Intracellular Divalent Cations Block Smooth Muscle K⁺ Channels

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The patch-clamp technique was used to examine the sensitivity of delayed rectifier K⁺ channels to changes in intracellular divalent cations (Mg²⁺ and Ca²⁺). During voltage-step and ramp depolarizations, a delayed rectifier K⁺ current (Iₖ,dec) was identified in renal, pulmonary, coronary, and colonic smooth muscle cells as a low-noise outward current that activated near −40 mV, was sensitive to 4-aminopyridine (4-AP), and was insensitive to charybdotoxin. During whole-cell voltage-clamp experiments in each of the cell types, the 4-AP-sensitive Iₖ,dec was significantly less in cells dialyzed with 10 mM Mg²⁺ as compared with cells in which no Mg²⁺ was added to the internal dialysis solution (P ≤ 0.05, n ≥ 4). In coronary artery cells, 100 μM 2,3-(aminomethyl)pyridine (an H₁ receptor agonist) or 10 μM ryanodine, agents that cause an increase in [Ca²⁺], also caused a significant reduction of the 4-AP-sensitive Iₖ,dec, similar to that produced by Mg²⁺. 4-AP (5 mM) significantly depolarized single renal arterial cells that were dialyzed with Mg²⁺-free solution but not those dialyzed with 10 mM Mg²⁺ (P < 0.01, n = 4). In outside-out patches of renal arterial smooth muscle cells, with 200 mM charybdotoxin in the patch pipette to block large conductance Ca²⁺-activated K⁺ channels, a 59 ± 10-picosiemens K⁺ channel that was sensitive to cytoplasmic Mg²⁺ was identified. In Mg²⁺-free solution, channel open probability was 0.028 ± 0.012 (n = 8) and 0.095 ± 0.011 (n = 8) at +40 and +80 mV, respectively. When the bath solution was changed to one containing 5 or 15 mM Mg²⁺, channel open probability was significantly reduced by 66% and 68% (+40 mV) or 93% and 96% (+80 mV), respectively. This decrease in the open probability of the delayed rectifier K⁺ channel resulted from a concentration- and voltage-dependent decrease in mean open time. At +40 mV, time constants for the open time distribution were significantly decreased from 5.5 ± 0.52 to 1.2 ± 0.14 milliseconds, whereas the closed time constant was significantly increased from 634 ± 11.1 to 829 ± 14.4 milliseconds (P < 0.01, n = 4). It is concluded that a 4-AP-sensitive delayed rectifier K⁺ channel in both vascular and visceral smooth muscle cells is modulated by changes in intracellular Ca²⁺ and Mg²⁺ that may alter membrane potential and the contractile state of smooth muscle. (Circulation Research 1993;73:24-34)

Key Words • Mg²⁺ • Ca²⁺ • renal artery • pulmonary artery • coronary artery • colon • delayed rectifier K⁺ current

The importance of Ca²⁺ and Mg²⁺ as regulators of vascular tone is well established. An increase in [Ca²⁺] not only initiates contraction but also initiates many other biochemical events necessary for excitation-contraction coupling of smooth muscle. An increase in [Mg²⁺] enhances enzymatic activity responsible for signal transduction and regulates bioenergetics and ion transport. In vascular smooth muscle cells, it has been recently demonstrated that during agonist stimulation there is not only an increase in [Ca²⁺], but also an increase in [Mg²⁺]. Thus, increases in both [Ca²⁺] and [Mg²⁺] may play a significant role in agonist-induced contraction and modulation of resting membrane potential.

Despite increasing evidence that links changes in membrane potential directly to changes in smooth muscle tone, there is a paucity of information on the nature of the ionic conductances responsible for the resting membrane potential in most types of smooth muscle. Various types of K⁺ channels in a number of different preparations are inhibited by intracellular divalent cations. One example of intracellular divalent cation inhibition is the property of inward rectification. Inward rectification is believed to be due to block of outward movement of K⁺ through K⁺ channels by intracellular divalent6-8 and monovalent9,10 cations by a mechanism that involves membrane depolarization driving the blocking ion into the open channel pore. Inward rectification has been described in heart,5,7 egg cells,11-13 skeletal muscle,14 vascular endothelial cells,15 and neurons16 and is believed to play a role in regulation of resting membrane potential and action potential repolarization.14 Although in smooth muscle many K⁺ channels exhibit the property of outward rectification, only a few reports of inward rectification have appeared.17-19

