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Open Channel Block of hKv1.5 by Psoralen from *Heracleum moellendorffii* Hance

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A furocoumarin derivative, psoralen (7*H*-furo[3,2-g][1]benzopyran-7-one), was isolated from the n-hexane fraction of *Heracleum moellendorffii* Hance. We examined the effects of psoralen on a human Kv1.5 potassium channel (hKv1.5) cloned from human heart and stably expressed in Ltk$^-$ cells. We found that psoralen inhibited the hKv1.5 current in a concentration-, use- and voltage-dependent manner with an IC_{50} value of 180 ± 21 nM at +60 mV. Psoralen accelerated the inactivation kinetics of the hKv1.5 channel, and it slowed the deactivation kinetics of the hKv1.5 current resulting in a tail crossover phenomenon. These results indicate that psoralen acts on the hKv1.5 channel as an open channel blocker. Furthermore, psoralen prolonged the action potential duration of rat atrial muscles in a dose-dependent manner. Taken together, the present results strongly suggest that psoralen may be an ideal antiarrhythmic drug for atrial fibrillation.

Key words: hKv1.5 Channel blocker, Psoralen, *Heracleum moellendorffii* hance

INTRODUCTION

A literature survey revealed that little pharmacological work has been carried out on *Heracleum moellendorffii* Hance, and only the antiproliferative constituents panaxynol and falcarindiol have been reported (Nakano *et al.*, 1998). Earlier investigations on the chemical constituents of *H. moellendorffii* mainly dealt with the isolation of coumarins (Zhang, 1981; Wu *et al.*, 1986).

The shape and duration of cardiac action potentials vary depending on the region of the heart where they are recorded. These regional differences result, in part, from the differential expression of K^+ channel genes within the myocardium (Sanguinetti and Keating, 1997). K^+ channels represent the most diverse class of ion channels in the heart. The inward K^+ currents regulate the resting membrane potential, whereas the voltage-gated K^+ (Kv) currents control the action potential duration (Roden and George, 1999). The Kv channels contribute to cell repolarization and regulate the action potential duration. Clinically, it is known that repolarization disorders in damaged tissues result in cardiac arrhythmias. Accordingly, Kv channels become major targets for the treatment of arrhythmias. The hKv1.5 channel is known to have the same electrophysiological and pharmacological properties as I_{KUR}, a current specific in a human atrium (Fedida *et al.*, 1998). The development of highly selective blockers for the hKv1.5 channel will lead to an ideal drug for the treatment of atrial fibrillations.

In the present study, we found that psoralen, one of the extracts from *Heracleum moellendorffii* Hance blocked the hKv1.5 channel current expressed in Ltk$^-$ cells in a concentration-, time-, voltage-, and use-dependent manner, and it induced the prolongation of action potentials in rat atrial muscles.

MATERIALS AND METHODS

General procedure

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer. The EI/MS (70 eV) spectrum was determined on a VG-VSEQ mass spectrometer (VG Analytical, UK). All chemicals and solvents were of

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analytical grade and used without further purification.

Plant materials

The roots *H. moellendorffii* Hance was collected in July 2002 at Soonchang, Chonbuk, Korea. A voucher specimen was deposited in the herbarium of the College of Pharmacy, Woosuk University (WSU-02-015).

Extraction and isolation

Air-dried plant materials (500 g) were finely ground and extracted with MeOH under 50°C. The solvent of the resultant MeOH extract (60 g) was successively portioned to give *n*-hexane (5 g), $CHCl_3$ (4 g), *n*-BuOH (5 g) and H_2O soluble fractions. The *n*-hexane soluble fraction was chromatographed over a silica gel column using a solvent system of $CHCl_3$-MeOH (45:1) as an eluent to give seven subfractions. Subfraction 3 was purified by a Lobar A column (*n*-hexane:EtOAc = 5:1) to yield compound **1** (12 mg).

Compound 1 (psoralen, Fig. 1)

EIMS m/z: 186 [M^+], 158, 130, 102., ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.79 (1H, d, *J*=9.7 Hz, H-4), 7.70 (1H, d, *J*=2.2 Hz, H-2'), 7.68 (1H, s, H-5), 7.48 (1H, d, *J*=1.0 Hz, H-8), 6.84 (1H, dd, *J*=2.2, 1.0 Hz, H-3'), 6.38 (1H, d, *J*=9.7 Hz, H-3). ^{13}C-NMR (400 MHz, $CDCl_3$) : δ 161.2 (C-2), 156.5 (C-7), 152.0 (C-8a), 147.0 (C-2'), 144.3 (C-4), 125.0 (C-6), 120.0 (C-5), 115.5 (C-4a), 114.8 (C-3), 100.0 (C-8), 106.5 (C-3').

