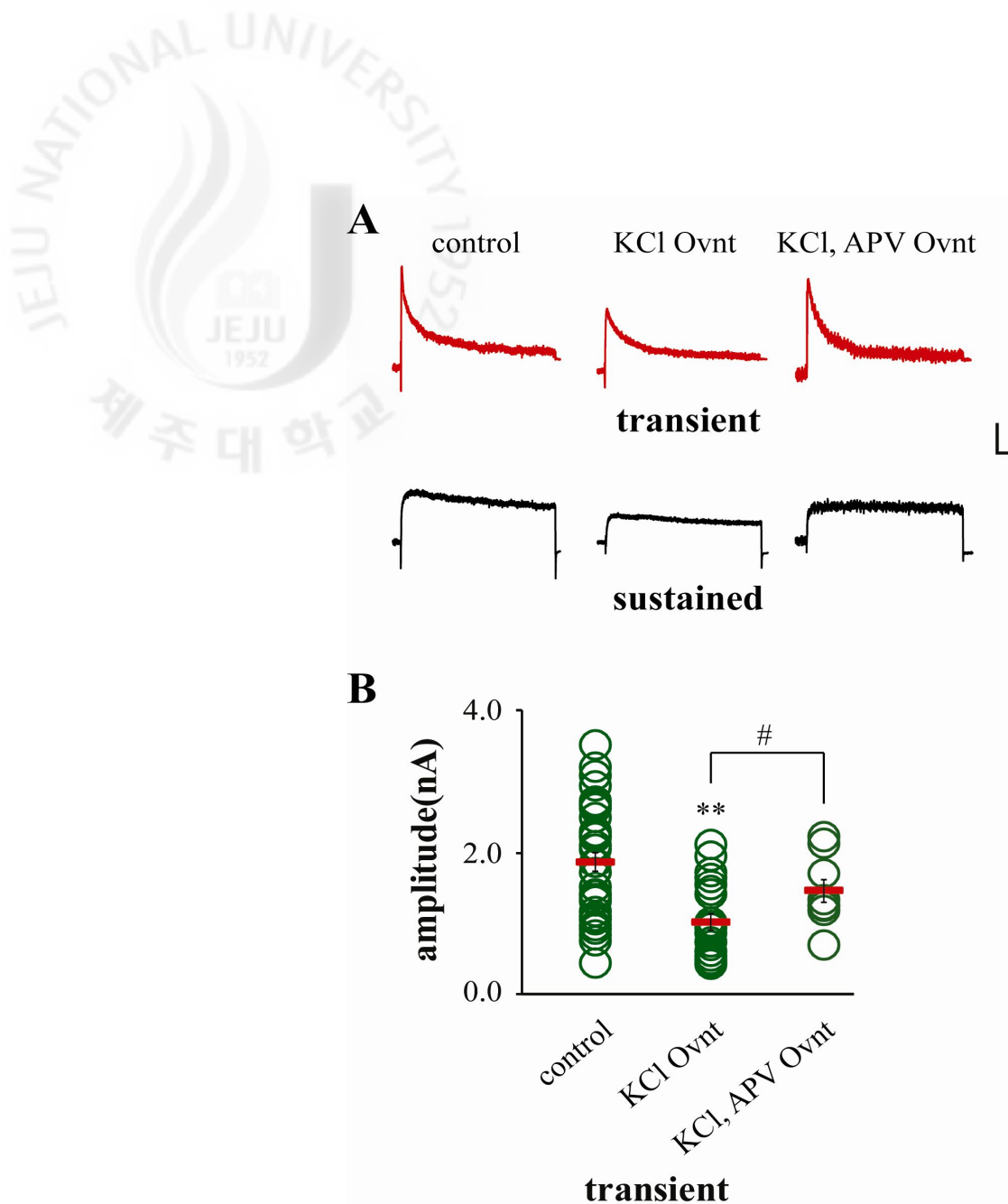
Figure 6. KCl Ovnt application significantly reduces somatic I_A peak amplitude of hippocampal neurons.

A. Example traces of A-type currents recorded in cultured hippocampal neurons. Applying KCl for 24 hrs induces the significant decrease of I_A peak, and NMDA receptor blocker (APV) prevents the significant decrease of peak amplitudes of I_A. Scale bars: 0.5 nA, 50 ms.

B. The averaged values of I_A peak with individual values. ** $p < 0.01$, # $p < 0.05$ by using student's t-test (unpaired).
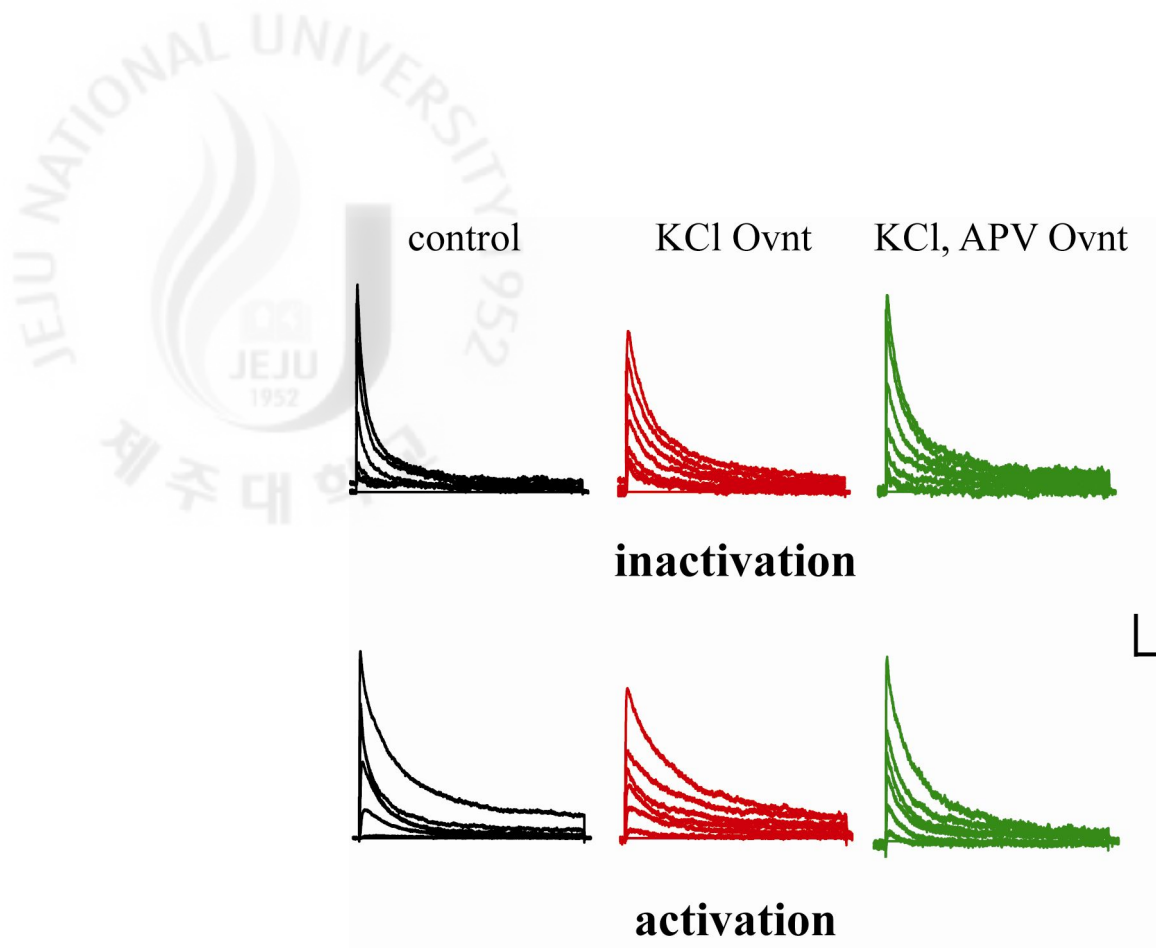
Figure 7. Example traces of inactivation and activation properties altered by KCl and KCl APV for 24 hours in pyramidal neurons.

