Whole-Cell and Single-Channel Calcium Currents of Isolated Smooth Muscle Cells From Saphenous Vein

A. Yatani, C.L. Seidel, J. Allen, and A.M. Brown

Whole-cell and single-channel calcium currents in enzymatically isolated dog saphenous vein cells were recorded by the patch-clamp method. Test pulses to negative potentials from holding potentials of -90 to -40 mV elicited currents that inactivated quickly and in a voltage-dependent manner (called I_low for low threshold). A second calcium current persisted even at relatively positive holding potentials of -30 to -10 mV, required stronger depolarizations for maximum current, and inactivated slowly (I_high for high threshold). I_high transported barium more than calcium, whereas I_low transported the two ions equally. Single-channel current records (90 mM barium) showed a larger conductance that activated at relatively positive potentials and a smaller (about one-third) conductance that activated at weak depolarizations. Nitrendipine suppressed I_high, and the effect was voltage dependent as observed in cardiac cells, although block of resting channels was much greater in vein cells (K_R=10^-8 M). Exposure to the stereoisomer (-)Bay K 8644 increased I_high but not I_low. The (-)Bay K 8644 also increased the channel activity and prolonged the open time of the larger conductance current. Thus, two types of calcium channels, differing in potential-dependence of activation and inactivation, calcium/barium selectivity, single-channel conductance, and sensitivities to dihydropyridines were identified in smooth muscle cells isolated from a large cutaneous vein. (Circulation Research 1987;60:523-533)

Voltage-dependent calcium channels in the surface membrane of smooth muscle cells are important for electrical activity and excitation-contraction coupling. In multicellular smooth muscle preparations, it has been difficult to obtain reliable voltage clamp measurements of membrane current. The problems in achieving rapid spatial and temporal control of membrane potential include the presence of a large series resistance that arises from the narrow extracellular cleft between the elongated cells. This series resistance can cause a significant voltage drop during current flow.

In recent years, it has proved possible to circumvent series resistance errors in voltage clamp measurements by using single cells, and calcium channel currents have been recorded in muscle cells from stomach, ileum, and urinary bladder. More recently, whole-cell patch-clamp recordings from vascular smooth muscle cells have been reported. However, despite the functional importance of the circulatory system, knowledge about calcium channels in vascular smooth muscle cells is sparse.

In the present investigation, we used the patch-clamp method to study the electrophysiologic properties of whole-cell and single-channel calcium currents in freshly isolated vascular smooth muscle cells obtained from dog saphenous vein and maintained for 1-2 days in culture. This preparation was selected for several reasons. First, the pharmacologic properties of the saphenous vein have been studied in great detail, but there have been few studies of the membrane electrical properties of peripheral vein cells. Second, the membrane properties of vascular smooth muscle cells differ markedly depending on vessel and species, and hence, the different types require examination. Third, the saphenous vein is widely used in coronary homografts, and information about the membrane electrical properties of these cells is very important.

Materials and Methods

Preparation of Single Cells

Adult mongrel dogs of either sex were anesthetized with sodium pentobarbital (30 mg/kg), and the saphenous veins were removed aseptically. At least two veins were needed for each preparation. All vessels were placed in 10 ml of Hanks' balanced salt solution (HBSS) containing 3% penicillin and 1% gentamicin and transported to the tissue culture facility. While under a laminar flow hood, the vessels were transferred to a 60-mm sterile petri dish that contained HBSS, cleaned of adhering tissue, slit open, and cut into pieces 1.5-2.0 cm long.