Cell culture and transfection

The method used to establish the hKv1.5 expression in a clonal mouse Ltk$^-$ cell line is the same as described previously (Snyders *et al.*, 1992; 1993). Transfected cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% horse serum and 0.25 mg/mL of G418, under a 5% CO_2 atmosphere. Before the experiment, subconfluent cultures were incubated with 2 μM dexamethasone for 12 h to induce the expression of the hKv1.5 channels. Cells were removed from the dish with a rubber policeman, a procedure that left the majority of the cells intact.

Rat atrial muscle preparation

To prepare rat atrial muscles, the heart of a rat was rapidly excised and then transferred to a dissection bath filled with Tyrode's solution oxygenated with a 97% oxygen and 3% carbon dioxide mixture. The atrial muscles were carefully dissected and mounted horizontally in a narrow channel of a tissue chamber, and they were continuously superfused with oxygenated Tyrode's solution at 37°C. The mural ends of these muscles were fixed with an insect pins to the bottom of the chamber coated with Sylgard. The portion of the muscles adjacent to the insect pin was pressed against the bottom of the chamber by stimulating electrodes, and these electrodes were used to elicit action potentials.

Electrical recordings

Kv currents were recorded by using the whole cell configuration of the gigaohm-seal patch clamp technique (Kwak *et al.*, 1999). Electrical signals were amplified with a patch clamp amplifier (Axopatch-1D, Axon Instruments, Foster). The currents were digitized by a signal converter (Digidata 1200, Axon Instruments) and stored on the hard disk of a computer. A micropipette with a resistance of 1-2 MΩ (Kimax-51, 1.5-1.8×10 mm) for current recording, was pulled out by a 2-stage pipette puller (PP-83, Narishige, Tokyo, Japan). The intracellular pipette-filling solution for the whole cell mode contained 100 mM KCl, 10 mM HEPES, 5 mM K_4BAPTA, 5 mM K_2ATP and 1 mM $MgCl_2$ (pH 7.2). The extracellular solution contained 130 mM NaCl, 4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES and 10 mM glucose (pH 7.35).

The action potentials were elicited by stimulating the cardiac cells with square pulses (1 Hz, lasting 1 ms, 20-30% above the threshold voltage) by a stimulator via a stimulus isolation unit (WPI, Sarasota, FL). The action potentials were recorded with a 3-M KCl-filled microelectrode (10-20 MΩ) connected to an amplifier (KS-700, WPI), and they were displayed on an oscilloscope (dual-beam storage 5113, Tektronix, Beaverton, OR). The tracings on the oscilloscope screen were photographed using 35-mm film, and they were also recorded on a chart recorder (RS 3400, Gould, Cleveland, OH). The rat atrial muscles were superfused with Tyrode's solution at a constant rate (5 mL/min). Tyrode's solution contained 137 mM NaCl, 5.4 mM KCl, 1.05 mM $MgCl_2$, 0.45 mM NaH_2PO_4, 11.9 mM $NaHCO_3$, 1.8 mM $CaCl_2$, and 5 mM dextrose.

Statistical analysis

The results are expressed as mean ± S.E.M. The student's *t*-test and analysis of variance (ANOVA) were used for statistical analysis of data. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

A selective block of the hKv1.5-like current in human atrial myocytes results in a significant prolongation of the action potential (Wang *et al.*, 1993, 1994). Blocking of the cardiac K^+ channels increases the action potential duration (Colatsky *et al.*, 1990; Roden, 1993). The *n*-hexane soluble fraction of *H. moellendorffii* Hance inhibited the hKv1.5 channel expressed in mouse Ltk$^-$ cells. Activity-guided column chromatography of the *n*-hexane soluble fraction

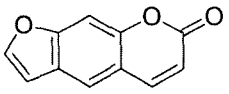

Fig. 1. Chemical structure of psoralen

focusing on the inhibition of the hKv1.5 current afforded one compound. This compound was readily elucidated as psoralen (7*H*-furo[3,2-g][1]benzopyran-7-one) by comparison of reported spectroscopic data from this plant (Matsumoto *et al.*, 1978). Psoralen has been used for the treatment of vitiligo (Kostovic *et al.*, 2003), fungi (Smith *et al.*, 2004) and cancer (Mariano *et al.*, 2002; Carneiro *et al.*, 2004). Recently, it was reported that psoralen (Fig. 1) inhibits the Kv1.3 channel in effecter T cells (Damjanovic *et al.*, 2004).

