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Comparison of the endogenous I_K currents in rat hippocampal neurons and cloned Kv2.1 channels in CHO cells

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Abstract

The Kv2.1 potassium channel is a principal component of the delayed rectifier I_K current in the pyramidal neurons of cortex and hippocampus. We used whole-cell patch-clamp recording techniques to systemically compare the electrophysiological properties between the native neuronal I_K current of cultured rat hippocampal neurons and the cloned Kv2.1 channel currents in the CHO cells. The slope factors for the activation curves of both currents obtained at different prepulse holding potentials and holding times were similar, suggesting similar voltage-dependent gating. However, the half-maximal activation voltage for I_K was ~20 mV more negative than the Kv2.1 channel in CHO cells at a given prepulse condition, indicating that the neuronal I_K current had a lower threshold for activation than that of the Kv2.1 channel. In adddition, the neuronal I_K showed a stronger holding membrane potential and holding time-dependence than Kv2.1. The Kv2.1 channel gave a U-shaped inactivation, while the I_K current did not. The I_K current also had much stronger voltage-dependent inactivation than Kv2.1. These results imply that the neuronal factors could make Kv2.1 channels easier to activate. The information obtained from these comparative studies help elucidate the mechanism of molecular regulation of the native neuronal I_K current in neurons.

Keywords: Activation; Inactivation; Ion channel; CHO cells; Patch-clamp

1. Introduction

Kv2.1 is a delayed rectifier voltage-dependent K^+ (Kv) channel that is highly expressed in the mammalian central nervous system (Murakoshi et al., 1997; Trimmer, 1991; Trimmer, 1993), especially in hippocampal neurons (Du et al., 1998; Misonou et al., 2005; Misonou et al., 2004; Misonou and Trimmer, 2004). This particular abundance presumably reflects a fundamental and general role of the Kv2.1 channel in these neurons. Kv2.1 regulates the somatodendritic excitability in the mammalian CNS, where it forms unique cell surface clusters on the soma and proximal dendrites of the hippocampal neurons (Misonou and Trimmer, 2004). Kv2.1 is also important in setting the resting membrane potential and shaping action potential repolarization to regulate the somatodendritic excitability and the process of synaptic integration in the central neurons (Bekkers, 2000; Du et al., 2000; Korngreen and Sakmann, 2000). Knockdown of Kv2.1 in rat hippocampal neurons, leads to an enhanced somatodendirtic excitability, especially during a high-frequency synaptic transmission (Du et al., 2000). As a Ca^{2+}- and metabolic state-sensitive K^+ channel, Kv2.1 plays important roles in neuronal pathophysiology. Excitatory synaptic activity, epileptic seizures and ischemia can lead to a graded enhancement of Kv2.1 activity by lowering the threshold for voltage-dependent activation and accelerating the activation kinetics (Misonou et al., 2004), suggesting that the Kv2.1 channel could confer neuroprotection in response to epileptic seizures