Long-term release of endogenous glutamate reduces I_A amplitude, and reduction of I_A is abolished by non-specific NMDAR blocker (APV). Scale bars: 0.5 nA, 50 ms.
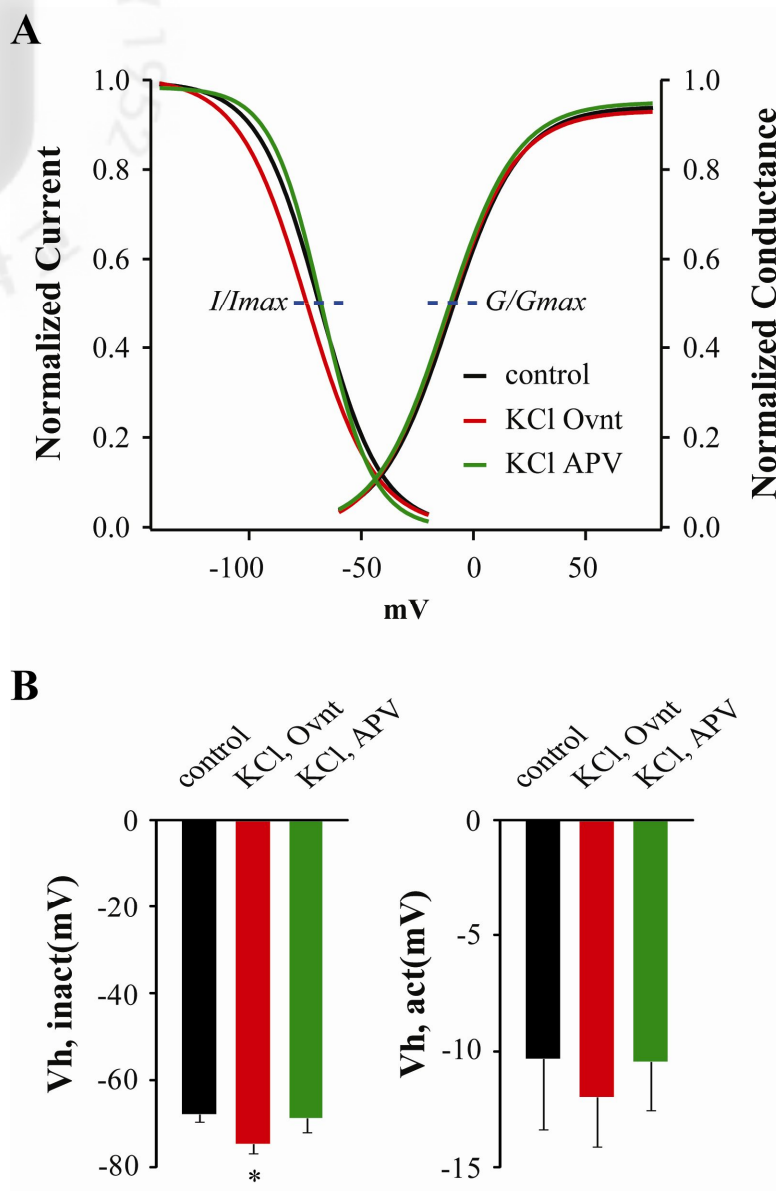
Figure 8. Long-term release of endogenous glutamate induces hyperpolarizing shift of inactivation curve in hippocampal neurons.

A. Inactivation and activation curves of I_A channels. This figure shows the effect of KCl Ovnt to induce the hyperpolarizing shift of inactivation curve, and APV application abolishes this effect of KCl Ovnt.

B. Averaged V_h values of inactivation and activation curves of I_A channels. * $p < 0.05$ by using student's t-test (unpaired).

3. Blocking synaptic NMDARs prevents Glutamate-induced reduction of I_A peak amplitude.

Endogenous glutamate activates NMDARs associated Ca^{2+} influx. Either non-specific (APV, 100 μM) or synaptic NMDAR blocker (MK801, 25 μM) was added in culture media for 24 hours to activate extra synaptic NMDARs (Fig 9).

In glutamate Ovnt application (5 μM, 24 hours), long-term exogenous glutamate significantly reduced peak amplitude of I_A (Fig 10A). The I_A of pyramidal cells was approximately decreased up to 53% in glutamate Ovnt group. The peak amplitude of I_A is 0.86 ± 0.16 nA (Fig 10B, $p < 0.01$, compared with control group). Applying 100 μM non-specific NMDARs blocker (APV) or 25 μM MK801 (with 20 mM KCl) with 5 μM glutamate in culture media abolished the reduction of I_A peak amplitude (Fig 10B, glutamate APV Ovnt $I_A = 1.64 \pm 0.27$ nA, $p = 0.46$, compared with control group, $p = 0.02$, compared with KCl Ovnt group, glutamate APV Ovnt $I_A = 1.57 \pm 0.14$ nA, $p = 0.14$, compared with control group, $p = 0.00$, compared with KCl Ovnt group). All peak amplitude data are contained in Table 1.

Figure 11 shows kinetic changes of I_A by long-term application of exogenous glutamate in hippocampal neurons of rats. Glutamate Ovnt application reduced the peak amplitude of inactivation. Glutamate Ovnt application also reduced peak amplitude of activation. Reduction of both inactivation and activation peak amplitude was abolished by 100 μM APV or 25 μM MK801.

In inactivation and activation curves, it was found that the I_A voltage ranges of inactivation and activation curves were slightly shifted 4 and 2 mV (respectively) to the hyperpolarized direction by the long-term application of exogenous glutamate (Fig 12A, inactivation $p = 0.24$, compared with control; activation $p = 0.58$, compared with control). However, there is

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no significant alteration by glutamate Ovnt application. Figure 12B shows half I_A voltage values (V_h) of inactivation (control V_h = - 67.69 ± 1.96 mV; glutamate Ovnt V_h = - 71.64 ± 2.64 mV, p = 0.24, compared with control) and activation (control V_h = - 10.32 ± 3.03 mV; glutamate Ovnt V_h = - 12.56 ± 2.46 mV, p = 0.58, compared with control) curves.

This finding suggests that the long-term application of exogenous glutamate to culture media altered I_A expression in hippocampal neurons. However, this application did not alter both inactivation and activation properties. All kinetic properties data are contained in Table 2.

control

without any application   recording
├───┼─────────────────────────────┤
                        5 min

glutamate Ovnt

5 μM glutamate   wash out   recording
├───────────────────────────────────┼───────────────┼───────────────┤
                24 hours   2 min

glutamate, APV Ovnt

5 μM glutamate, 100 μM APV   wash out   recording
├───────────────────────────────────┼───────────────┼───────────────┤
                24 hours   2 min