In the present study, we have investigated the actions of intracellular divalent cations on delayed rectifier K⁺ channels in several different types of vascular and visceral smooth muscles. Our results suggest that 4-aminopyridine (4-AP)-sensitive delayed rectifier K⁺ channels in each of these cells are inhibited by physiological concentrations of intracellular divalent cations. These

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results suggest the existence of a novel feedback pathway in smooth muscle by which intracellular divalent cations may regulate sarcloemmal ionic permeability. This pathway may play a significant role in agonist-induced contractions, in vascular tone, and in the etiology of certain pathophysiological disorders of smooth muscle cells.

**Materials and Methods**

**Electrophysiological Measurements**

Single smooth muscle cells from canine renal and pulmonary artery, colon, and rabbit coronary artery were enzymatically dissociated using previously described methods.²⁻³ Single cells were voltage-clamped, and membrane currents were measured using the whole-cell or inside-out configurations of the patch-clamp technique.²⁴ Patch pipettes for whole-cell and inside-out patch-clamp recordings were made from borosilicate glass capillaries and had resistances of 1 to 3 and 7 to 10 MΩ, respectively, when filled with the appropriate solutions. Voltage-clamp command potentials, either step or ramp depolarizations (from −80 to 80 mV for 4 seconds or from −80 to 40 mV for 2 or 4 seconds, respectively) were applied to the cells, and membrane currents were recorded using an Axopatch-1D patch clamp amplifier. Membrane current was monitored on a digital oscilloscope, digitized on-line, and stored on an IBM-AT computer. Whole-cell currents were digitized (2048 points) at either 2.0 (600-millisecond steps), 1.0 (5-second steps), or 0.5 (ramps) kHz and filtered at 0.5 kHz. Single-channel currents were digitized at 10 kHz and filtered at 1.0 kHz. Resting membrane potentials were measured in the I=0 position of the patch-clamp amplifier. Data analysis was performed with pCLAMP 5.5.1 or iPROC software (Axon Instruments, Burlingame, Calif). Values for channel open probability were obtained from 1-minute recordings of data. All experiments were performed at room temperature except those in coronary arterial smooth muscle cells (at 35°C).

**Solutions**

For all whole-cell voltage-clamp experiments examining outward currents, the bath solution contained (mM) NaCl, 130; NaHCO₃, 10; KCl, 4.2; KH₂PO₄, 1.2; MgCl₂, 0.5; CaCl₂, 1.5; D-glucose, 5.5; and HEPES, 10 (pH 7.4 with NaOH). The pipette solution for the whole-cell experiments contained (mM) potassium aspartate, 120; KCl, 20; MgCl₂, 0 or 10; EGTA, 0.1; ATP (magnesium or potassium salt), 5; and HEPES, 5 (pH 7.2 with KOH). When Mg²⁺ was omitted from the patch pipette solution, 10 mM BAPTA was substituted for the EGTA. For inside-out single-channel recordings, the bath solution contained (mM) KCl, 140; MgCl₂, 0, 5, or 15;

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**Fig 1.** Representative current-voltage relations for K⁺ current recorded from renal, coronary, pulmonary, and colonic smooth muscle cells. Panel A: Effects of 4-aminopyridine (4-AP, 5 mM) on outward current in renal smooth muscle cells during ramp or step depolarizations. Holding potential was −80 mV; voltage steps were applied in 20-mV steps from −20 to +80 mV. Scale bar: vertical, 750 pA; horizontal, 100 milliseconds. Panels B through D: Current-voltage relations for K⁺ current elicited by 4-second voltage ramps in the absence and presence of 4-AP (5 mM). Note the similarity in whole-cell current and 4-AP block in all four types of smooth muscle cells.
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Separation of Delayed Rectifier K⁺ Current and Ca²⁺-Activated K⁺ Current