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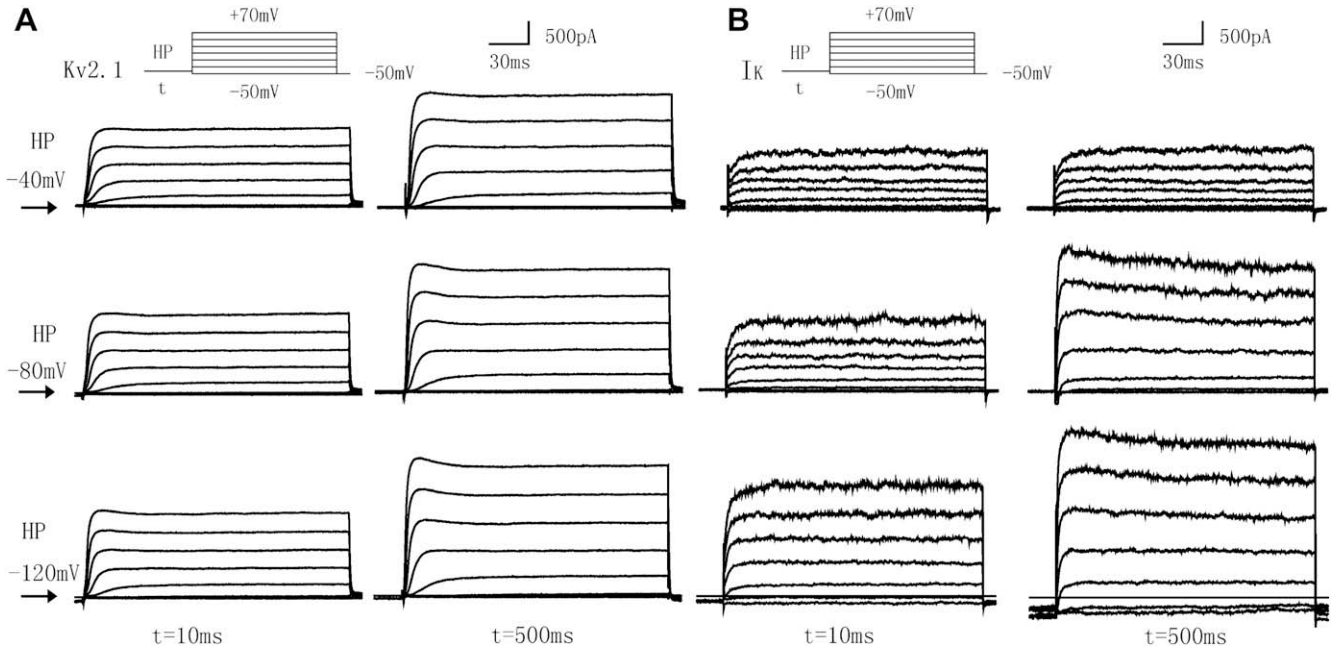

Fig. 1. Comparison of currents evoked from neuronal I_K (B) and Kv2.1 channels (A). The whole-cell currents were elicited by voltage steps from different holding potentials (e.g., −120, −80, −40 mV) and holding times (10, 100, 500, 1000 ms) to the test potentials stepped from −50 to +70 mV in 20-mV increments.

and ischemic insults. Recent work indicated that multiple phosphorylation sites on the Kv2.1 channel enable graded activity-dependent changes in the gating of Kv2.1, which makes the channel to change its function to maintain an optimal neuronal electrical activity in the face of a wide range of changes within the brain or the environment (Park et al., 2006).

In neurons, the outward voltage-dependent potassium currents could be split into two major components based on pharmacology and kinetics: a fast transient A-type, 4-aminopyridine sensitive I_A and a delayed rectifier, TEA sensitive I_K currents (Klee et al., 1995). Evidence indicates that Kv2.1 is a principal component of I_K in the pyramidal neurons of the cortex and hippocampus (Du et al., 2000; Malin and Nerbonne, 2002; Martina et al., 1998; Misonou and Trimmer, 2004; Murakoshi and Trimmer, 1999; Pal et al., 2003). The antibody that is specific for the Kv2.1 channel blocks the majority of the I_K current in hippocampal neurons (Murakoshi and Trimmer, 1999). The I_K currents of CA1 pyramidal neurons decreased significantly following treatment with antisense oligonucleotides which were directed against the Kv2.1 mRNA (Du et al., 2000). Kv2-specific dominant negative mutants selectively attenuate the I_K current (Malin and Nerbonne, 2002). These studies have indicated that Kv2.1 is the major component of I_K current in neurons. However, there is no report to systemically compare the electrical properties of I_K with the Kv2.1 channel currents. Therefore, we investigated the electrophysiological characteristics of the Kv2.1 channel current that was heterologously expressed in CHO cells, and we compared them with those of the native I_K current in hippocampal neurons using the patch-clamp technique in the voltage-clamp configuration.

