Calcium Channel Inhibitor, Verapamil, Inhibits the Voltage-Dependent K^+ Channels in Rabbit Coronary Smooth Muscle Cells

Eun A Ko,[a,#] Won Sun Park,[a,#] Youn Kyoung Son,[b] Jae-Hong Ko,[c] Tae-Hoon Choi,[d] In Duk Jung,[e] Yeong-Min Park,[e] Da Hye Hong,[a] Nari Kim,[a] and Jin Han*,[a]

[a] *National Research Laboratory for Mitochondrial Signaling, Department of Physiology, College of Medicine, Cardiovascular and Metabolic Disease Center, Biomarker Medical Research Center, FIRST Mitochondrial Research Group, Inje University; Busan 613–735, Korea:* [b] *University Paris-Sud 11, Faculté de Pharmacie; Châtenay-Malabry, France:* [c] *Department of Physiology, Chung-Ang University; Seoul 156–756, Korea:* [d] *Department of Physical Education, Andong Science College; Gyo-ri, Seohu-myeon, Andong-si 760–709, Korea: and* [e] *Department of Microbiology and Immunology and National Research Laboratory of Dendritic Cell Differentiation & Regulation, Medical Research Institute, Pusan National University, College of Medicine; Beom-eo Ri, Mulgum Eop, Yangsan 626–770, South Korea.*
Received August 3, 2009; accepted October 6, 2009

We investigated the effect of the phenylalkylamine Ca^{2+} channel inhibitor verapamil on voltage-dependent K^+ (Kv) channels in rabbit coronary arterial smooth muscle cells using a whole-cell patch clamp technique. Verapamil reduced the Kv current amplitude in a concentration-depenent manner. The apparent K_d value for Kv channel inhibition was 0.82 μM. Although verapamil had no effect on the activation kinetics, it accelerated the decay rate of Kv channel inactivation. The rate constants of association and dissociation by verapamil were $2.20\pm0.02\ \mu M^{-1}\ s^{-1}$, and $1.79\pm0.26\ s^{-1}$, respectively. The steady-state activation and inactivation curves were unaffected by verapamil. The application of train pulses increased the verapamil-induced Kv channel inhibition. Furthermore, verapamil increased the recovery time constant, suggesting that the inhibitory effect of this agent was use-dependent. The inhibitory effect of verapamil was not affected by intracellular and extracellular Ca^{2+}-free conditions. Another Ca^{2+} channel inhibitor, nifedipine (10 μM) did not affect the Kv current, and did not alter the inhibitory effect of verapamil. Based on these results, we concluded that verapamil inhibited Kv current in a state-, time-, and use-dependent manner, independent of Ca^{2+} channel inhibition.

Key words verapamil; voltage-dependent K^+ channel; coronary arterial smooth muscle cell

Influx *via* voltage-dependent Ca^{2+} channels is the one of the major mechanisms of Ca^{2+} influx for the initiation of vascular smooth muscle contraction.[1—3] Ca^{2+} channel blockers inhibit the entry of calcium into cardiac and vascular smooth muscle cells *via* voltage-dependent L-type Ca^{2+} channels, which results in the relaxation of vascular smooth muscle and prevention of coronary vasospasm.[4—6] Several Ca^{2+} channel blockers are used therapeutically in the management of hypertension as monotherapy or in combination with other antihypertensive agents.[7,8] Phenylalkylamine Ca^{2+} channel inhibitor verapamil has been used in the treatment of hypertension, cardiac arrhythmia, and coronary vasospasm, and is effective in angina pectoris.[9,10] However, the unexpected effects of verapamil on other channels limit its safe use.[11,12]

Vascular smooth muscles express four types of K^+ channels: voltage-dependent K^+ (Kv), Ca^{2+}-activated K^+ (BK_{Ca}), ATP-sensitive K^+ (K_{ATP}), and inward rectifier K^+ (Kir) channels.[13] Among these channels, Kv channel contributes to the regulation of membrane potential, and thereby basal tone, and agonist-induced changes in vascular diameter.[13—17] Therefore, it is important to examine the effect of verapamil and its mechanism of action on Kv channels to avoid unexpected consequences in vascular smooth muscle.

In the present study, we clarified the effect of verapamil on Kv channels and determined its detailed mechanism of action by a patch clamp technique in native coronary arterial smooth muscle cells. Here, we show that verapamil directly inhibited Kv channels in a state-, time-, and use-dependent manner.

