Papaverine Blocks hKv1.5 Channel Current and Human Atrial Ultrarapid Delayed Rectifier K⁺ Currents

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ABSTRACT

Papaverine, 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-isoquinoline, has been used as a vasodilator agent and a therapeutic agent for cerebral vasospasm, renal colic, and penile impotence. We examined the effects of papaverine on a rapidly activating delayed rectifier K⁺ channel (hKv1.5) cloned from human heart and stably expressed in Ltk⁻/H11002 cells as well as a corresponding K⁺ current (the ultrarapid delayed rectifier, Iₚ,Kur) in human atrial myocytes. Using the whole cell configuration of the patch-clamp technique, we found that papaverine inhibited hKv1.5 current in a time- and voltage-dependent manner with an IC₅₀ value of 43.4 μM at +60 mV. Papaverine accelerated the kinetics of the channel inactivation, suggesting the blockade of open channels. Papaverine (100 μM) also blocked Iₚ,Kur in human atrial myocytes. These results indicate that papaverine blocks hKv1.5 channels and native hKv1.5 channels in a concentration-, voltage-, state-, and time-dependent manner. This interaction suggests that papaverine could alter cardiac excitability in vivo.

Papaverine, 1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline, is a smooth muscle relaxant found in opium. It blocks the contractile response to excitatory agents (Tashiro and Tomita, 1970; Brading et al., 1983; Huddart et al., 1984). Because of those relaxant effects on smooth muscles, papaverine has been used as a vasodilator agent (Wilson and White, 1986; Franz et al., 1991; Newell et al., 1999) and as a therapeutic agent for renal colic (Jonsson et al., 1987) and penile impotence (Handelsman, 1990). It was proposed as an “ideal coronary vasodilator” (Wilson and White, 1996). Additionally, intra-arterial papaverine infusion has been used for prevention and treatment of vasospasm after subarachnoid hemorrhage (Flemming et al., 1999; Tsurushima et al., 2000). However, under certain clinical settings, such as cases of overdose, papaverine induced serious cardiovascular arrhythmias (Inoue et al., 1994). Pharmacological blockade of voltage-gated K⁺ channels (Kv channels) in cardiac muscle has been associated with adverse cardiac arrhythmias or beneficial antiarrhythmic action, suggesting that papaverine may interact with cardiac Kv channels. However, the effects of papaverine on cardiac Kv channels remain to be elucidated.

Kv channels represent a structurally and functionally diverse group of membrane proteins. These channels play an important role in determining the length of the cardiac action potential and are the targets for antiarrhythmic drugs (Colatsky et al., 1990). Multiple Shaker-like K⁺ channel α and β subunit genes have been cloned from human myocardium and contribute to its electrical activity (Deal et al., 1996). One of these, Kv1.5, is one of the more cardiovascular-specific Kv channel isoforms identified to date, although it has been found in other tissues (Tamkun et al., 1991a; Overturf et al., 1994; Mays et al., 1995; Deal et al., 1996). Cloned from human heart, it forms the molecular basis for an ultrarapid delayed rectifier K⁺ current (Iₚ,Kur). hKv1.5 currents expressed in heterologous expression systems are similar in their biophysical and pharmacological properties to Iₚ,Kur recorded in human atrial myocytes (Wang et al., 1993; Deal et al., 1996; Feng et al., 1997). Thus, hKv1.5 may form an important molecular target for the treatment of atrial tachyarrhythmias, which represent a major clinical problem with serious morbidity (Cobbe, 1994).

In the present study, we found that papaverine blocked hKv1.5 channel current stably expressed in Ltk⁻ cells and...
I\textsubscript{Kur} current in human atrial myocytes in a concentration-, time-, voltage-, and state-dependent manner.

**Materials and Methods**

**Isolation of Human Atrial Myocytes.** Specimens of human right atrial appendage were obtained from the hearts of four patients (range 1–4 years) undergoing cardiopulmonary bypass surgery. Samples were immersed in nominally Ca\textsuperscript{2+}-free Tyrode’s solution (100% O\textsubscript{2}, 37°C) of the following composition: 136.0 mM NaCl, 5.4 mM KCl, 1.0 mM MgCl\textsubscript{2}, 0.33 mM Na\textsubscript{2}HPO\textsubscript{4}, 10 mM dextrose, and 10 mM HEPES, pH adjusted to 7.4 with NaOH. The myocardial specimens were chopped with scissors into cubic pieces and placed in a 25-ml flask containing 10 ml of the Ca\textsuperscript{2+}-free Tyrode’s solution. The tissue was gently agitated by continuous bubbling with 100% O\textsubscript{2}. After an initial 5 min in this solution, the pieces were reincubated in a similar solution containing 200 U/ml collagenase (CLS II; Worthington Biochemicals, Freehold, NJ) and 4 U/ml protease (type XXIV; Sigma Korea, Seoul, South Korea). The first supernatant was removed after 45 min and was discarded. The pieces were then incubated in a fresh enzyme-containing solution. Microscopic examination of the medium was performed every 15 min to determine the number and the quality of isolated cells. When the yield seemed to be maximal, the cells were suspended in a storage solution of the following composition: 20 mM KCl, 10 mM KH\textsubscript{2}PO\textsubscript{4}, 10 mM glucose, 70 mM glutamic acid, 10 mM β-hydroxybutyric acid, 10 mM taurine, 10 mM EGTa, and 0.1% albumin, pH adjusted to 7.4 with KOH, and gently pipetted. Only quiescent rod-shaped cells showing clear cross-attachments were used.