2. Materials and methods

2.1. Neuronal cell culture

Hippocampal neurons were acutely dissociated according to a previous method (Brewer et al., 1993; Chen et al., 2005) with slight modification. All animal protocols were approved by the animal research ethical committee (Institute of Biophysics, CAS). Briefly, the hippocampi were dissected from neonatal Sprague–Dawley rats (aged within 24 h; Weitonglihua Animal Center, Beijing), and neurons were dissociated by incubation (7 min, 37 °C) in Trypsin–EDTA (GIBCO) and triturated in DMEM (Life Technologies) supplemented with 10% fetal bovine serum (Hyclone). The resulting hippocampal cells, at a density of 2×10^5 cells/cm^2 onto poly-L-lysine (Sigma) coated coverslips, were cultured in a humidified incubator in 5% CO_2 in air at 37 °C. The medium was replaced 7 h later with Neurobasal Medium, B-27 (GIBCO) and 0.5 mM glutamine without antibiotic solution. After 48 h, the medium was changed to Neurobasal Medium and B-27.

2.2. Cell culture and gene transfection

CHO-K1 cells were grown in Ham's F-12 nutrient mixture (Invitrogen, Co. Grand Island, NY) supplemented with 10% fetal bovine serum. Cells were grown in a 37 °C incubator with 5% CO_2 in a humidified air environment and passaged twice weekly using 0.05% trypsin with 0.5 mM EDTA in PBS(−) solution. For gene transfection, CHO-K1 cells were transferred to poly-L-lysine (Sigma) coated glass coverslips. After cell density reached 50–70% confluence, pEGFP (Clontech, Palo Alto, CA) was transiently coexpressed with the Kv2.1 channel gene (a generous gift from Dr. Wary, Leeds