MATERIALS AND METHODS

Cell Isolation New Zealand White rabbits (2.0—2.3 kg) of either sex were sacrificed with sodium pentobarbitone (50 mg/kg) and heparin (100 U/kg), which were injected simultaneously into the ear vein. The rabbit for the experiments was approved by the Institutional Animal Care and Use Committee (IACUC) of the Inje University, Korea. The hearts were removed, and the left anterior descending coronary artery was rapidly dissected and cleaned of connective tissue under a stereomicroscope. Single vascular smooth muscle cells were mechanically dispersed by triturating the arteries after two step enzyme treatment with papain (1 mg/ml) and collagenese (1.5 mg/ml) in Ca^{2+}-free normal Tyrode solution as described previously.[18] Cells were kept at 4 °C under Kraft–Brühe (KB) and used within the same day. KB solution contained (in mM): 70 KOH, 50 L-glutamate, 20 KH_2PO_4, 55 KCl, 20 taurine, 3 $MgCl_2$, 20 glucose, 10 *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.5 EGTA adjusted to pH 7.3 with KOH.

Electrophysiological Recordings Whole cell K^+ currents were recorded with patch-clamp technique using Axopatch 1C amplifier (Axon instruments, Union, CA, U.S.A.) and a DigiData 1322 interface (Molecular Devices, Sunnyvale, CA, U.S.A.). The patch pipettes were fabricated on a model PP-83 vertical puller (Narishige, Tokyo, Japan). The currents were filtered at 1—2 kHz and digitized at 2—4 kHz. The resistance of patch pipettes was 3—4 MΩ with filling of the pipette solution.

The extracellular solution for whole cell recording was

* To whom correspondence should be addressed. e-mail: phyhanj@inje.ac.kr
# These authors contributed equally to this work.

normal Tyrode solution contained (in mM); 143 NaCl, 5.4 KCl, 0.33 NaH_2PO_4, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 5 HEPES, and 16.6 glucose (adjusted with NaOH to pH 7.4). The pipette (intracellular) solution for whole cell current recording contained (in mM); 105 K-aspartate, 25 KCl, 5 NaCl, 1 $MgCl_2$, 4 Mg-ATP, 10 1,2-bis(*O*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) and 10 HEPES (adjusted with KOH to pH 7.25). All chemicals were purchased from Sigma (St. Louis, MO, U.S.A.).

Data Analysis Data analysis was performed by using the Origin 6.0 software (Microcal Software, Inc., Northampton, MA, U.S.A.). For statistical analysis of the data, we used the Student's *t*-test. The results are expressed as means±S.E. Values of $p<0.05$ were considered as significant.

As described previously,[19] the drug–channel interaction kinetics could be expressed with a first-order blocking scheme. The affinity constant (K_d) and Hill coefficient (n), which were obtained from data on concentration-dependence, were determined by the Hill equation:

$$f=1/\{1+(K_d/[D])^n\}$$

where f is the fractional current block ($f=1-I_{drug}/I_{control}$) at the test potential, and [D] is the concentration of the drug.

The activation curve was determined from tail currents elicited on return to −40 mV after depolarizing voltage from −60 to +80 mV in 10 mV increments. Activation curves were fit with the Boltzmann equation, as follows:

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

where V represents the test potential, $V_{1/2}$ is the mid-point of activation and k is the slope factor.

The steady-state inactivation was studied using a two-pulse voltage protocol; currents were measured at +40 mV, and the 7 s pre-pulses to potential varied from −70 to +40 mV in steps of 10 mV. The curves for steady-state inactivation were fit with the Boltzmann equation:

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

where V represents the test potential, $V_{1/2}$ is the mid-point potential, and k is the slope factor of the curve.

RESULTS

Inhibition of Kv Current by Verapamil Figure 1 shows the effect of verapamil on the Kv currents recorded with step-depolarizing pulses from −80 to +60 mV and a holding potential of −60 mV. The Kv current was rapidly inhibited by 3 μM verapamil within 1 min, and this inhibitory effect was completely abrogated by washout (data not shown). Figures 1C and D show the current–voltage (I–V) relationships of peak and steady-state Kv currents in the absence and presence of 3 μM verapamil, respectively. Verapamil reduced the both peak and steady-state current, but the extent of the reduction in the steady-state current was much more prominent than that in the peak current.

Verapamil Inhibits Kv Currents in a Dose-Dependent Manner Figure 2 shows representative Kv currents superimposed on the variable concentration of verapamil at 0.3, 1, 3, 10, and 30 μM to evaluate dose-dependency. The Kv currents were recorded by depolarizing pulses to +60 mV from a holding potential of −60 mV. As described in Fig. 1, Kv current inhibition by verapamil was greater for the steady-state current than for the peak current. For steady-state inhibition, a nonlinear least-squares fit of the Hill equation to the concentration–response data at +60 mV yielded a K_d value of 0.82±0.03 μM and a Hill coefficient of 1.10±0.04 (Fig. 2B).