**Cell Culture and Transfection.** The method used to establish hKv1.5 expression in a clonal mouse Ltk\textsuperscript{−} cell line is the same as that described previously (Snyders et al., 1992, 1993). The expression vector contains a dexamethasone-inducible murine mammary-tumor virus promoter that controls transcription of the inserted cDNA and a gene that confers neomycin resistance driven by the simian virus 40 early promoter. The cells used for the experiments reported in the present study displayed hKv1.5-specific mRNA expression after dexamethasone induction as evidenced by Northern blot analysis (Tamkun et al., 1991). Transfected cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% horse serum and 0.25 mg/ml G418 under 5% CO\textsubscript{2} atmosphere. The cultures were passaged every 3 to 5 days by the use of a brief trypsin treatment. Before experiments, subconfluent cultures were incubated with 2 μM dexamethasone for 12 h to induce expression of hKv1.5 channels. The cells were removed from the dish with a rubber policeman, a procedure that left the majority of the cells intact. The cell suspension was stored at room temperature (20–22°C) and was used within 12 h for all the experiments reported.

**Electrical Recording.** The intracellular pipette filling solution contained 100 mM KCl, 10 mM HEPES, 5 mM K\textsubscript{2}ATP, 5 mM K\textsubscript{3}ATP, and 1 mM MgCl\textsubscript{2} and was adjusted to pH 7.2 with KOH, yielding a final intracellular K\textsuperscript{+} concentration of ~145 mM. The bath solution contained 130 mM NaCl, 4 mM KCl, 1.8 mM CaCl\textsubscript{2}, 1 mM MgCl\textsubscript{2}, 10 mM HEPES, and 10 mM glucose and was adjusted to pH 7.35 with NaOH. All chemicals were purchased from Sigma Korea. Experiments were performed in a small volume (0.5 ml) bath mounted on the stage of an inverted microscope (model TE300; Nikon, Tokyo, Japan), perfused continuously at a flow rate of 1 ml/min. I\textsubscript{Kur} in human atrial myocytes and hKv1.5 currents in Ltk\textsuperscript{−} cells were recorded at room temperature (20–22°C) using the whole cell configuration of the patch-clamp technique (Hamill et al., 1981) with an Axopatch 200B patch-clamp amplifier (Axon Instruments., Inc., Foster City, CA). Currents were recorded at room temperature (21–23°C) and sampled at 1 to 10 kHz after anti-alias filtering at 0.5 to 5 kHz. Data acquisition and command potentials were controlled by pClamp 6.03 software (Axon Instruments, Inc.). To ensure voltage-clamp quality, electrode resistance was kept below 3 MΩ.

**Results**

Figure 1 shows superimposed tracings of potassium current through hKv1.5 channels expressed in mouse Ltk\textsuperscript{−} cells in control conditions and in the presence of papaverine. The membrane potentials were held at −80 mV and depolarizing pulses from −80 to +60 mV in 10-mV steps were applied every 20 s. Outward currents were followed by decaying outward tail currents upon repolarization to −50 mV. Under control conditions, depolarizations positive to −40 mV elicited outward currents that progressively increased with further depolarizations. The rate of activation was faster at more depolarized levels. The activation time constant was
1.4 ± 0.1 ms at +60 mV (n = 15). At +60 mV, after the current reached the maximum, it declined slowly during the maintained depolarization. Outward tail currents exhibited a dominant time constant of deactivation of 26.7 ± 3.2 ms (n = 15), as has been described previously (Snyders et al., 1992, 1993).

In the presence of papaverine (100 μM), both outward current during depolarizing steps and tail current were reduced compared with control condition (Fig. 1B). Figure 1C shows the effect of papaverine (100 μM) on the steady-state current-voltage (I-V) relationship for the hKv1.5 channel constructed by plotting the current amplitudes at the end of 250-ms depolarizing steps from -80 to +60 mV with 10-mV increments from a holding potential from -80 mV. The tail current was normalized to the largest value in each group and plotted against membrane potential. Data were fitted with eq. 1. Each point with vertical bar denotes the mean ± S.E.M. *p < 0.05, significantly different from current before papaverine.