Previous voltage-clamp studies in renal, pulmonary, coronary, and colonic smooth muscle cells demonstrated at least two components of macroscopic outward K⁺ current.²⁰⁻²²,²⁵ The separation of the two components of current was based on pharmacological and biophysical characteristics. In general, smooth muscle delayed rectifier K⁺ channels are sensitive to 4-AP and relatively insensitive to tetraethylammonium (TEA) and charybotoxin, whereas large conductance Ca²⁺-activated K⁺ channels are highly sensitive to TEA and charybotoxin and insensitive to 4-AP.²⁰⁻²⁶,²⁷ The delayed rectifier K⁺ current in our preparations (I_{K(d)}⁰) activates between -40 and -30 mV, is relatively small in amplitude, and displays voltage- and time-dependent activation.²⁰⁻²² I_{K(d)}⁰ is also 4-AP sensitive and relatively TEA (Kᵢ ≥ 1 mM) and charybotoxin insensitive.²⁰ The Ca²⁺-activated K⁺ current (I_{K(Ca)⁰}) activates at more positive potentials, is noisy and of large amplitude, exhibits voltage and time dependence, and is charybotoxin and TEA (Kᵢ = 250 μM) sensitive and 4-AP insensitive.²⁰⁻²² I_{K(Ca)⁰} and I_{K(Ca)} are carried by single channels with conductances between 51 and 63 and between 113 and 147 pico siemens, respectively, in a physiological K⁺ gradient and between 53 and 130 and between 215 and 232 pico siemens, respectively, in symmetrical 140 mM K⁺.²⁰⁻²²,²⁸ In cell-attached single-channel experiments, the renal artery large-conductance Ca²⁺-activated K⁺ channel was sensitive to 250 μM TEA and insensitive to 4-AP (5 mM).²⁰ Delayed rectifier K⁺ channels were inhibited by 4-AP (5 mM) but were insensitive to 250 μM TEA.²⁰

Statistics

Results are expressed as mean±SEM. Statistical significance was evaluated using Student’s t test for unpaired observations. Differences were considered significant at P<.05; n corresponds to the number of cells examined. Membrane currents were measured from the zero current level. Outward current measurements, which reflect an average of the sustained outward current, were normalized to cell capacitance, which was calculated assuming a specific capacitance of 1 μF/cm².

Results

Macroscopic K⁺ Currents in Different Isolated Smooth Muscle Cells

Fig 1 illustrates representative whole-cell currents measured from renal, coronary, pulmonary, and colonic smooth muscle cells. Panel A illustrates the effect of 4-AP (5 mM) on whole-cell currents in canine renal arterial smooth muscle cells. During both voltage-step (inset) and ramp depolarizations, 4-AP significantly inhibits I_{K(d)}. The noisy component of the outward current that represents I_{K(Ca)} is similar after drug application, suggesting that 4-AP has no significant effect on I_{K(Ca)}. Panels B through D illustrate whole-cell current tracings elicited by similar voltage-ramp protocols in three other smooth muscle cell preparations: rabbit coronary, canine pulmonary, and colonic cells. In each preparation both I_{K(d)} and I_{K(Ca)} were evident, illustrating the similarity that exists
between the macroscopic outward currents in four distinctively different smooth muscle cell preparations. In each type of cell, 4-AP significantly inhibited $I_{K(\text{Ca})}$, with little noticeable effects on $I_{K(\text{Na})}$. This selective effect of 4-AP on $I_{K(\text{Ca})}$ while having little effect on $I_{K(\text{Ca})}$ is consistent with results obtained in other non-smooth muscle cell preparations. Although the selective block of $I_{K(\text{Na})}$ by 4-AP may not be complete, it can still be used as a pharmacological tool to investigate the effects of intracellular divalent cations on $I_{K(\text{Ca})}$. In some cases, however, the measurements of 4-AP-sensitive current may be underestimates of the total $I_{K(\text{Ca})}$, or the remaining current may reflect a 4-AP-insensitive current that activates with a voltage dependence similar to that of $I_{K(\text{Ca})}$.