Kinetics of Kv Current Inhibition by Verapamil To evaluate the effects of verapamil on the activation time constant, the rising phase of each trace was measured by fitting a

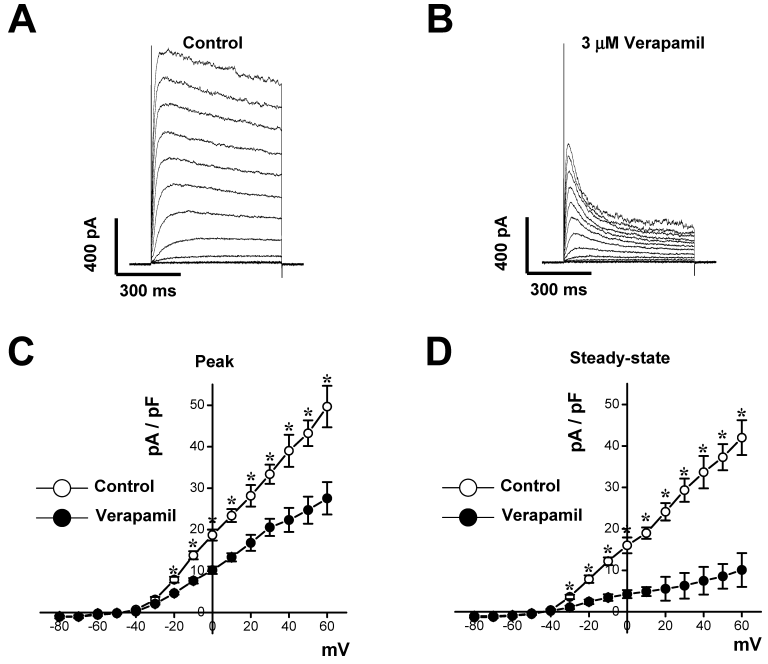


Fig. 1. Effects of Verapamil on Kv Channels from Coronary Arterial Smooth Muscle Cells

The Kv currents were elicited by a 600 ms depolarizing pulse from −80 to +60 mV in increments of 10 mV at a holding potential of −60 mV. Representative Kv currents without (A) and with (B) 3 μM verapamil. I–V relationship of the peak (C) and steady-state (D) Kv currents in the absence (○) and presence (●) of verapamil. $n=5$. $*p<0.05$.

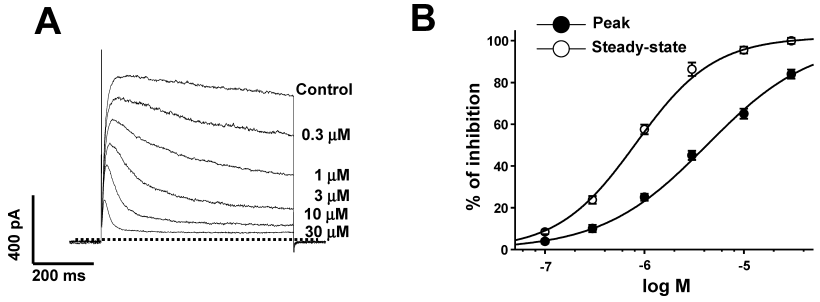
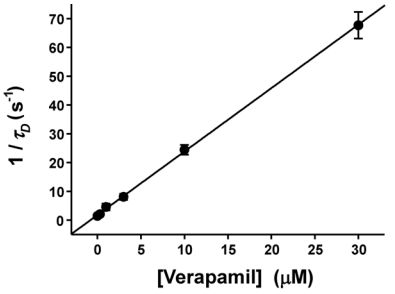


Fig. 2. Dose–Response Curves for the Inhibition of Kv Currents by Verapamil

(A) Representative current traces were elicited in the absence of verapamil (control) and in the presence of 0.3, 1, 3, 10, and 30 μM verapamil. (B) Average concentration-dependent inhibition of Kv currents at peak (●) and steady-state (○), normalized to the current in the absence of each drug. The curve was fit to the Hill equation. Whole-cell Kv currents were elicited by 600-ms depolarization to +60 mV from a holding potential of −60 mV. n=4.



