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Original Paper

Identification of Verapamil Binding Sites Within Human Kv1.5 Channel Using Mutagenesis and Docking Simulation

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Key Words
Verapamil • Kv1.5 • Open-channel block • Atrium • Effective refractory period

Abstract
Background/Aims: The phenylalkylamine class of L-type Ca^{2+} channel antagonist verapamil prolongs the effective refractory period (ERP) of human atrium, which appears to contribute to the efficacy of verapamil in preventing reentrant-based atrial arrhythmias including atrial fibrillation. This study was designed to investigate the molecular and electrophysiological mechanism underlying the action of verapamil on human Kv1.5 (hKv1.5) channel that determines action potential duration and ERP in human atrium. ***Methods:*** Site-directed mutagenesis created 10 single-point mutations within pore region of hKv1.5 channel. Whole-cell patch-clamp method investigated the effect of verapamil on wild-type and mutant hKv1.5 channels heterologously expressed in Chinese hamster ovary cells. Docking simulation was conducted using open-state homology model of hKv1.5 channel pore. ***Results:*** Verapamil preferentially blocked hKv1.5 channel in its open state with IC_{50} of 2.4±0.6 μM (n = 6). The blocking effect of verapamil was significantly attenuated in T479A, T480A, I502A, V505A, I508A, L510A, V512A and V516A mutants, compared with wild-type hKv1.5 channel. Computer docking simulation predicted that verapamil is positioned within central cavity of channel pore and has contact with Thr479, Thr480, Val505, Ile508, Ala509, Val512, Pro513 and Val516. ***Conclusion:*** Verapamil acts as an open-channel blocker of hKv1.5 channel, presumably due to direct binding to specific amino acids within pore region of hKv1.5 channel, such as Thr479, Thr480, Val505, Ile508, Val512 and Val516. This blocking effect of verapamil on hKv1.5 channel appears to contribute at least partly to prolongation of atrial ERP and resultant antiarrhythmic action on atrial fibrillation in humans.

W. Ding and A. Tano contributed equally to this work.

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Introduction

In the heart, the action potential duration (APD) is a key determinant of the effective refractory period (ERP) [1], and prolongation of ERP is expected to be effective in preventing the occurrence of reentrant-based cardiac arrhythmias [2]. APD is determined by the balance between depolarizing inward currents, such as voltage-gated Na^+ and L-type Ca^{2+} currents, and repolarizing outward K^+ currents, including ultra-rapidly, rapidly and slowly activating delayed rectifier K^+ currents (I_{Kur}, I_{Kr} and I_{Ks}, respectively) [2]. I_{Kur}, I_{Kr} and I_{Ks} are encoded by KCNA5 (Kv1.5), KCNH2 (ERG [ether-a-go-go-related gene], Kv11.1) and KCNQ1 (Kv7.1)/KCNE1 (mink) genes, respectively [3]. The expression levels of ion channels exhibit species- and tissue-dependent variations in the heart [4]. For example, in humans, whereas I_{Kr} and I_{Ks} are both functionally expressed in the atrium and ventricle [5, 6], I_{Kur} is predominantly expressed in the atrium [4, 7-9]. It is therefore expected that the pharmacological blockade of I_{Kur} has a prolonging effect on atrial APD but a minimal effect on ventricular APD in the human heart [10].

Currently, three classes of structurally different L-type Ca^{2+} channel antagonists, namely dihydropyridines, benzothiazepines and phenylalkylamines, are used to treat hypertension and tachyarrhythmia in the clinical settings [11]. Of note, these three classes of Ca^{2+} channel antagonists have different effects on APD and ERP in cardiac muscle. For example, a previous study using guinea-pig ventricular myocytes showed that whereas the dihydropyridine nisoldipine only shortens APD in a concentration-dependent manner, the phenylalkylamine verapamil prolongs APD at lower concentrations but shortens it at higher concentrations [12]. These phenomena have been assumed to arise from Ca^{2+} channel antagonists acting on not only L-type Ca^{2+} channel but also K^+ channels in different manners [12].

It is also important to note that verapamil prolongs the ERP in human atrial myocardium [13, 14]. Multiple K^+ currents, including I_{Kur}, I_{Kr} and I_{Ks}, are important in determining the atrial ERP in humans [2]. Previous studies have shown that verapamil inhibits both human Kv1.5 (hKv1.5) and human ERG (hERG) channels [14, 15], which is assumed to contribute to the prolonging effect of verapamil on ERP in the human atrium. Furthermore, clinical evidence has shown that verapamil is effective in reducing the recurrence of atrial fibrillation after electrical cardioversion [16, 17]. Taken together, it seems likely that the blockade of hKv1.5 and hERG channels by verapamil prolongs the atrial ERP and thereby exerts an antiarrhythmic effect on the reentrant-based atrial fibrillation in humans. The molecular target for the verapamil action within hERG channels has been examined by site-directed mutagenesis, which shows that Ser620 and Ser631, which lie near the internal mouth of channel pore in the S5-S6 linker, are critically involved in mediating the blocking action of verapamil [14]. However, little information is available concerning the molecular basis for the blocking effect of verapamil on hKv1.5 channel.

The present study was undertaken to examine the effects of verapamil on wild-type and mutant hKv1.5 channel to predict the blocking site(s) within hKv1.5 channels, using site-directed mutagenesis and computational docking simulation.

Materials and Methods

Complementary DNA and site-directed mutagenesis

The full-length complementary DNA (cDNA) encoding the human Kv1.5 (hKv1.5) channel was subcloned into the mammalian expression vector pcDNA3.1, which was kindly provided by Dr. David Fedida (University of British Columbia, Vancouver, Canada). Polymerase chain reaction (PCR)-based site-directed mutagenesis was applied to introduce 10 single-point mutations into hKv1.5 cDNA using a Quikchange-II-XL Kit (Agilent Technologies, Santa Clara, CA, USA), as previously described [18-20]. All mutations were verified by sequencing and designated as T479A, T480A, A501V, I502A, V505A, I508A, A509G, L510A, V512A and V516A.

