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Effect of Genistein, a Tyrosine Kinase Inhibitor, on the Cloned Rat Brain Potassium Channel Kv1.5

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The effect of genistein, widely used as a specific tyrosine kinase inhibitor, on rat brain Kv1.5 channels which were stably expressed in Chinese hamster ovary cells was investigated using the whole-cell patch-clamp technique. Genistein inhibited Kv1.5 currents at +50 mV in a concentration-dependent manner, with an IC_{50} of 54.7±8.2 μM and a Hill coefficient of 1.1±0.2. Pretreatment of Kv1.5 with protein tyrosine kinase inhibitors (10 μM lavendustin A and 100 μM AG1296) and a tyrosine phosphatase inhibitor (500 μM sodium orthovanadate) did not block the inhibitory effect of genistein. The inhibition of Kv1.5 by genistein showed voltage-independence over the full activation voltage range positive to 0 mV. The activation (at +50 mV) kinetics was significantly delayed by genistein: time constant for an activation of 1.4±0.2 msec under control conditions and 10.0±1.5 msec in the presence of 60 μM genistein. Genistein also slowed the deactivation of the tail currents, resulting in a crossover phenomenon: a time constant of 11.4±1.3 msec and 40.0±4.2 msec under control conditions and in the presence of 60 μM genistein, respectively. Inhibition was reversed by the application of repetitive depolarizing pulses, especially during the early part of the activating pulse. These results suggest that genistein directly inhibits Kv1.5 channels, independent of phosphotyrosine-signaling pathway.

Key Words: Genistein, Tyrosine kinase inhibitor, Kv1.5, Closed channel block

INTRODUCTION

Voltege-gated K^+ channels have been known to be involved in forming action potentials, controlling membrane excitability, neuronal firing patterns, neurotransmitter release, volume regulation and cell proliferation. Therefore, gating, conductance and kinetics of K^+ channel have received the most attention. Protein kinases play an important role in regulating the activity of a variety of ion channels. In modulation of ionic channel activity by protein kinases, phosphorylation and dephosphorylation are crucial processes (Levitan, 1994; Siegelbaum, 1994; Jonas & Kaczmarek, 1996). K^+ channels have been known to be modulated by serine/threonine and tyrosine phosphorylation (Levitan, 1994; Siegelbaum, 1994; Jonas & Kaczmarek, 1996). While serine/threonine phosphorylation of K^+ channels by protein kinase C (PKC) and protein kinase A (PKA) is well known (Levitan, 1994; Siegelbaum, 1994; Jonas & Kaczmarek, 1996), K^+ channels are shown to be modulated by tyrosine phosphorylation via receptor and non-receptor tyrosine kinases (Huang et al, 1993; Timpe & Fantl, 1994; Holmes et al, 1996a; Holmes et al, 1996b; Jonas & Kaczmarek, 1996; Jonas et al, 1996; Aniksztejn et al, 1997; Fadool & Levitan, 1998). Because of the above reasons, the intensive research on the role of protein tyrosine kinase (PTK) in the regulation of ion channel, especially K^+ channel, is expected.

Genistein, an isoflavone abundant in vegetables, has widely been used in the study of PTK phosphorylation because it has a broad inhibitory action on both receptor and non-receptor tyrosine kinases (Akiyama et al, 1987). In the heart, PTK pathway has been known to play a potentially important role in mediating the cell swelling-induced increase of Cl^- and delayed rectifier K^+ currents in canine cardiac myocytes (Sorota, 1995; Zhou et al, 1997) and in modulating cellular hypertrophy (Morgan & Baker, 1991; Sadoshima & Izumo, 1993). Therfore, PTK inhibitors have widely been used in the study on the involvement of PTK pathway in the heart. However, accumulating data have shown that PTK inhibitors have a direct action on ion channels in a phosphorylation-independent manner. Independent of PTK activity, genistein inhibits directly voltage-gated K^+ currents in the pulmonary arterial cells of rats and rabbits (Smirnov & Aaronson, 1995), in the ventricular cells of guinea pig (Washizuka et al, 1998), cloned Kv3.1 expressed in Chinese hamster ovary (CHO) cells (Choi et al, 2006), and voltage-gated L-type Ca^{2+} currents in guinea pig ventricular cells (Chiang et al, 1996). The possibility of direct blocking actions of genistein and tyrphostin 23, a selective PTK inhibitor, on Ca^{2+} channel has also been described in the vascular smooth muscle cells of rabbit ear artery (Wijetunge et al, 1992). AG1478, ano-

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ABBREVIATIONS: PTK, protein tyrosine kinase; PKC, protein kinase C; PKA, protein kinase A.

ther tyrosine kinase inhibitor, inhibits directly the cloned Kv1.5 channel (Choi et al, 2002).