MK801Ovnt, glutamate Ovnt

20 mM KCl, 25 μM MK801   5 μM glutamate   wash out   recording
├───────────────────────┼───────────────┼───────────────┼───────────────┤
      24 hours   24 hours   2 min
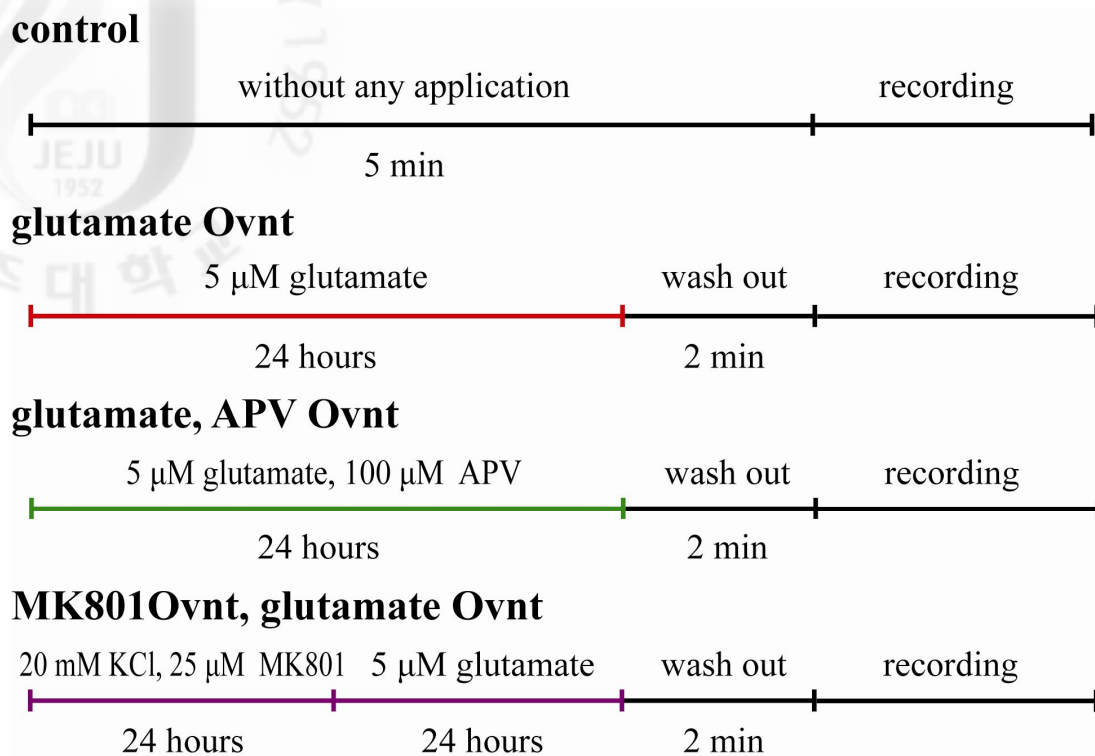
Figure 9. Experimental protocols of control, glutamate Ovnt, glutamate APV Ovnt and MK801 (with KCl) glutamate Ovnt groups.
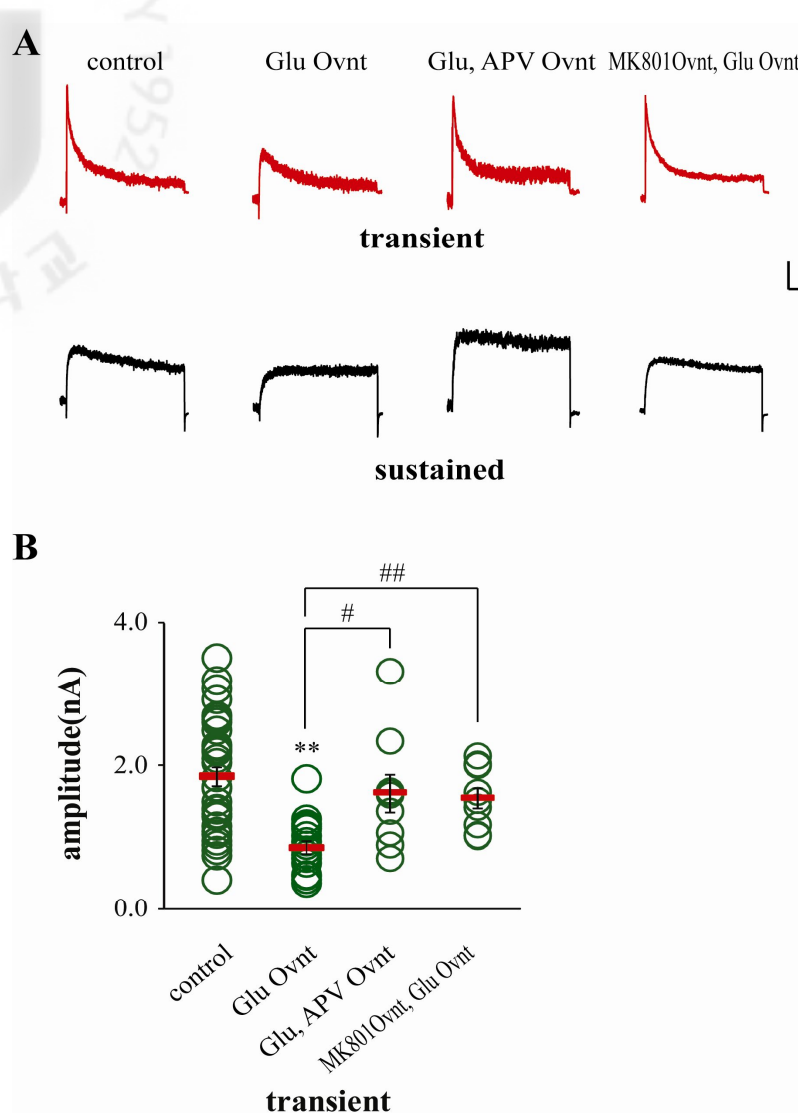
Figure 10. Glutamate Ovnt application significantly reduces the peak amplitude of somatic I_A and blocked by APV, MK801 of hippocampal neurons.

A. Example traces of A-type currents recorded in cultured hippocampal neurons. Applying glutamate for 24 hrs induces significant decrease of I_A, and then both non-specific (APV) and synaptic (MK801 with KCl) NMDAR blocker prevent this effect. Scale bars: 0.5 nA, 50 ms.

B. The averaged values of I_A peak with individual values. ** $p < 0.01$, # $p < 0.05$, ## $p < 0.01$ by using student's t-test (unpaired).

control   Glu Ovnt   Glu, APV Ovnt   MK801Ovnt, Glu Ovnt

inactivation
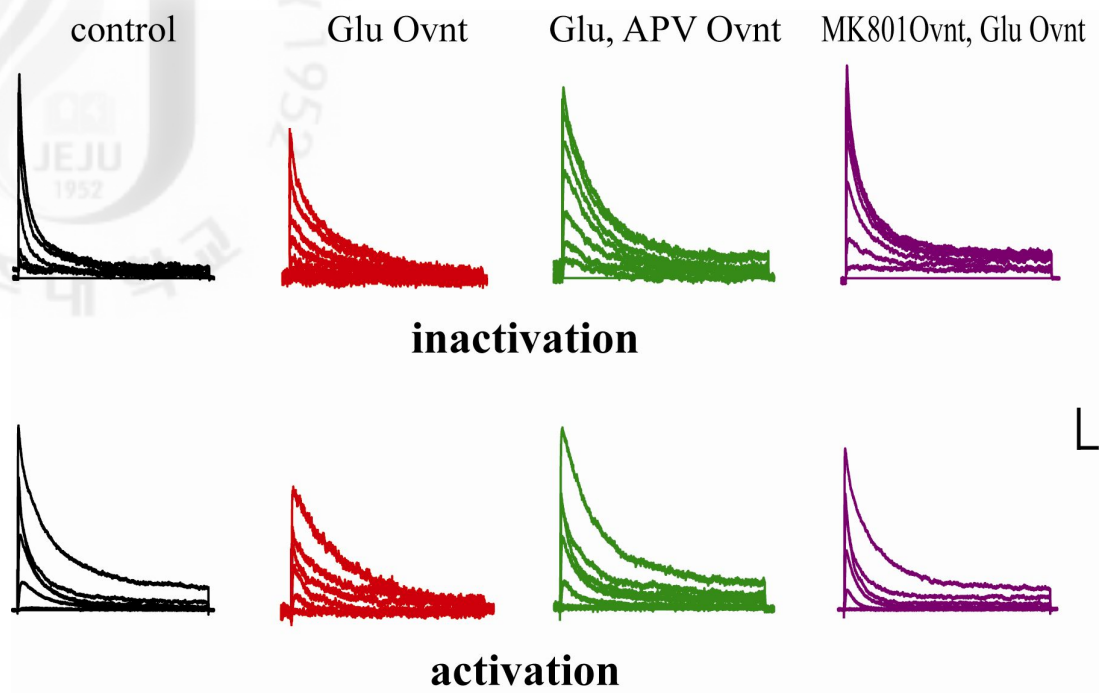
activation

Figure 11. Example traces of inactivation and activation properties.