Cell culture and transfection

Chinese hamster ovary (CHO) cells were maintained at 37°C in DMEM/Ham's F-12 medium supplemented with 10% fetal bovine serum and antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin) in a humidified atmosphere with 5% CO_2. The CHO cells were seeded onto small glass coverslips (3×5 mm) in 35-mm culture dishes 24 h before transfection. Wild-type or mutant hKv1.5 channel cDNA (0.5 μg) was transiently transfected together with GFP cDNA (0.5 μg), using the Lipofectamine transfection reagent (Invitrogen Life Technologies, Carlsbad, CA, USA).

Whole-cell patch-clamp recordings and data analyses

Whole-cell patch-clamp technique [21] was applied to GFP-positive CHO cells using an EPC-8 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany), approximately 48 h after transfection. The glass coverslips with adherent CHO cells were transferred to a recording chamber mounted on the stage of inverted microscope (ECLIPSE TE-2000U; Nikon, Tokyo, Japan) and superfused continuously at 23-25°C with normal Tyrode solution containing (in mM); 140 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 5.5 glucose and 5.0 HEPES (pH adjusted to 7.4 with NaOH). Patch electrode was fabricated from thick-walled glass capillaries (outer diameter, 1.5 mm, inner diameter 0.9 mm; Narishige Scientific Instrument Lab., Tokyo, Japan) using a horizontal microelectrode puller (P-97; Sutter Instrument Co., Novato, CA, USA) and had a resistance of 2.5-4.0 MΩ, when filled with pipette solution containing (in mM); 70 potassium aspartate, 40 KCl, 10 KH_2PO_4, 1 $MgSO_4$, 3 adenosine 5'-triphosphate (disodium salt; Sigma, St Louis, MO, USA), 0.1 guanosine 5'-triphosphate (dilithium salt; Sigma), 5 EGTA and 5 HEPES (pH adjusted to 7.2 with KOH).

CHO cells were depolarized from a holding potential of -80 mV to test potentials of -50 to +50 mV in 10-mV steps for 300 ms, followed by repolarization to -40 mV. The amplitude of hKv1.5 current was measured at the initial and end points of 300-ms depolarizing steps (initial and late currents, respectively). The concentration-response relationships for the inhibitory effect of verapamil on hKv1.5 current was determined by measuring the reduction in the late current amplitudes caused by verapamil relative to the control at a test potential of +30 mV (percent inhibition). This percent inhibition was fitted with a Hill equation as follows: percent inhibition = $1/(1+([IC_{50}]/[D])^{nH})$, where IC_{50} is the concentration of verapamil causing a half-maximal inhibition, [*D*] is the concentration of verapamil, and n_H is the Hill coefficient. The time constant for the development of verapamil-induced blockade of hKv1.5 current during 300-ms depolarizing steps was evaluated by fitting a single exponential function to the current ratio ($I_{Verapamil}/I_{Control}$), obtained by dividing the hKv1.5 current in the presence of verapamil ($I_{Verapamil}$) with that in its absence ($I_{Control}$) at test potentials of ≥-10 mV [18, 20, 22, 23]. Membrane currents were usually low-pass filtered at 1 kHz and sampled at 5 kHz through an LIH-1600 analogue-to-digital converter, using Patchmaster software (HEKA Elektronik). Series resistance was compensated up to 80% to minimize the voltage errors. The zero-current level is indicated to the left of current traces by a horizontal line.

Stock solutions of verapamil (Sigma) were made in DMSO (Sigma), stored in aliquots at -20°C, and diluted to 0.1 to 100 μM in external bath solution (normal Tyrode solution) before use. The concentration of DMSO in the final solution was ≤0.1% (v/v), which was confirmed to have no effect on hKv1.5 current.

Computer docking simulation

Homology modeling of the hKv1.5 channel, verapamil docking, calculation of the binding free energy and two- and three-dimensional representation were conducted using the Molecular Operating Environment (MOE) 2016.0802 (Chemical Computing Group, Inc., Quebec, Canada), as previously described [19, 20]. An open-state homology model of the hKv1.5 channel pore domain was constructed using the modeling software MOE-Homology Model, using the 2.9 Å-crystal structure of the Kv1.2 channel as a template (Protein Data Bank: 2A79) [24], which is approximately 90% homologous in amino acid sequence to the pore domain with hKv1.5 channel. Verapamil was docked to the hKv1.5 model using the Dock in the MOE program. We adopted the Amber10:EHT force-field to set force-field parameters and then calculated the verapamil binding energy to the hKv1.5 model. During the docking simulation, the channel structure was kept rigid, while ligand (verapamil) was allowed to be flexible [25]. The calculated free energy of binding (*S* score) was used to assess the verapamil docking to the channel, and the amino acids within 4.5 Å of the docked compound were identified as having potential contact [22, 23].

Data and statistical analyses

Results are expressed as the means±SEM, and the number of cells is indicated by *n*. The error bars in the figures indicate SEM with *n* given in parentheses. The statistical power analysis was conducted for each experimental protocol using the StatMate (version 2.0, GraphPad Software, La Jolla, CA, USA) with a statistical power of 0.8 at a significance level (α) of 0.05 (two-tailed). This predicted that a group size of $n = 5$ would allow detection of a difference of 30% between group means in current ratio between initial and late currents, measured in the absence and presence of verapamil. On the other hand, a group size of $n = 6$ was required to detect a difference of 20% between group means in the percent inhibition of wild-type and mutant hKv1.5 channel by verapamil. Statistical comparisons were evaluated using either Student's *t*-test or ANOVA with the Dunnett post hoc test (Prism Version 5.0; GraphPad Software), as appropriate. We used two-tailed hypothesis testing for all tests. *P* values of <0.05 were considered to indicate statistical significance.