In the present study, in order to determine the direct modulation of Kv1.5 channel by genistein in a phosphorylation-independent manner, the effects of genistein on the cloned rat Kv1.5 channels expressed in CHO cells, using the patch-clamp technique were examined.

METHODS

Stable transfection and cell culture

Rat brain Kv1.5 channels stably expressed in CHO cells were used in the present study (Choi et al, 2000; Choi et al, 2005). The method to establish Kv1.5 expression in CHO cells was as follows. Briefly, the cDNA of Kv1.5 (Swanson et al, 1990) was subcloned into plasmid expression vector pCR3.1 (Invitrogen Corporation, San Diego, CA, USA) and pRc/CMV (Invitrogen Corporation, San Diego, CA, USA). CHO cells were cultured in Iscove's modified Dulbecco's medium (IMDM; Life Technologies, Grand Island, NY, USA), supplemented with 10% fetal bovine serum, 2 mM glutamine, 0.1 mM hypoxanthine and 0.01 mM thymidine, under a 95% humidified air-5% CO_2 environment at 37°C. Kv1.5 expression vector was added to CHO cells for transfection using FuGENE^{TM}6 reagent (Boehringer Mannheim, Indianapolis, IN, USA) and lipofectamine reagent (Invitrogen Corporation), respectively. Transfected cells were exposed to 0.5 mg/ml G418 (Invitrogen Corporation), and antibiotic-resistant cells were selected and maintained in a fresh IMDM containing 0.5 mg/ml G418. By using a brief trypsin/EDTA (Life Technologies) treatment, transfected CHO cells were passed every 4~5 days and were seeded onto glass coverslips (diameter: 12 mm, Fisher Scientific, Pittsburgh, PA, USA) in a Petri dish 24 h before use. For electrophysiological recording, cells-attached coverslips were transferred to a continually perfused (1 ml/min) recording chamber (RC-13, Warner Instrument Corporation, Hamden, CT, USA).

Electrophysiological recordings

At room temperature (22~24°C), whole-cell current of Kv1.5 was recorded and stored using the patch-clamp technique (Hamill et al, 1981) with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA, USA) and a Digidata 1200A acquisition board (Axon Instruments)-equipped IBM compatible computer. Currents were sampled at 5 kHz and filtered at 2 kHz (four-pole Bessel filter). Pulse generation and data acquisition were controlled using pClamp 6.05 software (Axon Instruments). Patch electrodes were fabricated using PG10165-4 glass capillary tubing (World Precision Instruments, Sarasota, FL, USA). Liquid junction potentials between external and pipette solution were offset. Whole-cell capacitative current compensation and 80% series resistances compensation were done without any leakage compensation. The whole-cell currents and series resistance with about 1~6 nA and 1.8~3.3 MΩ, respectively, were used for analysis, and any significant voltage error was avoided.

Solutions and drugs

The pipette solution contained (in mM) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 EGTA, and pH was adjusted to 7.3 with KOH. The bath solution contained (in mM) 140 NaCl, 5 KCl, 1.3 $CaCl_2$, 1 $MgCl_2$, 20 HEPES, 10 glucose, and pH was adjusted to 7.3 with NaOH. Sodium orthovanadate (Calbiochem, San Diego, CA, USA) was dissolved in distilled water to prepare 50mM stock solutions. Genistein (Calbiochem, San Diego, CA, USA), lavendustin A (Calbiochem), Daidzein (Calbiochem) and AG1296 (Calbiochem) were dissolved in dimethyl sulfoxide (DMSO) to yield 50 mM stock solutions. The final concentration of DMSO was less than 0.1%, and this concentration had no effect on Kv1.5 currents.