The smooth muscle cells were isolated by enzyme dispersion, essentially as described by Ives et al except that periods of exposure to enzyme were lengthened to increase cell yield. Briefly, the vessels were placed in a sterile Erlenmeyer flask that contained 12...
ml of HBSS to which was added 0.2 mM CaCl₂ and enzymes obtained from Sigma Chemical Co., St. Louis, Mo., as follows: trypsin inhibitor (type I) 0.38 mg/ml, elastase 0.125 mg/ml (90–95 units/mg), collagenase 1.25 mg/ml (200 units/mg), bovine albumin 2 mg/ml, and HEPES 3.58 mg/ml (pH 7.4). The vessels were incubated with agitation in a water bath at 37° C for 90 minutes. At the end of this time, the enzyme solution was discarded and replaced with fresh solution, and the incubation was continued for 120 minutes. After this final incubation, any remaining pieces of vessel were disrupted mechanically by forcing them repeatedly through a 15-gauge needle. The final homogeneous solution was filtered through a 100-μm screen, and the pellet was resuspended in 10 ml of complete Dulbecco’s modified Eagle’s medium that contained 10% fetal calf serum, 1% glutamine, 3% penicillin, and 1% gentamicin. A sample was taken for cell counting (hemocytometry) and for plating onto polylysine-coated glass slides in 30-mm petri dishes at a concentration of 2 × 10⁶ cells per dish. The remaining cells were centrifuged again at 250 xg for 10 minutes, and the pellet was resuspended in Tyrode solution to give an approximate concentration of 1.5 × 10⁶ cells/ml. The cells were oval (typical dimensions 10 × 15 μm) or spherical (diameter 5–10 μm). These cells were examined immediately after resuspension in Tyrode solution (freshly dispersed). The cells in the petri dishes were placed in a standard tissue culture incubator maintained at 37° C and equilibrated in 5% CO₂/95% air. After one or two days, those cells that were attached to the coverslip and had not spread were examined electrophysiologically.

**Electrophysiology**

**Measurement of Whole-Cell Currents.** After isolation, saphenous vein cells were electrically quiescent and did not exhibit any spontaneous activity. The resting membrane potential measured in Tyrode solution ranged from −50 to −70 mV (−60 ± 3 mV, n = 10).

The membrane potentials were recorded with patch electrodes filled with potassium aspartate internal solution. The cell was perfused with normal Tyrode solution.

Whole-cell currents were recorded following the procedure of Hamill et al., using a List EPC7 patch-clamp amplifier. The patch pipettes had a tip resistance of 3–5 MΩ, and the input resistances of the cells were between 5 and 10 GΩ. The pipette capacitance to ground was neutralized after a seal was formed. The series resistance was computed from the time constant of the transient after the membrane was ruptured. In 30 cells, the average cell capacitance was 20 ± 5 pF, the average capacitative transient was 200 ± 10 microseconds, and the series resistance was 10 MΩ or less. Since the calcium channel currents always were less than 300 pA, the effects of series resistance on these currents were negligible. The series resistance could be compensated subsequently to enhance further the speed of the clamp. Tail currents were not evaluated because deactivation occurred too quickly and the current surges were usually too large for voltage clamp control. The experimental chamber (0.2 ml) was placed on a microscope stage, and external solutions were superfused at a rate of 2 ml/min by gravity. The solutions in the chamber exchanged in less than 30 seconds. The normal Tyrode solution (in mM) was NaCl 135, KCl 5.4, MgCl₂ 1.0, CaCl₂ 1.8, glucose 10, and HEPES 5 (pH 7.4). The patch pipette solution (in mM) was K aspartate 110, KCl 20, ATP 2, EGTA 5, MgCl₂ 1, and HEPES 5 (pH 7.3). For comparison, some experiments were performed without EGTA.

To record optimal calcium channel currents, outward potassium currents were suppressed using a cesium-rich pipette solution (in mM): Cs aspartate 110, CsCl 20, ATP 2, EGTA 5, MgCl₂ 1, and HEPES 5 (pH 7.3). The external solution (in mM) was CaCl₂ or BaCl₂ 20, tetraethylammonium chloride (TEA) 115, 4-aminopyridine 5, MgCl₂ 1, glucose 10, and HEPES 10 (pH 7.4). The ionic conditions provided isolation of calcium channel currents from other membrane currents. The membrane potentials were corrected for a liquid junction potential of −10 mV between pipette and bath solution. All experiments were performed at room temperature (20–22° C). Membrane currents were sampled at 10 kHz, and the records were stored for subsequent analysis on a PDP 11/23 computer. Since leak currents were negligibly small, no subtraction was made in the present study. The inactivation curves were fitted to Boltzmann nonlinear least-squares fitting method, using a modified Marquardt-Levenberg nonlinear squares method.

**Measurement of Single-Channel Currents.** Single-channel calcium currents were measured in the cell-attached patch configuration. The patch pipette solution (in mM) was BaCl₂ 90, glucose 10, and HEPES 10 (pH 7.4). To put the patch-membrane potential on an absolute scale, the composition of the external solution (in mM) was K aspartate 130, EGTA 5, MgCl₂ 1, glucose 20, and HEPES 5 (pH 7.4). The unitary currents were filtered at 5 or 10 kHz, digitized at 10–20 kHz, stored, and analyzed using a PDP 11/23 computer. Capacity and leak currents were subtracted digitally. The single-channel currents were analyzed at 5 kHz using the method described by Lux and Brown.