Results

Open-channel blocking effect of verapamil on hkv1.5 channel

We first investigated the inhibitory effect of verapamil on hKv1.5 channel heterologously expressed in CHO cells. The hKv1.5 current was elicited by 300-ms depolarizing voltage-clamp steps to test potentials of -50 to +50 mV applied from a holding potential of -50 mV. The hKv1.5 current activated rapidly upon depolarizations positive to -30 mV and exhibited a stable level or minimal decline during depolarizing steps under control conditions (Fig. 1A, left panel), similar to that described previously [18-20, 22, 23, 25]. Bath application of 10 μM verapamil evoked a progressive decline in hKv1.5 current during

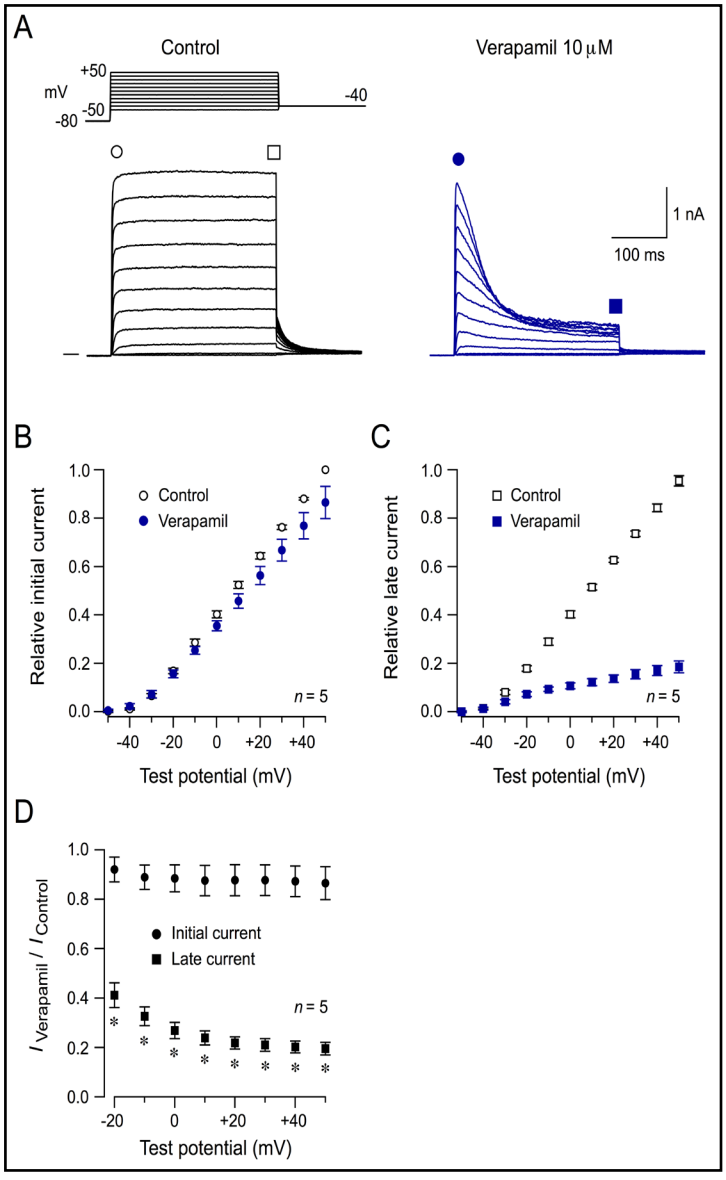

Fig. 1. Inhibitory effect of verapamil on hKv1.5 current. (A) Superimposed current traces of hKv1.5 current activated by 300-ms depolarizing voltage-clamp steps to test potentials of -50 to +50 mV applied from a holding potential of -50 mV, before and 5 min after exposure to 10 μM verapamil. (B and C) Initial (B) and late (C) current levels during test potentials measured in the absence and presence of verapamil. (D) Current ratio of initial and late currents, measured by dividing the hKv1.5 current in the presence of verapamil by that in its absence. *, P<0.05 compared with the initial current ratio.

depolarizing steps (Fig. 1A, right panel). To characterize the blocking action of verapamil on hKv1.5 current, we measured the initial and late current levels during depolarizing steps in the absence and presence of verapamil. Whereas the initial current levels were not appreciably changed (Fig. 1B), the late current levels were greatly reduced by the presence of verapamil (Fig. 1C). This observation was confirmed by measuring the current ratio in the presence and absence of verapamil ($I_{Verapamil}/I_{Control}$) for initial and late current levels of hKv1.5 current at each test potential. As summarized in Fig. 1D, the late current ratio was significantly smaller than the initial current ratio at each test potential. Thus, the blocking action of verapamil on hKv1.5 channel was found to proceed during depolarizing steps.

To quantitatively evaluate the time course of verapamil blockade of hKv1.5 current during depolarizing steps, the current ratio was obtained by dividing the hKv1.5 current in the presence of 10 μM verapamil with that in its absence ($I_{Verapamil}/I_{Control}$) and was fitted with a single exponential function, yielding the time constant for the channel block (τ) [18, 20, 22, 23]. As shown in Fig. 2A, the current ratio progressively decreased with τ of 102.2 ms from approximately 1.0 to about 0.3 during a 300-ms depolarizing step. As summarized in Fig. 2B, the time constant for channel block was smaller at moderate or strong depolarizations (+20 to +50 mV) than at mild depolarization (-10 mV). These results show that verapamil blockade of the hKv1.5 channel progresses rapidly at strong depolarizations where the open probability of the channel is