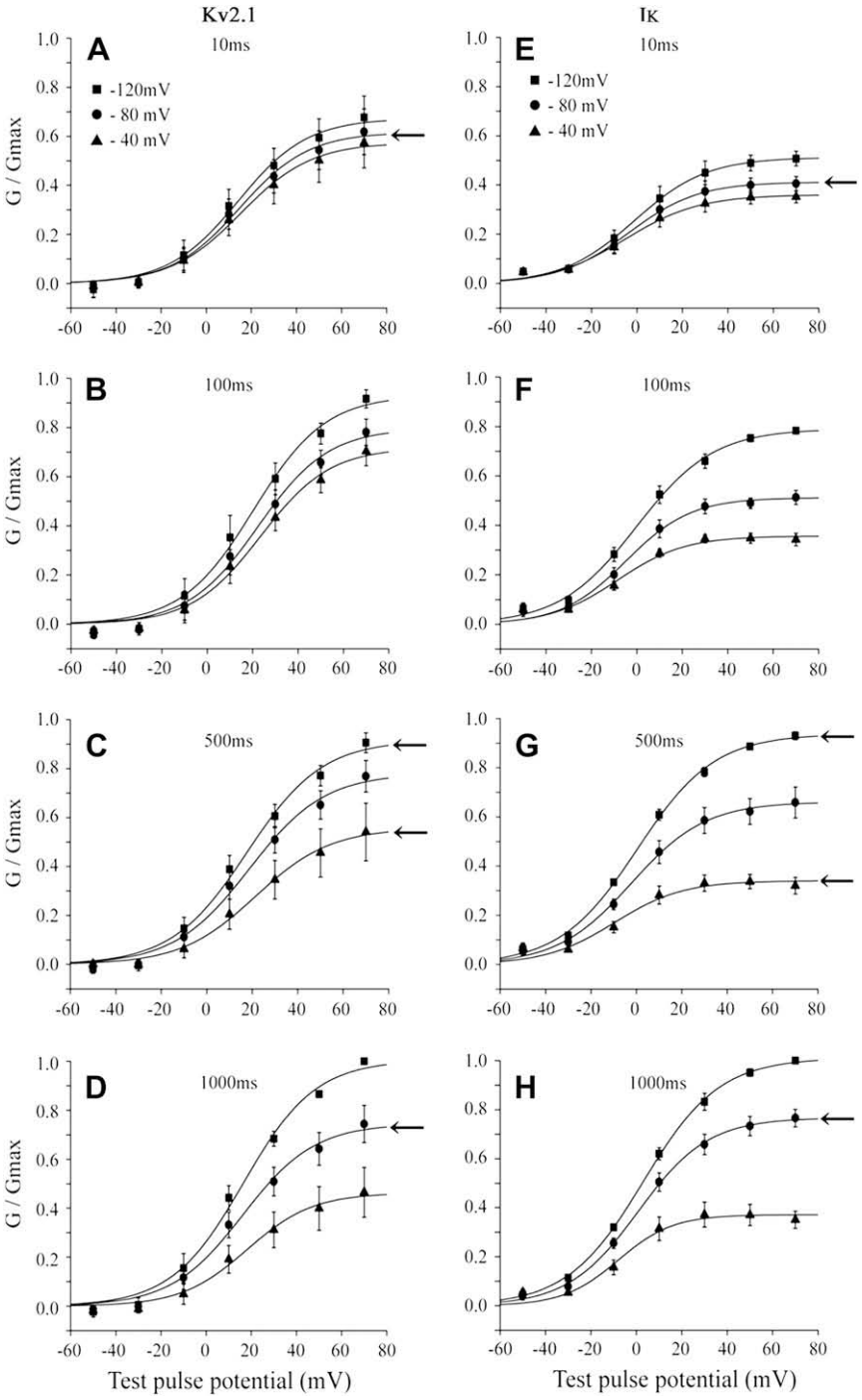

Fig. 2. Comparison of voltage-dependent activation for neuronal I_K and Kv2.1 channels. The normalized conductance values (G/G_{max}) are plotted as a function of the membrane voltage for Kv2.1 (A–D) and neuronal I_K current (E–H). The data were fitted with the Boltzmann equation with parameters in Tables 1 and 2. The prepulse holding potentials were −40 (▲), −80 (●) and −120 (■) mV at different holding time of 10, 100, 500 and 1000 ms ($n = 4–15$).

University) that is in mammalian expression vectors pCDNA3 at a ratio of 5:1 (weight /weight) using LipofectAMINE Plus™ reagent (Invitrogen). Cells were used for electrophysiological studies for 1–3 days after the transfection.

2.3. Whole-cell patch-clamp recordings

Voltage-clamp recordings were performed on the hippocampal pyramidal neurons between 6 and 10 days *in vitro* by using the EPC-9 or EPC-10 patch-clamp amplifiers (HEKA, Germany). Pipette and membrane capacitances were compensated automatically with the amplifier. The membrane potential was at −30 mV after whole-cell configuration was formed. Currents were corrected offline for a linear leak current measured at −90 mV. A program package Pulse + Pulsefit (HEKA, Germany) was used for data acquisition and analysis. Hanks' Balanced salts solution (HBSS, Sigma) was taken as extracellular solution (in mM): 1.3 $CaCl_2$, 0.8

Table 1
Biophysical parameters for activation curves of the Kv2.1 channels

HT (ms)[a]	10			100			500			1000		
HP (mV)[b]	−40	−80	−120	−40	−80	−120	−40	−80	−120	−40	−80	−120
$V_{1/2}$ (mV)	15.4	14.9	14.2	23.6	22.4	20.4	21.4	18.7	17.7	18.1	16.3	16.5
k	15.7	15.2	15.3	15.2	15.4	15.8	16.5	16.5	16.6	14.6	15.9	15.9
P_{max}	0.57	0.62	0.67	0.72	0.80	0.93	0.56	0.78	0.92	0.46	0.75	1.00

[a] HT: Holding time.
[b] HP: Holding potential.

$MgSO_4$, 5.4 KCl, 0.4 KH_2PO_4, 136.9 NaCl, 0.3 Na_2PO_4, 10 D-glucose and 4.2 $NaHCO_3$. The intracellular solution contained (in mM): 140 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1 EGTA, 2 Na_2ATP, and 10 HEPES at pH 7.3. The steady-state outward I_K current (+70 mV) was measured as mean value in a range from 85% to 100% of the current trace. For neurons, a conditioning prepulse of −40 mV for 100 ms was given before each test pulse to delete the majority of I_A current component. All experiments were performed at room temperature (22–25 °C).

2.4. Statistical analysis

Data were expressed in mean and standard error of mean (mean ± S.E.M.) throughout the text. One-way analysis of variance (ANOVA) was used to detect statistical significance between more than two means when appropriate. Statistical significance was set at a $P < 0.05$ if not otherwise mentioned below.