**Intracellular Mg$^{2+}$ Blocks Delayed Rectifier $K^+$ Current**

On the basis of the pharmacological sensitivity of $I_{K(\text{Ca})}$ to 4-AP and its lack of effect on $I_{K(\text{Ca})}$, we used 4-AP as a pharmacological tool to isolate $I_{K(\text{Ca})}$ in whole-cell voltage-clamp experiments. When the concentration of Mg$^{2+}$ was varied in the dialyzing whole-cell patch pipette, a dramatic effect on $I_{K(\text{Ca})}$ was observed. Fig 2 illustrates the effects of varying [Mg$^{2+}$] on whole-cell currents in canine renal and colonic smooth muscle cells elicited by voltage-ramp protocols applied from −80 to +40 mV. When Mg$^{2+}$ was omitted from the intracellular dialysis solution (5 mM K$_2$-ATP), $I_{K(\text{Ca})}$ was robust and inhibited by 5 mM 4-AP (panels A and B). The 4-AP-sensitive difference current for both cell types is illustrated at the bottom of panels A and B. When the intracellular dialysis solution contained 10 mM Mg$^{2+}$ (5 mM Mg$^{2+}$-ATP, panels C and D), very little 4-AP-sensitive $K^+$ current was observed. The difference current under these conditions was close to the zero current level (bottom of panels C and D). Mean current-voltage relations for the 4-AP-sensitive difference current for renal and colonic smooth muscle cells under these conditions are shown in Fig 3. At potentials between −30 and +15 mV, where $I_{K(\text{Ca})}$ is predominantly active, a significantly larger 4-AP-sensitive $K^+$ current was observed in both renal (panel A) and colonic (panel B) cells when Mg$^{2+}$ was omitted from the dialysis solution, as compared with cells dialyzed with a solution containing 10 mM Mg$^{2+}$ (n=4). In Fig 4, similar effects of [Mg$^{2+}$] on $I_{K(\text{Ca})}$ were observed in coronary (panel A) and pulmonary (panel B) arterial smooth muscle cells. The mean current-voltage relations for the effects of 0 and 10 mM Mg$^{2+}$ on $I_{K(\text{Ca})}$ are shown in Fig 5. In renal (panel A), coronary (panel B), pulmonary (panel C), and colonic (panel D) smooth muscle cells at potentials more positive than −15 mV, significantly less $I_{K(\text{Ca})}$ was observed in cells dialyzed with 10 mM intracellular Mg$^{2+}$ compared with cells in which Mg$^{2+}$ was omitted from the dialysis solution. These results demonstrate that changes in [Mg$^{2+}$] can alter the activity of $I_{K(\text{Ca})}$ in both vascular and visceral smooth muscle cells.

**Intracellular Ca$^{2+}$ Blocks Delayed Rectifier $K^+$ Current**

To test whether changes in [Ca$^{2+}$], might exert an inhibitory effect similar to that produced by Mg$^{2+}$ on $I_{K(\text{Ca})}$, the effects of 2-(2-aminoethyl)pyridine (AEP) and ryanodine, agents that release intracellular Ca$^{2+}$ in smooth muscle via different mechanisms, were examined in isolated coronary smooth muscle cells. AEP, a histamine H$_1$ receptor agonist, causes an increase in inositol-1,4,5-trisphosphate (IP$_3$), which releases Ca$^{2+}$ from the sarcoplasmic reticulum, whereas ryanodine releases intracellular Ca$^{2+}$ in vascular smooth muscle by locking the Ca$^{2+}$ release channel in the open state. Figs 6A and 6B show the effects of AEP (100 μM) and ryanodine (10 μM) on $I_{K(\text{Ca})}$. These experiments were performed in the presence of low internal Mg$^{2+}$ (0.5 mM Mg$^{2+}$) and 1 mM TEA to inhibit $I_{K(\text{Ca})}$. During control conditions, $I_{K(\text{Ca})}$ activated between −40 and −20 mV. Absent from the control recordings was the large noisy $I_{K(\text{Ca})}$ that was present in Fig 1B. Application of AEP or ryanodine caused a significant reduction in $I_{K(\text{Ca})}$. The AEP- and ryanodine-sensitive currents (Fig 6) were very similar to one another, and they closely resemble the 4-AP-sensitive currents observed in renal and colonic smooth muscle cells (Fig 2). Moreover, the
**FIG 4.** Effect of intracellular Mg\(^{2+}\) on the delayed rectifier K\(^{+}\) current (I\(_{K(d)}\)) recorded from coronary and pulmonary artery cells. 
Panel A: With both step and ramp depolarizations, 10 mM intracellular Mg\(^{2+}\) significantly inhibits I\(_{K(d)}\) as compared with 0 mM Mg\(^{2+}\). Holding potential was −60 mV; voltage steps were applied in 20-mV steps from −20 to +60 mV. Panel B: Similar results are obtained with Mg\(^{2+}\) in pulmonary artery cells. Holding potential was −70 mV; voltage steps were applied in 15-mV steps from −25 to +80 mV.

