Korean J Physiol Pharmacol 2017;21(2):259-265
https://doi.org/10.4196/kjpp.2017.21.2.259

KJPP

Original Article

Chronic Ca^{2+} influx through voltage-dependent Ca^{2+} channels enhance delayed rectifier K^+ currents via activating Src family tyrosine kinase in rat hippocampal neurons

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ARTICLE INFO

Received December 21, 2016
Revised January 20, 2017
Accepted January 23, 2017

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Key Words
A-type K^+ channels
Delayed rectifier K^+ channel
NMDA receptors
Src family tyrosine kinase
Voltage-dependent Ca^{2+} channel

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ABSTRACT Excessive influx and the subsequent rapid cytosolic elevation of Ca^{2+} in neurons is the major cause to induce hyperexcitability and irreversible cell damage although it is an essential ion for cellular signalings. Therefore, most neurons exhibit several cellular mechanisms to homeostatically regulate cytosolic Ca^{2+} level in normal as well as pathological conditions. Delayed rectifier K^+ channels (I_{DR} channels) play a role to suppress membrane excitability by inducing K^+ outflow in various conditions, indicating their potential role in preventing pathogenic conditions and cell damage under Ca^{2+}-mediated excitotoxic conditions. In the present study, we electrophysiologically evaluated the response of I_{DR} channels to hyperexcitable conditions induced by high Ca^{2+} pretreatment (3.6 mM, for 24 hours) in cultured hippocampal neurons. In results, high Ca^{2+}-treatment significantly increased the amplitude of I_{DR} without changes of gating kinetics. Nimodipine but not APV blocked Ca^{2+}-induced I_{DR} enhancement, confirming that the change of I_{DR} might be targeted by Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs) rather than NMDA receptors (NMDARs). The VDCC-mediated I_{DR} enhancement was not affected by either Ca^{2+}-induced Ca^{2+} release (CICR) or small conductance Ca^{2+}-activated K^+ channels (SK channels). Furthermore, PP2 but not H89 completely abolished I_{DR} enhancement under high Ca^{2+} condition, indicating that the activation of Src family tyrosine kinases (SFKs) is required for Ca^{2+}-mediated I_{DR} enhancement. Thus, SFKs may be sensitive to excessive Ca^{2+} influx through VDCCs and enhance I_{DR} to activate a neuroprotective mechanism against Ca^{2+}-mediated hyperexcitability in neurons.

INTRODUCTION

Dynamic changes of cytosolic Ca^{2+} level under physiological conditions are essential for cellular signalings of protein synthesis and signal transmission of neurons in central nervous system (CNS). However, due to its cytotoxic effects, excessive influx of Ca^{2+} and subsequent rapid elevation of $[Ca^{2+}]_i$ can induce irreversible damage of neurons or result in pathological dysfunctions such as epileptic seizure. Hence, mammalian neurons exhibit several protective mechanisms to homeostatically regulate membrane excitability and cytosolic Ca^{2+} level. Several types of voltage dependent ion channels are involved in these neuroprotective processes via regulating cation flow. In its original description based on neuronal excitability, intrinsic excitability can be defined as the ability to fire action potentials (APs), which are determined by voltage-dependent K^+ and Na^+ channels (K_V and Na_V channels) [1]. Therefore, cation channels especially sensitive to membrane potential are key factors to

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Author contributions: Y.S.Y. and S.C.J. performed electrophysiological experiments. Y.S.Y. and D.K.K. performed statistical analysis. S.Y.E. and Y.S.Y. drafted the manuscript. S.C.J. designed, supervised and coordinated the study and wrote the manuscript.

regulate excitability in most neurons even though they are inhibitory. In particular, several subtypes of Kv channels can determine resting membrane potential and effectively induce cation outflow to downregulate neuronal excitability in many conditions of membrane depolarization [2].