Data analysis

Analysis of data was carried out using pClamp 6.05 software (Axon Instruments) and Origin 6.1 software (Microcal Software, Inc., Northampton, MA, USA). The concentratration-dependent curve for current inhibition by genistein was fitted to a following equation:

$$I\ (\%)=1/[1+(IC_{50}/[D])^n] \quad (1)$$

in which I (%) is the percentage current inhibition ($I(\%)=[1-I_{drug}/I_{control}]\times 100$) at test potential, [D] represents various drug concentrations, IC_{50} is a concentration of half-maximal inhibition, and n is the Hill coefficient. Activation curves were fitted with a Boltzmann equation:

$$y=1/[1+\exp(-(V-V_{1/2})/k)] \quad (2)$$

where k represents the slope factor, V the test potential and $V_{1/2}$ the voltage at which the conductance was half-maximal. The activation kinetics was calculated by fitting with a single exponential function immediately after the transient capacitative current on depolarizing pulses. The steady-state voltage dependence of inactivation was investigated by using a two-pulse voltage protocol; currents were measured by a 250-ms test potential to +50 mV, while 20-s preconditioning pulses were varied from −60 to 0 mV, stepped by 10 mV, in the absence and presence of drugs. The experimental points were calculated as shown in equation 3.

$$\text{Normalized } I=(I-I_c)/(I_{max}-I_c) \quad (3)$$

in which I_{max} represents the current measured at the most hyperpolarized preconditioning pulse and I_c represents a non-zero current which was not inactivated at the most depolarized 20-s preconditioning pulse. This non-zero residual current was subtracted from the actual value. The resulting steady-state inactivation data were fitted with a Boltzmann equation:

$$y=1/[1+\exp(V-V_{1/2})/k] \quad (4)$$

where V is the preconditioning potential, and $V_{1/2}$ and k represent the potential corresponding to the half-inactivation point (in mV) and slope value (in mV), respectively. The activation and deactivation kinetics were determined by fitting the sum of the exponentials:

$$y=B+A_1\exp(-t/\tau_1)+A_2\exp(-t/\tau_2)+\cdots+A_n\exp(-t/\tau_n) \quad (5)$$

in which τ_1, τ_2, and τ_n are the time constants; A_1, A_2,

and A_n are the amplitudes of each component of the exponential; and B is the baseline value.

Results were expressed as means±SE. Student's *t*-test and analysis of variance (ANOVA) were used for statistical analysis. A value of $p<0.05$ was considered statistically significant.

RESULTS

Concentration-dependent inhibition of Kv1.5 by genistein

Fig. 1A shows the superimposed-original Kv1.5 current traces obtained by 250-ms depolarizing pulse to +50 mV under control conditions and in the presence of various concentrations of genistein. As described previously (Choi et al, 2000; Choi & Hahn, 2005), Kv1.5 currents under control conditions, were characterized by rapid activation and then slow inactivation while a depolarizing pulse was maintained. When switched to solutions containing different concentrations of genistein, inhibition of Kv1.5 reached a steady state within 1 min. The washout by perfusion of drug-free solution was also achieved within 1 min, and currents recovered to 94.1±1.5% of control value (n=7), indicating that the effect of genistein was largely reversible upon washout. Rapid inhibition and recovery by genistein suggests that the effect is protein kinase-independent and probably due to a direct interaction between the drug and Kv1.5 channel. Genistein-induced inhibition of Kv1.5 was characterized by both slowing of the apparent rate of activation and reduction of peak current amplitudes in a concentration-dependent manner. To elucidate the concentration dependence of genistein action on Kv1.5, the effects of genistein on the peak amplitudes of Kv1.5 currents at 250-ms depolarizing pulse of +50 mV as a reflection of the drug effects on Kv1.5 were studied. As shown in Fig. 1B, a nonlinear least-squares fit of the Hill equation to the concentration-response data, which correspond to the percentage inhibition of currents, yielded an apparent IC_{50} of 54.7±8.2 μM and a Hill coefficient of 1.1±0.2 (n=5).

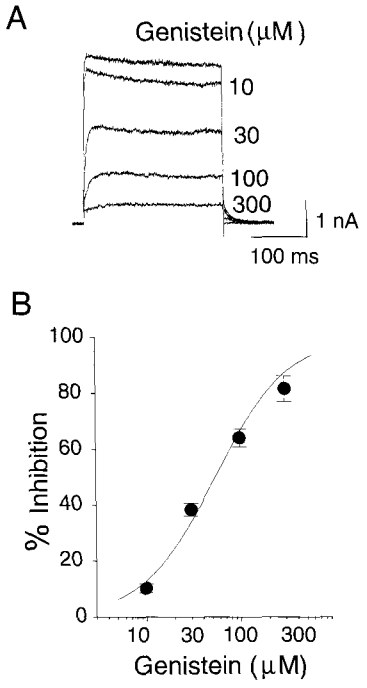

Fig. 1. Concentration-dependence of genistein-induced inhibition of Kv1.5 currents. (A) Superimposed current traces were produced by applying 250-msec depolarizing pulses from a holding potential of −80 mV to +50 mV every 20 sec in the absence and presence of 10, 30, 100, and 300 μM genistein, as indicated. (B) Concentration-dependent inhibition curve by genistein. Peak amplitudes of Kv1.5 currents during the series of depolarizing pulses were used as an index of inhibition, and percentage inhibition was plotted against various concentrations of genistein. The solid line is fitted to the data points by the Hill equation, which yielded an IC_{50} of 54.7±8.2 μM and a Hill coefficient of 1.1±0.2 (n=5).