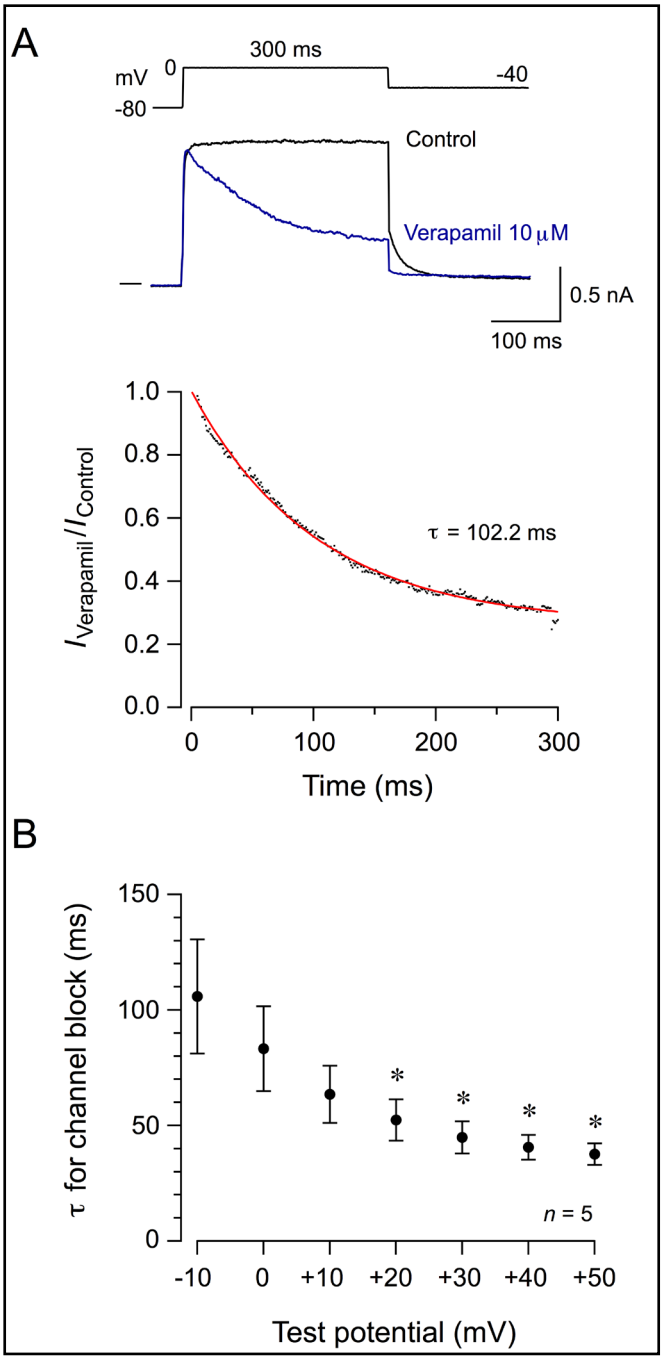

Fig. 2. Time constant of verapamil blockade of hKv1.5 current during depolarization. (A) Superimposed hKv1.5 currents recorded during the depolarizing step to 0 mV in the absence and presence of verapamil (upper panel). The current ratio was obtained by dividing hKv1.5 current in the presence of verapamil ($I_{Verapamil}$) by that in control ($I_{Control}$) and fitted with a single exponential function, yielding the time constant for channel blockade (τ). (B) Time constant (τ) for channel blockade at various test potentials. *, P<0.05 versus -10 mV.