Fig. 2 shows the effects of psoralen on the hKv1.5 currents expressed in the Ltk$^-$ cells. Under control conditions, depolarization positive to -20 mV elicited outward currents that progressively increased with further depolarizations. At +60 mV, after the current reached the maximum, it declined slowly during the maintained depolarization (Fig. 2Aa). In the presence of psoralen (1 μM), both the outward current during the depolarizing steps and the tail current were reduced compared to those of the control group (Fig. 2Ab). Figure 2B shows the effect of psoralen (1 μM) on the steady-state current-voltage relationship for the hKv1.5 channel constructed by plotting the current amplitudes at the end of 250-ms depolarizations as a function of the test pulse voltage. In the presence of psoralen (1 μM), an inhibition of steady-state currents was observed through the whole voltage range over which hKv1.5 was activated (n = 6). The dose-response curve of psoralen on the block of the hKv1.5 current is shown in Fig. 2C. Plots of the steady-state current as a function of psoralen concentration were fitted to the Hill equation. For the psoralen-induced block, the half-maximal inhibitory concentration (IC_{50}) and Hill coefficient were 180 ± 21 nM and 1.39 ± 0.17, respectively (n = 6).

To quantify the voltage dependence of the psoralen-induced inhibition of the hKv1.5 current, the relative current $I_{psoralen}/I_{control}$ was plotted as a function of the membrane potential (Fig. 3). In the presence of psoralen (1 μM), the blocking action on the hKv1.5 current increased between -40 and 0 mV, which corresponds to the voltage range of channel opening (Snyders *et al.*, 1993). This suggests that a psoralen-induced inhibition of the hKv1.5 current occurs preferentially after channels are open. However, the inhibition of hKv1.5 channels by psoralen in the range of voltages between 0 and +60 mV, where channels are fully activated, did not show a voltage dependence (n = 6, ANOVA, $p<0.05$).

As shown in Fig. 4, the effect of psoralen on the deactivation kinetics of hKv1.5 was determined during a

