Verapamil Diminishes Action Potential Changes During Metabolic Inhibition by Blocking ATP-Regulated Potassium Currents

Shinichi Kimura, Arthur L. Bassett, Hongying Xi, and Robert J. Myerburg

Verapamil has beneficial effects on ischemic myocardium, including reduction in electrophysiological derangements, prevention of intracellular K+ loss, and preservation of high-energy phosphates, but the mechanisms underlying these actions are not clear. Recent studies have demonstrated a role of ATP-regulated K⁺ (K<sub>ATP</sub>) current in action potential shortening and K⁺ loss during ischemia and metabolic inhibition. Therefore, we studied the effects of verapamil on K<sub>ATP</sub> current in feline ventricular myocytes to test the hypothesis that the drug prevents ischemic electrophysiological disturbances by affecting the K<sub>ATP</sub> channel. Membrane potentials and currents were recorded using standard patch-clamp techniques. During 15-minute superfusion with 1 mM CN<sup>−</sup>, action potential duration measured at 90% repolarization was reduced from 259 ± 12 to 98 ± 15 msec (62% reduction) in the absence of verapamil and from 266 ± 11 to 183 ± 16 msec (31% reduction) in the presence of 2 μM verapamil (p < 0.01). In inside-out membrane patches, the K<sub>ATP</sub> current, activated in the absence of ATP, was significantly suppressed by intracellular application of 2 μM verapamil, but the single-channel conductance was not changed. Verapamil did not change the mean open and closed times of the channel within bursts (e.g., the mean open time was 1.92 ± 0.18 and 1.82 ± 0.21 msec in the absence and presence of 2 μM verapamil, respectively), but it shortened the mean lifetime of bursts from 41.1 ± 3.5 to 24.9 ± 2.8 msec (p < 0.01) and prolonged the closed time between bursts from 39.4 ± 4.6 to 78.5 ± 5.1 msec (p < 0.01). As a result, the open-state probability of the channel was significantly reduced from 0.31 ± 0.04 to 0.03 ± 0.01 (p < 0.01). We suggest that these effects of verapamil on the K<sub>ATP</sub> channel in isolated ventricular myocytes provide, in part, an explanation for its amelioration of electrophysiological disturbances and K⁺ loss during ischemia. (Circulation Research 1992;71:87–95)

**KEY WORDS** • patch clamp • ventricular myocytes • cyanide • ischemia

Verapamil has been demonstrated to prevent arrhythmias occurring during experimental ischemia and reperfusion. However, the precise mechanism of this action remains unsettled. A major action of verapamil is to block Ca<sup>2+</sup> entry through the L-type Ca<sup>2+</sup> channel during excitation, and this effect is thought to suppress Ca<sup>2+</sup>-dependent slow responses that may be generated in ischemic myocardium. However, verapamil has been shown to have multiple pharmacological actions under ischemic conditions, including a reduction in the size of the experimental infarct zone, metabolic protection of the ischemic tissues, a lessening of ischemia-induced conduction disturbances, and a reduction in the rate of extracellular K⁺ accumulation during ischemia. Our previous study demonstrated that verapamil also lessens action potential changes and conduction disturbances in the epicardium during the early phase of ischemia in coronary-perfused cat left ventricular preparations. The role of the ATP-regulated K⁺ (K<sub>ATP</sub>) current in action potential shortening during ischemia and metabolic inhibition has received much attention recently. Since Noma showed that this current is activated during exposure to CN<sup>−</sup> in guinea pig ventricular cells, data demonstrating the involvement of K<sub>ATP</sub> current in action potential shortening and K⁺ loss during ischemia have been accumulated. A recent study provided indirect evidence that several antiarrhythmic drugs, including verapamil, may block K<sub>ATP</sub> currents. The present investigation derives from our prior characterization of the action of verapamil in ischemia and was designed to directly test the hypothesis that verapamil reduces electrophysiological abnormalities during metabolic inhibition by modifying K<sub>ATP</sub> channels.