Verapamil (μM)	τ_D (ms)	
0	686.12 ± 26.75	Control
0.3	471.76 ± 45.57	P < 0.05
1	218.06 ± 43.34	P < 0.05
3	123.54 ± 14.02	P < 0.05
10	40.89 ± 2.72	P < 0.05
30	14.77 ± 0.95	P < 0.05

Fig. 3. Time Constant of Inhibition as a Function of the Drug Concentration

Time constants (τ_D) were estimated from single exponential fits to the decay phase of the tracings shown in Fig. 2A. The time courses of the current during inactivation were fit to a single (control) or double (presence of verapamil) exponential function. The apparent rate constants for association (k_{+1}) and dissociation (k_{-1}) were obtained from $1/\tau_D = k_{+1}[D] + k_{-1}$, $K_d = k_{-1}/k_{+1}$. n=4.

monoexponential curve at +60 mV under additive increases in the concentration of verapamil. The time constants for activation of the Kv current were 11.98±0.15 (n=5) and 12.21±0.19 (n=4) ms in the absence and presence of 3 μM verapamil, respectively. These results suggest that the activation process was not significantly altered by verapamil. However, Fig. 2A shows that the decay rate of the Kv current accelerated significantly with increases in the concentration of varapamil. In the Kv current with verapamil treatment, the inactivation processes were composed of fast and slow components. The slow component is regarded as an intrinsic inactivation process in vascular smooth muscle.[17] Therefore, the fast component was thought to represent the time constant for drug-induced blockade of the Kv current (τ_D). Figure 3 shows τ_D at +40 mV plotted against the various concentrations of verapamil (raw τ_D values were shown in the table). From this fit, an apparent association (k_{+1}) of 2.20±0.02 $\mu M^{-1} s^{-1}$, and dissociation rate constant (k_{-1}) of 1.79±0.26 s^{-1} were obtained. The K_d value derived on the basis of a first-order reaction between the drug and channel[20—22] was 0.81±0.001 μM in the presence of verapamil. This value is very close to the K_d value of 0.82 μM obtained from the concentration–response curve shown in Fig. 2.

Effects of Verapamil on the Activation and Steady-State Inactivation of the Kv Current The activation and inactivation curves were measured in the absence and presence of 3 μM verapamil to investigate whether the inhibition was due to a shift in the activation and inactivation curves. The activation curves were obtained using the tail current with a typical two-pulse protocol in the absence and presence of verapamil. The application of verapamil did not affect steady-state activation with a voltage of half-maximal activation potential ($V_{1/2}$) and slope value (k) of −13.58±0.67 mV and 8.60±0.52, respectively, for the control and −13.70±0.63 mV and 7.97±0.33, with verapamil (Fig. 4A).

The steady-state inactivation of Kv was investigated using a typical two-pulse protocol in the absence and presence of verapamil. The inactivation curve of Kv current was not changed by verapamil; the potential of half-maximal activation ($V_{1/2}$) and the slope value (k) were −34.68±0.51 mV and 9.33±0.40, respectively, under control conditions and −35.26±0.50 mV and 8.90±0.44, respectively, in the presence of verapamil (Fig. 4B). The lack of an effect on steady-state inactivation may be explained as an inability of verapamil to interact with the channel in the inactivated state.

Use-Dependent and Recovery Kinetics of Kv To evaluate the use-dependent inhibition of Kv by verapamil, we recorded the Kv current at two different frequencies, 1 Hz and 2 Hz, in the absence and presence of verapamil (3 μM, Fig. 5). Under control conditions, the Kv currents were slightly reduced by the both 1 Hz and 2 Hz stimulation. For example, the peak amplitude of the Kv current measured after a train of 20 depolarizing pulses was decreased by 14% at a frequency of 1 Hz and by 22% at a frequency of 2 Hz in the absence of verapamil. However, in the presence of verapamil, the Kv current showed greater decay than the control until it reached a steady-state block. For example, the peak amplitude of the Kv current after 20 depolarizing pulses was reduced by 53.20±0.64% at 1 Hz and by 39.35±0.31% at 2 Hz in the presence of verapamil. These results suggested that the recovery of the channel blocked by verapamil was slower than that of the control. To explain this result more clearly, we investigated the recovery time from inactivation using a double-pulse protocol in which the interpulse interval was varied from 20 to 7000 ms. Figure 6 shows the representative curves, which were fit with a single exponential, in