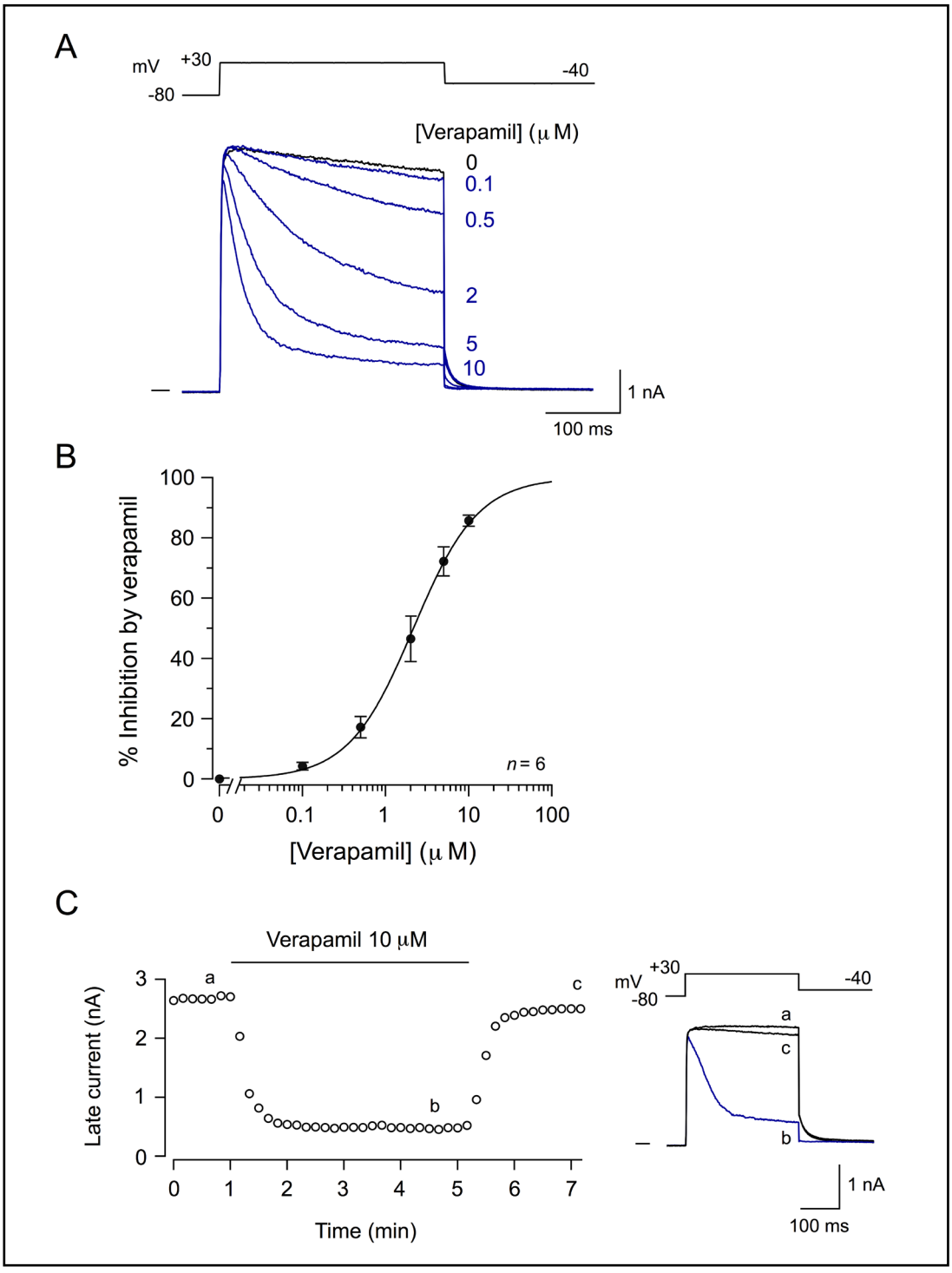

Fig. 3. Concentration-dependence and reversibility of the blocking action of verapamil on hKv1.5 current. (A) Superimposed hKv1.5 currents during depolarizing voltage-steps to +30 mV in the absence and presence of increasing concentrations of verapamil from 0.1 to 10 μM applied in a cumulative manner. (B) The percent inhibition of hKv1.5 current measured at the end of test potential ($(1 - I_{Verapamil}/I_{Control}) \times 100$) was plotted as a function of verapamil concentrations. The smooth curve through the data points represents a least-squares fit of Hill equation. (C) Time course of the changes in the late current amplitude of hKv1.5 current by exposure to 10 μM verapamil. Inset, superimposed hKv1.5 currents recorded at time points a, b and c.

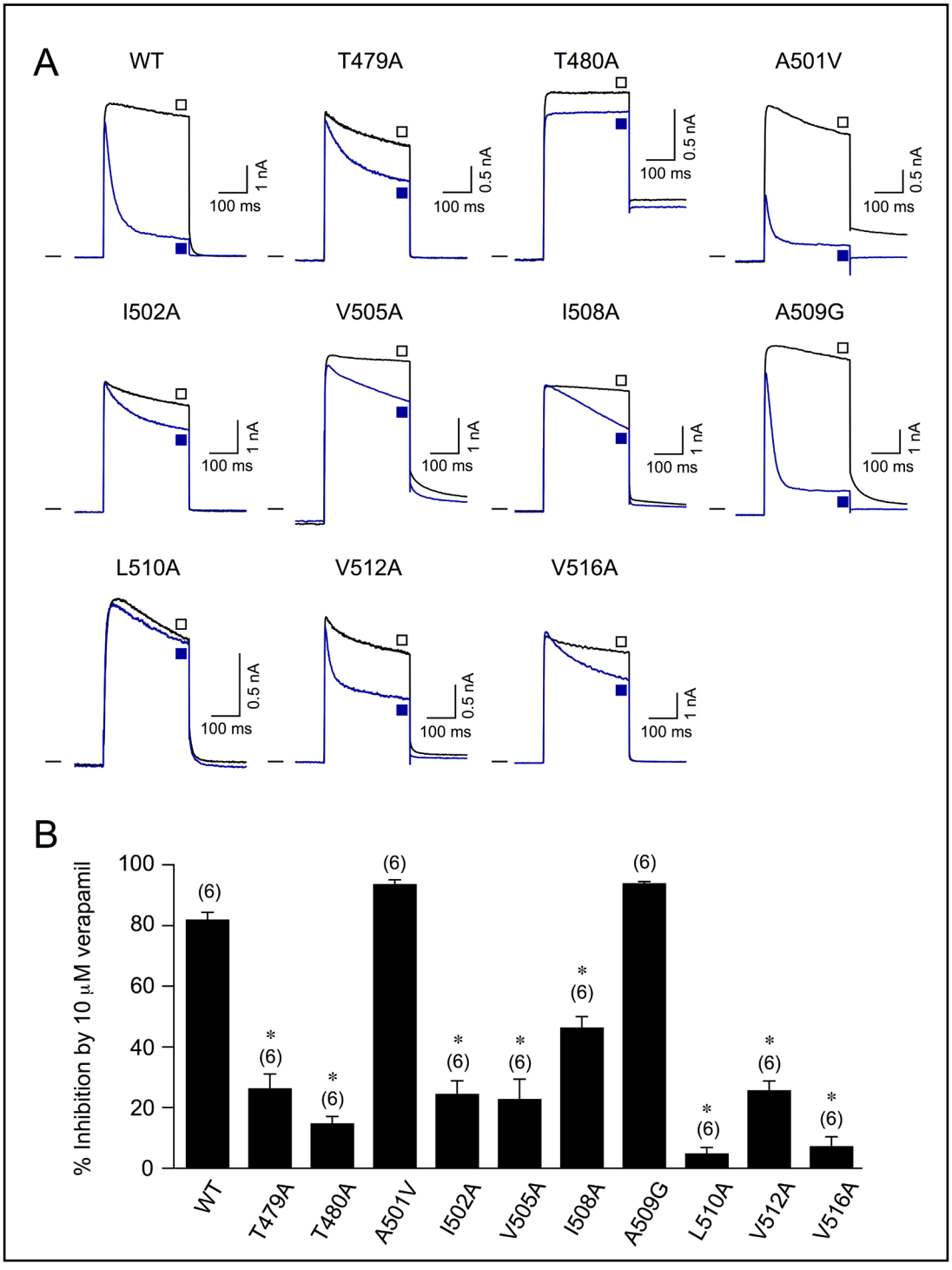

Fig. 4. Blocking effect of verapamil on wild-type and various mutant hKv1.5 channels. (A) Superimposed hKv1.5 current traces recorded from wild-type (WT) and mutant (T479A, T480A, A501V, I502A, V505A, I508A, A509G, L510A, V512A and V516A) channels during depolarizing steps to +30 mV in the absence (open squares) and presence (filled squares) of 10 μM verapamil. (B) Percent inhibition of wild-type (WT) and various mutant hKv1.5 channels by 10 μM verapamil. The number of experiments was indicated in parentheses. *, P<0.05 compared with wild-type channel.