Among the many types of K^+ channels expressed in CNS neurons, Kv2.1 is a major component of delayed rectifier K^+ channel (I_{DR} channel), exhibiting sustained outward K^+ currents [3-5]. This subtype plays a direct role in lowering membrane potential thus inhibiting AP initiation as well as limiting repetitive APs firing. Thus, the hyperpolarization of membrane potential and blockade of depolarization by Kv2.1 can prevent hyperexcitability that activates cytotoxic cascades and induces neuronal damage [6,7]. Previous studies showed that Kv2.1 channels are sensitive to changes in cytosolic Ca^{2+} level. For example, cytosolic Ca^{2+} increase and subsequent calcineurin activation affects the gating kinetics of I_{DR} channels via dephosphorylating Kv2.1 channels, since calcineurin-dependent dephosphorylation of Kv2.1 decreases the threshold for I_{DR} channel opening and disrupts channel clustering, resulting in changes of activation kinetics [1,8]. Kv2.1 channels have many serine, threonine, and tyrosine phosphorylation sites. These participate in the regulation of I_{DR} channel kinetics; for e.g., PKA-mediated phosphorylation in serine/threonine sites induces the changes of gating kinetics but not membrane expression of Kv2.1 channels [9,10]. Dynamic changes of gating kinetics by de- or phosphorylation of Kv2.1 channels seem to be minor but significant for regulating K^+ outflow and membrane excitability under physiological conditions. However, other studies have recently demonstrated that under several pathological conditions such as epileptic seizures and ischemic damages, acute increment of cytosolic Ca^{2+} rapidly potentiates I_{DR}, and thus suppresses hyperexcitability [11-13]. These results suggest that rapid changes of cytosolic Ca^{2+} may stimulate more effective signaling pathways by which I_{DR} channels directly regulate membrane excitability. However, whether excessive Ca^{2+} influx rapidly and potentially enhances I_{DR} remains unclear.

In the present study, we confirmed that dissociated hippocampal neurons of rats showed significant increase of I_{DR} after high Ca^{2+} treatment, which could enhance synaptic activities and membrane excitability. The Ca^{2+}-mediated I_{DR} upregulation was sensitive to activity of voltage-dependent Ca^{2+} channels (VDCCs) but not NMDA receptors (NMDARs), suggesting a neuroprotective signaling pathway that is not targeted by postsynaptic Ca^{2+} influx via NMDARs. In the additional experiment, we confirmed that the contribution of Src family tyrosine kinase (SFK) was necessary for upregulating I_{DR} under high Ca^{2+} condition, suggesting that the increased expression of I_{DR} channels via SFK activation is a protective mechanism against excitotoxicity and pathogenic processes in neurons.

METHODS

Animal preparation and hippocampal primary culture

Hippocampal primary cultures were prepared from embryonic 20-day SD rats. After pregnant rats were deeply anesthetized, the embryos were removed and transferred to an ice-cold normal tyrode solution containing the following (in mM): 140 NaCl, 5.4 KCl, 2.3 $MgCl_2$, 10 HEPES, 5 glucose. The pH level was adjusted to 7.4 with NaOH. Isolated hippocampi from embryonic rat brains were transferred to ice-cold minimal essential medium (MEM) containing Earle's salts and glutamine with 10% fetal bovine serum, 0.45% glucose, 1 mM sodium pyruvate, 25 μM glutamate and antibiotics, and the hippocampi were triturated. The cells were counted and seeded on glass coverslips coated with poly-L-lysine (Sigma-Aldrich) at a density of 9×10^4 cells/ml and transferred to an incubator gassed with 95% O_2 and 5% CO_2 at 37°C. After 7 hours, the whole plating medium was exchanged to Neurobasal medium (Sigma-Aldrich) containing B-27 (Invitrogen), and a half of the medium was replaced with new media once at DIV 5. Detail protocols for cell preparation and recording techniques were as previously reported [14,15]. All experiments and procedures with animals were approved by the Animal Care and Use Committee of Jeju National University.