Drugs that block ion channels often alter the voltage dependence. Therefore, we analyzed the voltage dependence of activation from the peak amplitude of the decaying tail currents in the absence or presence of papaverine (100 μM) (Fig. 1D). The sigmoidal voltage dependence was fitted with eq. 1, resulting in half-activation voltages of -16.7 ± 1.2 mV (n = 6) and -26.3 ± 1.5 mV (n = 6; p < 0.01), without and with papaverine (100 μM), respectively. The slope factors were not significantly different (6.2 ± 0.5 mV for control and 5.9 ± 0.7 mV for papaverine; n = 6).

The block of hKv1.5 by papaverine in a concentration-dependent manner was shown in Fig. 2A. Steady-state currents were measured at the end of depolarizing pulse of +60 mV to construct the concentration-response curve (Fig. 2B). Plots of steady-state current as a function of papaverine concentration confirmed the Hill coefficient. The concentration of papaverine that produced half-maximal inhibition was approximately 100 μM. Papaverine (100 μM) was used in all subsequent experiments.
concentration were fitted to the Hill equation. For papaverine-induced block, a half-maximal inhibitory concentration (IC50) and Hill coefficient were 43.4 nM and 1.4, respectively (n = 6).

Figure 3 shows superimposed recordings of 250-ms depolarizations of −60 mV followed by repolarizing pulse of −50 mV in the absence and in the presence of papaverine (100 nM). Under control conditions, hKv1.5 current decay was well fitted to a single exponential function with a time constant of 126 ± 12 ms (n = 6). In the presence of papaverine (100 nM), a new component of rapid inactivation was added (Fig. 3A). The time constant of the rapid component was concentration-dependent and was 41 ± 10 ms (n = 6). In contrast, the time constant of slow inactivation was not modified by papaverine.

To quantify the voltage dependence of papaverine block, the relative current I/papaverine/I/control was plotted as a function of membrane potential (Fig. 3B). In the presence of papaverine (100 nM), the blockade increased steeply between −30 mV and 0 mV, which corresponds to the voltage range of channel opening (Snyders et al., 1993). These data suggest that papaverine binds primarily to the open state of the hKv1.5 channel. Between 0 and +60 mV, the block of hKv1.5 channel continued to increase with a shallow voltage dependence. It is unlikely that the shallow voltage dependence of block observed at membrane potentials positive to 0 mV was due to channel gating, because hKv1.5 activation had reached saturation over this voltage range (Snyders et al., 1992, 1993). At physiological pH, papaverine is predominantly present in the charged form with a pKd value of 8.07.

Thus, this shallow voltage dependence could be due to the influence of the transmembrane electrical field on the interaction between the charged form of papaverine and the channel receptor. The fractional electrical distance (δ) that is the fraction of electrical field sensed by a single charge at the receptor site was calculated from Woodhull (1973) model. The solid line in Fig. 3B represents a fit of this Boltzmann equation to the data points positive to 0 mV. Using this analysis, we obtained δ value of 0.13 ± 0.02 (n = 6) in the presence of papaverine (100 nM).

We next examined the effects of papaverine on I Kur in human atrial myocytes. These currents display a number of similarities to those of hKv1.5 (Fedida et al., 1993; Wang et al., 1993). I Kur was obtained by 250-ms depolarizing pulses ranging from −80 to +60 mV from a holding potential of −50 mV and then repolarizing to 0 mV at 30-s interval. A 100-ms prepulse was introduced 10 ms before each depolarizing pulse to inactivate transient outward current (Wang et al., 1993). Depolarizing pulses applied to atrial myocytes elicited I Kur and this current showed outward rectification (Fig. 4A). These currents were inhibited by papaverine (100 nM) in a voltage-dependent manner (Fig. 4B). The I-V relation for I Kur in five cells is shown in Fig. 4C and indicates voltage-dependent block by papaverine. In Fig. 4D, the normalized current decrease with papaverine is plotted as a function of the voltage.

**Discussion**

The current generated by hKv1.5 channels is similar in voltage dependence, kinetics, and pharmacological sensitiv-
A. Normalized hKv1.5 currents

Fig. 3. Effects of papaverine on kinetics of hKv1.5 channel. A, time-dependent block during a depolarization to +60 mV followed by the repolarizing pulse of −50 mV in the presence of 100 μM papaverine. The tracings were normalized to each peak current. B, voltage-dependent block of hKv1.5 by papaverine. Relative currents expressed as I_{papaverine}/I_{control} at each depolarizing potential from data obtained in the absence and the presence of papaverine (100 μM). Steady-state current amplitude, normalized to control, is plotted as a function of test potentials. This voltage dependence was fitted (continuous line) with eq. 2, which yielded δ, values of 0.14 (n = 6). The dashed line represents activation curves in the absence of papaverine. It was superimposed to show that the activation is saturated at the potential range, suggesting the shallow voltage dependence is not due to the intrinsic gating of the channel but the effect of papaverine (see text).