**Materials and Methods**

**Cell Isolation**

Domestic cats of either sex, weighing 2.5–4.0 kg, were anesthetized with sodium pentobarbital (30 mg/kg i.p.), and heparin sodium (400 IU/kg) was injected intravenously. The heart was excised and mounted on a Langendorff apparatus and perfused via the aorta with a modified Tyrode’s solution (37°C) containing (mM)
NaCl 143, KCl 4, CaCl2 1.8, MgCl2 0.5, NaHPO4 0.33, glucose 5.5, and HEPES 5.5 (pH 7.4 with NaOH) and gassed with 100% O2. After a 5-minute equilibration period, the preparation was perfused with Ca2+-free Tyrode’s solution (otherwise identical to the above solution) for 5 minutes, followed by perfusion with 2 mg/ml (300 units/ml) collagenase (type II, Worthington Biochemical Co., Freehold, N.J.) and 0.3 mg/ml protease (type XIV, Sigma Chemical Co., St. Louis, Mo.) dissolved in Tyrode’s solution containing 50 μM Ca2+. Exposure to the enzymes was continued until the solution flowed freely (12–14 minutes), after which the collagenase and protease were washed out with a solution containing (mM) KOH 70, KCl 40, glutamic acid 50, tauro20, KH2PO4 10, MgCl2 0.5, glucose 11, EGTA 0.5, and HEPES 10 (pH 7.4 with KOH). Small pieces of left ventricular tissue were dissected from the endocardial and epicardial surfaces with fine scissors and blades. After the tissues had been minced, single cells were separated from tissue pieces by passing them through nylon mesh (150 μm).

Electrical Stimulation and Recording

Isolated cells were introduced into a superfusion chamber (1 ml in volume) on the stage of an inverted microscope and were superfused with Tyrode’s solution (37°C) at a rate of 3 ml/min. Action potential studies were performed using low-resistance (2–4 MΩ) suction pipettes in the whole-cell recording mode.28 The pipettes were forged by a micropipette puller (model p-87, Sutter Instrument Co., Novato, Calif.) and were heat-polished before use. The electrode potential was adjusted to give zero current between the pipette solution and bath solution immediately before each cell was attached. After a gigaseal (>5 GΩ) was formed by gentle suction, the cell membrane under the electrode tip was broken by further application of negative pressure. The pipette solution contained 140 mM KCl, 4 mM MgCl2, and 10 mM HEPES (pH 7.2 with KOH). The cells were stimulated by passing depolarizing currents (1-msec pulse width, twice diastolic threshold) through the pipette at a rate of 1 Hz. After a 10-minute equilibration period, cells were superfused with glucose-free Tyrode’s solution containing 1 mM NaCN for 15–30 minutes, during which action potentials were monitored (control group). In the verapamil-treated group, the cells were superfused with Tyrode’s solution containing 1 mg/l (2 μM) verapamil for 20 minutes and then superfused with Tyrode’s solution containing both CN− and verapamil.

Single KATP current signals were recorded in inside-out membrane patches.22 The pipette and bath solutions had the same composition (mM): KCl 140, HEPES 5, and EGTA 1 (pH 7.4 with KOH). Inside-out membrane patches were obtained with the microelectrodes having a resistance of 3–5 MΩ. After obtaining a >5-GΩ seal, the pipette tip was briefly passed out and in through the solution–air interface, resulting in an inside-out patch. Channel activity was recorded in the absence of ATP at pH 7.4 at room temperature. Patches were subjected to test potentials ranging from −80 to 80 mV. After control measurements, patches were exposed to verapamil (1, 2, or 10 μM), and the measurements were repeated. Since we often observed the rundown phenomenon for KATP channels, the channel activity was reexamined after washout of verapamil. Data obtained from the experiments in which the channel activity did not reappear (>50% of baseline activity) were discarded, because it is unclear whether the suppression of channel activity was due to the effect of verapamil or channel rundown.