**FIG 5.** Graphs showing the mean current-voltage relation for the delayed rectifier K\(^{+}\) current (I\(_{K(d)}\)) in the presence of 0 and 10 mM Mg\(^{2+}\) in renal (panel A), coronary (panel B), pulmonary (panel C), and colonic (panel D) smooth muscle cells. As shown in all panels, 10 mM Mg\(^{2+}\) (△) significantly reduced I\(_{K(d)}\) when compared with 0 mM Mg\(^{2+}\) (●) (*P<.05, **P<.01, n≥4).
inhibition of $I_{K_{(d)}}$ by AEP is prevented by pretreatment of cells with 4-AP. To test if the inhibitory effects of AEP may be the result of a direct action on $I_{K_{(d)}}$, we examined the effects of AEP in the absence of TEA in cells dialyzed with an intracellular solution containing the Ca$^{2+}$ chelator BAPTA (15 mM). In the presence of internal BAPTA, AEP caused no significant inhibition of $I_{K_{(d)}}$, nor was there any increase of $I_{K_{(d)}}$, which acts as a "sensor" for increases in intracellular Ca$^{2+}$ (Fig 6C). These data suggest that the ability of AEP to

Fig 7. Effect of 4-aminopyridine (4-AP) on resting membrane potential recorded from renal arterial cells dialyzed with either 0 or 10 mM Mg$^{2+}$. Panel A: In a cell dialyzed with no Mg$^{2+}$, application of 4-AP (5 mM) depolarized this cell from $-50$ to $-34$ mV. The delay in depolarization was due to the slow wash in and equilibration of 4-AP. Panel B: In a cell dialyzed with 10 mM Mg$^{2+}$, 4-AP had no effect on resting membrane potential ($-33$ mV).

inhibit $I_{K_{(d)}}$ can be attributed to an elevation of [Ca$^{2+}$]. Thus, changes in either intracellular Mg$^{2+}$ or Ca$^{2+}$ may regulate the activity of $I_{K_{(d)}}$.

**Effect of 4-AP and Intracellular Mg$^{2+}$ on Membrane Potential**

It has been demonstrated that, in vascular smooth muscle cells, [Mg$^{2+}$] is increased from 0.5 mM to approximately 3.0 mM during agonist stimulation. To examine if Mg$^{2+}$ inhibition of $I_{K_{(d)}}$ might possibly contribute to agonist-induced changes in resting membrane potential, we investigated the effects of 4-AP and [Mg$^{2+}$] on resting membrane potential of canine renal arterial smooth muscle cells. In an earlier study, the resting membrane potential of isolated canine renal arterial cells ranged between $-35$ and $-70$ mV with an average value of $-51.8\pm2.1$ mV. Fig 7 shows the effects of 4-AP (5 mM) on resting membrane potential in cells that were dialyzed with either Mg$^{2+}$-free or 10 mM Mg$^{2+}$-containing solution. In panel A, the resting membrane potential is shown for a cell that was dialyzed with Mg$^{2+}$-free solution. On application of 4-AP, the cell depolarized from $-50$ to $-34$ mV. In a number of cells dialyzed with Mg$^{2+}$-free solution, 4-AP (5 mM) caused a significant depolarization of the resting membrane potential ($21.5\pm5.3$ mV, $P<.05$, n=4). In panel B, the resting membrane potential is shown for a cell dialyzed with 10 mM Mg$^{2+}$. The resting membrane potential in this cell ($-33$ mV) did not change in the presence of 4-AP. In four cells dialyzed with solutions containing 10 mM Mg$^{2+}$, a small but statistically insignificant depolarization ($6.5\pm11.6$ mV, n=4) was produced by 4-AP. These data suggest that $I_{K_{(d)}}$ and its modulation by [Mg$^{2+}$] may contribute to changes in the resting membrane potential of isolated smooth muscle cells.