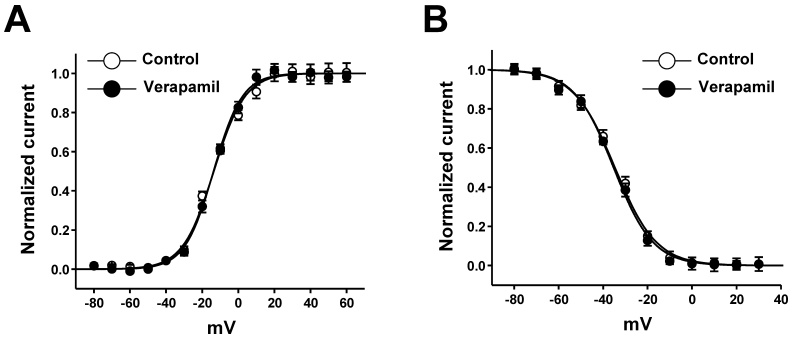


Fig. 4. Effects of Verapamil on Activation and Steady-State Inactivation of Kv Channels

(A) Activation curves in the absence (○) and presence (●) of verapamil. $n=6$. (B) Steady-state inactivation curves in the absence (○) and presence (●) of verapamil. $n=6$. Curves were fit to the Boltzmann equation (see Materials and Methods).

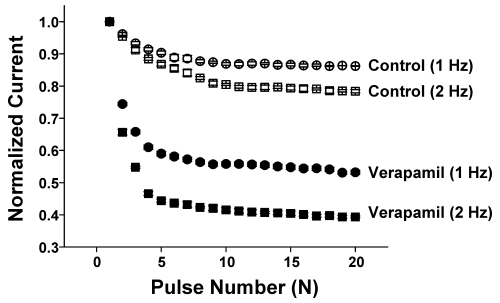


Fig. 5. Use-Dependent Inhibition of Kv Current by Verapamil

Twenty repetitive depolarizing pulses from −60 to +40 mV for 150-ms each were applied in the absence (○, □) and presence of verapamil (●, ■) at frequencies of 1 Hz and 2 Hz. Each peak current obtained at by frequencies of 1 Hz or 2 Hz was normalized to the peak current at the first pulse and then plotted against the number of pulses. $n=4$.

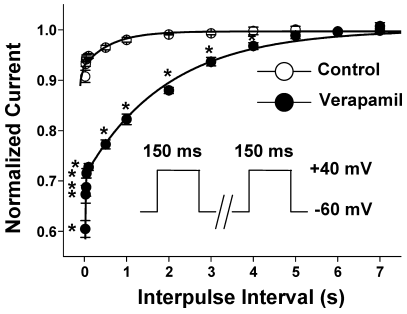


Fig. 6. Effects of Verapamil on the Kinetics of Kv Recovery from Steady-State Inactivation

The degree of recovery was measured by following a double-pulse protocol; the first pre-pulse of a 150-ms depolarizing potential of +40 mV from a holding potential of −60 mV was followed by a second identical pulse after increasing time intervals between 20 ms and 7000 ms (inset). All cycles of the double-pulse protocol were 10 s. The peak currents elicited by the second test pulse were normalized against the peak currents obtained by the first pre-pulse and plotted as a function of the various interpulse intervals. The solid line represents the recovery kinetics of Kv current in the absence (○) and presence of verapamil (●). $n=5$. $*p<0.05$.

the absence and presence of verapamil. The recovery time constant was slower in the presence of verapamil, *i.e.*, 0.76±0.21 for control *versus* 1.96±0.12 s with verapamil. This result suggested that the recovery rate of Kv channels from inhibition by verapamil was slower than the transition rate between the open and closed states in the absence of verapamil, which may indicate that verapamil inhibits Kv channels with a use-dependent block.

Inhibitory Effects of Verapamil in the Absence of Extracellular Ca^{2+} or in the Presence of Another Ca^{2+} Channel Inhibitor To confirm that the inhibitory effect of verapamil on Kv channels was not a result of Ca^{2+} channel inhibition, its effects on Kv currents were recorded in the absence of extracellular Ca^{2+}, or in the presence of another Ca^{2+} channel inhibitor, nifedipine (10 μM). As shown in Fig. 7A, extracellular Ca^{2+}-free conditions did not alter the verapamil-induced inhibition of Kv current (Normal extracellular solution+verapamil: 80.11±3.72%, Ca^{2+}-free extracellular solution+verapamil: 77.32±4.41% inhibition, respectively, Fig. 7B). Pretreatment with another Ca^{2+} channel inhibitor, nifedipine, did not affect the basal Kv current; however, additional application of verapamil inhibited the Kv currents to a similar to that observed with verapamil alone (Verapamil alone: 80.32±2.88%, verapamil+nifedipine: 78.91±3.09% inhibition, respectively, Figs. 7C, D). These results indicated that the inhibitory effect of verapamil is not related to the inhibition of Ca^{2+} channels.