3. Results

3.1. Activation properties of neuronal I_K current and expressing Kv2.1 channels

Outward potassium currents were recorded either from CHO cells transiently expressing the recombinant rat Kv2.1 channels or from cultured rat hippocampal neurons using the whole-cell mode of the patch-clamp technique. The test potentials were stepped from −50 to +70 mV with a 20 mV increment from the prepulse holding potentials of −40, −80 and −120 mV at different holding times of 10, 100, 500 and 1000 ms. Both currents were enhanced by more negative and longer period prepulse holding potentials (Fig. 1). The voltage-dependent activation curves of the Kv2.1 channels and neuronal I_K currents were constructed by fitting the data to a modified Boltzmann equation: $G/G_{max} = P_{max}/\{1 + \exp[-(V - V_{1/2})/k]\}$, where G_{max} is the maximum conductance (usually achieved for holding time of 1000 ms at −120 mV) among all of the prepulse conditions, P_{max} is the maximum open probability of the channel at a given holding potential and holding time, V is the membrane potential, $V_{1/2}$ is the voltage at half-maximal activation, and k is the slope factor. Activation curves for neuronal Kv2.1 and I_K channels are shown in Fig. 2. The slope factor (k), a measure of the sensitivity of channels to membrane potential, was around 16 mV for both the neuronal I_K and Kv2.1 channels in the range of holding time from 10 to 1000 ms and holding potential from −120 to −40 mV (Tables 1 and 2), suggesting a similar voltage sensitivity of activation kinetics for both the I_K and Kv2.1 channels.

Despite these similarities, there are several differences. First, despite the wide range of holding time and potential, the value of $V_{1/2}$ was within the range between −9.7 and +3.0 mV in neurons and within the range between 14.2 and 23.6 mV for the Kv2.1 channels (Tables 1 and 2). This result indicated that the neuronal I_K current could be activated at much more negative voltage than that of Kv2.1 channel. Second, the neuronal I_K showed a stronger prepulse holding time-dependence than that shown in the Kv2.1. At holding potential of −80 mV, the P_{max} of the neuronal I_K increased from 0.41 to 0.77 (Table 2, and the curves indicated by the arrows in Fig. 2E and H), while it only increased from 0.62 to 0.75 for the Kv2.1 (Table 1, and the curves indicated by the arrows in Fig. 2A and D) in the range of holding time prolonging from 10 to 1000 ms. Besides, the outward K^+ current produced by I_K for a test pulse of +50 mV from a prepulse holding potential of −120 mV, increased almost 2-fold (from 718 ± 79 to 1349 ± 155 pA, $n = 4$) when the holding time was increased from 10 to 500 ms, while it only increased 1.4-fold (from 1380 ± 132 to 1946 ± 93 pA, $n = 5$) for the Kv2.1 channel at the same condition (see Fig. 1 for representative current traces). Third, the neuronal I_K showed a stronger holding membrane potential dependence than that shown in the Kv2.1 channels. For instance, at holding time of 500 ms, the P_{max} increased from 0.34 to 0.94 for neuronal I_K when the holding potential changed from −40 to −120 mV (Table 2 and the curves indicated by the arrows in Fig. 2G), while it only

Table 2
Biophysical parameters for activation curves of the neuronal I_K currents

HT (ms)	10			100			500			1000		
HP (mV)	−40	−80	−120	−40	−80	−120	−40	−80	−120	−40	−80	−120
$V_{1/2}$ (mV)	−5.1	−3.6	−1.0	−8.5	−4.8	−0.2	−9.7	−2.2	0.4	−8.1	−0.7	3.0
k	16.3	15.3	15.8	14.8	14.3	17.0	15.1	16.4	17.0	12.0	15.4	16.7
P_{max}	0.36	0.41	0.51	0.38	0.51	0.79	0.34	0.66	0.94	0.37	0.77	1.00