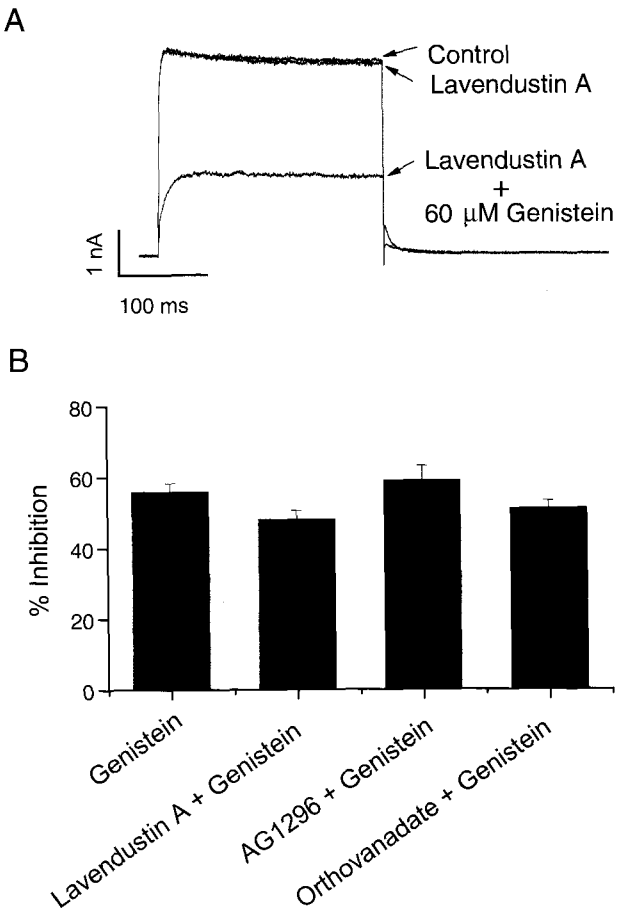

Fig. 2. Different protein kinase inhibitors do not affect the inhibition of Kv1.5 induced by genistein. Superimposed original currents were produced by applying 250-msec depolarizing pulses from a holding potential of −80 mV to +50 mV every 20 sec. (A) The control current, the current recorded after a 10-min exposure to 10 μM lavendustin A, and the current measured after a further 3-min treatment with 60 μM genistein are shown. The same protocol was used for cells treated with 100 μM AG1296 and 500 μM sodium orthovanadate (original current traces are not shown). (B) Peak amplitudes of Kv1.5 currents during 250-msec depolarizing pulses under each set of experimental conditions (A) were normalized to that of the control currents, and displayed as the percentage inhibition to show the effects of lavendustin A, AG1296, sodium orthovanadate, and genistein (n=5, respectively).

The effects of other PTK inhibitors on the inhibition of Kv1.5 induced by genistein

The Kv1.5 has multiple consensus sites for phosphorylation by PKC, PKA and PTK (Swanson et al, 1990; Tseng-Crank et al, 1990). Furthermore, human Kv1.5 channel in HEK cells is down-regulated by tyrosine phosphorylation (Holmes et al, 1996b). Therefore, it was tested whether the inhibition of Kv1.5 by genistein is mediated through PTK by using other types of structurally dissimilar PTK inhibitors (lavendustin A, AG1478) and protein tyrosine phosphatase inhibitor (sodium orthovanadate). Fig. 2A shows the effects of lavendustin A on the inhibition of Kv1.5 induced by genistein. A 8-min exposure to 10 μM lavendustin A did not induce statistically significant inhibition of Kv1.5 (3.1$\pm$1.5% inhibition, n=5). Subsequent application of genistein (60 μM) inhibited the peak amplitude of Kv1.5 by 47.9$\pm$2.5% (n=5). A similar series of experiments with AG1296 and sodium orthovanadate were carried out, and genistein inhibited Kv1.5 by 58.8$\pm$4.2% (n=5) and 51.0$\pm$2.2% (n=5), respectively, whereas 100 μM AG1296 and 500 μM sodium orthovanadate did not induce statistically significant inhibition of Kv1.5 (4.2$\pm$1.7% inhibition, n=5 and 5.8$\pm$1.5% inhibition, n=5, respectively). As seen in Fig. 2B, the summary of results indicate that the inhibition values of Kv1.5 peak current by genistein after pretreatment of various inhibitors were not significantly different from that of genistein alone (55.8$\pm$2.4%, n=5). These results strongly suggest that genistein inhibits Kv1.5 currents, independent of phosphorylation or dephosphorylation processes.