Long-term application of exogenous glutamate reduces I_A amplitude by activates synaptic NMDAR, and reduction of I_A is abolished by both non-specific NMDAR blocker (APV) or synaptic NMDAR blocker (MK801 with KCl). Scale bars: 0.5 nA, 50 ms.
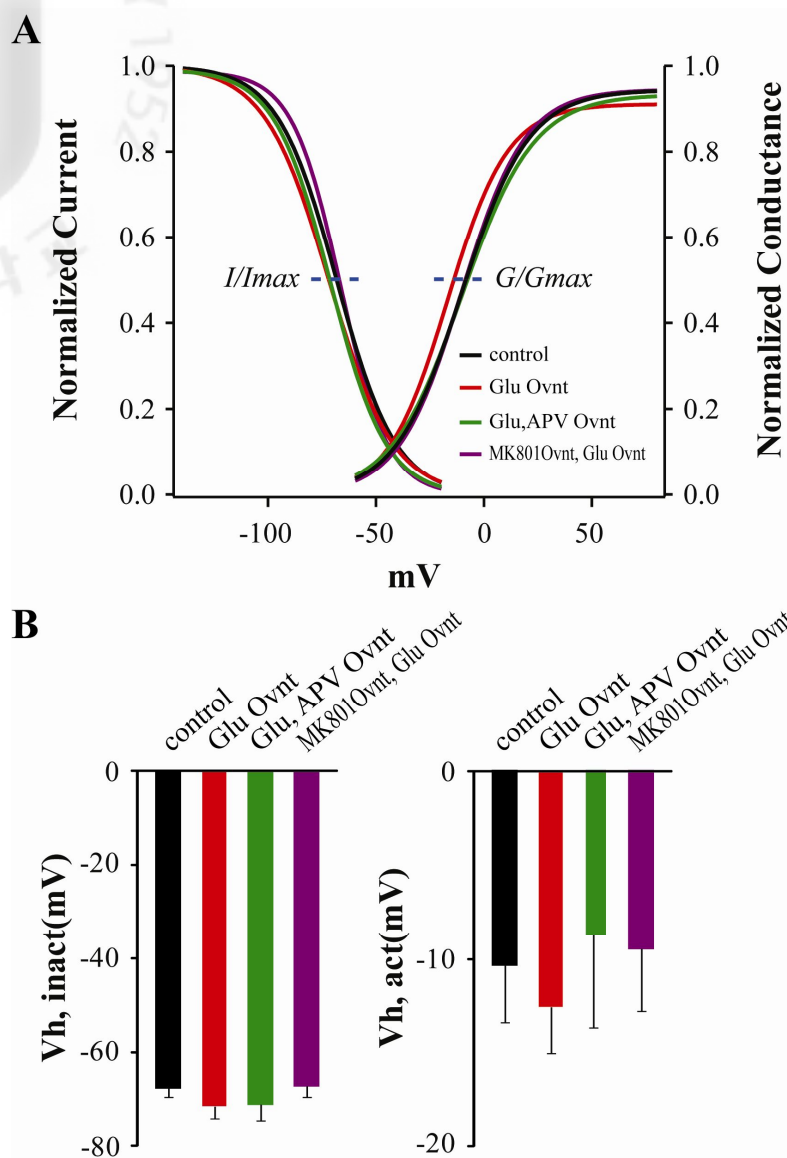
Figure 12. Inactivation and activation curves of I_A channels in hippocampal neurons.

A. Inactivation and activation curves of I_A channels. This figure shows the effect of glutamate Ovnt on inactivation and activation properties.

B. Averaged V_h values of inactivation and activation curves of I_A channels.

4. Activation of synaptic NMDA receptors are obviously required for the activity-dependent reduction of I_A.

AMPA receptors mediate the postsynaptic depolarization that initiates neuronal firing (David and Roger, 2003), and Mg^{2+} ions to block NMDARs are detached by activation of AMPA receptor in synapses. But extra-synaptic site is no existence of APMARs. Thus, the effects of the specific activation of either synaptic or extra-synaptic NMDARs by using Mg^{2+}-free recording solution were tested. To examine dominant contribution sites of NMDARs, non-specific (APV, 100 μM) or synaptic (MK801, 25 μM) NMDAR blocker in culture media for 24 hours to selectively block either synaptic or extra synaptic NMDARs (Fig 13).

In acute glutamate application (5 μM, 5 mins) in Mg^{2+} or Mg^{2+}-free recording solution, the short-term application of exogenous glutamate significantly reduced peak amplitude of I_A (Fig 14A). The peak of $I_A$ amplitudes were decreased in 37% (Mg^{2+}) and 31% (Mg^{2+}-free) in acute glutamate group. The peak amplitude of I_A is 1.18 ± 0.13 nA (Fig 14B, Mg^{2+}, $p = 0.001$, compared with control group) and 1.14 ± 0.17 nA (Fig 14B, Mg^{2+}-free, $p = 0.003$, compared with control group). Applying 25 μM MK801 (with KCl for 24 hours before acute glutamate application) to culture media abolished the reduction of I_A peak amplitude (MK801 acute glutamate group $I_A = 1.69 \pm 0.24$ nA, $p = 0.52$, compared with control group, $p = 0.08$, compared with acute glutamate group). All peak amplitude data are contained in Table 1.

Figure 15 shows kinetic changes of I_A by the short-term application of exogenous glutamate in hippocampal neurons of rats. Either inactivation or activation properties of I_A was measured by using different voltage protocols in control group and in acute glutamate in Mg^{2+}-containing recording solution, acute glutamate in Mg^{2+}-free recording solution or MK801 acute glutamate solution. Acute glutamate application reduced the peak amplitude of

inactivation and activation curves. And the reduction of both inactivation and activation peak amplitude was abolished by 25 μM MK801 with KCl.

In inactivation and activation curves, it was found that the I_A voltage ranges of inactivation curve was shifted approximately 10 mV to the hyperpolarized voltage direction by the short-term application of exogenous glutamate in Mg^{2+}-free contained recording solution. (Fig 16A, $p = 0.03$, compared with control). Figure 16B shows half I_A voltage values (V_h) of inactivation (control $V_h = - 67.69 \pm 1.96$ mV; acute glutamate (Mg^{2+}) $V_h = - 65.75 \pm 3.45$ mV, $p = 0.63$, compared with control; acute glutamate (Mg^{2+}-free) $V_h = - 77.40 \pm 3.29$ mV, $p = 0.03$, compared with control) and activation (control $V_h = - 10.32 \pm 3.03$ mV; acute glutamate (Mg^{2+}) $V_h = - 13.28 \pm 2.78$ mV, $p = 0.48$, compared with control; acute glutamate (Mg^{2+}-free) $V_h = - 14.93 \pm 3.17$ mV, $p = 0.31$, compared with control) curves. The hyperpolarizing-shift of inactivation curve was abolished by 25 μM MK801 pretreatment for 24 hours (inactivation $V_h = - 67.86 \pm 2.91$ mV, $p = 0.96$, compared with control group, $p = 0.05$, compared with acute glutamate (Mg^{2+}-free) group; activation $V_h = - 12.84 \pm 2.37$ mV, $p = 0.53$, compared with control group, $p = 0.61$, compared with acute glutamate (Mg^{2+}-free) group).

This finding suggests that the activation of synaptic, but not extra-synaptic NMDARs alters I_A expression and inactivation property in hippocampal neurons. All kinetic properties data are contained in Table 2.

control

without any application   recording

5 min

acute Glu(Mg²⁺+)

5 μM glutamate(Mg²⁺+)   wash out   recording

5 min   2 min

acute Glu(Mg²⁺-)