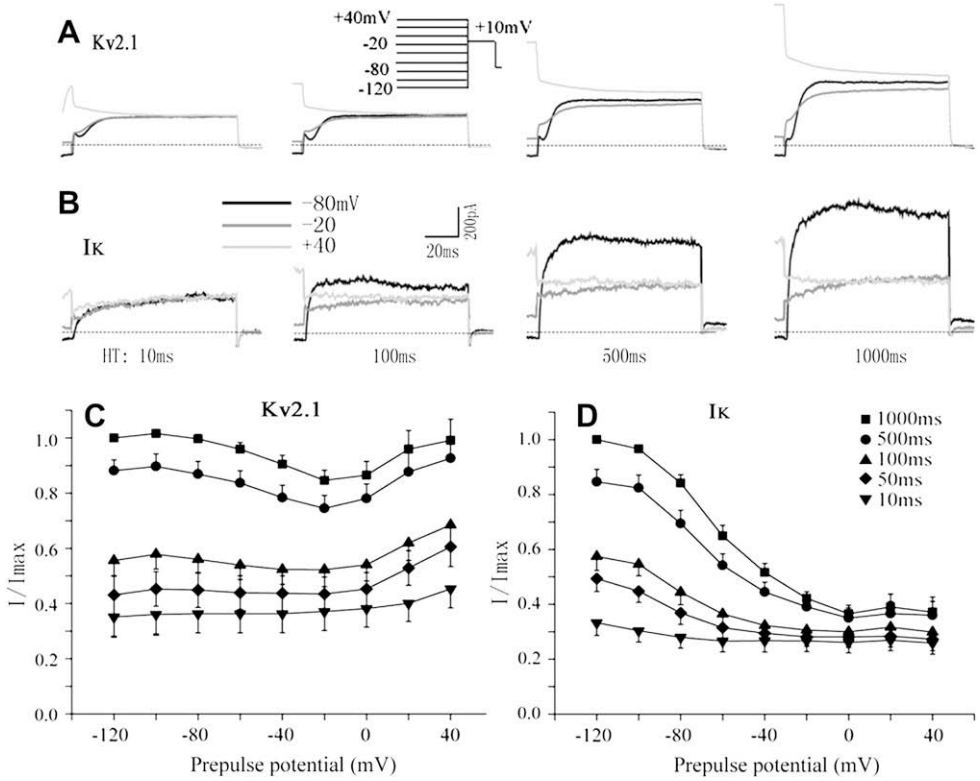

Fig. 3. Comparison of voltage-dependent inactivation for neuronal I_K and Kv2.1 channels. Inactivation of the currents for the neuronal I_K (B) and Kv2.1 channels (A) were elicited by a test potential of +10 mV from three different conditioning prepulses (−40, −80 and −120 mV) with holding periods of 10, 100, 500 and 1000 ms. The normalized current (I/Imax) is plotted against the conditioning prepulse for the neuronal I_K (D) and Kv2.1 col for getting the inactivation curves.

increased from 0.56 to 0.92 for Kv2.1 channel at the same condition (Table 1 and the curves indicated by the arrows in Fig. 2C). At holding time of 500 ms and test potential of +50 mV, neuronal I_K current increased 2.6-fold (from 572 ± 102 pA to 1465 ± 292 pA, $n = 6$) when the holding potential was changed from −40 to −120 mV, while the Kv2.1 current only increased 1.6-fold (from 1091 ± 275 to 1788 ± 237 pA, $n = 6$) at the same holding time (see Fig. 1 for representative current traces).

3.2. Inactivation properties of Kv2.1 channels and neuronal I_K currents

Conventionally, inactivation of a channel is characterized by the steady-state inactivation curve which is constructed by setting a prepulse holding time long enough to achieve the steady-state inactivation of the channel. However, a real stimulus to the neurons is in a range from several milliseconds at most for a typical action potential stimulus to a range of several milliseconds to minutes for slow potentials that are generated and graded at a localized membrane. Therefore, we compared the inactivation curve of the channels from 10 to 1000 ms. The protocol is as follows, the conditioning prepulse potentials started from −120 up to +40 mV for different durations of 10, 100, 500 and 1000 ms, respectively, with 20 mV increments, following by a test pulse of +10 mV with 100-ms duration. When the conditioning prepulse was at short period (e.g., 10 ms), both the neuronal I_K currents and Kv2.1 channel current almost did not change even the conditioning prepulse potential changed from −40 to −120 mV, suggesting that short period could not change the inactivation state of both I_K and Kv2.1. When the holding times from 50 to 1000 ms were applied, the inactivation process of both current became obviously dependent on the different conditioning prepulses. For the neuronal I_K, the current amplitude decreased as the conditioning prepulse was progressively depolarized. For the Kv2.1 channel, the current amplitude gradually decreased only when the conditioning prepulse was more negative than approximately −20 mV. When the conditioning prepulse was more positive than −20 mV, the Kv2.1 current amplitude began to increase again, indicating a U-shape inactivation (Fig. 3). This phenomenon is more clearly shown in Fig. 3C and D, where the normalized current amplitudes were plotted against the conditioning prepulse potential to emphasize the fraction of current elicited from each conditioning prepulse. Thus, the voltage-dependent inactivation of the Kv2.1 channels was not monotonic, but a U-type, with a lesser inactivation at strong depolarization.