Voltage-independent inhibition of Kv1.5 by genistein

Fig. 3 shows the effect of 60 μM genistein on Kv1.5 current-voltage (I-V) relations. Under control conditions, the I-V relationship was sigmoidal for depolarizing pulses between -30 and 0 mV, and almost linear for depolarizing pulses greater than 0 mV (Fig. 3A and C). As shown in Fig. 3B and C, the inhibition of steady-state currents by 60 μM genistein was observed in the entire voltage range over which Kv1.5 was activated. By plotting the % inhibition versus potential (Fig. 3D), genistein-induced inhibition of Kv1.5 currents was voltage-independent across the voltage range (from 0 mV to +50 mV) over which Kv1.5 was fully activated. Least squares linear regression of the data yielded a value approximately equal to zero for the slope of the line (ANOVA, n=5, $p<0.05$)

Effects of genistein on activation and deactivation kinetics of Kv1.5

Fig. 4A shows superimposed original current traces of Kv1.5 obtained in the absence and presence of 60 μM genistein. The onset of activation of the current elicited by a 250-msec depolarizing pulse of +50 mV was clearly leng-

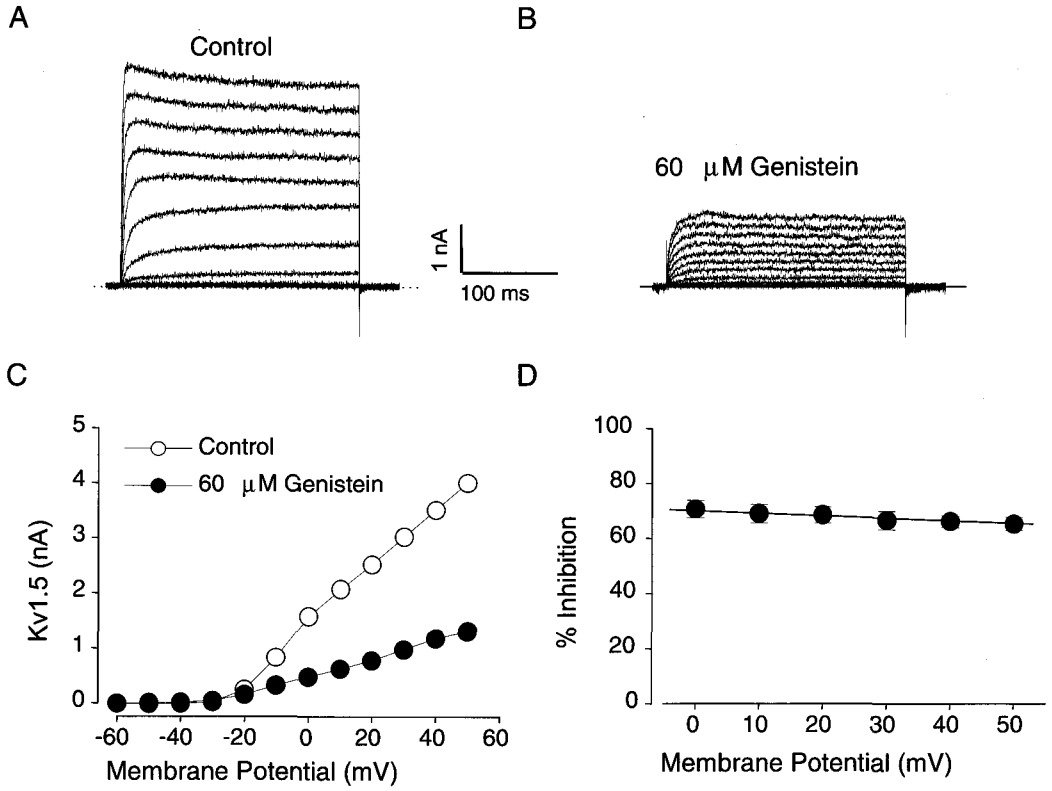