**Drugs**

Stereoisomer (−)-Bay K 8644 and nitrendipine, which were kindly provided by Miles Laboratories (New Haven, Conn.), were prepared as 10 mM stock solution in 100% ethanol or polyethylene glycol. The final concentration of solvents was less than 0.01% and had no effect on calcium channel currents.

**Results**

The characteristics of the total membrane currents in Tyrode solution and the effects of the drugs on the calcium currents were qualitatively similar in freshly dispersed and primary cultured cells; therefore, the observations from these different preparations were combined in the present study.
The Membrane Currents in Venous Cell

When the cell was in normal Tyrode solution and the pipette contained K aspartate, depolarizing voltage steps from a holding potential of $-50\,\text{mV}$ elicited a time-dependent inward current followed by an outward current (figure 1A). At test potentials between $-20$ and $+20\,\text{mV}$, a net inward current was evident. Voltage steps to more positive potentials activated further outward currents while decreasing the apparent peak inward current. At potentials more positive than $+20\,\text{mV}$, the latter reached its peak value and then declined slowly to a steady-state level. The current-voltage (I-V) relations of the peak outward current, the peak outward current, and the late current at 100 milliseconds into the clamp pulse are plotted in Figure 1B.

The existence of multiple types of calcium channels has been demonstrated in several cell types, including those from dorsal root ganglia, sensory neurons, heart, and clonal pituitary cells. We have examined the calcium channel currents by applying depolarizing test pulses to various test potentials from different holding potentials. For kinetic analysis of calcium channel currents, all experiments were performed using sodium, potassium-free external solution, and cesium aspartate-filled pipettes. Figure 2A shows the difference between calcium currents ($I_{\text{Ca}}$) elicited from two different holding potentials at test potentials of $-10$, $0$, and $+30\,\text{mV}$. When the membrane potential was held at $-80\,\text{mV}$, test pulses between $-30$ and $0\,\text{mV}$, elicited a time-dependent inward current that inactivated within 50 milliseconds. When the potential was held at $-30\,\text{mV}$, the rapidly inactivating inward current was not activated, and a slowly inactivating current appeared at more positive test potentials.

At the $0\,\text{mV}$ test potential, the amplitude of peak $I_{\text{Ca}}$, and its initial rate of inactivation were greater when the holding potential was $-80\,\text{mV}$ than $-30\,\text{mV}$; however, the amplitudes of the currents from both holding potentials were nearly identical at the end of the clamp pulse. These observations suggest that the fast inactivating current contributes mostly to the peak current and little to the late current. In this report, we refer to this current as the low threshold calcium current ($I_{\text{Ca,low}}$). For larger steps to more potentials positive than $+30\,\text{mV}$, the magnitude of the currents from both holding potentials showed a similar amplitude and inactivation time course. The current that is activated at high threshold potential is referred to as the high-threshold calcium current ($I_{\text{Ca,high}}$).

Figure 2B compares the peak $I_{\text{Ca}}$ I-V relation for the two current components. Both current components were observed consistently in every cell, but there was considerable variation in their relative magnitudes.

Both current components also were seen when barium was the charge carrier; however, the currents at positive potentials inactivated more slowly than when calcium was the charge carrier. Figure 3 shows the I-V relations with barium, elicited from a holding potential of $-80\,\text{mV}$. At test potentials between $-30$ and $-10\,\text{mV}$, the rapidly inactivating current ($I_{\text{Ca,low}}$) was present. When potentials were held at levels more positive than $-30\,\text{mV}$, the current was inactivated completely. At test potentials more positive than $0\,\text{mV}$, the slowly inactivating current ($I_{\text{Ca,high}}$) was present. The I-V curve...
obtained from a holding potential of −80 mV displayed a hump near 0 mV.

In Figure 4, we compared the effects of barium and calcium ions on \( I_{\text{low}} \) and \( I_{\text{high}} \). At a test potential of +30 mV, changing the bath solution from barium to calcium decreased the amplitude of the current by \( \approx 50\% \), and the current inactivated much faster in calcium solution (figure 4B). In contrast, the currents elicited by a test potential of −10 mV were almost identical in amplitude and kinetics in both solutions (Figure 4A). Assuming that differences between the shifts in gating produced by barium or calcium are offset by shifts in respective reversal potentials,\(^26\) the results suggest that barium is more permeable in high-threshold calcium channels, and barium and calcium are equally permeable in low-threshold calcium channels.