high [18, 19, 22, 23, 25]. These properties are consistent with verapamil preferentially affecting the hKv1.5 channel in its open state. Taken together, verapamil appears to act as an open-channel blocker of the hKv1.5 channel.

We then examined the concentration-dependence of verapamil blockade of the hKv1.5 current. The hKv1.5 current was activated by depolarizing steps to +30 mV in the absence and presence of verapamil at concentrations ranging from 0.1 to 10 μM (Fig. 3A). As demonstrated in Fig. 3B, the inhibitory effect of verapamil on hKv1.5 current was concentration-dependent with an IC_{50} of 2.4±0.6 μM (n = 6). In another set of experiments, the amplitude of hKv1.5 current was recovered to the control level after washing out of 10 μM verapamil (Fig. 3C). Similar results were obtained in five other cells, thus showing that verapamil blocks the hKv1.5 current in a reversible manner.

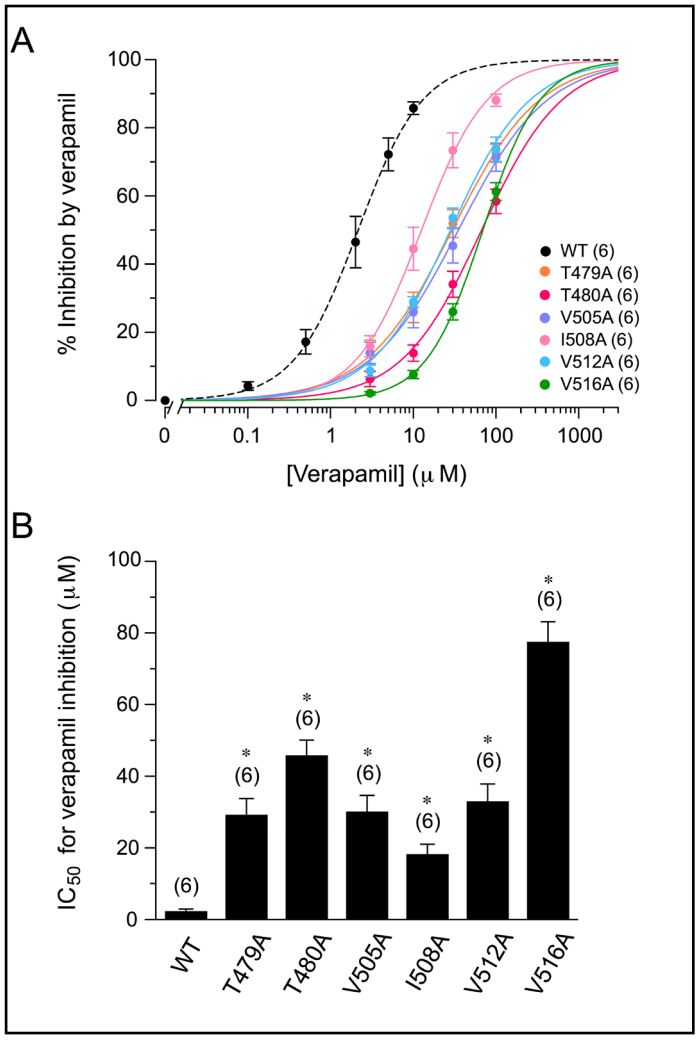

Fig. 5. Changes in IC_{50} for verapamil block caused by mutant hKv1.5 channels. (A) Concentration-response relationships for the blocking effect of verapamil on wild-type (WT) or mutant (T479A, T480A, V505A, I508A, V512A and V516A) channels. Smooth curves through the data points represent a least-squares fit of Hill equation, yielding IC_{50} values. (B) IC_{50} for verapamil blockade of wild-type (WT) and mutant (T479A, T480A, V505A, I508A, V512A and V516A) channels. The number of experiments is indicated in parentheses. *, P<0.05 compared with wild-type channel.

Mutational analysis for verapamil blockade of hKv1.5 channel

To identify the blocking site of verapamil within the channel, we examined the blocking effects of 10 μM verapamil on various mutant hKv1.5 channels. Fig. 4A shows the superimposed traces of hKv1.5 currents recorded from wild-type and various mutant channels during depolarizing step to +30 mV. As summarized in Fig. 4B, the percent inhibition by verapamil was significantly attenuated in T479A, T480A, I502A, V505A, I508A, L510A, V512A and V516A, but was not affected in A501V or A509G mutant channels, compared with the wild-type channel.

Because Thr479, Thr480, Val505, Ile508, Val512 and Val516 are believed to face toward the central cavity of the channel pore [22, 23], their mutations, namely, T479A, T480A, V505A, I508A, V512A and V516A, are selected for further characterization of the blocking potency of verapamil on hKv1.5 channel. The IC_{50} was obtained by constructing the concentration-response relationships for the blocking effect of verapamil on mutant channels (Fig. 5A). As summarized in Fig. 5B, IC_{50} values for verapamil blockade of mutant hKv1.5 channels

ranged from 18.3±2.7 μM (*n* = 6) in I508A to 77.6±5.6 μM (*n* = 6) in V516A, which are significantly larger than that (2.4±0.6 μM, *n* = 6; Fig. 3B) for wild-type hKv1.5 channel, thus confirming that the blocking potency of verapamil was attenuated in these 6 mutant channels.