B. Percentage block

Papaverine induced an initial fast decline of the hKv1.5 current (IKur) recorded in human atrial myocytes (Wang et al., 1993), dog ventricle (Jeck and Boyden, 1992), and rat atria (Boyle and Nerbonne, 1991). In fact, the hKv1.5 channel protein has been located in human atrial and ventricular myocardium (Mays et al., 1995). However, electrophysiological studies have indicated the absence of hKv1.5-like current in human ventricular myocytes (Konarzewska et al., 1995; Li et al., 1996). All these results suggest that IKur is the native counterpart to hKv1.5 channels in human atria (Tamkun et al., 1991; Snyders et al., 1993; Wang et al., 1993; Deal et al., 1996). In particular, selective block of hKv1.5-like current in human atrial myocytes results in a significant prolongation of the open state of hKv1.5 channels (Wang et al., 1993). Block of cardiac K⁺ channels increases the action potential duration (Colatsky et al., 1990; Hondeghem and Snyders, 1990; Roden, 1993). However, it is noteworthy that there is a difference in the shape of the steady I-V plots between hKv1.5 (Fig. 1C) and IKur (Fig. 4C). Also there is a difference in the shape of percentage of block between hKv1.5 (Fig. 3B) and IKur (Fig. 4D). It seems that IKur was contaminated by leak current as indicated by the slope in the I-V curve before the activation of the channels. It is also possible that there is more than one type of K⁺ current in the atrial cells and that papaverine is blocking more than just the Kv1.5 current. Therefore, it is likely that the voltage dependence in Fig. 4D may be contaminated by other currents.

Papaverine induced an initial fast decline of the hKv1.5 current during a depolarization in addition to the slow inactivation process that characterizes this current at positive potentials (Fig. 3A), suggesting that papaverine binds to the open state of hKv1.5 channels. Moreover, the interaction of papaverine with the hKv1.5 channels was voltage-dependent (Fig. 3B), reaching a higher degree of block at more positive membrane potentials. These results are also consistent with an open channel block mechanism, because the probability of opening increases at more positive membrane potentials. The δ value based on Woodhull’s voltage-dependent block obtained for papaverine is very similar to those described previously for other hKv1.5 blocking agents (Snyders et al., 1992; Rampe et al., 1993a,b; Valenzuela et al., 1995, 1996, 1997; Yang et al., 1995; Delpont et al., 1996; Caballero et al., 1997; Franqueza et al., 1998), which suggests that all these compounds share the same receptor site in hKv1.5 channels. Open channel blockers mimic fast inactivation. Papaverine resulted in earlier activation of hKv1.5 current and shifted the midpoint of activation (Fig. 1D), similar to the effects of Kvβ subunits on hKv1.5 currents (England et al., 1995a,b; Ubele et al., 1996). This effect may result from the charge of papaverine, because a simple accumulation of positive charges at the inner surface of the channel reduces the effective membrane potential (Gilbert and Ehrenstein, 1969).

Papaverine has been shown to prolong QT interval and ventricular tachycardia (Inoue et al., 1994). Many commonly used drugs, including antiarrhythmic, antihistamine, antipsychotic, and antibiotic agents are associated with drug-induced LQTS. Most of these drugs either block human ether-a-go-go-related gene-dependent K⁺ current (IKr) in ventricular myocytes or inhibit liver enzymes that are important for metabolic degradation of other drugs that block IKr. Indeed, papaverine blocks human ether-a-go-go-related gene current with somewhat higher IC_{50} value than that for Kv1.5 (H. Choe, Y. K. Lee, and Y. G. Kwak, unpublished data). Therefore, it is most likely that papaverine may induce arrhythmia through block of IKr in ventricular myocytes. Nonetheless, it is also true that selective block of hKv1.5-like
current in human atrial myocytes results in significant prolongation of the action potential (Wang et al., 1993). Thus, the block of hKv1.5 and IKur by papaverine could affect cardiac excitability (Cobbe, 1994). In summary, this report is the first to detail the effects of papaverine on voltage-gated K channels in the heart. We find that papaverine blocks both a cloned cardiac channel (hKv1.5) expressed in Ltk cells and a rapidly IKur in human atrial cells. The effects of papaverine on these currents were shown to be concentration-, time-, voltage-, and state-dependent in a qualitatively similar manner.

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References


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