Fig. 3. Inhibition of Kv1.5 currents by genistein is independent of voltage. The Kv1.5 currents were produced by applying 250-msec depolarizing pulses from -60 mV to +50 mV in 10-mV increments every 10 sec, from a holding potential of -80 mV. The original current traces under control conditions and after the addition of 60 μM genistein are shown in (A) and (B), respectively. (C) Resultant I-V relationships taken from the peak amplitudes of Kv1.5 currents. (D) Percentage current inhibition from data in (C).

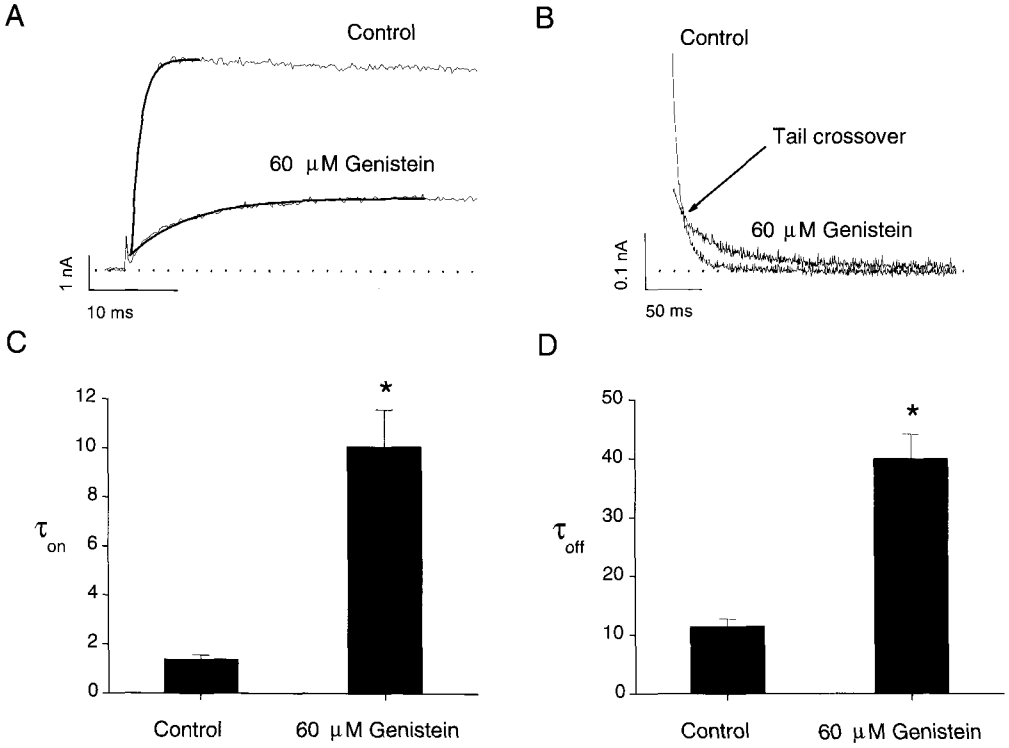

Fig. 4. Effects of genistein on activation and deactivation kinetics of Kv1.5. (A) Superimposed original current traces produced by a 250-msec depolarizing pulse of +50 mV from a holding potential of −80 mV, under control conditions and in the presence of 60 μM genistein. (B) Tail currents were recorded during a 250-msec repolarizing pulse of −40 mV after a 250-msec depolarizing pulse of +50 mV from a holding potential of −80 mV, in the absence and presence of 60 μM genistein. When the two tail currents in the absence and presence of genistein were superimposed, a tail crossover phenomenon (indicated by the arrow) was observed. (C, D) Summary data obtained from (A) and (B). τ_{on} and τ_{off} represent the activation and deactivation time constants, respectively. The solid lines over the current traces represent the monoexponential least-squares fit of the data. The dotted lines represent a zero current. The symbol * indicates a statistically significant difference (n=4, $p<0.05$).