DISCUSSION

The results of the present study indicated that verapamil affects Kv channels, and its mechanism of action was determined in rabbit coronary arterial smooth muscle cells. The main findings of this study were that verapamil inhibits Kv channels in a state-, time-, and use-dependent manner. In addition, the inhibition of Kv channels by verapamil had no effect on the voltage-dependent activation and inactivation curves.

Several observations indicated that the inhibitory effect of verapamil on Kv channels was not mediated by the inhibition of Ca^{2+} channel but was the result of direct action on Kv channels. (1) Another Ca^{2+} channel inhibitor, nifedipine, had no effect on the Kv channels and did not alter the inhibitory effect of verapamil. Furthermore, the deletion of extracellular Ca^{2+} did not alter the inhibitory effect of verapamil (Fig. 7). (2) The inhibitory time course of verapamil is very short, reaching the steady-state inhibition of Kv channels within 1 min. Therefore, the inhibitory effect of verapamil on Kv channels did not involve Kv-related signaling mechanisms, but was the result of its direct action.

The inhibitory effect of verapamil on Kv channels is most likely due to an interaction in the open state. Figures 2 and 3 suggest that the time course of the Kv current was accelerated by verapamil in a concentration-dependent manner, even though the activation time course was not changed. Furthermore, the peak amplitude of the Kv current was affected rel-

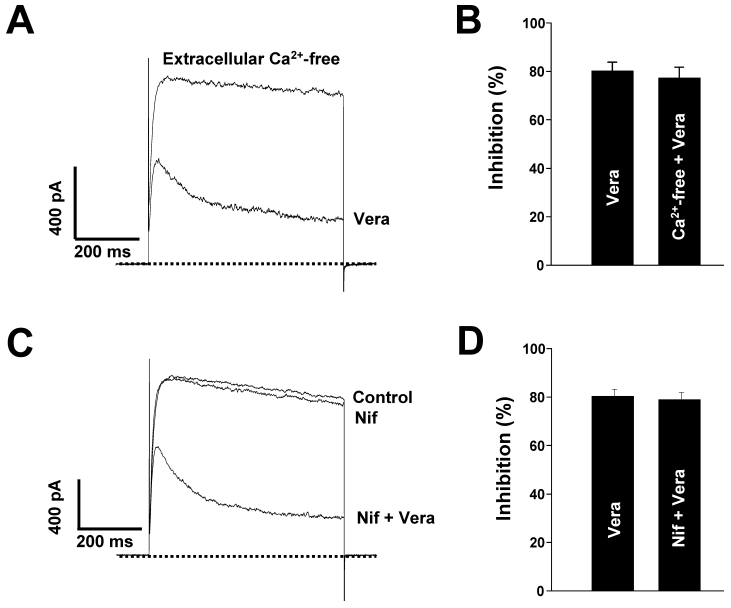


Fig. 7. Effects of Extracellular Ca^{2+}-Free Conditions or Another Ca^{2+} Channel Blocker (Nif) on Verapamil (Vera)-Induced Inhibition of Kv Channels

(A) Representative currents in the absence of extracellular Ca^{2+} (n=4). These results are summarized in (B). The verapamil-induced inhibition of the Kv currents was measured at steady-state. (C) Representative currents were obtained under control conditions, in the presence of 10 μM nifedipine, and with an additional application of 10 μM verapamil (n=4). These results are summarized in (D). All current recordings were obtained with a depolarizing pulse at +60 mV from a holding potential of −60 mV.

atively little by verapamil at the first onset of depolarization. In addition, verapamil did not alter the activation or steady-state inactivation curves, suggesting that this agent interacts with Kv channels in the open state, and not the inactivated state (Fig. 4).

A number of classes of Ca^{2+} channel blockers such as dihydropyridine, phenylalkylamine, and benzothiazepine have been reported to date. Among these, phenylalkylamine was reported to reduce myocardial oxygen demand, and coronary vasospasm. Phenylalkylamine is also very effective in the treatment of angina pectoris.[23] Verapamil, which is a specific phenylalkylamine Ca^{2+} channel inhibitor, causes vasodilation and in turn lowers arterial blood pressure. However, effects of verapamil on other channels have also been reported. For example, verapamil was shown to inhibit ultra-rapid delayed rectifier K^+ current in human atrial myocytes,[24] delayed rectifier K^+ currents in guinea pig cardiac myocytes,[25] the human *Ether-á-go-go* Related Gene (HERG) K^+ channels expressed in HEK 293 cells,[26] rapidly activating delayed rectifier K^+ channels in cloned human heart cells[11] and large-conductance Ca^{2+}-activated K^+ channels in rat aortic myocytes.[27] Although these reports also, but partially, explained the inhibitory mechanism of verapamil on delayed rectifier K^+ channel, the data were originated from cardiac cells, not vascular smooth muscle. Furthermore, verapamil effect on aortic smooth muscle was restricted to large-conductance Ca^{2+}-activated K^+ channels. In this reason, neither the effect of verapamil nor its mechanism of action on Kv channels from native coronary arterial smooth muscle cells has been determined, even though verapamil has been used as an inhibitor of Ca^{2+} channels. Considering its clinical relevance, the identification of unexpected effects of verapamil on other types of channel is necessary in order to avoid misinterpretation of data.