5 μM glutamate(Mg²⁺-)   wash out   recording

5 min   2 min

MK801, acute Glu

20 mM KCl, 25 μM MK801   5 μM glutamate   wash out   recording

24 hours   5 min   2 min
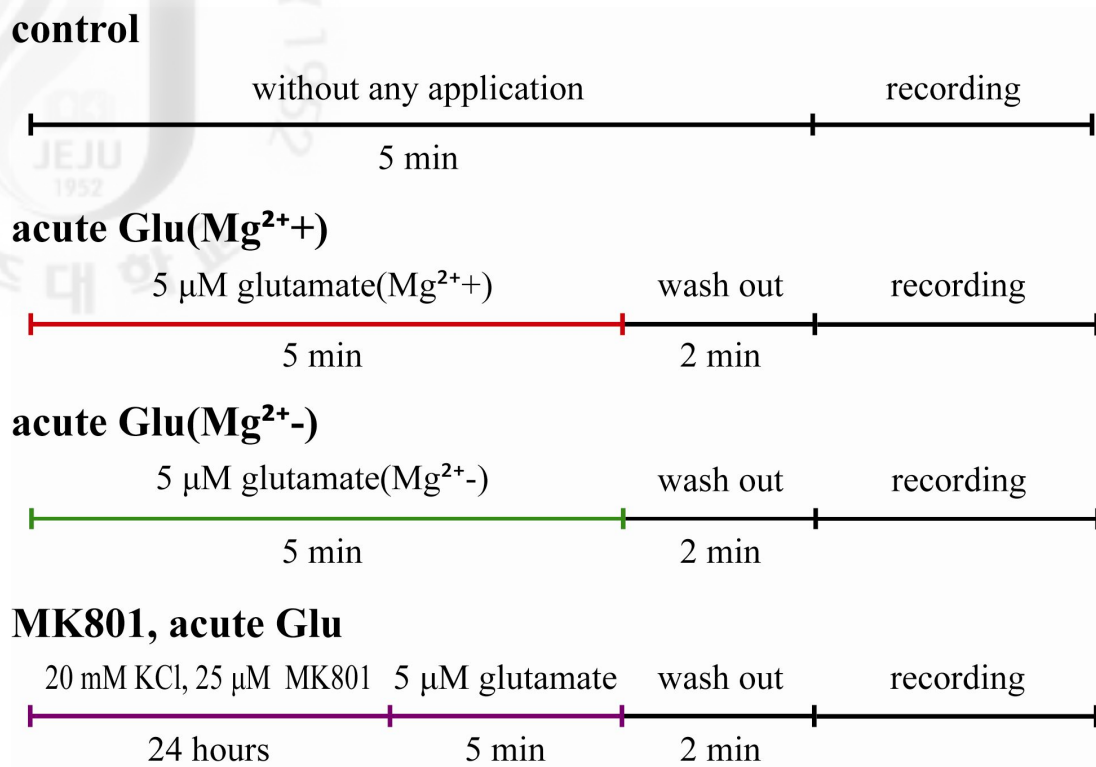
Figure 13. Experimental protocols of control, acute glutamate (Mg^{2+}+), acute glutamate (Mg^{2+}-) and MK801 acute glutamate groups.
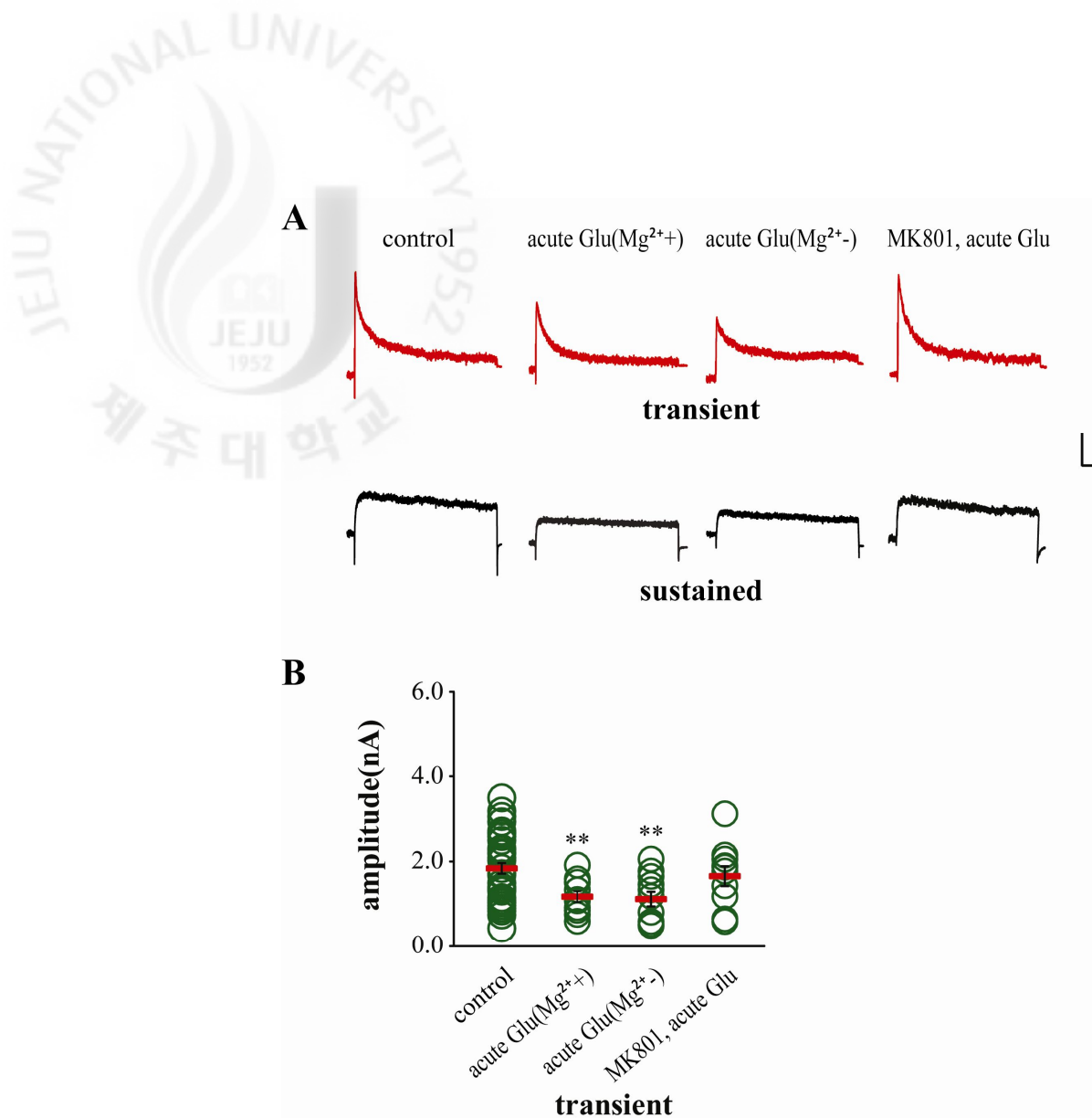
Figure 14. Acute glutamate application in Mg^{2+} or Mg^{2+}-free recording solutions significantly reduces somatic I_A peak amplitude and this effect is blocked by APV or MK801 treatment.

A. Example traces of A-type currents recorded in cultured hippocampal neurons. Applying glutamate (Mg^{2+}-free recording solutions) for 5 min induces significant decrease of I_A and sustained currents, and MK801 (with KCl) pretreatment blocks the significant decrease of transient amplitude in cultured hippocampal neurons. Scale bars: 0.5 nA, 50 ms.

B. The averaged values of I_A peak with individual values. ** $p < 0.01$ by using student's t-test (unpaired).

control   acute Glu(Mg²⁺+)   acute Glu(Mg²⁺-)   MK801, acute Glu

inactivation
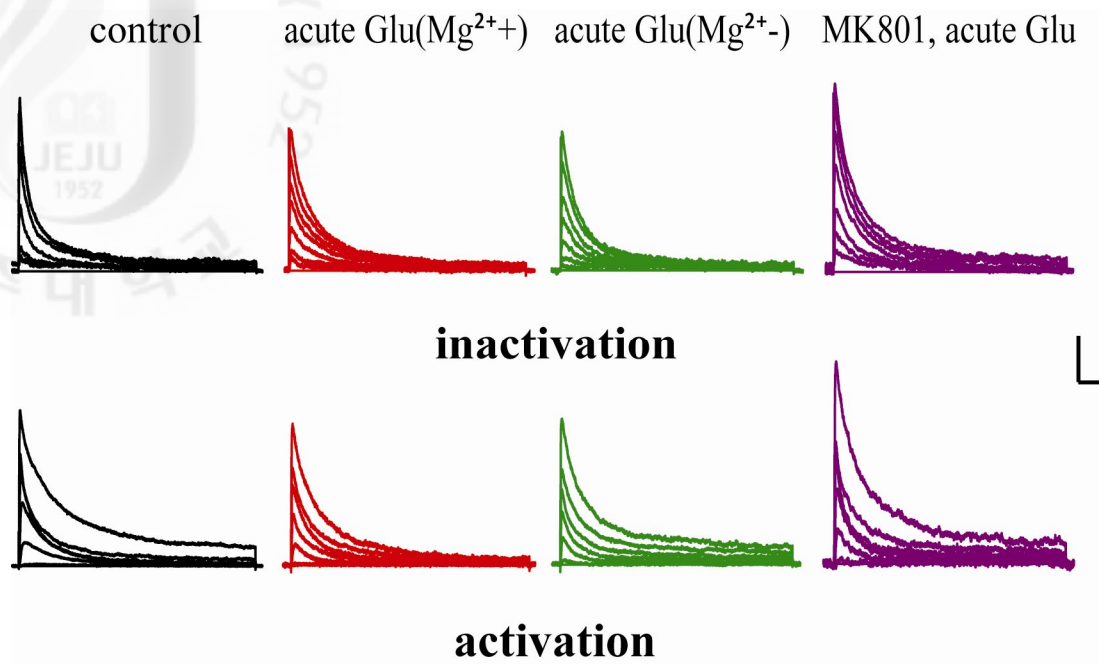
activation

Figure 15. Example traces of inactivation and activation properties of I$_A$ altered by acute glutamate application.