Data Acquisition and Analysis

Membrane potentials and single-channel currents were recorded through an Axopatch-1D patch-clamp amplifier (Axon Instruments, Inc., Burlingame, Calif.) or a Dagan 8900 patch-clamp amplifier (Dagan Corp., Minneapolis, Minn.). Voltage and current data were displayed on an oscilloscope (Tektronix, Beaverton, Ore.) and were stored on a videocassette recorder (Betamax, Sony, Tokyo) through an analog-to-digital converter (PCM-1C, Medical Systems Corp., Greenvale, N.Y.) at a conversion rate of 40 kHz. The signals for single-channel currents were filtered off-line through an eight-pole low-pass Bessel filter (model 902-LPF, Frequency Devices Inc., Haverhill, Mass.) with cutoff frequency of 0.5–5 kHz at −3 dB, digitized at a conversion rate of 30 kHz with a 12-bit Labmaster analog-to-digital converter (Axon Instruments), and analyzed with a set of software programs (pCLAMP, Axon Instruments) and an IBM-AT personal computer. Action potentials were played back on the chart recorder (Gould Inc., Altamonte Springs, Fla.) at a paper speed of 125 mm/sec for analysis. For analysis of kinetics and open-state probability of single channels, segments of data (60 seconds) were displayed on screen, after which baseline and single-channel amplitudes were fitted by eye to each recording. A “50% threshold” criterion was used to detect events with the help of manual confirmation. The amplitude histograms were formed using the averaged amplitude of openings, including the baseline and open levels, and were expressed by a sum of several Gaussian distributions using a least-squares algorithm. The difference of the means of two adjacent Gaussian peaks was taken as a measure of the unitary current amplitude. The number of the nonzero Gaussian peaks was taken as the number of channels in the patch, although this method may not be accurate at the low open-state probabilities. Open-state probability (Popen) was calculated using the formula

\[
P_{\text{open}} = \frac{I}{(N \times \text{threshold})}
\]

where I is mean patch current, N is the number of channels in patch, and threshold is the unit amplitude of the single-channel current. Open-state probability was determined at a membrane potential positive to the reversal potential by 50 mV; at membrane potentials negative to the reversal potential it is conceivable that openings of the inward rectifier K+ channel may interfere with the measurement.14 For kinetic analysis, only recordings containing one active KATP channel were used.

All results are expressed as mean±SEM. Statistical significance was evaluated by analysis of variance with repeated measurements or Student’s unpaired t test, where appropriate. Differences with values of p<0.05 were considered to be significant.

Results

Effect of Verapamil on Action Potential Changes Induced by CN−

Since our previous studies29,30 demonstrated that endocardial and epicardial cells respond differently to
metabolic inhibition induced by CN⁻ and that endocardial and epicardial K<sub>ATP</sub> channels have different sensitivity to ATP, the present study was designed to generate data separately for endocardial and epicardial cells. Most data presented were obtained from epicardial cells, since K<sub>ATP</sub> current is activated to a greater extent during exposure to 1 mM CN⁻ in cells of epicardial origin.29

In epicardial myocytes, verapamil (2 µM) reduced action potential duration at the plateau level measured at 0 mV (from 172±12 to 108±13 msec, p<0.01) and slightly prolonged action potential duration measured at 90% repolarization (from 263±13 to 286±15 msec, p<0.05). Figure 1 shows a representative experiment of action potential changes in epicardial cells during superfusion with Tyrode’s solution containing 1 mM CN⁻ in the absence and presence of 2 µM verapamil. In the absence of verapamil, a 15-minute superfusion with 1 mM CN⁻ markedly reduced action potential duration, without affecting action potential amplitude and resting potential. When the cells were treated with verapamil for 20 minutes before, and also during, exposure to CN⁻, action potential shortening was significantly reduced. Figure 2 shows the cumulative time course of changes in action potential duration measured at 0 mV and 90% repolarization during 15 minutes of exposure to 1 mM CN⁻ in the absence and presence of verapamil. At 15 minutes, action potential duration at 90% repolarization was reduced from 259±12 to 98±15 msec (62±5% reduction) in the absence of verapamil and from 266±11 to 183±16 msec (31±7% reduction) in the presence of verapamil (n=10, p<0.01).

Figure 3 shows that the protective effect of verapamil on action potential shortening was also observed in endocardial cells when the cells were exposed to a higher concentration of CN⁻ (5 mM); 1 mM CN⁻ had little effect on endocardial action potentials, as previously reported.29,30

When verapamil was added after the action potential was maximally shortened by CN⁻, action potential duration began to lengthen, but it did not return to the control value (Figure 4). For comparison, we also examined the effect of glibenclamide, a specific blocker of K<sub>ATP</sub> current, on action potential duration after exposure to CN⁻. Glibenclamide (10 µM) also partially reversed action potential duration, but the effect was greater than that of verapamil. The difference may result from the blocking action of verapamil on the L-type Ca<sup>2+</sup> current, which probably shortened action potential duration.