The Kv channel expressed in coronary vascular smooth muscle is one of the major channels involved in the regulation of the resting membrane potential, and it also mediate the physiological changes in agonist-induced vascular tone.[3,22,28] Therefore, elucidation of unexpected effects of chemical agents on Kv channels is an important area of research. Indeed, several previous studies have revealed unexpected effects of various agents on Kv channels. For example, the protein kinase C (PKC) inhibitors staurosporine and bisindolylmaleimide(I) were shown to directly inhibit Kv channels in the coronary artery, mesenteric artery, and cell lines stably expressing Kv subtypes.[17,21,22,29,30] The protein kinase A (PKA) inhibitor H-89 and the tyrosine kinase inhibitor genistein also inhibited Kv channels in rabbit coronary arterial smooth muscle cells.[31,32] Together with the results of previous studies, the findings presented here provide information that will be important when using these agents to study Kv channels.

The Kv currents can be classified as the delayed rectifier outward K^+ current (I_K) and transient outward K^+ current (I_A) based on the differences in the voltage dependence of activation and/or inactivation, and pharmacological properties in vascular smooth muscle cells.[15,33] The I_K showed a relatively slow activation and inactivation kinetics in comparison to the I_A.[34,35] Although the detailed molecular subtypes of Kv channel remains to be determined in vascular smooth muscle, at least, it has been known that Kv1.2, Kv1.5 and Kv2.1 alpha subunits were regarded as main subtypes of I_K since these subtypes showed the similar current kinetics to the native I_K in vascular smooth muscle cells.[36,37] Therefore, we suggest that verapamil may inhibit Kv1.2, Kv1.5 and/or Kv2.1 subtypes in native coronary arterial smooth muscle cells. To make a clear, it needs to further experiment whether verapamil inhibits Kv current using the overexpressed HEK-293 cells with Kv1.5 (Kv1.2 or Kv2.1) subtype or whether knock down of Kv1.5 (Kv1.2 or Kv2.1) using siRNA can abolish the verapamil effect in coronary arterial smooth muscle cells.

In the present study, we found that verapamil directly inhibits the Kv current of smooth muscle cells from rabbit coronary artery in a state-, time-, and use-dependent manner. Furthermore, the inhibitory effects of verapamil are completely independent of Ca^{2+} channel inhibition. Therefore, caution is required when using verapamil to inhibit Ca^{2+} channels in the vascular system.

Acknowledgements This research was supported by Basic Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (R0A-2007-000-20085-0, R13-2007-023-00000-0, KRF-2008-313-E00046, and KRF-2008-355-E00014).