The short-term application of exogenous glutamate in Mg^{2+} or Mg^{2+}-free recording solutions reduces I$_A$ amplitude, and the reduction of I$_A$ is abolished by synaptic NMDAR blocker (MK801 with KCl). Scale bars: 0.5 nA, 50 ms.
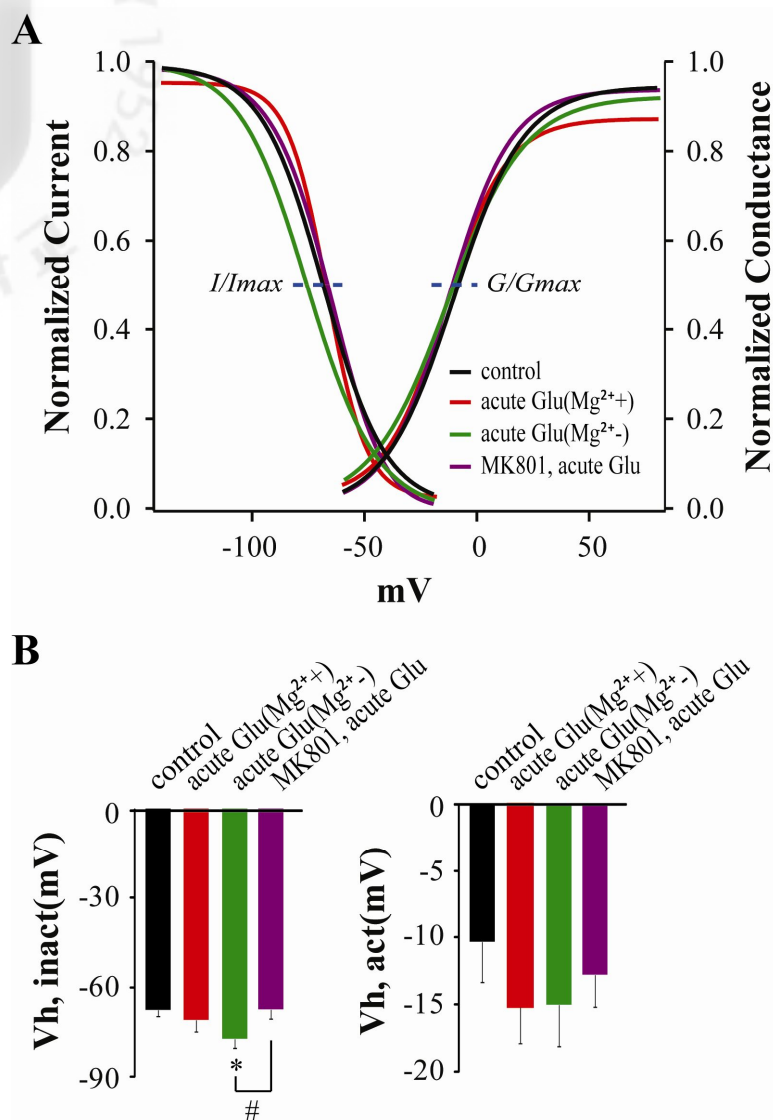
Figure 16. Exogenous glutamate induces hyperpolarizing shift of inactivation curve in hippocampal neurons.

A. Inactivation and activation curves of I_A channels. This figure shows the effect of acute glutamate (Mg^{2+}-free recording solution) induces the hyperpolarizing shift of inactivation curve, and the alteration of inactivation curve is abolished by MK801 (with KCl).

B. Averaged V_h values of inactivation and activation curves. * $p < 0.05$, # $p < 0.05$ using student's t-test (unpaired).

conditions	I_A amplitude (nA)	p-value
control	1.87 ± 0.13	
acute KCl	1.24 ± 0.14	0.002
KCl Ovnt	1.03 ± 0.12	0.000
KCl AVP Ovnt	1.47 ± 0.16	0.039 (compared with KCl Ovnt)
glutamate Ovnt	0.86 ± 0.09	0.000
glutamate APV Ovnt	1.64 ± 0.27	0.020 (compared with glutamate Ovnt)
MK801 glutamate Ovnt	1.57 ± 0.14	0.001 (compared with glutamate Ovnt)
acute glutamate (Mg^{2+})	1.18 ± 0.13	0.001
acute glutamate (Mg^{2+}-free)	1.14 ± 0.17	0.003
MK801 acute glutamate (Mg^{2+}-free)	1.69 ± 0.24	0.079 (compared with acute glutamate (Mg^{2+}-free))

Table 1. Averaged peak amplitude of I_A in condition.

conditions	Vh (mV, inact / act)	p-value (inact / act)
Control	- 67.69 ± 1.96 / - 10.32 ± 3.03	
acute KCl	- 68.07 ± 2.40 / - 17.85 ± 2.45	0.903 / 0.068
KCl Ovnt	- 74.66 ± 2.08 / - 11.95 ± 2.15	0.020 / 0.666
KCl AVP Ovnt	- 68.68 ± 3.19 / - 10.45 ± 2.08	0.145 / 0.622 (compared with KCl Ovnt)
glutamate Ovnt	- 71.64 ± 2.64 / - 12.55 ± 2.46	0.242 / 0.575
glutamate APV Ovnt	- 71.12 ± 3.61 / - 8.67 ± 5.00	0.909 / 0.504 (compared with glutamate Ovnt)
MK801 glutamate Ovnt	- 67.17 ± 2.37 / - 9.40 ± 3.35	0.226 / 0.470 (compared with glutamate Ovnt)
acute glutamate (Mg^{2+})	- 65.75 ± 3.45 / - 13.28 ± 2.78	0.634 / 0.483
acute glutamate (Mg^{2+}-free)	- 77.40 ± 3.29 / - 14.93 ± 3.17	0.029 / 0.313
MK801 acute glutamate (Mg^{2+}-free)	- 67.86 ± 2.91 / - 12.84 ± 2.37	0.049 / 0.610 (compared with acute glutamate (Mg^{2+}-free))

Table 2. Kinetic properties of I_A in each condition.

IV. Discussion

Voltage-dependent A-type K^+ channels in hippocampal neurons are primary regulators of membrane excitability (Hoffman and Johnston, 1998). Modulation of these channels dynamically and selectively control signals propagation through dendrites. Somatodendritic A-type currents 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 (Hoffman et al., 1997; Goldberg et al., 2003; Cai et al., 2004; Kim et al., 2005). Several evidences indicate that I_A channels are involved in LTP. First, heteropodatoxin-3, a selective blocker of the Kv4 channels, reduces the threshold for LTP induction (Ramakers and Storm, 2002). Second, mitogen-activated protein kinase inhibitors PD098059 or U0126 shift I_A activation curve to more hyperpolarized potentials and suppress the induction of LTP (Watanabe et al., 2002). Third, a hyperpolarized shift in the inactivation curve of A-type currents occurs with LTP induction (Frick et al., 2004). Fourth, deletion of Kv4.2 gene results in a lower threshold than that of wild-type littermates for LTP induction (Chen et al., 2006).

Glutamate is a principle excitatory neurotransmitter in the CNS, acting via NMDA receptors, non-NMDA (AMPA and kainite) receptors, and metabotropic glutamate receptors (Ozawa et al. 1998). 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 (Malenka and Bear 2004). Furthermore, 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 A-type currents is shifted toward more hyperpolarized potentials following glutamate treatment (Lei *et al*., 2008).

In the present study, the down-regulation of I_A channels in hippocampal neurons by activation of NMDA receptors is studied. Both short- and long-lasting activation of synaptic transmission induce the reduction of A-type currents, indicating that the expression level of somatic I_A channels may be targeted by synaptic activities of neurons for higher efficiency of EPSP-AP coupling. Activation of NMDA receptors and the subsequent Ca^{2+} influx through activated NMDA receptors are critical for the glutamate-induced reduction of I_A channels expression. Long-lasting activation of synaptic transmission but not short-lasting induces the hyperpolarizing shift of inactivation kinetics of I_A channels, suggesting a possibility that additional kinetic changes of I_A channels contribute to somatic regulations induced by stronger synaptic activities.

Several evidences indicate NMDA receptors are heteromeric complexes of NR1 and the NR2A-NR2D subunits, resulting in a tetrameric or pentameric complex (Dingledine *et al*., 1999). In addition, it has been reported that the pharmacological blockade of NMDAR subtypes proposed that the NR2A type is responsible for inducing LTP (Tang *et al*., 1999; Wong *et al*., 2002), whereas the NR2B type induces LTD (Liu et al., *2004*; Massey et al., *2004*). In this study, with blocking synaptic NMDA receptors, glutamate did not show any influence on somatic A-type currents in Mg^{2+}-free solution. This means that only synaptic NMDA receptors play may critical roles to modulate somatic I_A channels for input-output correlations in hippocampal neurons.

Consequently, synaptic NMDA receptors act as potent factors to regulate somatic signaling processes induced by synaptic transmission and decide somatic responses reflecting synaptic plasticity through I_A channels regulation.