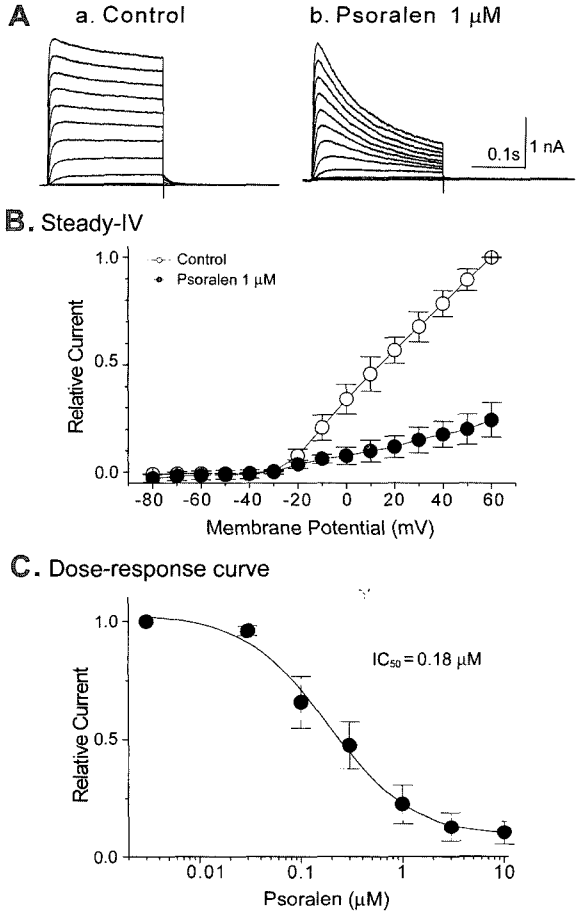

Fig. 2. Effects of psoralen on the hKv1.5 current expressed in a Ltk$^-$ cell line. hKv1.5 current traces were recorded before (Aa) and 20 min after an exposure to 1 μM psoralen (Ab). The voltage protocol consisted of 250-ms depolarizing pulses from -80 to +60 mV with 10-mV increments from a holding potential of -80 mV and repolarization to -50 mV for 400 ms. Steps were repeated at 20-s intervals. B, the resultant current-voltage (I-V) relationship of the steady-state current taken at the end of the depolarizing pulses in the absence and presence of 1 μM psoralen. C, concentration-response relationships of the hKv1.5 block by psoralen. Steady-state currents taken at the end of the depolarizing pulse of +60 mV were normalized to the control to construct the dose-response curve. Data were fitted with the Hill equation. Each point of the vertical bar denotes the mean ± S.E.M.

repolarizing step of -50 mV after a depolarizing step to +60 mV. In the presence of psoralen (300 nM), the initial tail current was reduced and the subsequent decline of the current was slowed, which resulted in a crossover phenomenon.

We examined the use-dependence of the psoralen-induced inhibition of the hKv1.5 channel. Original current traces, under control conditions and in the presence of 1 μM psoralen, were produced by 15 repetitive applications of depolarizing pulses at two different frequencies, 1 and 2 Hz (Fig. 5A). As shown in Fig. 5B, the peak amplitude of the hKv1.5 current decreased slightly by 4.9 ± 0.3% (n =

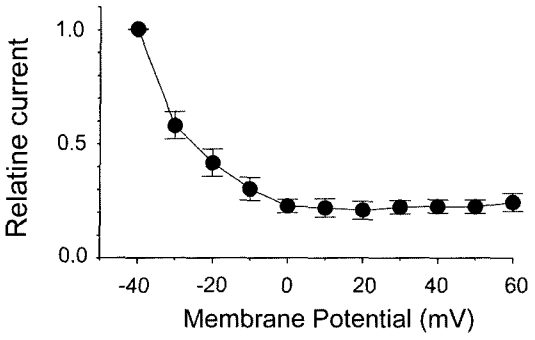

Fig. 3. Voltage-dependent block of hKv1.5 expressed in Ltk⁻ cells by psoralen. The voltage protocol consisted of 250-ms depolarizing pulses from -40 to +60 mV with 10-mV increments from a holding potential of -80 mV and repolarization to -50 mV for 400 ms. Steps were repeated at 20-s intervals. Relative currents were obtained by $I_{psoralen}/I_{control}$ at each depolarizing potential in the absence and presence of 1 μM psoralen. The steady-state current amplitude, normalized to that of the control, was plotted as a function of the test potential. Each point on the vertical bar denotes the mean ± S.E.M.

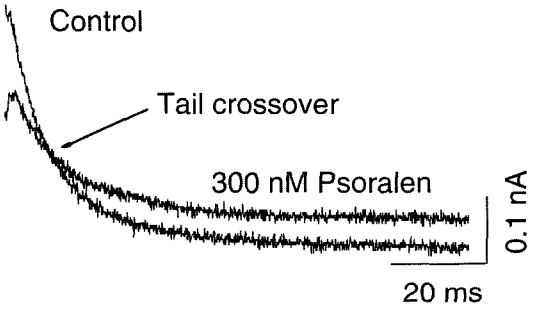

Fig. 4. Effect of psoralen on the deactivation kinetics of hKv1.5 expressed in Ltk⁻ cells. Deactivation kinetics were investigated during a repolarizing step of -50 mV for 400 ms after a 250 ms depolarizing step to +60 mV from a holding potential of -80 mV. By superimposing the tail currents in the absence and presence of 300 nM psoralen, a tail crossover phenomenon (indicated by the arrow) was observed.

4) at a frequency of 1 Hz, and by 8.4 ± 0.3% (n = 4) at a frequency of 2 Hz under control conditions. In the presence of 1 mM psoralen, the peak amplitude of hKv1.5 current was not significantly reduced after the first pulse. The subsequent trace showed a progressive decrease in the peak amplitude of hKv1.5 to a steady level. The extent of the steady-state block was 26.2 ± 2.8% (n = 4) and 53.4 ± 3.1% (n = 4) at 1 and 2 Hz, respectively. Thus, psoralen exhibited a use-dependent inhibition of hKv1.5 with little tonic block.