**Mg$^{2+}$ Blocks Single Delayed Rectifier K$^+$ Channels**

We next examined the effects of Mg$^{2+}$ on the single-channel currents responsible for $I_{K_{(d)}}$. We have previously shown that the conductance of 4-AP-sensitive
FIG 8. Recordings showing the effects of Mg$^{2+}$ on delayed rectifier $K^+$ channels in inside-out patches of renal arterial smooth muscle cells. Charybdotoxin (200 nM) was in the patch pipette. Holding potential was +60 mV. All single-channel openings are upward deflections of current. Panel A: In the presence of 0 Mg$^{2+}$, long openings of delayed rectifier $K^+$ channels (>750 milliseconds) and large-conductance Ca$^{2+}$-activated $K^+$ channels were recorded. Open probability of the delayed rectifier $K^+$ channel was 0.78. On the application of 15 mM Mg$^{2+}$ to the bath, the delayed rectifier $K^+$ channels were inhibited (open probability, 0.05) while the large-conductance Ca$^{2+}$-activated $K^+$ channels were activated. Panel B: This patch contained only delayed rectifier $K^+$ channels with short openings (<50 milliseconds). Open probability in 0 Mg$^{2+}$ was 0.06. Application of 15 mM Mg$^{2+}$ decreased channel open probability to 0.004.

delayed rectifier $K^+$ channels in renal arterial cells is 57±6 picoSiemens, whereas the Ca$^{2+}$-activated $K^+$ (BK) channels have a conductance of 130±17 picoSiemens. Inside-out patches from both vascular and visceral smooth muscle cells were studied so that Mg$^{2+}$ could be directly applied to the cytoplasmic surface of the patches. In these experiments, the pipette contained 200 nM charybdotoxin to reduce the activity of large-conductance BK channels. Fig 8 shows single-channel currents recorded from two inside-out patches from a renal arterial smooth muscle cell at a holding potential of +60 mV. Both types of channel activity were observed in some patches (panel A) despite the presence of charybdotoxin, whereas in other membrane patches, only the smaller conductance delayed rectifier channel openings were observed (panel B). Typically, in Mg$^{2+}$-free solution, delayed rectifier channel openings were either of long duration with a mean open time greater than 750 milliseconds (panel A, small conductance channel) or of much shorter duration with a mean open time of less than 50 milliseconds (panel B). The shorter duration openings were the most frequently observed. When 15 mM Mg$^{2+}$ was washed into the bath, channel open probability significantly decreased with no noticeable change in single-channel conductance (Fig 8). Consistent with previous reports, Mg$^{2+}$ increased the open probability of BK channels in some patches.33,34

Fig 9 shows the current-voltage relation for the 4-AP- and Mg$^{2+}$-sensitive delayed rectifier $K^+$ channel (panel A) and summarizes the effects of Mg$^{2+}$ on channel open probability (panel B) and mean open times (panel C). The single-channel slope conductance of the delayed rectifier $K^+$ channels in Fig 8B was 66 picoSiemens, and the mean slope conductance was 59±10 picoSiemens (n=8). At potentials of +20, +40, +60, and +80 mV, 5 and 15 mM Mg$^{2+}$ significantly decreased channel open probability in a concentration-dependent manner (Fig 9B, n=4). The Mg$^{2+}$-dependent block was caused by a decrease in mean open time of delayed rectifier $K^+$ channels. At potentials of +40, +60, and +80 mV, 15 mM Mg$^{2+}$ significantly decreased mean open time 43%, 72%, and 84%, respectively (Fig 9C, n=4). Similar results were observed when delayed rectifier $K^+$ channels were recorded from inside-out patches of colonic smooth muscle cell membranes (n=4, data not shown). These data are consistent with a voltage-dependent blocking mechanism in which Mg$^{2+}$ is driven into the channel pore by depolarization, since the driving force for outward movement of Mg$^{2+}$ is increased with depolarization.

At +40 mV with 15 mM internal Mg$^{2+}$, the open probability of delayed rectifier $K^+$ channels is near 0 (Fig 9B); however, the mean open time of the channel is approximately 2 milliseconds (Fig 9C). A simple mechanism that involves changes in open time distribution
and closed time distributions of the delayed rectifier K⁺ channel.