4. Discussion

Due to its high threshold for voltage-dependent activation and slow activation kinetics, it has been suggested that the Kv2.1 channels may not act during the duration of a single action potential or a single excitatory event which may end within 10 ms (Du et al., 2000; Malin and Nerbonne, 2002).

However, the neuronal I_K has a lower threshold than that of the Kv2.1 channel and a more negative voltage for activation. For example, the $V_{1/2}$ for neuronal I_K current is over −20 mV negative to that of the Kv2.1 at a holding potential of −80 mV and 500 ms. These results suggest that, when Kv2.1 is expressed in the neurons, the neuronal factors could make it easier to activate. So, the Kv2.1 channels could play more important roles in neuronal excitability than expected from the properties of the Kv2.1 channels along. Consistent with this result, the blockade of I_K current has been shown to broaden the single action potential duration (data not shown).

In spite of the Kv2.1 channels as the major component of the I_K currents, significant difference was found in the activation and inactivation properties between them. What are the origins of this heterogeneity in their activation and inactivation properties? One possibility is that channel phosphorylation may be responsible. Serine phosphorylation was shown to shift the voltage dependence of activation in the Kv2.1 channels (Murakoshi et al., 1997). So the difference on the phosphorylation condition of the Kv2.1 in CHO cells and in hippocampal neurons may provide a physiological mechanism for changes in the inactivation and activation of Kv2.1 channels. Another intriguing possibility is that auxiliary subunits may be responsible. Differential subunit assemblies of homologous pore-forming subunits could provide a mechanism for the functional diversity of the channels. Furthermore, interactions with auxiliary subunits or posttranslational modification introduce a greater variability in the channel kinetics. These kinetic differences cause alterations in the I_K and Kv2.1 currents, which would translate into differences in the cellular excitability. This mechanism may allow cells to regulate their excitability based on the physiological demand for that cell. A variety of regulatory subunits may interact with the pore complex either statically or dynamically. Although α-subunits form the pore, many channels function as complexes that require cytoplasmic and transmembrane auxiliary subunits. To date, little is known regarding the way in which such components bind α-subunits and modulate the channel action. The Kv2.1 channel complexes behave as large macromolecular complexes with an apparent oligomeric size of 650 kDa, while a subunit of Kv2.1 is only 100 kDa (Chung and Li, 2005), implying that the neuronal Kv2.1 channels function as complexes which require cytoplasmic and/or transmembrane auxiliary proteins to regulate their function. In addition, an appropriate localization of Kv2.1 channels is crucial for local regulation of electrical excitability, such as by possibly placing the channels in proximity to local signaling pathways which modulate channel activity and/or cell surface expression (Lim et al., 2000; O'Connell et al., 2006). Finally, hippocampal neurons express several types of Kv channels. It is possible that the delayed rectifier I_K current is from the combination of these Kv channel. However, as Kv2.1 channel dominated the expression, we think that other types of Kv channels have minor contribution to the I_K current. Comparisons of native I_K current with the cloned Kv2.1 current demonstrated several similarities in their electrophysiological and pharmacological characteristics, but they were nevertheless distinguishable. As dynamic modulation of ion channels underlies the neuronal plasticity, further study is needed to gain insight into how neurons can modulate the Kv2.1 channel to make it possess properties of the I_K currents.

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References

Bekkers JM. Distribution and activation of voltage-gated potassium channels in cell-attached and outside-out patches from large layer 5 cortical pyramidal neurons of the rat. J Physiol 2000;525:611–20.