Molecular docking of verapamil to hKv1.5 channel

Because verapamil was expected to preferentially affect the hKv1.5 channel in its open state, we conducted a docking simulation to predict the potential binding modes of verapamil within the pore cavity of the hKv1.5 channel using an open-state homology model of the hKv1.5 channel constructed from the Kv1.2 channel structure [24]. At the lowest free energy of binding (-8.41 kcal/mol), verapamil was positioned within the central cavity of the channel pore (Fig. 6A). The side chains of Thr479, Thr480, Val505, Ile508, Ala509, Val512, Pro513 and Val516 from at least one subunit of the tetramer were predicted to be located within 4.5 Å distance of verapamil (Fig. 6B). This binding pose of verapamil formed arene-H interactions with the side chain of Val512 in the hKv1.5 channel at a distance of 2.96 Å (Fig. 6B). Thus, the present docking simulation predicted that verapamil occupies the central cavity of the channel pore and has contact with multiple amino acids that reside between the base of the ion selectivity filter and near the C-terminal end of the S6 domain.

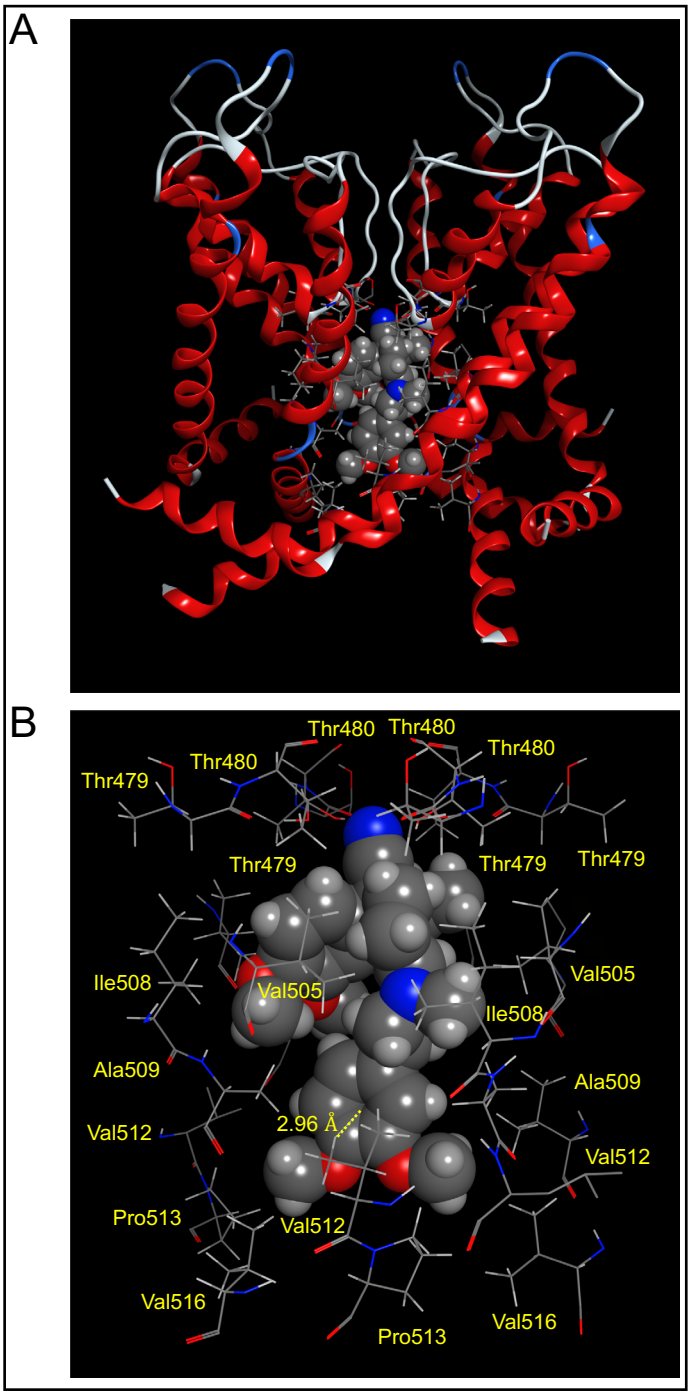

Fig. 6. Docking simulation for verapamil within the pore cavity of hKv1.5 channel. (A) The side view of the hKv1.5 channel pore region with verapamil binding at the lowest free energy of binding (-8.41 kcal/mol). (B) A close-up view of the binding area of verapamil shown in panel (A). The amino acids predicted to reside within 4.5 Å of verapamil are shown as a skeletal model, and verapamil is drawn as a space-filling model.