**Effects of Verapamil on K<sub>ATP</sub> Currents**

To test the hypothesis that verapamil blocks K<sub>ATP</sub> current, we examined the direct effect of verapamil on K<sub>ATP</sub> channel activity using the inside-out membrane patch mode. Figure 5 shows a representative experiment demonstrating the effects of ATP and verapamil on the openings of K<sub>ATP</sub> channels in an inside-out patch obtained from an epicardial cell. When the membrane potential was depolarized to 50 mV positive to the reversal potential (0 mV) in the absence of ATP, the channels opened frequently (control). When 1 mM ATP
was applied to the internal face of the patch membrane (i.e., when ATP was added to the bath solution), channel openings were almost completely abolished. The channel openings reappeared after ATP was washed out. Subsequent intracellular application of 2 μM verapamil also inhibited channel openings. When verapamil was washed out, channel openings reappeared. Results similar to those shown in Figure 5 were confirmed in both endocardial and epicardial cells ($n=20$ for each). However, external application of verapamil (i.e., addition of the drug to the pipette solution) did not affect channel activity (data not shown).

Although verapamil reduced channel openings, it did not alter single-channel conductance. The top panel of Figure 6 shows segments of recordings that include openings and closings of a single channel (clusters of bursts) at different test potentials, and the bottom left panel of Figure 6 shows the amplitude histograms constructed from the data obtained at 60 mV positive to the reversal potential. The current amplitude at 60 mV was 2.55 pA in the absence of 2 μM verapamil and 2.49 pA in the presence of verapamil. The current–voltage relations obtained before and after application of verapamil are shown in the bottom right panel of Figure 6.

**Effect of Verapamil on Channel Kinetics**

To estimate the effect of verapamil on the kinetic properties of the $K_{ATP}$ channel, the open- and closed-time distributions were analyzed for recordings from patches containing only one functional $K_{ATP}$ channel. Figure 7 shows open- and closed-time distributions obtained from segments of recordings that included the openings and closings of the channel (clusters of bursts). Data were obtained at the membrane potential of 50 mV positive to the reversal potential and sampled at a 0.5-msec interval with a low-pass filter of 5 kHz. Both open- and closed-time distributions were fitted to a single exponential function. The time constants of open and closed times were 2.32 and 0.65 msec, respectively, in the absence of verapamil and 2.13 and 0.61 msec, respectively, in the presence of verapamil. No significant effects of verapamil on the channel kinetics within bursts were observed. Similarly, we constructed the histograms of the lifetime of bursts to examine the effect of verapamil on burst duration (Figure 8). Data were obtained from 60-second recordings with a low-pass filter of 0.5 kHz; the test potential was 50 mV positive to the reversal potential. The histograms were plotted using a 20-msec bin for open-time distribution and a

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**Figure 3.** Panel A: Recordings showing action potential changes in endocardial cells during superfusion with 5 mM CN⁻ in untreated and verapamil-treated groups. Panel B: Line graphs showing the time course of the change in action potential duration measured at 90% repolarization (APD90) after exposure to CN⁻. Relative changes from the control values (before exposure to CN⁻) are plotted in the right panel.
40-msec bin for closed-time distribution. Verapamil shortened the mean lifetime of bursts from 66 to 24 msec and prolonged the closed time between bursts from 43 to 79 msec. Mean open and closed times within bursts and lifetime of bursts obtained from six experiments are summarized in Table 1. Since verapamil shortened the mean lifetime of bursts and prolonged the closed time between bursts, it reduced the open-state probability of the KATP channel. Figure 9 shows the effect of 2 μM verapamil on open-state probability of the KATP channel calculated from 60-second recordings. Verapamil reduced the open-state probability from 0.31±0.04 to 0.03±0.01 (n=10, p<0.01).

The inhibitory effect of verapamil on KATP channel activity was concentration dependent. Figure 10 shows the concentration–response relation for inhibition of KATP by verapamil. The channel activity was expressed by normalizing the open-state probability to that found in drug-free solution. The concentration–response curve was fitted to the logistic equation

\[ y = \frac{1}{1 + (10^a/10^b)^3} \]  

where a is the concentration of verapamil that produces half-maximal inhibition of the channel, and b is the slope factor. The concentration of verapamil that produced half-maximal inhibition of the channel was 1.59 μM, and the slope factor was 1.50.

**Discussion**

The present study demonstrates that verapamil lessens action potential shortening during metabolic inhibition induced by CN⁻ and reduces KATP current in isolated inside-out patch membranes. It is concluded from these observations that the blocking action of verapamil on KATP channels may partly contribute to the verapamil-induced reduction in action potential shortening during ischemia and metabolic inhibition.