V. Reference

Alonso G., Widmer H., Clustering of Kv4.2 potassium channels in postsynaptic membrane of rat supraoptic neurons: an ultrastructural study., *Neuroscience* **77**:617–621(1997)

Anderson A.E., Adams J.P., Qian Y., Cook R.G., Pfaffinger P.J., Sweatt J.D., Kv4.2 phosphorylation by cyclic AMP-dependent protein kinase., *J. Biol. Chem.* **275**:5337-5346(2000)

Birnbaum S.G., Varga A.W., Yuan L.L., Anderson A.E., Sweatt J.D., and Schrader L.A., Structure and function of Kv4-family transient potassium channels., *Physiol. Rev.* **84**:803–833(2004)

Bliss T.V. and Collingridge G.L., A synaptic model of memory: long-term potentiation in the hippocampus., *Nature* **361**:31–39(1993)

Cai X., Liang C.W., Muralidharan S., Kao J.P., Tang C.M., Thompson S.M., Unique roles of SK and Kv4.2 potassium channels in dendritic integration., *Neuron* **44**:351–364(2004)

Chavez-Noriega L.E., Halliwell J.V., Bliss T.V., A decrease in firing threshold observe dafter induction of the EPSP-spike (E-S) component of long-term potentiation in rat hippocampal slices., *Exp. Brain Res.* **79**:633–641(1990)

Chen L., Huang L.M., Protein kinase C reduces Mg^{2+} block of NMDA-receptor channels as a mechanism of modulation., *Nature* **356**:512-523(1992)

Chen X., Yuan L.L., Zhao C., Birnbaum S.G., Frick A., Jung W. E., Schwarz T.L., Sweatt J.D. and Johnston D, Deletion of Kv4.2 gene eliminates dendritic A-type K^+ current and enhances induction of long-term potentiation in hippocampal CA1 pyramidal neurons., *J. Neurosci.* **26**:12143–12151(2006)

Cooper E.C., Milroy A., Jan Y.N., Jan L.Y. and Lowenstein D.H., Presynaptic localization of Kv1.4-containing A-type potassium channels near excitatory synapses in the hippocampus., *J. Neurosci.* **18**:965–974(1998)

David S.B., Roger A.N., AMPA receptors trafficking at excitatory synapses., Neuron **40**:361-379(2003)

Dingledine R., Borges K., Bowie D. and Traynelis S.F., The glutamate receptor ion channels. , *Pharmacol Rev* **51**:7– 61(1999)

Frick A., Magee J. and Johnston D., LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites., *Nat. Neurosci.* **7**:126–135(2004)

Goldberg J.H., Tamas G., Yuste R., Ca^{2+} imaging of mouse neocortical interneurone dendrites : Ia-type K^+ channels control action potential backpropagation., *J. Physiol.* **551**:49–65(2003)

Gulbis J.M., Zhou M., Mann S., MacKinnon R., Structure of the cytoplasmic beta subunit-T1 assembly of voltage-dependent K$^+$ channels., *Science* **289**:123-127.(2000)

Gutman G.A., Chandy K.G., Adelman J.P., Aiyar J., Bayliss D.A., Clapham D.E., Covarrubias M., Desir G.V., Furuichi K., Ganetzky B., Garcia M. L., Grissmer S., Jan L.Y., Karschin A., Kim D., Kuperschmidt S., Kurachi Y., Lazdunski M., Lesage F., Lester H.A., McKinnon D., Nichols C.G., O'Kelly I., Robbins J., Robertson G.A., Rudy B., Sanguinetti M., Seino S., Stuehmer W., Tamkun M.M., Vandenberg C.A., Wei A., Wulff H. and Wymore R.S., International Union of Pharmacology. XLI. Compendium of voltage-gatedion channels: potassium channels., *Pharmacol. Rev.* **55**:583–586(2003)

Hammond R.S., Lin L., Sidorov M.S., Wikenheiser A.M., Hoffman D.A., Protein kinase a mediates activity-dependent Kv4.2 channel trafficking., *J. Neurosci.* **28**:7513-7519(2008)

Hardingham G.E. and Bading H., The Yin and Yang of NMDA receptor signaling., *Trends Neurosci.* **26**:81–89(2003)

Hoffman D.A. and Johnston D., Downregulation of transient K$^+$ channels in dendrites of hippocampal CA1 pyramidal neurons by activation of PKA and PKC., *J. Neurosci.* **18**:3521-3528

Hoffman D.A., Magee J.C., Colbert C.M. and Johnston D., K$^+$ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons., *Nature* **387**:869–875(1997)

Jerng H.H., and Covarrubias M., K$^+$ channel inactivation mediated by the concerted action of

the cytoplasmic N- and C- terminal domains., *Biophys. J.* **72**:163–174(1997)

Jerng H.H., Pfaffinger P.J., Covarrubias M., Molecular physiology and modulation of somatodendritic A-type potassium channels., *Mol. Cell. Neurosci.* **27**:343–369(2004)

Khaliq Z.M. and Bean B.P., Dynamic, nonlinear feedback regulation of slow pacemaking by A-type potassium current in ventral tegmental area neurons., *J. Neurosci.* **28**:10905–10917(2008)

Kim J., Wei D.S. and Hoffman D.A., Kv4 potassium channel subunits control action potential repolarization and frequency dependent broadening in hippocampal CA1 pyramidal neurons., *J. Physiol.* **569**:41–57(2005)

Kim J., Jung S.C., Clemens A.M., Petralia R.S. and Hoffman D.A., Regulation of dendritic excitability by activity-dependent trafficking of the A-type K^+ channel subunit Kv4.2 in hippocampal neurons., *Neuron* **54**:933–947(2007)

Lei Z., Deng P. and Xu Z. C., regulation of Kv4.2 channels by glutamate in cultured hippocampal neurons., *J. Neurochem.* **106**:182-192(2008)

Lin L., Wei S., Wikenheiser A.M., Faith K., Hoffman D.A., KChIP4a regulates Kv4.2 channel trafficking through PKA phosphorylation., *Mol. Cell. Neurosci.* **43**:315-325(2010)

Liu L., Wong T.P., Pozza M.F., Lingenhoehl K., Wang Y., Sheng M., Auberson Y.P. and Wang Y.T., Role of NMDA receptor subtypes in governing thedirection of hippocampal

synaptic plasticity., *Science* **304**:1021–1024(2004)

Lissin D.V., Carroll R.C., Nicoll R.A., Malenka R.C. and von Zastrow M., Rapid, activation-induced redistribution of ionotropic glutamate receptors in cultured hippocampal neurons., *J. Neurosci.* **19**:1263–1272(1999)

Malenka R.C. and Bear M.F., LTP and LTD: an embarrassment of riches., *Neuron* **44**:5–21(2004)

Mark L.M., Gary L.W. and Peter B.G., Voltage-dependent block by Mg^{2+} of NMDA responses in spinal cord neurons., *Nature* 309:261-263(1884)

Massey P.V., Johnson B.E., Moult P.R., Auberson Y.P., Brown M.W., Molnar E., Collingridge G.L. and Bashir Z.I., Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J. Neurosci.* **24**:7821–7828(2004)

Misonou H., Mohapatra D.P., Park E.W., Leung V., Zhen D., Misonou K., Anderson A.E. and Trimmer J.S., Regulation of ion channel localization and phosphorylation by neuronal activity., *Nat. Neurosci.* **7**:711–718(2004)

Ozawa S., Kamiya H. and Tsuzuki K., Glutamate receptors in the mammalian central nervous system., *Prog. Neurobiol.* **54**:581–618(1998)

Papazian D.M., Potassium channels: some assembly required., *Neuron* **23**:7-10(1999)

Ramakers G.M., Storm J.F., A postsynaptic transient K$^+$ current modulated by arachidonic acid regulates synaptic integration and threshold for LTP induction in hippocampal pyramidal cells., *Proc. Natl. Acad. Sci. USA* **99**:10144–10149(2002)

Rhodes K.J., Strassle B.W., Monaghan M.M., Bekele-Arcuri Z., Matos M.F. and Trimmer J.S., Association and colocalization of the Kvbeta1 and Kvbeta2 beta-subunits with Kv1alpha-subunits in mammalian brain K$^+$ channel complexes., *J. Neurosci.* **17**:8246–8258(1997)