Drugs treatment

To electrophysiologically observe the changes of I_{DR} in cultured hippocampal neurons, high Ca^{2+} condition was induced by doubling Ca^{2+} concentration of culture media (normal 1.8 mM) up to 3.6 mM (with $CaCl_2$, Sigma-Aldrich) for 24 hours. APV (100 mM, Sigma-Aldrich) and nimodipine (10 mM, Sigma-Aldrich) were used with high Ca^{2+} treatment to block NMDARs and VDCCs, respectively, in some experiments. H89 was added to block the activation of PKA and ryanodine receptors and IP_3 receptors of ER were blocked by Ryanodine and 2AP, respectively. Blockers of kinases and receptors were obtained from Sigma Aldrich. Caffeine or 4CMC (Sigma-Aldrich) were used to test the effects of CICR via ryanodine receptor activation. Details of drugs treatment are mentioned in results and legends of figures.

Electrophysiology

For patch-clamp recordings of dissociated hippocampal neurons, DIV 6~8 cultured cells 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 level was adjusted to 7.4 with NaOH, and bubbled with 95% O_2 and 5% CO_2. TTX (0.5 μM, Tocrise) was added to the recording solution to block the Nav channels. The patch pipettes (4~6 MΩ) were filled with an internal solution containing the following (in mM): 20 KCl, 125 K^+-gluconate,

4 NaCl, 10 HEPES, 0.5 EGTA, 4 ATP, 0.3 tris-GTP, and 10 phosphocreatin. pH and osmolarity were adjusted to 7.2 and 280~300 mOsm, respectively. In all experiments, neurons showing 7~11 pF whole cell capacitance were used. Whole cell recording parameters were monitored throughout each experiment and recordings where series resistance (6~20 MΩ) varied by more than 10% were rejected.

All recordings were performed at room temperature, low-pass filtered at 5 kHz, and digitized at 10 kHz by a Digidata 1322A convertor. Voltage-clamping whole cell patches, command pulse generation and data recording were performed using Axopatch 200B and pClamp 8 software. Thick-walled, filamented patch electrodes had tip resistance of 3~6 MOhm. For adjusting whole-cell parameters, membrane capacitance and series resistance were manually compensated in Axopatch 200B during applying command pulses. Series resistance varied between 8~30 MOhm, and recordings where series resistance varied by more than 10% were rejected. No electronic compensation for series resistance was employed during currents recording. Pulse commands to record sustained currents in voltage-clamping mode are described in detail in results and figures. Sustained K^+ currents were digitally isolated using a prepulse protocol after subtracting leak currents. Peak currents were measured at +60 mV. All experimental data were additionally analyzed using IGOR pro software.

Statistical analysis

Further data and statistical analysis was performed using SPSS and Excel software and data were expressed as the mean value±standard error (SEM). The Student's *t*-test was used, and statistical difference between groups was indicated for p values of <0.05 or 0.01.

RESULTS

In physiological regulation of neuronal excitability, the dominant role of small but sustained K^+ outflow through I_{DR} channels is to determine and stabilize sub- and suprathreshold membrane potentials. However, signaling-mediated changes of its kinetics suggest a possibility that I_{DR} channels may also be targeted by hyperexcitable conditions. In the present study, we tested K^+ outflow through I_{DR} channels in response to hyperexcitable condition induced by high Ca^{2+} overnight treatment (3.6 mM $CaCl_2$, for 24 hours) in cultured hippocampal neurons. This experimental condition not only induces the downregulation of A-type K^+ channels (I_A channels) but also enhances synaptic Ca^{2+} influx by remodeling NMDARs composition, resulting in the hyperexcitability of hippocampal neurons [14,16]. After treating high Ca^{2+} to culture media for 24 hours, neurons were transferred to a recording chamber and washed in normal ACSF solution for electrophysiological measurement. In results, the current density of I_{DR} channels was significantly increased by high Ca^{2+} treatment, as shown in Fig. 1 (Control=112.62±10.28 pA/pF, n=13; high Ca^{2+}=171.05±18.30 pA/pF, n=12, p=0.02). This indicates that I_{DR} channels may be upregulated under hyperexcitable conditions.