Recently, Haworth et al \(^\text{27}\) suggested that verapamil might block KATP currents, based on its block of rubidium flux in ATP-depleted rat ventricular cells. The present study, using cell-free inside-out membrane patches, directly demonstrates that verapamil inhibits the KATP channel. The characteristics of KATP currents in feline ventricular cells in the present study are similar to those previously reported by us \(^\text{30,31}\) and to those reported for other mammalian heart cells \(^\text{32-36}\). The possibility of channel rundown influencing the effects of verapamil was ruled out by confirming that the effect on channel activity was reversed by washout of the drug.

Our data and those of other investigators \(^\text{37}\) show that verapamil shortens action potential duration at the plateau level but prolongs late repolarization. The former action is through inhibition of the L-type Ca\(^{2+}\) currents, but the mechanisms of the latter action are unknown. Although the role of KATP channels under physiological conditions is unclear, KATP channel openers have been shown to shorten action potential duration under normal conditions \(^\text{38}\). Thus, prolongation of late repolarization by verapamil may result from the inhibition of KATP current. The suppressive effect of verapamil on KATP currents also partly explains its reduction of shortening of action potential duration during CN⁻-induced metabolic inhibition. Glibenclamide, a specific KATP channel blocker, reversed CN⁻-shortened action potentials; its effect was greater than that of verapamil. The lesser effect of verapamil may reflect its blockade of L-type Ca\(^{2+}\) current.

The precise mechanisms by which verapamil inhibits KATP currents are uncertain. Our data demonstrated
that verapamil did not change the single-channel conductance of the $K_{ATP}$ channel. It did not change the mean open and closed times within bursts. However, it did reduce the mean lifetime of bursts and prolonged the closed time between bursts. Therefore, verapamil reduced the open-state probability of the $K_{ATP}$ channel.

A recent study by Undrovinas et al.\(^\text{39}\) demonstrated that quinidine blocks $K_{ATP}$ currents in rat ventricular cells in a manner similar to that of verapamil observed in the present study. It is not unexpected that quinidine, a Na"+ channel blocker, and verapamil, a Ca"+ channel blocker, influence the function of the $K_{ATP}$ channel, since Na"+, Ca"+, and K"+ channels share partial homologous features\(^\text{40}\) and most antiarrhythmic drugs are not specific for single-channel types.\(^\text{39}\)

The exact site of action of verapamil is uncertain. We found that $K_{ATP}$ currents were inhibited by intracellular application of verapamil but not by extracellular application (addition of verapamil to the pipette solution), suggesting that the binding site of verapamil is inside the cell membrane. When verapamil is added to the pipette solution, it may penetrate the patch membrane and reach the intracellular binding site of the $K_{ATP}$ channel, but it may be diluted by the large amount of the bath solution. A dual–binding site model responsive to the ATP/ADP ratio has been proposed for the cardiac $K_{ATP}$ channel.\(^\text{25,41,42}\) $K_{ATP}$ channel openers (e.g., pinacidil) and closers (e.g., glibenclamide) apparently compete with ATP at these sites. Verapamil blocks cardiac Ca"+ channels probably by binding to $\alpha_1$ subunits.\(^\text{43}\) It is unknown whether the drug acts at a similar location in the $K_{ATP}$ channel.

The mechanisms by which action potential duration is shortened and K"+ efflux is increased during myocardial ischemia have been the subject of extensive prior studies. Conventional voltage-clamp studies have revealed that hypoxia increases the background outward K"+ current.\(^\text{44}\) Subsequently, Noma\(^\text{18}\) discovered large K"+ currents that are sensitive to intracellular ATP concentration in guinea pig cardiac cells; this current is activated when intracellular ATP is depleted. Since discovery of this current, much attention has been focused on the role of the $K_{ATP}$ current in action potential shortening and increase in K"+ efflux during ischemia and metabolic inhibition. Although it is argued that half-maximal inhibition of $K_{ATP}$ channel by ATP occurs in the micromolar range in inside-out patches, while the measured ATP concentration at the time of action potential shortening is still at, or near, normal levels (millimolar range), evidence has been accumulated that indicates the involvement of $K_{ATP}$ currents in action potential shortening during ischemia and metabolic inhibition in