Discussion

The present patch-clamp experiments demonstrate that verapamil preferentially blocks the hKv1.5 channel in its open state in a concentration-dependent manner with an IC_{50} of 2.4±0.6 μM (Fig. 1, 2 and 3). The site-directed mutagenesis analysis suggests that several amino acids within the pore region, namely Thr479, Thr480, Ile502, Val505 Ile508 Leu510, Val 512 and Val516, are involved in mediating the blocking action of verapamil (Fig. 4 and 5). The computer docking simulation using an open-state homology model of the Kv1.5 channel also supports the view that verapamil resides within the central cavity of the channel pore and has contact with Thr479, Thr480, Val505 Ile508, Ala509, Val 512, Pro513 and Val516 (Fig. 6). Thus, the results obtained by mutational and docking simulation studies are in good agreement, and the amino acids commonly detected by these two experimental approaches, namely Thr479, Thr480, Val505, Ile508, Val 512 and Val516, are highly likely to provide binding sites for verapamil. Indeed, previous studies have shown that Thr479, Thr480 (located at the base of the ion selectivity filter), Val505, Ile508, Val 512 and Val516 (located within S6 domain) within hKv1.5 channel face toward the central cavity of the channel pore and provide the putative binding sites for many clinical and experimental drugs that act as open-channel blockers of the hKv1.5 channel, such as propofol [18, 20], S0100176 [22], AVE0118 [23], vernakalant [25] and bupivacaine [26]. A recent study found that Val417 and Ile420 within S6 domain of the hKv1.3 channel (which correspond to Val505 and le508 in the hKv1.5 channel, respectively) play an important role in mediating the blocking action of verapamil on the hKv1.3 channel [27]. It thus seems likely that valine and isoleucine at these positions are commonly important in verapamil block of hKv1.3 and hKv1.5 channels, which are members of the Shaker familay of voltage-gated K^+ channels [3].

Previous mutational studies have shown that the inhibitory actions of propofol, S0100176, AVE0118, vernakalant and bupivacaine on hKv1.5 channel are also significantly reduced by mutations of Ile502 and/or Leu510 [18, 20, 22, 23, 25, 26]. Because these two amino acids are predicted to point away from the inner cavity of the hKv1.5 channel [22, 23, 25], it remains unclear whether verapamil can have direct contact with these amino acids. The side chain of Ile502 is predicted to face toward Leu437 and Leu441 in the adjacent S5 subunit and thereby forms a hydrophobic interface with the adjacent subunit [25, 28]. It is therefore probable that hydrophobic moieties of verapamil protrude into the hydrophobic subunit interface and approach the side chain of Ile502 residue. On the other hand, a previous study suggested that the substitution of the large hydrophobic leucine at position 510 with a much smaller alanine alters the orientation of the side chain of the nearby Ile508 and Val512 and thereby hampers the interaction of hKv1.5 channel with AVE0118 [23]. A similar allosteric effect of L510A mutation may occur in the blocking action of verapamil on hKv1.5 channel. In this way, these two amino acids, namely Ile502 and Val510, are likely to play some functional roles in providing a structural basis for the development of verapamil blockade of the hKv1.5 channel.

Verapamil preferentially affects the L-type Ca^{2+} channel in its open state [11, 29] and binds to multiple amino acids in the S6 segment of domain IV in Cav1.2 (α_{1C}) channel, namely Tyr1463, Ala1467 and Ile1470 [30]. There are some (approximately 30%) similarities in the amino acid sequences in S6 segments between the hKv1.5 channel and α_{1CII} subunit of the L-type Ca^{2+} channel [15]. On the other hand, verapamil blocks the hERG channel by affecting specific amino acids (Ser620 and Ser631) located within S5-S6 linker [14]. Future studies are required to elucidated whether or not a similar molecular mechanism is involved in the blocking actions of verapamil on these three cation channels.

A previous study also demonstrated that verapamil acts as an open-channel blocker of the hKv1.5 channel heterologously expressed in human embryonic kidney (HEK) cells with a much lower potency (IC_{50} of 45 μM) [15] than was observed in the present experiments (IC_{50} of 2.4 μM; Fig. 3B). This discrepancy may arise from differences in experimental conditions, such as the method of evaluating the blocking potency ($^{86}Rb^+$ efflux versus K^+ current measurements), and cell types used (HEK versus CHO cells).

Electrophysiological experiments on atrial trabeculae isolated from patients with chronic atrial fibrillation showed that the pharmacological blockade of I_{Kur} significantly prolongs APD and ERP, suggesting that I_{Kur} could represent a potential target for prolonging the ERP in the treatment of atrial fibrillation [10, 31-33]. It is interesting to note that atrial fibrillation is accompanied by an alteration of the atrial electrophysiological properties (electrical remodeling), such as a shortening of atrial APD and ERP, which favors the maintenance of arrhythmias by facilitating multiple-circuit reentry [2, 34, 35]. The administration of verapamil attenuates atrial fibrillation-induced shortening of atrial ERP in humans, which has been suggested to arise from the inhibition of outward K^+ currents [13]. Indeed, verapamil has been shown to reduce the recurrence of atrial fibrillation after electrical cardioversion in the clinical setting [16, 17]. Taken together, the inhibitory effect of verapamil on hKv1.5 channel appears to be involved at least partly in the efficacy of verapamil in the prevention of atrial fibrillation.

In the clinical settings, the maximum concentrations of free verapamil in the serum has been reported to be approximately 80 nM [36], and at this concentration verapamil has a modest effect on the hKv1.5 current (Fig. 3A, B). However, because the localized levels in the heart during infusion are likely even higher [15], verapamil may inhibit I_{Kur} to some extent and thereby contributes to prevention of atrial fibrillation-induced shortening of atrial APD and ERP. It is, however, of interest to elucidate to what extent the verapamil-induced blockade of the hKv1.5 channel contributes to the reduction in recurrence of atrial fibrillation in the clinical setting. Of note, verapamil potently (IC_{50} = 0.143 μM) blocks the hERG channel [14], a molecular correlate of I_{Kr} in humans [3]. Because I_{Kr} is responsible for repolarization of the human atrial action potential [30], the verapamil-induced blockade of I_{Kr} could also contribute to prolongation of atrial APD and ERP in humans.

Conclusion

In conclusion, verapamil acts as an open-channel blocker of hKv1.5 channel, probably by acting on specific amino acids within the pore region of the hKv1.5 channel, and this blocking effect is expected to contribute at least in part to the efficacy of verapamil in the treatment of atrial fibrillation. Our study thus elucidates one important molecular and electrophysiological mechanism that seems to be involved in the prolonging effect of verapamil on human atrial ERP, associated with antiarrhythmic action on reentrant-based atrial arrhythmias.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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