thened by the drug: time constants for activation under control conditions and in the presence of 60 μM genistein were 1.4±0.2 msec (n=4) and 10.0±1.5 msec (n=4), respectively (Fig. 4C). The significant increase of the activation time constant in comparison with control values ($p<0.05$) indicates that the activation kinetics of Kv1.5 was significantly modified by genistein. Fig. 4B shows the superimposed original tail current traces of Kv1.5 elicited by a 250-msec repolarizing pulse of −40 mV after a 250-msec depolarizing pulse of +50 mV from a holding potential of −80 mV under control conditions and in the presence of 60 μM genistein. Under control conditions, the tail current was completely deactivated. On the other hands, the initial peak amplitude of the tail current decreased in the presence of 60 μM genistein, and the subsequent decline of the current was significantly slowed, resulting in a tail crossover phenomenon: time constants for deactivation under control conditions and in the presence of 60 μM genistein were 11.4±1.3 msec (n=4) and 40.0±4.2 msec (n=4), respectively (Fig. 4D).

Reversial use-dependent inhibition of Kv1.5 by genistein

Fig. 5A shows the original current traces of Kv1.5 channel in the absence and presence of 60 μM genistein which were obtained by 15-repetitive application of 150-ms depolarizing pulses to +50 mV at 1 Hz. Trains of stimuli were separated from each other by 2 min intervals. Under control conditions, the peak amplitude of Kv1.5 currents decreased slightly by 4.2±0.2%, compared with the peak current value measured at the first pulse (n=5) (Fig. 5B). In the presence of 60 μM genistein, however, there was a reduction in the peak amplitude elicited by the first depolarizing pulse which averaged 55.2±2.7% inhibition (Fig. 5A, n=5). Thereafter, the current amplitude was almost unaffected during the application of the train of pulses. Moreover, the amplitude of the Kv1.5 current increased by 17.9±1.1%, compared with the peak current value measured at the first pulse (n=5), and this increase was apparent at approximately 15 msec after the start of the depolarization pulses (Fig. 5B). These results suggest that there was little use-dependence of genistein action, and that repetitive depolarization relieved the genistein-induced inhibition of Kv1.5, which is known as a process of reverse use-dependence.

DISCUSSION

Genisteinis, an isoflavone compound isolated from the fermentation broth of *Pseudomonas*, is a tyrosine kinase inhibitor, because it potently exerts an inhibitory action on

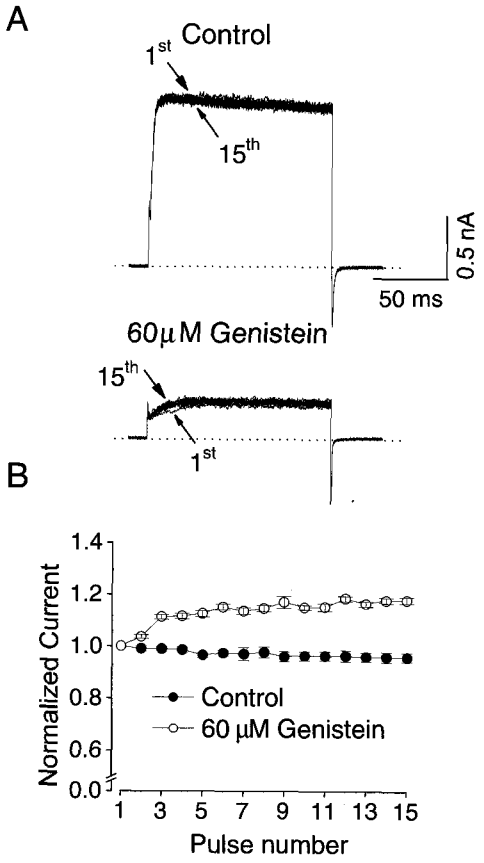

Fig. 5. Effects of repetitive depolarization on genistein-induced inhibition of Kv1.5 currents. (A) Repetitive depolarizing pulses (125 msec) from a holding potential of −80 mV to +50 mV were applied at 1 Hz, under control conditions (n=5) and in the presence of 60 μM genistein (n=5). (B) The peak amplitudes of currents at each pulse were normalized to the peak amplitudes of currents obtained at the first pulse, and were then plotted against the pulse numbers.

both receptor and non-receptor tyrosine kinases. However, it is known to poorly inhibit serine/threonine kinases. Consequently, it has widely been used to investigate the role of PTK in the modulation pathways of ion channel/receptor function.

The Kv1.5 channel (*Shaker*-type K^+ channel family) has been cloned from rat brain and human heart (Swanson et al, 1990), and is known to play an important role in cardiac action potential repolarization. The Kv1.5 has multiple consensus sites for phosphorylation by PKC, PKA and PTK (Swanson et al, 1990; Tseng-Crank et al, 1990) and can be highly modulated by phosphorylation-involved signaling systems (Li et al, 1996).