Salkoff L., Baker K., Butler A., Covarrubias M., Pak M.D., and Wei A., An essential 'set' of K$^+$ channels conserved in flies, mice and humans., *Trends Neurosci.* **5**:161–166(1992)

Serodio P., Kentros C. and Rudy B., Identification of molecular components of A-type channels activating at subthreshold potentials., *J. Neurophysiol.* **72**:1516–1529(1994)

Sewing S., Roeper J., Pongs O., Kv beta1 subunit binding specific for shaker- related potassium channel alpha subunits., *Neuron* **16**:455-463(1996)

Sheng M., Tsaur M.L., Jan Y.N. and Jan L.Y., Subcellular segregation of two A-type K$^+$ channel proteins in rat central neurons., *Neuron* **9**:271–284(1992)

Song W.J., Tkatch T., Baranauskas G., Ichinohe N., Kitai S.T., and Surmeier D.J. Somatodendritic depolarization-activated potassium currents in rat neostriatal cholinergic interneurons are predominantly of the A-type and attributable to coexpression of Kv4.2 and

Kv4.1 subunits., *J. Neurosci.* **18**:3124–3137(1998)

Tang Y.P., Shimizu E., Dube G.R., Rampon C., Kerchner G.A., Zhuo M., Liu G and Tsien J.Z., Genetic enhancement of learning and memory in mice., *Nature* **401**:63– 69(1999)

Watanabe S., Hoffman D.A., Migliore M. and Johnston D., Dendritic K+ channels contribute to spike-timing dependent longterm potentiation in hippocampal pyramidal neurons., *Proc. Natl. Acad. Sci. USA* **99**:8366–8371(2002)

Wong W., Newell E.W., Jugloff D.G., Jones O.T., Schlichter L.C., Cell surface targeting and clustering interactions between heterologously expressed PSD-95 and the *Shal* voltage-gated potassium channel, Kv4.2., *J. Biol. Chem.* **277**:20423–20430(2002)

Zhang W. and Linden D.J., The other side of the engram: experience-driven changes in neuronal intrinsic excitability., *Nat. Rev.* **4**: 885–900(2003)

Ⅵ. Abstract in Korean

흥분성 신경전달 물질인 글루탐산은 중추신경계의 정상적인 기능에 중요한 역할을 하지만, 병적 조건하에서 과분비가 일어나면 신경세포를 과흥분시키고 흥분독성을 야기한다. 최근의 연구에서 이와같은 신경세포의 흥분성에 A-type K^+ channel의 작용이 중요하다는 사실이 보고되었다. A-type K^+ channel은 역치 이상의 전압 영역에서 순간적으로 활성화 후에 빨리 비활성화가 되는 특성을 갖는다. 또한 excitatory postsynaptic potential의 형성에 중요한 역할을 하고, 활동전위의 전파를 제한하고, 수상돌기에서의 활동전위 형성을 막는 등의 작용을 하여 신경세포막 흥분성 조절에 중요한 역할을 한다. 최근의 연구에서 신경세포 외부에 생화학적으로 외인성 글루탐산을 처리한 결과 세포막의 A-type K^+ channel의 발현이 감소함을 확인하였으나, 실제 생리적 활동 중인 신경세포에서의 흥분성에 대한 A-type K^+ channel의 조절에 대한 증거를 제시하지 못했다는 한계를 갖고 있다. 따라서 본 연구에서는 신경세포의 생리학적 흥분상황 유도를 통해 내인성 glutamate 분비하여 해마 신경세포에서의 흥분성에 대한 A-type K^+ channel의 조절작용이 어떻게 나타나는지 확인하였다. 내인성 글루탐산 분비를 유도하기 위해 recording solution(10 mM, 2 min) 또는 cluture media(20 mM, 24 hours)에 고농도의 KCl을 처리하여 A-type K^+ current(I_A)의 크기 및 기질 변화를 정상 대조군과 비교, 관찰하였다. 내인성 글루탐산 분비를 단시간 또는 장시간 유도한 결과 정상 대조군에 비하여 I_A의 크기가 유의하게 감소되었고, 또한 글루탐산 수용체 중 하나인 NMDA 수용체를 APV(100 μM)를 이용하

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여 차단하였을 때 내인성 글루탐산 효과가 사라짐을 확인하였다. 이는 A-type K$^+$ channel의 발현 조절에 내인성 글루탐산의 분비유도를 통한 NMDA 수용체의 활성화가 중요함을 의미한다. 반면 A-type K$^+$ channel의 기질변화를 확인한 결과 장시간 내인성 글루탐산 분비를 유도한 그룹에서만 inactivation 특성이 과분극 전위 영역으로 이동이 일어남을 확인하였다.

신경세포에는 연접내, 연접외 모두 NMDA 수용체가 존재하고 있고, 많은 연구에서 그 역할이 다르다는 것이 보고되었다. 따라서 외인성 글루탐산을 이용하여 연접내외의 NMDA 수용체의 활성화를 유도하고 A-type K$^+$ channel의 발현에 어떠한 역할을 하는지를 확인하였다. NMDA 수용체의 선택적 차단을 위하여 고농도의 KCl로 연접내에 글루탐산 분비 유도 후 연접내의 NMDA 수용체를 활성화 시키고 활성화된 NMDA 수용체만 차단하는 MK801(25 μM)을 처리하여 연접내의 NMDA 수용체만 선택적으로 차단하였다. 이후 culture media에 외인성 글루탐산(5 μM, 24 hours)을 처리하여 A-type K$^+$ channel의 발현이 어떻게 나타나는지 확인하였다. 그 결과 외인성 글루탐산은 A-type K$^+$ channel의 발현 감소를 일으켰고, 이러한 외인성 글루탐산의 효과는 연접내의 NMDA 수용체를 차단할 때 사라짐을 확인하였다. 이는 A-type K$^+$ channel의 발현조절이 연접내 NMDA 수용체의 활성화를 통해서만 일어남을 의미한다.

연접내는 AMPA 수용체의 존재로 마그네슘 이온에 대한 NMDA 수용체의 차단효과를 제거할 수 있지만 연접외에는 AMPA 수용체의 부재로 NMDA 수용체가 마그네슘에 의해 차단된다. 따라서 연접내의 NMDA 수용체만 선택적으로 차단하고 마그네슘을 제거한 recording solution에 외인성 글루탐산(5 μM, 5 min)을 처리하여 연접외의 NMDA 수용체를 충분히 활성화 시킨 후 A-type K$^+$

channel의 발현량을 확인하였다. 연접외에 존재하는 NMDA 수용체의 선택적 활성화는 A-type K$^+$ channel의 발현에 영향을 주지 못하였고, 이는 A-type K$^+$ channel 채널의 조절에 연접외의 NMDA 수용체 활성화가 영향을 주지 못함을 의미한다.

이상의 결과에서 고농도 KCl을 통한 내인성 글루탐산의 분비유도가 A-type K$^+$ channel의 세포막 발현을 정상 대조군에 비해 감소시키는 것을 확인하였다. 또한 내인성 글루탐산은 NMDA 수용체의 활성화를 통하여 A-type K$^+$ channel의 발현 조절에 작용함을 확인하였고, 특히 연접내에 존재하는 NMDA 수용체의 활성화를 통해서만 A-type K$^+$ channel의 세포막 발현이 감소함을 확인하였다. 이러한 결과는 신경세포의 연접신호전달 특성이 A-type K$^+$ channel을 통해 세포의 흥분성을 조절할 수 있다는 것을 의미한다.

감사의 글

어느덧 2년이 지나 졸업논문을 쓰게 되니 지난 시간이 떠오르며 감회가 새롭습니다. 처음 석사과정에 진학하면서 여러 가지 걱정들로 주저하였지만 시간이 지나고 나니 연구를 할 수 있다는 것이 굉장한 행복임을 느낍니다.

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마지막으로 못난 아들 때문에 아직도 고생하시는 어머니... 김옥희 여사님 걱정 마세요. 금방 장가갈게요. 고맙고 사랑합니다. 그리고 하나뿐인 동생 은림아 오빠라고 도움이 못 되는 거 같아 미안하고 고맙다. 화북에 계신 할머니 증손자 태어날 때까지 만수무강 하세요. 또 하늘에 계신 아버지 보고 계시죠. 정말 보고 싶고 고맙습니다.

지난 2 년은 꿈만 같던 시간이었습니다. 모든 일이 완벽할 수는 없는지라 아쉬움이 남는 것은 사실이지만 현재의 부족함은 남은 시간 동안 채워 발전하는 사람이 되도록 노력하겠습니다.