**Discussion**

We have described the effects of changes in internal divalent cation concentration on delayed rectifier K⁺ channels in four distinctively different smooth muscle cell preparations. In each type of cell, increasing [Mg²⁺] significantly reduced the magnitude of whole-cell I_{K(d)} AEP and ryanodine, drugs that release intracellular Ca²⁺, also inhibited I_{K(d)} in coronary arterial cells. Since the activation range of I_{K(d)} is close to the average resting membrane potential of smooth muscle cells, these results suggest that divalent cations may play a role in regulating membrane potential. This hypothesis was directly examined by testing the effects of 4-AP on resting membrane potential in the presence or absence of internal Mg²⁺. 4-AP significantly depolarized canine renal arterial cells when Mg²⁺ was omitted from the dialyzing pipette, while exerting little effect on membrane potential when I_{K(d)} was inhibited by 10 mM internal Mg²⁺. Thus, I_{K(d)} may play a role in the regulation of a smooth muscle cell's resting membrane potential. These results suggest a novel pathway that may be involved in the regulation of membrane potential both at rest and during agonist-induced contraction of smooth muscle.

In smooth muscle, agonists such as norepinephrine, angiotensin II, and AEP activate phospholipase C, initiating a rapid breakdown of membrane phosphoinositides. One of the earliest measurable biochemical events resulting from the hormone binding to its receptor is the phospholipase C-mediated hydrolysis of phosphatidylinositol 4,5-bisphosphate to yield IP₃ and diacylglycerol. IP₃ has been demonstrated to cause Ca²⁺ release from nonmitochondrial intracellular sites, presumably the sarcoplasmic reticulum; diacylglycerol has been shown to activate protein kinase C. Both the release of intracellularly stored Ca²⁺ and the activation of protein kinase C play important roles in the initiation and regulation of vasocostrictor responses in smooth muscle. Although the present study has emphasized the inhibitory effects of intracellular Mg²⁺ on I_{K(d)}, our results suggest that intracellular Ca²⁺ produces similar effects on I_{K(d)}, since this current was reduced by maneuvers that release intracellular Ca²⁺ (ie, ryanodine and H⁺ receptor stimulation). The inhibition of I_{K(d)} by H⁺ receptor stimulation was not observed in cells dialyzed with the Ca²⁺ chelator BAPTA. Since intracellular Ca²⁺ appears to have opposite effects on I_{K(d)} and I_{K(Ca)}, one would predict that agonists releasing intracellular Ca²⁺ may have complex voltage-dependent effects on outward current similar to those observed with H⁺ receptor stimulation of outward currents in rabbit coronary artery cells.

Electrically, the binding of an agonist may cause (1) an increase in open probability of voltage-dependent Ca²⁺ channel, (2) activation of an inward nonselective cation current, or (3) activation of a Cl⁻ current. All of these effects would tend to depolarize the smooth muscle cell and initiate or maintain contraction. The inhibitory effects of Ca²⁺ and Mg²⁺ on delayed rectifier K⁺ channels demonstrated here may represent another mechanism by which contraction is modulated. It should be noted that this pathway would also tend to oppose...
the membrane hyperpolarization associated with a rise in $\left[\text{Ca}^{2+}\right]_i$ due to activation of BK channels. 41

Since agonist activation of vascular smooth muscle cells is expected to cause an increase in intracellular $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$, our data suggest that this would produce significant inhibition of $I_{\text{K(Ca)}}$, resulting in depolarization, the activation of voltage-dependent $\text{Ca}^{2+}$ channels, and an increase in vascular tone. Then as the cell's homeostatic mechanisms allow $\text{Ca}^{2+}$ and $\text{Mg}^{2+}$ to return to their resting levels, $I_{\text{K(Ca)}}$ would reactivate, driving the membrane potential of the cell close to its resting level and reducing the contractile state of the vascular smooth muscle. Not only would this scheme benefit tonic smooth muscle, it could also modulate a phasic smooth muscle like the colon. For example, the upstroke component of the colonic slow wave is believed to be due to an increase in the permeability of the membrane to $\text{Ca}^{2+}$. $\text{Ca}^{2+}$-dependent inhibition of $I_{\text{K(Ca)}}$ may augment the depolarization and contribute to the development of the plateau phase of the slow wave.