The following evidences in the present study indicate that genistein inhibits Kv1.5 channels directly in a concentration-dependent manner, but independent of phosphotyrosine-signaling pathway.

First, Kv1.5 inhibition was detected rapidly within 10 s of the application of the drug, and reached a steady state within 1 min. Its effect was also highly and rapidly reversible within 1 min. This rapid time course of inhibition and reversibility is not in concordance with the slow time course of channel modulation by PTK inhibition shown in other study (Timpe & Fantl, 1994). Therefore, the rapid inhibition of Kv1.5 by genistein can not simply be explicable by the inhibition of an intracellular PTK.

Second, pretreatment with the structurally dissimilar PTK inhibitors (lavendustin A, AG1296) and protein tyrosine phosphatase inhibitor (sodium orthovanadate) had no effect on the genistein-induced inhibition of Kv1.5, and they by themselves did not modify the current kinetics of Kv1.5. In the present study, the pipette solution did not contain ATP. Therefore, this non-phosphorylating condition also supports the direct action of genistein.

Third, although the down- or up-regulation of Kv1.5 by PTK is controversial (Timpe & Fantl, 1994; Holmes et al, 1996b), genistein-induced inhibition of Kv1.5 does not seem to be the same with the observed pattern of Kv1.5 suppression by protein phosphorylation. Inhibition of the Kv1.5 current by tyrosine phosphorylation has been characterized by a reduction of peak current amplitude with little change in kinetics of activation (Timpe & Fantl, 1994). Taken together, the present results indicate that genistein directly inhibits Kv1.5 without affecting PTK activity. However, we cannot completely exclude the possibility that the inhibition of Kv1.5 by genistein results indirectly from other unknown pathway.

An important observation in the present study is that genistein caused a marked slowing of the apparent current activation. The present results on genistein are similar to those of a previous study on the inhibition of neuronal Kv1.1 currents by fluoxetine (Tytgat et al, 1997) and characteristics of inhibition induced by an ion channel-blocker that binds channels in the closed state (Campbell et al, 1993). A pore blocker usually exhibits use-dependent blockage of a channel, however, use-dependent inhibition by genistein was not observed in our experiment. In contrast, repetitive depolarization induced the reversal of inhibition during the early part of the activating pulse. This result agrees well with previous reports (Kirsch et al, 1986; Campbell et al, 1993; Choi et al, 2001a), which showed that repetitive depolarizing pulses reversed the block in a manner dependent on the duration and interval of pulses. These results suggest that genistein inhibits Kv1.5 through a preferential interaction with the closed state of the channel, and that inhibition may be reversed by opening the channel at positive potentials. Other evidence favoring closed-channel binding includes the voltage-independent effects of genistein on Kv1.5 at potentials positive to 0 mV, when channel activation reached saturation. This explanation could be applied to genistein, because genistein is predominantly in an uncharged form at physiological pH (pH=7.3), thus unaffected by the transmembrane electric field. Under such situation, the voltage is expected to be independent of the action of the drug.

The fact that genistein slowed the time course of deactivating tail currents, thus inducing a crossover phenomenon, dose not support the closed-channel binding mechanism, since the tail crossover is usually observed in open channel blocks (Valenzuela et al, 1996; Choi et al, 2000; Choi et al, 2001b). The tail crossover and the slowing of activation could possibly be explained by the fact that the binding of genistein to the closed state alone produces a large energy barrier to bidirectional transitions between the closed and open states, thereby slowing both the opening and the closing rates. However, we do not know the exact mechanism by which genistein inhibits Kv1.5 channels. The effects of genistein on Kv1.5 may be complex, and

it is unlikely that inhibition can be accounted for by a simple closed-channel blocking mechanism.

In conclusion, the present study shows that genistein, a potent PTK inhibitor, inhibits directly cloned rat brain Kv1.5 channels independent of PTK activity. On the basis of the present study, together with previous reports on the direct actions of PKC, PKA, and PTK inhibitors on ion channels (Wijetunge et al, 1992; Smirnov & Aaronson, 1995; Chiang et al, 1996; Washizuka et al, 1998; Choi et al, 2002; Choi et al, 2006), one should be cautious in the use of these kinds of drugs in physiological experiments, designed to determine the role of protein kinases in the modulation of ion channels. Alternatively, this study provides a pharmacological tool for the development of a specific ion-channel blocker.

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