**Voltage Dependence of Inactivation**

The experiments in Figure 2 suggested that \( I_{\text{low}} \) was completely available at a holding potential of −80 mV but was inactivated at −30 mV. We examined the voltage dependence of this current in more detail and compared it with the high-threshold current component. Figure 5 compares the inactivation curves for both current components. Currents elicited by test pulses to −10 mV (which are predominantly although not exclusively \( I_{\text{low}} \)) were maximum at −80 mV and were reduced to about one-third at a holding potential of −50 mV (Figure 5A). The currents elicited by test pulses of +40 mV (\( I_{\text{high}} \)) became inactivated when holding potentials were more positive than −30 mV (Figure 5B). The peak current amplitudes were measured at each potential and were normalized to the currents measured with the most negative prepulses. The resulting amplitude-potential curves were fit by a Boltzmann distribution.\(^27\)

\[
h_m = \frac{1}{1 + \exp \left( \frac{V_m - V_h}{k} \right)}
\]

where \( V_h \) is the midpotential and \( k \) is the slope factor of the curve. In 8 experiments in calcium solution, the inactivation curve for \( I_{\text{low}} \) had a midpotential of

![Figure 2. Two components of calcium currents. Panel A: Currents elicited by depolarizing steps from −80 mV (○) and −30 mV (●) are compared at each test potential. Panel B: I-V curves obtained from −30 mV (○) and the difference between peak current amplitude obtained from a holding potential of −80 mV and −30 mV (●). The external solution contained 20 mM Ca.](image)

![Figure 3. I-V relations of barium current. The external solution contained 20 mM Ba. Inset figure shows currents elicited by increments of 10 mV depolarizing pulses from −20 to +20 mV from a holding potential of −80 mV.](image)
-46 ± 4 mV and a slope factor of 5.5 ± 0.8 mV and for \( I_{\text{high}} \) a midpotential of \(-15 ± 2.5\) mV and a slope factor of \(6 ± 0.9\) mV.

**Effects of Dihydropyridine Calcium Channel Drugs**

The dihydropyridines, which are used clinically to treat hypertension and angina, are the most potent blockers of smooth muscle excitation-contraction coupling. We examined the effects of the calcium channel blocker nifedipine and the calcium channel agonist (-)Bay K 8644 on calcium currents in veins cells.

Figure 6 compares the susceptibility of two current components to blockade by nifedipine (10^{-8} M). Currents elicited from -80 mV to +20 mV, where the current is mainly \( I_{\text{low}} \), were blocked 50% by nifedipine (Figure 6B). The half-time of current inactivation at +20 mV, during the clamp pulse before and after the drug, was not significantly changed (<<8%, \( n = 3 \)). When currents were elicited by steps from -80 to -10 mV (mainly \( I_{\text{low}} \)), nifedipine produced a small decrease in current and perhaps a slight increase in rate of inactivation (Figure 6A). The I-V curves before and after nifedipine are shown in Figure 6C. In the presence of nifedipine, at positive test potentials (> +10 mV) the current was decreased to about 50% of control (45 ± 8%, \( n = 6 \)), but the potential at which the current was maximum (+30 mV) was not changed.

The block of \( I_{\text{Ca}} \) by nifedipine is much stronger when the cardiac membrane is held at depolarized potentials, and therefore, we examined the voltage-dependent block of nifedipine (10^{-8} M) on \( I_{\text{high}} \) (test potential +30 mV). At a holding potential of -50 mV, nifedipine decreased the current to ≈ 40% of control (Figure 7A), but when the membrane potential was held at -20 mV, nifedipine inhibited the current almost completely (Figure 7B). The effects of nifedipine (10^{-8} M) on the voltage dependence of \( I_{\text{high}} \) is plotted in Figure 7C. The curves were normalized to currents measured with the most negative pre-pulses (-80 mV). In the presence of nifedipine, the curve was shifted to more negative potentials. In 3 experiments, 10^{-8} M nifedipine shifted the curve by 18 ± 2 mV without changing the slope factor.