There are many reports of divalent ions regulating the activity of $K^+$ channels in a number of different preparations. For example, internal $\text{Mg}^{2+}$, $\text{Ca}^{2+}$, and $\text{Na}^+$ have been described to cause the property of rectification observed in many cell types. 6, 10, 12-16 In smooth muscle, the BK channel that underlies $I_{\text{K(Ca)}}$ is inhibited by increasing intracellular $\text{Ba}^{2+}$ or $\text{Na}^+$. 42, 43 $\text{Ba}^{2+}$ block of BK channels has been attributed to the $\text{Ba}^{2+}$ ion's being trapped in the channel pore, thus reducing this channel’s long open state. 40 This causes a decrease in open probability and mean open time while having no effect on single-channel conductance. Block of renal arterial and colonic delayed rectifier $K^+$ channels by $\text{Mg}^{2+}$ is also due to a decrease in mean open time and open probability, which is similar to the $\text{Ba}^{2+}$ block of BK channels. Although some change in surface charge is expected when using the concentrations of internal $\text{Mg}^{2+}$ tested in our experiments, such effects cannot account for the block of $I_{\text{K(Ca)}}$, since such a mechanism is inconsistent with the observed voltage-dependent decrease in mean open time observed (Fig 9). It is also noteworthy that changes in internal $\text{Mg}^{2+}$ of this magnitude in squid axon produce insignificant shifts in activation and inactivation of $I_{\text{K(Ca)}}$. 44 $\text{Mg}^{2+}$ block of smooth muscle delayed rectifier $K^+$ channels is quite different from that of the inward rectifier of heart, egg cells, skeletal muscle, endothelial cells, or the neuronal A current, 5-16 which is caused when the divalent ion causes a flicker block of the single channel and thereby decreases the amount of current flow. 45

The mechanism of block of $I_{\text{K(Ca)}}$ by $\text{Mg}^{2+}$ in our experiments could potentially involve a phosphorylation-dependent effect of $\text{Mg}^{2+}$-ATP, a phosphorylation-independent effect of $\text{Mg}^{2+}$-ATP, 46 or it could be due to a direct effect of $\text{Mg}^{2+}$ on the channel. There is currently little information available concerning regulation of delayed rectifier $K^+$ channels in smooth muscle by any of these three potential mechanisms. Our whole-cell
experiments cannot rule out the possibility that Mg^{2+} is involved in inhibition of I_{K_{Ca}} and might involve a phosphorylation-dependent or independent mechanism. However, the fact that the effects of Mg^{2+} on single delayed rectifier K^+ channels in inside-out membrane patches were observed in the absence of cytoplasmic ATP argues that the inhibition is caused by a direct effect of Mg^{2+}. This is also more consistent with the observation that Ca^{2+} also is able to produce inhibition of these channels. Future studies are required to reveal the exact nature of the inhibitory mechanism involved in divergent cation block.

Based on the results with agents that release intracellular Ca^{2+} and Mg^{2+}, our data suggest that modulation of I_{K_{Ca}} activity may modulate K^+ channel activity near the resting membrane potential and thus influence the contractile state of smooth muscle cells. If the smooth muscle is vascular in origin, this could be physiologically important in various forms of hypertension (renal or pulmonary) or coronary vasospasm. It has been postulated that the ouabain-insensitive component of resting membrane potential of smooth muscle cells from blood vessels is spontaneously hyperpolarized animals and that [Ca^{2+}]i can be elevated in spontaneously hypertensive vessels to near 1 \mu M. If both of these events take place, not only would the degree of Ca^{2+}-dependent block of delayed rectifier K^+ channels be increased, but the open probability of voltage-dependent Ca^{2+} channels would be greater. This could lead to an increased contractile state of the blood vessel and augment the pathophysiological condition. In the pulmonary vasculature, inhibition of I_{K_{Ca}} by [Ca^{2+}] may be partially responsible for the membrane depolarization associated with hypoxic pulmonary vasoconstriction. Block of I_{K_{Ca}} may also play a role in various gastrointestinal disturbances. For example, irritable bowel syndrome is described as a disorder of intestinal motility that is manifested by a decrease in colonic slow-wave frequency. Thus, an increase in slow-wave duration, through inhibition of I_{K_{Ca}} presumably by an excitatory agonist (i.e., acetylcholine, which increases [Ca^{2+}]), would exacerbate the diseased state, increasing the plateau phase and further decreasing the slow-wave frequency, thereby causing strong contractions of the colon and intense intestinal pain. Thus, both the Ca^{2+} and Mg^{2+}-dependent inhibition of delayed rectifier K^+ channels in both vascular and visceral smooth muscle may represent an important element in excitation-contraction coupling in the normal and diseased state.

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References
29. Adams DJ, Nonner W. Voltage-dependent potassium channels: gating, ion permeation, and block, in Cook NS, ed. Potassium
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