Depolarization of membrane potential activates two major Ca^{2+} influx pathways: VDCC and NMDAR. High Ca^{2+}-enhanced neuronal excitability is reportedly dependent on Ca^{2+} influx via NMDARs, hence it was necessary to confirm if the increase of I_{DR} was dependent on NMDARs [16]. APV (100 μM) or nimodipine (10 μM) with high Ca^{2+} treatment was applied to culture media for 24 hours before recording. APV-induced NMDARs block showed no effects on the high Ca^{2+}-enhanced I_{DR} in this experiment; whereas, nimodipine, a VDCCs antagonist, significantly inhibited the effect of high Ca^{2+} treatment on I_{DR} (Fig. 1, APV=151.55±12.56 pA/pF, Nimodipine=96.03±9.34 pA/pF, p=0.48, 0.008 respectively, compared with high Ca^{2+}). Thus, I_{DR} upregulation may be triggered by Ca^{2+} influx through VDCCs rather than NMDARs. Furthermore, because it is well known that Ca^{2+}-induced Ca^{2+} release (CICR) is responsive to Ca^{2+}

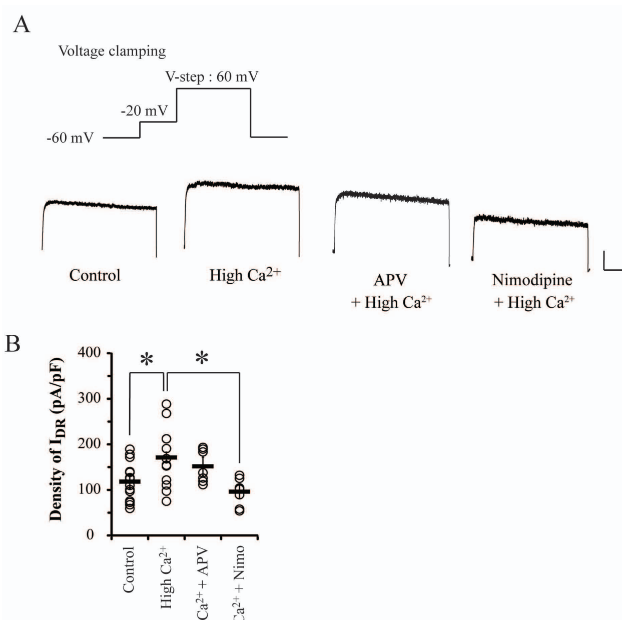

Fig. 1. I_{DR} is enhanced under high Ca^{2+} condition in cultured hippocampal neurons, showing the dependence on VDCCs but not NMDARs. (A) The experimental protocol of voltage clamping to record I_{DR} in hippocampal neurons and example traces of I_{DR}. High Ca^{2+} condition was induced by doubling Ca^{2+} concentration ($CaCl_2$, 3.6 mM) of culture media for 24 hours (High Ca^{2+}) and then neurons were washed in normal ACSF during recording. Either APV (100 μM, APV+High Ca^{2+}) or nimodipine (10 μM, Nimodipine + High Ca^{2+}) was added to culture media for 24 hours under high Ca^{2+} condition. Scale bars 500 pA, 100 ms. (B) Individuals (circles) and averaged (square bars) densities of I_{DR}. Currents were adjusted with whole cell capacitance for measuring current densities of each group. Error bars represent SEM. *p<0.05.

influx, we also tested if it might participate in I_{DR} upregulation resulted from Ca^{2+} influx via VDCCs. In Table 1, blocking of two major receptors of endoplasmic reticulum, ryanodine receptors (Ryanodine) and IP_3 receptors (2APB), showed no effects on enhanced I_{DR} under high Ca^{2+} condition. Additionally, caffeine or 4CMC, agonists of ryanodine receptors, showed no enhancement effects on I_{DR}, unlike high Ca^{2+} treatment. These results strongly indicated that Ca^{2+} influx through VDCCs may directly regulate I_{DR} channels regardless of CICR activation.