REFERENCES

1) Itoh T., Kanmura Y., Kuriyama H., Suzuki H., *Br. J. Pharmacol.*, **83**, 243—258 (1984).
2) Goto K., Kasuya Y., Matsuki N., Takuwa Y., Kurihara H., Ishikawa T., Kimura S., Yanagisawa M., Masaki T., *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 3915—3918 (1989).
3) Shimizu S., Yokoshiki H., Sperelakis N., Paul R. J., *J. Vasc. Res.*, **37**, 16—25 (2000).
4) Kuga T., Sadoshima J., Tomoike H., Kanaide H., Akaike N., Nakamura M., *Circ. Res.*, **67**, 469—480 (1990).
5) McDonald T. F., Pelzer S., Trautwein W., Pelzer D. J., *Physiol. Rev.*, **74**, 365—507 (1994).
6) Sugiura T., Kondo T., Kureishi-Bando Y., Numaguchi Y., Yoshida O., Dohi Y., Kimura G., Ueda R., Rabelink T. J., Murohara T., *Hypertension*, **52**, 491—498 (2008).
7) Mecca T. E., Love S. D., *J. Cardiovasc. Pharmacol.*, **20**, 678—682 (1992).
8) Abernethy D. R., Schwartz J. B., *N. Engl. J. Med.*, **341**, 1447—1457 (1999).
9) Rang H. P., Dale M. M., Ritter J. M., Moore P. K., "Pharmacology," 5th ed., Churchill, Livingstone, U.K., 2003.
10) Grossman E., Messerli F. H., *Prog. Cardiovasc. Dis.*, **47**, 34—57 (2004).
11) Rampe D., Wible B., Fedida D., Dage R. C., Brown A. M., *Mol. Pharmacol.*, **44**, 642—648 (1993).
12) DeCoursey T. E., *J. Gen. Physiol.*, **106**, 745—779 (1995).
13) Ko E. A., Han J., Jung I. D., Park W. S., *J. Smooth Muscle Res.*, **44**, 65—81 (2008).
14) Smirnov S. V., Aaronson P. I., *J. Physiol.*, **457**, 431—454 (1992).
15) Nelson M. T., Quayle J. M., *Am. J. Physiol. Cell. Physiol.*, **268**, 799—822 (1995).
16) Shimoda L. A., Sylvester J. T., Sham J. S., *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **274**, 842—853 (1998).
17) Park W. S., Son Y. K., Han J., Kim N., Ko J. H., Bae Y. M., Earm Y. E., *J. Cardiovasc. Pharmacol.*, **45**, 260—269 (2005).
18) Ko E. A., Park W. S., Earm Y. E., *Pflügers Arch.*, **449**, 195—204 (2004).
19) Snyders D. J., Yeola S. W., *Circ. Res.*, **77**, 575—583 (1995).
20) Snyders D. J., Tamkun M. M., Bennett P. B., *J. Gen. Physiol.*, **101**, 513—543 (1993).
21) Choi B. H., Choi J. S., Jeong S. W., Hahn S. J., Yoon S. H., Jo Y. H., Kim M. S., *J. Pharmacol. Exp. Ther.*, **293**, 634—640 (2000).
22) Park W. S., Son Y. K., Ko E. A., Ko J. H., Lee H. A., Park K. S., Earm Y. E., *Life Sci.*, **77**, 512—527 (2005).
23) Prisant L. M., *Heart Dis.*, **3**, 55—62 (2001).
24) Gao Z., Lau C. P., Chiu S. W., Li G. R., *J. Mol. Cell. Cardiol.*, **36**, 257—263 (2004).
25) Zhang S., Sawanobori T., Hirano Y., Hiraoka M., *J. Cardiovasc. Pharmacol.*, **30**, 489—496 (1997).
26) Zhang S., Zhou Z., Gong Q., Makielski J. C., January C. T., *Circ. Res.*, **84**, 989—998 (1999).
27) Harper A. A., Catacuzzeno L., Trequattrini C., Petris A., Franciolini F., *J. Membr. Biol.*, **179**, 103—111 (2001).
28) Leblanc N., Wan X., Leung P. M., *Am. J. Physiol. Cell Physiol.*, **266**, 1523—1537 (1994).
29) Choi J. S., Hahn S. J., Rhie D. J., Jo Y. H., Kim M. S., *Naunyn Schmiedebergs Arch. Pharmacol.*, **359**, 256—261 (1999).
30) Kim A., Bae Y. M., Kim J., Kim B., Ho W. K., Earm Y. E., Cho S. I., *Eur. J. Pharmacol.*, **483**, 117—126 (2004).
31) Son Y. K., Park W. S., Kim S. J., Earm Y. E., Kim N., Youm J. B., Warda M., Kim E., Han J., *Biochem. Biophys. Res. Commun.*, **341**, 931—937 (2006).
32) Ko E. A., Park W. S., Son Y. K., Kim D. H., Kim N., Kim H. K., Choi T. H., Jung I. D., Park Y. M., Han J., *Vascul. Pharmacol.*, **50**, 51—56 (2009).
33) Iida H., Jo T., Iwasawa K., Morita T., Hikiji H., Takato T., Toyo-Oka T., Nagai R., Nakajima T., *Br. J. Pharmacol.*, **146**, 49—59 (2005).
34) Beech D. J., Bolton T. B., *J. Physiol.*, **418**, 293—309 (1989).
35) Halliday F. C., Aaronson P. I., Evans A. M., Gurney A. M., *Br. J. Pharmacol.*, **116**, 3139—3148 (1995).
36) Kerr P. M., Clément-Chomienne O., Thorneloe K. S., Chen T. T., Ishii K., Sontag D. P., Walsh M. P., Cole W. C., *Circ. Res.*, **89**, 1038—1044 (2001).
37) Lu Y., Hanna S. T., Tang G., Wang R., *Life Sci.*, **71**, 1465—1473 (2002).