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**Figure 6.** Top panel: ATP-regulated K"+ current recordings at test potentials of 80, 40, -40, and -80 mV in the absence (control) and presence of verapamil (2 $\mu$M). Bottom panel: Amplitude histograms (left) and the current–voltage relation (right) in the absence and presence of verapamil.
intact cells. This evidence includes the following: 1) The concentration of ATP to block $K_{\text{ATP}}$ currents is much higher in open-cell attached patches (a more physiological condition) than in isolated inside-out membrane patches.\textsuperscript{33,45} 2) The density of $K_{\text{ATP}}$ channels in the cardiac membrane is high.\textsuperscript{34,45} Therefore, the effects of channel opening on the action potential might occur with the activation of only a few channels. 3) Activation of $K_{\text{ATP}}$ channels is modulated by various metabolites such as ADP and $H^+$.\textsuperscript{31,41} Regarding the latter, we recently demonstrated that acidosis increases the open-state probability of $K_{\text{ATP}}$ channels,\textsuperscript{34} indicating that $K_{\text{ATP}}$ channels may be activated by a slight fall in intracellular ATP concentration together with a rise in intracellular $H^+$. In addition, action potential shortening during metabolic inhibition is reversed by glibenclamide or tolbutamide, blockers of $K_{\text{ATP}}$ channels.\textsuperscript{29} Thus, it is quite likely that activation of the $K_{\text{ATP}}$ channel contributes to the shortening of action potential duration and to the increase in $K^+$ efflux during ischemia and metabolic inhibition. Verapamil has been demonstrated to have various effects on ischemic myocardium, including reductions in electrophysiological disturbances,\textsuperscript{9–15} decreased size of experimental infarction,\textsuperscript{2} decreased accumulation of extracellular $K^+$ and $H^+$,\textsuperscript{16} and preservation of high-energy phosphates.\textsuperscript{6–8} These effects have been ascribed to the blocking action of verapamil on $Ca^{2+}$ entry, which may reduce energy depletion by coronary vasodilation and negative inotropic effect. Previous investigators,\textsuperscript{9–12} including us,\textsuperscript{13,17} have suggested that the mechanism for the salutary action of verapamil on electrophysiological disturbances during ischemia is its preservation of ATP stores by blocking $Ca^{2+}$ entry through the L-type $Ca^{2+}$ channels. This mechanism may be involved in the prevention of action potential shortening by verapamil during metabolic inhibition observed in the present study. However, our data provide evidence for another mechanism by which verapamil may exert beneficial effects on ischemic myocardial cells; it may reduce dispersion of refractoriness by lessening action potential shortening through blocking the $K_{\text{ATP}}$ channel, thereby preventing arrhythmias during ischemia.

**TABLE 1.** Time Constants of Open and Closed Times Before and After Exposure to $2 \times 10^{-6}$ M Verapamil

<table>
<thead>
<tr>
<th></th>
<th>Control (msec)</th>
<th>Verapamil (msec)</th>
</tr>
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<tbody>
<tr>
<td>Mean open time within bursts</td>
<td>1.92±0.18</td>
<td>1.82±0.21</td>
</tr>
<tr>
<td>Mean closed time within bursts</td>
<td>0.68±0.12</td>
<td>0.60±0.14</td>
</tr>
<tr>
<td>Mean lifetime of bursts</td>
<td>41.1±3.5</td>
<td>24.9±2.8*</td>
</tr>
<tr>
<td>Mean closed time between bursts</td>
<td>39.4±4.6</td>
<td>78.5±5.1*</td>
</tr>
</tbody>
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Control, absence of verapamil. Values are mean±SEM. *p<0.01 vs. control.
FIGURE 9. Line graph showing open-state probability calculated in the absence of verapamil (control), in the presence of verapamil, and after washout of verapamil (n=10).

References

FIGURE 10. Concentration–response curve showing the inhibition of ATP-regulated K+ channel activity by verapamil. The channel activity was expressed by normalizing the open-state probability to that in drug-free solution. The concentration of verapamil that produced half-maximal inhibition of the channel was 1.59 μM.

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Verapamil diminishes action potential changes during metabolic inhibition by blocking ATP-regulated potassium currents.
S Kimura, A L Bassett, H Xi and R J Myerburg

_Circ Res._ 1992;71:87-95
doi: 10.1161/01.RES.71.1.87

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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