Although the current density of I_{DR} was increased, the gating kinetics of I_{DR} channels was not affected under high Ca^{2+} condition. In Fig. 2, the increase rate of I_{DR} positively reflected the increase rates of command pulse potentials in all experimental groups. At +80 mV injection, significant differences of I_{DR} amplitudes were observed between control and high Ca^{2+} treated neurons (Fig. 2A and B, Control=139.82±15.62 pA/pF; High Ca^{2+}=226.79±17.34 pA/pF, p=0.02). The current density of I_{DR} under high Ca^{2+} condition was enhanced up to 60% in each injection range from –40 to 80 mV compared with the control group. However, in the analysis of conductance compensated by each membrane potential, activation properties of I_{DR} channels were consistent in all groups, indicating that the gating kinetics of I_{DR} channels may not be subjected to high Ca^{2+} condition (Fig. 2C and D, V_h of activation: Control=12.38±2.62 mV; High Ca^{2+}=22.21±4.4 mV, p=0.1). This result suggests that the upregulation of I_{DR} channels under high Ca^{2+} condition may not be dependent on their dephosphorylation by calcineurin activated by Ca^{2+} signaling. Additionally, both antagonists, APV and nimodipine, showed no effects on the activation kinetics of I_{DR} channels (V_h of activation; APV group=10.11±1.91; Nimodipine group=14.92±1.63, p=0.55 and 0.45, respectively).

Previously, Ledoux et al. [17] reported that the enhancement of intracellular Ca^{2+} concentration induced the elevation of K^+ efflux through small conductance Ca^{2+} channels (SK channels). Because of possible change in intracellular Ca^{2+} concentration under the experimental condition, it was necessary to confirm whether the enhanced density of I_{DR} by high Ca^{2+} was contaminated by the flow of SK channels even though the two channels exhibit totally different gating mechanisms. In Fig. 3, the effect of 100 nM apamin with high Ca^{2+} was tested by adding to culture media for 24 hours to block SK channels. The addition of apamin did not influence the increased density of I_{DR} by high Ca^{2+} treatment, indicating that upregulating I_{DR} is not via the activation of SK channels (179.37±26.56 pA/pF, p=0.04 and 0.81, as compared with control and high Ca^{2+}, respectively).

Ca^{2+} signaling cascades activated by Ca^{2+} influx through synaptic NMDARs induce the phosphorylation of protein kinase A (PKA) resulting in the downregulation of I_A in hippocampal neurons [15,18]. Although it is still under argument, it is possible that PKA regulates the turnover rate of proteins from plasma membrane and currents through various channels. In this study, PKA was not involved in the cellular processing to enhance I_{DR} under high Ca^{2+} condition (Table 1, +H89). Rather, we confirmed that SFKs have a potentially important role in I_{DR} upregulation under high Ca^{2+} condition (Fig. 3). Adding SFK blocker, PP2 (1 μM), to culture media with high Ca^{2+} showed no enhancement of I_{DR}, with similar levels as in control neurons that were not treated with any drugs (Ca^{2+}+PP2=99.01±10.52 pA/pF, p=0.43 and 0.02 compared with the control and high Ca^{2+}, respectively). Furthermore, PP2 alone showed no effect on the density of I_{DR}, indicating that high Ca^{2+} condition possibly activate SFKs to prevent neuronal excitotoxicity or damage (123.46±20.23 pA/pF, p=0.63 compared with the control).

DISCUSSION

Neurons in CNS exhibit dynamic regulatory signalings to protect from hyperexcitable conditions that result in neuronal damage. Herein, we reported upregulation of I_{DR} channels, as a neuroprotective regulator, which increased K^+ outflow under hyperexcitable condition induced by high Ca^{2+} overnight treatment in cultured hippocampal neurons. The high Ca^{2+}-induced enhancement of I_{DR} was dependent on Ca^{2+} influx through VDCCs but not NMDARs and was not affected by CICRs that synergistically increase intracellular Ca^{2+} level (Fig. 1 and Table 1). These results indicate that hippocampal neurons may homeostatically modulate I_{DR} channels in response

Table 1. The densities of I_{DR} recorded in hippocampal neurons under each condition

	I_{DR} density (pA/pF) average	n	p value, compared with	
			Control	high Ca^{2+}
Control	118.11±10.91	14	-	-
High Ca^{2+}	171.05±18.30	12	0.021*	-
High Ca^{2+}+Ryanodine	180.93±27.97	11	0.039*	0.776
High Ca^{2+}+2APB	157.54±22.42	8	0.106	0.663
Caffeine	99.88±11.91	11	0.292	0.006**
4CMC	118.24±10.47	8	0.993	0.049*
High Ca^{2+}+H89	192.84±36.08	7	0.027*	0.579

*p<0.05, **p<0.01.