Further evidence for pharmacological dissimilarity of \( I_{\text{low}} \) and \( I_{\text{high}} \) was obtained from an experiment with (-)Bay K 8644, a dihydropyridine that has been demonstrated to increase calcium channel current. Figure 8 shows the I-V relations before and after (-)Bay K 8644 (10^{-7} M). The drug increased \( I_{\text{high}} \) but had little effect on \( I_{\text{low}} \). Although \( I_{\text{high}} \) was increased at all test potentials, the effects were larger at relatively negative potentials (compare at +20 and +60 mV). A comparison of the current traces revealed that the amplitude of \( I_{\text{high}} \) was increased strongly by the drug.

Although \( I_{\text{low}} \) and \( I_{\text{high}} \) had different sensitivity to dihydropyridines, both components were blocked by the organic calcium channel blockers cobalt and cadmium. At concentrations of 3 mM cobalt and 10^{-4} M cadmium, both current components were blocked almost completely.

**Single Calcium Channel Currents and Dihydropyridine Drug Effects**

Single-channel currents through calcium channels were measured in cell-attached recordings to examine the elementary currents that contribute to the whole-
cell calcium currents in vein cells. The patch pipettes were filled with 90 mM barium because barium was found to be useful in blocking single-channel potassium current and because barium ions have been shown to permeate calcium channels more than calcium ions. As suggested by whole-cell recordings, two types of single-channel currents were observed. Figure 9 illustrates an example of single calcium channel currents that show some of the features that distinguish the two types of calcium channels. Activity of previously observed types of smooth muscle calcium channel was elicited by depolarizing voltage-clamp steps to −10 mV from a holding potential of −40 mV. The brief channel openings of ≈1.3 pA amplitude occurred in clusters throughout the pulse, with occasional sweeps dominated by long-lasting openings. When the same patch was hyperpolarized to −80 mV and pulses were applied to the same test potential of −10 mV, openings of ≈0.4 pA amplitude appeared in addition to a large conductance of the opening channel. These generally are grouped as one burst of activity that tends to occur near the beginning of each pulse. Figure 10A shows unitary currents from a patch containing both types of channels. At test potential 0 mV, the single channel mean amplitude histogram was fitted by the sum of two Gaussian curves (solid line). This indicated the existence of two populations with mean amplitude of 0.34 pA and 0.85 pA (Figure 10B). The current–voltage relations for both types of channels are shown in Figure 10C. In the potential range that was investigated, the I-V relation for both types of single-channel current was linear. The slope conductance was 7–9 pS for the small channel type, considerably smaller than the conductance of the large type (18–24 pS). As would be expected for ionic currents that have positive equilibrium potentials, the amplitudes of both types of single-channel currents decreased with increasing depolarization. The current measurement could not be extended to more positive potentials because the signal-to-noise ratio became too small for accurate measurement.

We tested the effects of (−)Bay K 8644 on single calcium channel currents to examine the mechanism of its action at the molecular level. Figure 11A shows current traces under control conditions. The patch contained both types of calcium channels, and at a test potential of 0 mV, from a holding potential of −80 mV, activity was mainly openings of small channels.
Figures 8 and 9. Effects of Bay K 8644 (10^{-7} M) on I_{low} and I_{high}. The external solution contained 20 mM Ca. Currents were elicited by 10 mV increments from -30 to +20 mV in Panel A and from +30 to +50 mV in Panel B. The lower traces show results after the drug. Holding potential was -80 mV. Panel C: I-V curves before (o) and after Bay K 8644 (●).

Examples of channel activity from the same patch 3 minutes after perfusion of (-)Bay K 8644 at concentration of 10^{-7} M, which produced a large increase of \( I_{\text{high}} \) in whole cell calcium currents, are shown in Figure 11B. With (-)Bay K 8644, depolarization to the same test potential increased the channel activity and largely promoted openings of the large conductance type channel with many long-lasting openings. The averaged current was increased \( \approx \) threefold. The histograms for open time in the absence and in the presence of the drug are shown in Figure 11C and D, respectively. Under control conditions the open time histogram was fitted by a single exponential (\( \tau = 0.6 \pm 0.06 \) millisecond). In the presence of (-)Bay K 8644, the open time histogram was composed of the sum of two exponentials, one that had a short time constant (\( \tau_1 = 0.55 \pm 0.1 \) millisecond), as in the absence of the drug, and an additional component with a time constant \( \approx 10 \) times as long (\( \tau_2 = 5.4 \pm 0.3 \) milliseconds).