Brewer GJ, Torricelli JR, Evege EK, Price PJ. Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. J Neurosci Res 1993;35:567–76.

Chen X, Chi S, Liu M, Yang W, Wei T, Qi Z, et al. Inhibitory effect of ganglioside GD1b on K^+ current in hippocampal neurons and its involvement in apoptosis suppression. J Lipid Res 2005;46:2580–5.

Chung JJ, Li M. Biochemical characterization of the native Kv2.1 potassium channel. FEBS J 2005;272:3743–55.

Du J, Haak LL, Phillips-Tansey E, Russell JT, McBain CJ. Frequency-dependent regulation of rat hippocampal somato-dendritic excitability by the K^+ channel subunit Kv2.1. J Physiol 2000;522:19–31.

Du J, Tao-Cheng JH, Zerfas P, McBain CJ. The K^+ channel, Kv2.1, is apposed to astrocytic processes and is associated with inhibitory postsynaptic membranes in hippocampal and cortical principal neurons and inhibitory interneurons. Neuroscience 1998;84:37–48.

Klee R, Ficker E, Heinemann U. Comparison of voltage-dependent potassium currents in rat pyramidal neurons acutely isolated from hippocampal regions CA1 and CA3. J Neurophysiol 1995;74:1982–95.

Korngreen A, Sakmann B. Voltage-gated K^+ channels in layer 5 neocortical pyramidal neurones from young rats: subtypes and gradients. J Physiol 2000;525:621–39.

Lim ST, Antonucci DE, Scannevin RH, Trimmer JS. A novel targeting signal for proximal clustering of the Kv2.1 K^+ channel in hippocampal neurons. Neuron 2000;25:385–97.

Malin SA, Nerbonne JM. Delayed rectifier K^+ currents, I_K, are encoded by Kv2 alpha-subunits and regulate tonic firing in mammalian sympathetic neurons. J Neurosci 2002;22:10094–105.

Martina M, Schultz JH, Ehmke H, Monyer H, Jonas P. Functional and molecular differences between voltage-gated K^+ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. J Neurosci 1998; 18:8111–25.

Misonou H, Mohapatra DP, Menegola M, Trimmer JS. Calcium- and metabolic state-dependent modulation of the voltage-dependent Kv2.1 channel regulates neuronal excitability in response to ischemia. J Neurosci 2005; 25:11184–93.

Misonou H, Mohapatra DP, Park EW, Leung V, Zhen D, Misonou K, et al. Regulation of ion channel localization and phosphorylation by neuronal activity. Nat Neurosci 2004;7:711–8.

Misonou H, Trimmer JS. Determinants of voltage-gated potassium channel surface expression and localization in Mammalian neurons. Crit Rev Biochem Mol Biol 2004;39:125–45.

Murakoshi H, Shi G, Scannevin RH, Trimmer JS. Phosphorylation of the Kv2.1 K^+ channel alters voltage-dependent activation. Mol Pharmacol 1997;52:821–8.

Murakoshi H, Trimmer JS. Identification of the Kv2.1 K^+ channel as a major component of the delayed rectifier K^+ current in rat hippocampal neurons. J Neurosci 1999;19:1728–35.

O'Connell KM, Rolig AS, Whitesell JD, Tamkun MM. Kv2.1 potassium channels are retained within dynamic cell surface microdomains that are defined by a perimeter fence. J Neurosci 2006;26:9609–18.

Pal S, Hartnett KA, Nerbonne JM, Levitan ES, Aizenman E. Mediation of neuronal apoptosis by Kv2.1-encoded potassium channels. J Neurosci 2003;23:4798–802.

Park KS, Mohapatra DP, Misonou H, Trimmer JS. Graded regulation of the Kv2.1 potassium channel by variable phosphorylation. Science 2006;313:976–9.

Trimmer JS. Immunological identification and characterization of a delayed rectifier K^+ channel polypeptide in rat brain. Proc Natl Acad Sci U S A 1991;88:10764–8.

Trimmer JS. Expression of Kv2.1 delayed rectifier K^+ channel isoforms in the developing rat brain. FEBS Lett 1993;324:205–10.