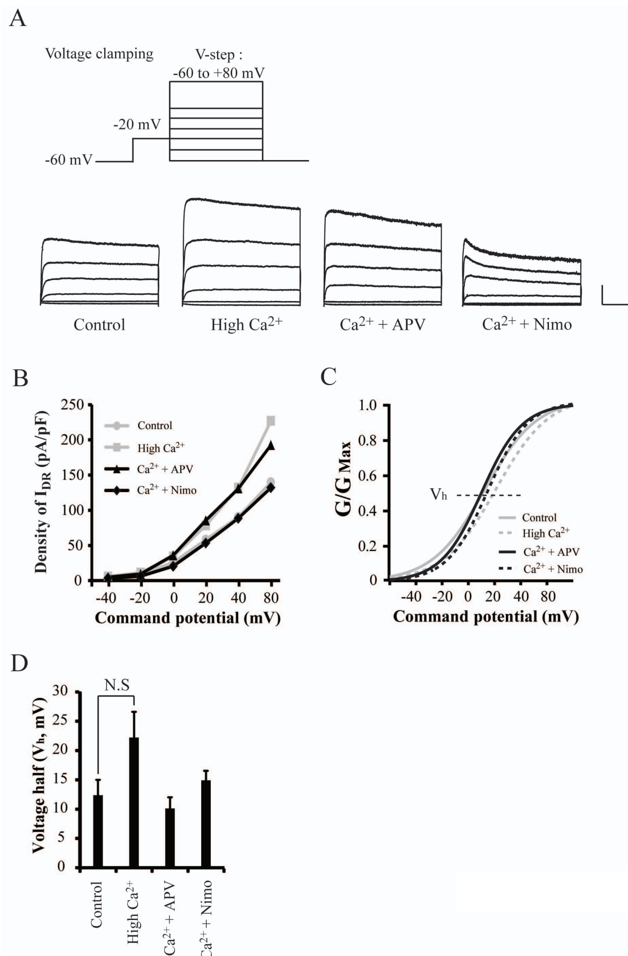

Fig. 2. The enhancement of I_{DR} under high Ca^{2+} condition does not involve the significant alteration of activation properties of I_{DR} channels. The activation property was measured at –60 to 80 mV with 20 steps after prepulse injection (–20 mV, 200 ms duration). (A) The experimental protocol of voltage clamping to measure the activation kinetics of I_{DR} channels and example traces of I_{DR}. The amplitude of I_{DR} is increased by steps of voltage clamping. However, the constant rate of increment indicates no changes of I_{DR} activating kinetics. Scale bars 500 pA, 100 ms. (B) Averaged densities of I_{DR} at each clamping voltage (–40 to 80 mV). (C) and (D) Boltzmann fitted curves of gating kinetics of I_{DR} activation and averaged values of voltage half (V_h, dotted line in C). The significant change of activation curves was not observed. Error bars represent SEM.