Similar results were obtained over a -20 to 0 mV range.
In this report, we describe the initial characterization of net membrane currents and the properties of calcium channels of vascular smooth muscle cells isolated from dog saphenous vein. Under physiological conditions, in which the pipette was filled with potassium ions and the external solution was Tyrode solution of net membrane currents and the properties of calcium channels of vascular smooth muscle cells. Although the voltage dependence of activation and inactivation differs from other smooth muscle cell types, the time course of the current, an increase in current amplitude by barium substitution for calcium, and the sensitivity to the dihydropyridine agonist Bay K 8644 were similar to previous observations. In its kinetics and lack of discrimination between calcium and barium, in vein cells seems very similar to the fast-inactivating calcium channel described in heart cells and in vertebrate sensory and dorsal root ganglion neurons.

More recently, two components of calcium currents that have properties similar to those we have described but different sensitivity to dihydropyridines have been found in vascular smooth muscle cells and in the smooth muscle cell line A10. The only obvious difference is that the rapidly inactivating calcium channel in A10 cells conducts calcium ions much more than barium ions, while in vein cells the inwardly rectifying component I_{inw} was similar with either ion.

In vein cells, I_{inw} was more sensitive to block by nitrendipine, and the potency of the block was enhanced by depolarizing the holding potential, as observed in cardiac cells. From the results shown in Figures 6 and 7, we could estimate dissociation constants for nitrendipine binding in the resting and inactivated state by using an approach described by Bean et al., assuming one-to-one binding of drug to the resting and inactivated states. The dissociation constant for binding to the inactivated state (K_i) can be calculated using the equation:

$$\Delta V_h = k \ln \left( \frac{(1 + (N)/K_i)}{(1 + (N)/K_a)} \right)$$

where $\Delta V_h$ is the shift of the midpoint of the steady-state availability curve, k is the slope factor of the inactivation of the curve (k = 6 mV), (N) is nitrendipine concentration, and $K_i = 10^{-8}$ M. Using the shift ($\Delta V_h$) of 18 mV caused by $10^{-8}$ M nitrendipine gave a value for $K_i$ of $0.25 \times 10^{-9}$ M. This value was similar to that measured for the high-affinity binding site for nitrendipine in vascular tissue.

Although holding potential-dependent block of nitrendipine was similar in vein and cardiac cells, the potency of nitrendipine for the resting channel was much greater in veins. For example, a $K_i$ value of

**Discussion**

In this report, we describe the initial characterization of net membrane currents and the properties of calcium channels of vascular smooth muscle cells isolated from dog saphenous vein. Under physiological conditions, in which the pipette was filled with potassium ions and the external solution was Tyrode solution, net membrane current seemed to be composed of two time- and voltage-dependent current components: an inward current carried by calcium ions and an outward current carried mainly by potassium ions. There was no evidence of the presence of TTX-sensitive inward sodium currents nor of inwardly rectifying potassium currents. Since the outward current was readily blocked by external application of the potassium channel blocker TEA and by replacing the potassium ions in the pipette solution with cesium ions, these cells provided a useful system in which to study calcium channels of vascular smooth muscle.

This study demonstrates the existence of two distinct populations of calcium channels in mammalian venous cells. The two components of current that can be distinguished kinetically had different selectivity for calcium and barium, different single-channel currents, and different sensitivities to dihydropyridine drugs. These components are the I_{inw}, which activated only at negative holding potentials and inactivated rapidly, and the slowly inactivating component I_{inw}, which was present even at positive holding potentials. The latter component in vein cells appears to be identical to the calcium currents described in single smooth muscle cells in recent years. Although the voltage dependence of activation and inactivation differs from other smooth muscle cell types, the time course of the current, an increase in current amplitude by barium substitution for calcium, and the sensitivity to the dihydropyridine agonist Bay K 8644 were similar to previous observations. In its kinetics and lack of discrimination between calcium and barium, in vein cells seems very similar to the fast-inactivating calcium channel described in heart cells and in vertebrate sensory and dorsal root ganglion neurons.
7.3 x 10^{-7} M was reported for resting calcium channels of cardiac cells, which is almost two orders of magnitude greater than the value reported here. This observation suggests that membrane potential is not the sole determinant of dihydropyridine binding affinities.