In atrial myocytes, a prolongation of the action potential duration resulting in an increase of cardiac refractoriness represents one modality of antiarrhythmic action (class III action) (Vaughan-Williams, 1989). Therefore, potassium channels that open in the voltage range of the plateau are potential molecular targets of class III antiarrhythmic agents.

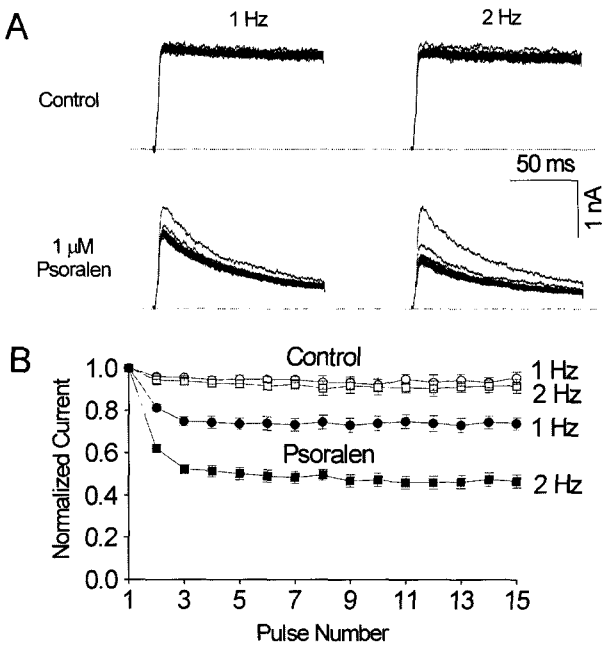

Fig. 5. Use-dependent inhibition of hKv1.5 expressed in Ltk⁻ cells by psoralen. A, original current traces obtained from 15 repetitive applications of 125 ms-depolarizing pulses of +60 mV from a holding potential of –80 mV at two different frequencies, 1 and 2 Hz, in the absence and presence of 1 μM psoralen. The dotted lines represent zero current. B, plot of the normalized peak amplitudes of currents under control conditions and in the presence of 1 μM psoralen at every pulse versus the pulse numbers in the pulse train. Each point on the/of the vertical bar denotes the mean ± S.E.M.

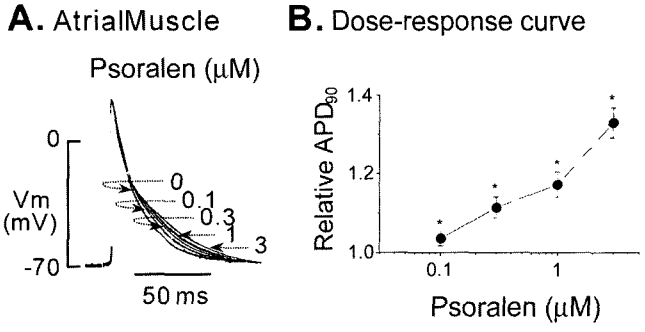

Fig. 6. Effects of psoralen on the action potential duration (APD) in rat atrial muscles. A, representative tracings of action potentials in the absence and presence of various concentrations of psoralen. B, concentration-dependent relationship of psoralen-induced APD prolongation (n = 5). APD_{90} was measured at 90% repolarization. Each point on the vertical bar denotes the mean ± S.E.M. *p<0.05 compared to control.

Therefore, we next examined the effects of psoralen (1 μM) on the action potential duration (APD) in rat atrial muscles. Figure 6A and 6B show the effects of psoralen on the APD in atrial muscles. Psoralen prolonged the APD of atrial myocytes in a dose-dependent manner.

In the present study, psoralen preferentially interacted

with the open state of the hKv1.5 channel in the following manner. First, psoralen accelerated the rate of hKv1.5 current decay with little effect on the initial activation kinetics. Second, the blockade produced by psoralen was voltage-dependent and increased steeply in the voltage range of channel activation. Third, psoralen slowed the deactivation of the tail current, thus inducing a tail cross-over phenomenon. This tail crossover phenomenon suggests an interaction between psoralen and the open state of the hKv1.5 channel. Fourth, the effects of psoralen were use-dependent. These phenomena are also cited as evidences of an open-channel blocking mechanism.

In summary, psoralen acted on the hKv1.5 channel as an open channel blocker. Furthermore, psoralen prolonged the action potential duration of rat atrial muscles in a dose-dependent manner. Taken together, these findings indicate that psoralen may be an ideal antiarrhythmic drug for atrial fibrillation.

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