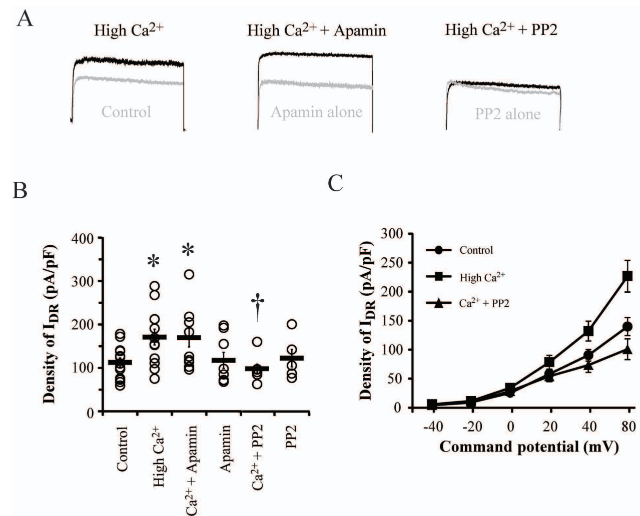

Fig. 3. SFKs are participated in I_{DR} enhancement under high Ca^{2+} condition. (A) Example traces of I_{DR} in each group. To confirm the contribution of SK channels, apamin (100 nM, Ca^{2+}+Apamin) was added to culture media. PP2 (1 μM) abolished the Ca^{2+}-induced enhancement of I_{DR}, indicating the involvement of SFK in I_{DR} upregulation. (B) Individual (open circles) and averaged values (square bars) of I_{DR} densities. Error bars represent SEM. *p<0.05 compared with control and †p<0.05 compared with High Ca^{2+}. (C) Averaged densities of I_{DR} at each clamping voltage (–40 to 80 mV). Error bars represent SEM.

to Ca^{2+} influx through VDCCs. Thus, neuronal excitability is consequently downregulated by the enhancement of K^+ outflow via I_{DR} channels. Furthermore, I_{DR} enhancement was dependent on tyrosine kinases of Src family, indicating the possibility of an unique signaling cascade for the downregulation of excitability to prevent neuronal damages under hyperexcitable or pathogenic conditions.

High Ca^{2+} treatment for 24 hours used in this study (i.e. doubling Ca^{2+} concentration in culture media), is reported to mediate changes in the subunit composition of synaptic NMDARs, I_A density and Ca^{2+}-calmodulin dependent kinase activity [14,16,19]. Increased Ca^{2+} influx through NMDARs and subsequent I_A downregulation critically increases neuronal excitability due to changes in membrane resistance, firing rates of APs and after-hyperpolarizing potentials resulting from direct effects of the cationic flow through channels or receptors [16,19]. These effects of high Ca^{2+} condition possibly result in irreversible damage to hippocampal neurons. However, hippocampal neurons showing Ca^{2+}-induced downregulation of Kv4.2 channels had no effect on the resting membrane potential even though suprathreshold properties of membrane excitability were significantly changed [19]. This phenomenon is consistent with previous results in hippocampal neurons pretreated with high Ca^{2+}, indicating that membrane potentials may also be targeted by a regulatory mechanism to prevent excitotoxicity [14]. In this study, the enhancement of I_{DR} strongly suggested a neuroprotective mechanism related with I_{DR} channels and Ca^{2+} influx through VDCCs (Fig. 1). I_{DR} channels contribute to the restoration of depolarized membrane potential to resting membrane potential in cardiac cells, determining refractory period as well as heart rate. In nervous system, these channels seem to contribute to the regulation of somatodendritic excitability. Kv2 family mediated with I_{DR} acts as a dominant regulatory factor for membrane excitability in hippocampal and cortical pyramidal neurons [20-22]. Experimental evidence indicates that the genetic manipulation of Kv2.1 directly affects intrinsic excitability, showing epileptiform activity, and that Kv2.1 knock-out mice exhibit defects in spatial learning [23].

These pathogenic outcomes are likely due to the hyperexcitability of neurons during development, as I_{DR} channels are targeted by cellular signaling pathways of various growth factors in young brain [24]. Therefore, enhanced I_{DR} under high Ca^{2+} condition observed in this study may be a rapid but effective neuroprotective modulation to prevent entry into pathogenic stages.