Single-channel recordings also distinguished the two types of calcium channels in venous cells. In addition to the differences in their conductance, differences also existed in the voltage dependence of the two types of calcium channels. With barium in the pipette, clamp pulses to -10 mV from a holding potential of -40 mV evoked channel activity of large current amplitude, which was similar to previously described smooth muscle calcium channel currents. The pattern of activity also was similar and was typified by brief openings in cluster throughout the voltage-clamp pulses. The other type of channel activity, which had not been described previously in smooth muscle cells, showed strikingly different patterns and appeared only with pulses from holding potentials more negative than -50 mV. The latter type of calcium channel had much smaller unitary current amplitude and generally was grouped as one burst of activity that occurred near the beginning of each pulse. The pattern of channel activity seems kinetically similar to the T-type calcium channel in dorsal root ganglia (DRG) neurons and ventricular cells. More recently, Worley et al have reported the existence of two types of calcium channel in an excised patch with different conductances (8 pS and 15 pS) in the rabbit mesenteric artery. Although the calcium channel conductances were similar, their sensitivity to the dihydropyridine calcium channel blocker appeared different from our preparation. Nisoldipine (30 nM) completely blocked the 15 pS channel and reduced the 8 pS channel to ~20% of control. We have not examined the effects of dihydropyridine calcium channel blockers on single-channel currents, but our whole-cell data predict that smaller conductance channels are insensitive to nanomolar concentrations of nitrindipine. Since the difference may be due to different experimental conditions, comparative studies under identical conditions are required.

Although more detailed analysis is necessary, these two types of calcium channel activity seem to agree with those of whole-cell calcium currents. The calcium channel with smaller conductance seems to correspond to I_{low} because the current decayed rapidly and its peak amplitude was much smaller than that of I_{high}.

We estimated channel densities using whole-cell I-
V relations for $I_{low}$ and $I_{high}$ (Figure 2) after correcting for a shift of 6 mV in surface charge effects between 90 mM barium and 20 mM calcium. The unitary conductance for smaller conductance channels was $8 \pm 0.5$ pS. Assuming a probability of opening $P_o$ of $0.4$ at 0 mV and using the maximum conductance $g_{max}$ for whole-cell $I_{low}$, we estimate that there are between 200 and 400 channels/cell. The variability of $I_{low}$ could change the density by a factor of 2–3. For $I_{high}$, $P_o$ is $0.1$ at 0 mV, and with a unitary conductance of $24 \pm 2$ pS, values between 1,200 and 2,000 channels/cell.

In addition to differences in kinetics, unitary conductance, and channel density, the two types of calcium channel differed in their responsiveness to $(-\Delta )$Bay K 8644. As observed in the whole-cell recordings, $(-\Delta )$Bay K 8644 in the single-channel recordings strongly enhanced the channel activity with large conductance by prolonging the open time. Our results with $(-\Delta )$Bay K 8644 are consistent with findings in other types of cells.

In the present study, we have demonstrated the presence of two distinct calcium channels in vascular smooth muscle cells dissociated from dog saphenous vein. The channels had different single-channel conductances and different sensitivities to dihydropyridine drugs. These two types of current also appear to be carried through distinct types of single-channel populations because some membrane patches contained only one type of single-channel activity while others contained both. The high-threshold calcium channel, which had the larger conductance, would deliver the bulk of the calcium ions to the cytoplasm when those ions are required for such cell functions as contraction. In contrast, it is not clear whether the existence of $I_{low}$ has some special significance for the function of neuronal cells. Caffrey et al. recently reported the existence of high-threshold calcium-channel currents in smooth muscle cells from guinea pig aorta that resemble the large-conductance single-channel currents we recorded. The low threshold of channels was not identified, however. Although more comprehensive studies under identical conditions are required, these findings indicate that vascular smooth muscle is heterogeneous, just as smooth muscle cells are in general. In preliminary results, application of norepinephrine (1 to $10 \times 10^{-6}$ M) had no effect on either $I_{low}$ or $I_{high}$, although it is still possible that the current through this channel could be increased through modulation by some hormones and neurotransmitters. In addition, since organic calcium channel antagonists are used widely in the management of cardiovascular disorders, differences in the pharmacology of $I_{low}$ and $I_{high}$ in vascular smooth muscle are of great interest for understanding the clinical pharmacology of calcium channel blockers.

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