As a critical regulator of neuronal excitability, I_{DR} channels are dominantly targeted by Ca^{2+}-mediated signaling in the manner of activity- and use-dependence. Even though I_{DR} channels are highly phosphorylated in neurons, the expression and gating kinetics of these channels are bidirectionally subjected to activity-dependent dephosphorylation, which is mediated by glutamatergic transmission [11,13,25]. The dephosphorylation of these channels can rapidly and homeostatically suppress neuronal excitability under pathological conditions via the enhancement of K^+ outflow. Dephosphorylation of I_{DR} channels under anoxic stress is dependent on Ca^{2+} influx through NMDARs that are activated by excess glutamate release in synapses [25]. Thus, overexcitability of glutamatergic synapses in various pathological conditions possibly activates neuroprotective signalings to dephosphorylate and upregulate I_{DR} channels. However, we observed that enhanced I_{DR} under high Ca^{2+} condition was clearly dependent on Ca^{2+} influx through VDCCs but not NMDARs. This is evidenced by the effect of nimodipine to abolish the enhancement of I_{DR} (Fig. 1). Despite previous reports on the effects of VDCC blockers to inhibit I_{DR}, nimodipine, one of the dihydropyridines, showed no direct inhibition of I_{DR} in this study (data not shown) [26-28]. Because I_A downregulation is dependent on NMDARs under high Ca^{2+} condition, I_{DR} enhancement may be a possible mechanism of compensatory regulation in hippocampal neurons that are chronically exposed to high Ca^{2+} [14]. I_{DR} channels are predominantly regulated by calcineurin-dependent dephosphorylation, which is accompanied by changes of threshold as well as activation kinetics [1,8]. In this study, however, we confirmed that high Ca^{2+}-induced upregulation of I_{DR} involved only the increase of I_{DR} peak but not the alteration of gating kinetics, suggesting the possibility of another signaling mechanism distinctive to calcineurin-dependent dephosphorylation. Moreover, the bidirectional and homeostatic upregulation of I_{DR} observed in neurons is due to the overexcitation of glutamatergic synapses, hence, the enhancement of I_{DR} peak without a kinetic change may be originated from Ca^{2+} influx via VDCCs rather than NMDARs [11,13,25].

The upregulation of I_{DR} peak without changes of gating kinetics suggests the involvement of specific modulatory signalings of kinases, which can be activated under high Ca^{2+} condition (Fig. 1 and 2). Previous reports focusing on regulatory mechanisms of KV2.1 channel indicate that these channels possess a large number of kinase binding sites such as serine, threonine, and tyrosine, and are usually affected by all except threonine [9,10]. In particular, tyrosine kinases, such as Src and Fyn, seem to play an important role in the regulation of KV2.1 channel expression. Although, tyrosine 124 phosphorylated by src regulates channels activities, tyrosine 810 phosphorylation influences the intracellular trafficking, regulating Kv2.1 channels expression [29-31]. Furthermore, growth differentiation factor 15 (GDF15)-induced upregulation of Kv2.1 channels expression was also abolished by the inhibition of Src-mediated phosphorylation of TGFβ receptors [24]. These previous reports indicate that SFKs are dominantly mediated with the cellular regulation of Kv2.1 channel expression, while the gating kinetics of I_{DR} channels is specifically targeted by serine kinases. Thus, the upregulation of I_{DR} by high Ca^{2+} treatment observed in this study may be originated from the modulatory function of tyrosine kinases. Fig. 2 clearly shows that PP2 blocked the enhancement of I_{DR} under high Ca^{2+} condition, indicative of SFKs' roles in I_{DR} channels for preventing hyperexcitability.

Because excessive Ca^{2+} influx is sufficient to cause hyperexcitability and irreversible damage to hippocampal neurons, resulting in pathological conditions, a number of subcellular signalings in neurons target the expression and gating kinetics of Kv channels which actively regulate membrane excitability. The current study confirmed that a neuroprotective regulatory mechanism involving I_{DR} channels in hippocampal neurons possibly plays a role to suppress neuronal excitability under various pathogenic conditions. Although further study is needed to clarify the relationship(s) between Ca^{2+} influx via VDCCs and SFK signaling in I_{DR} upregulation, VDCC-mediated upregulation of I_{DR} suggests another signaling pathway to actively regulate membrane excitability of hippocampal neurons.

ACKNOWLEDGEMENTS

This work was supported by the Academic Research Foundation of Jeju National University Institute of medical science in 2014.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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