Calcium-Activated Chloride Current in Rabbit Coronary Artery Myocytes

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Abstract Whole-cell patch-clamp techniques were used to study enzymatically dispersed epicardial coronary artery smooth muscle cells. Depolarization voltage pulses of 500-millisecond duration from -60 mV (118 mmol/L CsCl, 22 mmol/L tetraethylammonium chloride, and 5 mmol/L EGTA pipette solution) elicited inward L-type calcium currents (I_{ca}). When EGTA was omitted from the pipette solution, an outward current was superimposed on the calcium current, and repolarizing voltage steps produced an inward tail current (I_{r}). The amplitude of these inward currents was proportional to the I_{ca} amplitude from -30 to +50 mV. The time course of decay of the current was well fit by a single exponential equation. The time constant (τ) of this equation did not change with the size of I_{r} but was clearly voltage dependent (shorter at more negative potentials). Changing the chloride reversal potential from -1.3 to -39.7 mV by anion substitution using methanesulfonate as the chloride replacement in the pipette solution shifted the zero current level of I_{r} from 0.9±0.56 to -33.1±0.85 mV. The tail current was blocked by nifedipine (10^{-4} mol/L) and by isosmolar calcium substitution with barium in the bath solution and was enhanced by the dihydropyridine agonist Bay K 8644 (10^{-4} mol/L). I_{r} was also blocked by the chloride channel blockers DIDS (10^{-4} mol/L) and niflumic acid (10^{-5} mol/L). Caffeine (10^{-2} mol/L), which releases intracellular calcium stores, caused an inward current at holding potentials (-60 mV), which was inhibited by DIDS. Caffeine also inhibited subsequent attempts to elicit I_{r} by depolarizing pulses (88% reduction in I_{r}). Bay K 8644 potentiated both I_{ca} and I_{r} elicited by depolarizing pulses in the presence of caffeine; however, I_{r} remained much smaller than control (before caffeine), despite an I_{ca} that exceeded control values. These results suggest that rabbit coronary artery myocytes possess a calcium-activated chloride conductance, which is activated by depolarizing voltage pulses and the resultant calcium current. Calcium released from intracellular stores in response to inward calcium currents (calcium-induced calcium release) appears to be the primary trigger for activation of the chloride current. This chloride current may contribute to the depolarization observed in response to agonists, which elicit the release of intracellular calcium stores. (Circ Res. 1994;75:742-750.)

Key Words • Ca^{2+} channels • Cl^{-} channels • vascular smooth muscle cells • calcium-induced calcium release

One of the initial events in agonist activation of vascular smooth muscle cells is a transient rise in intracellular calcium, which is due primarily to release of stored calcium. Membrane potential changes associated with agonist activation may then regulate subsequent fluctuations in intracellular calcium levels by affecting the rate of entry of calcium across the plasma membrane. These two events are highly interrelated, because the initial elevation of intracellular calcium has the potential to stimulate any of several calcium-activated ion conductances including potassium, chloride, and a nonselective cation channel, which may itself be highly permeable to calcium. These conductances may also be intermittently activated by spontaneous release of calcium from the sarcoplasmic reticulum, and this process could conceivably contribute to "resting" potential. Factors such as the number, location, regulation, and calcium and voltage sensitivity of these channels must play an important role in determining the way in which membrane potential is altered in response to a given stimulus.

It is well established that membrane depolarization is an important contributor to both the initiation and maintenance of vascular smooth muscle contraction. The mechanism by which vascular smooth muscle is depolarized in response to vasoactive substances is not completely understood and may involve alterations in multiple ion conductances. Potential mediators of depolarization include (1) inhibition of a previously active potassium conductance, (2) activation of a nonselective cation conductance, and (3) activation of a chloride conductance. Certain inward calcium currents themselves can contribute to membrane depolarization, although the magnitude of these currents is generally small. Exceptions to this are tissues that are capable of firing calcium-dependent action potentials, such as the portal vein and cerebral blood vessels. Many vascular tissues, including the coronary artery, are actually capable of firing action potentials under conditions in which outward potassium currents are blocked by compounds such as tetraethylammonium (TEA). The role of the chloride current in regulation of membrane excitability is yet to be clearly defined.

Calcium-activated chloride currents have been carefully characterized at the whole-cell current level in several tissues, including Xenopus oocytes, AT-20 and GH, pituitary cells, rat lacrimal glands, undifferentiated human colonic cells, ventricular myocytes, and portal vein myocytes. This current has been implicated in initiating depolarization of portal
vein to norepinephrine\(^3\) and arterial vascular smooth muscle in response to norepinephrine\(^1\) and endothelin.\(^4\)

The calcium-activated chloride current has not, however, been systematically characterized in arterial vascular smooth muscle by the whole-cell patch-clamp technique.

In the present study, we attempt to characterize an inward tail current that is seen after L-type calcium currents elicited by depolarizing pulses in freshly dispersed rabbit coronary artery myocytes. We present evidence that the two currents are activated over a similar voltage range and parallel each other in magnitude. Tail currents display multiple characteristics (blocker sensitivity, calcium dependence, and reversal potential), which suggest that they represent a calcium-activated chloride conductance. Furthermore, these currents appear to be highly dependent on the release of calcium from intracellular stores. These data support the concept that a calcium-activated chloride current may underlie a component of agonist-induced depolarization and extend the potential means of activation of this current to include calcium-induced calcium release.

Materials and Methods

Cell Isolation

Rabbit coronary artery myocytes were prepared in a manner similar to that previously described by this laboratory.\(^27\) Briefly, hearts from New Zealand White rabbits (1.5 to 2.0 kg) were perfused retrograde (25 mL/min) on a modified Langendorff apparatus with nominally calcium-free buffer containing (mg/mL) collagenase B 0.05 (Boehringer Mannheim), protease 0.01 (type XXIV, Sigma Chemical Co.), and trypsin inhibitor 0.017 (type II-S, Sigma Chemical Co) for 10 minutes at 37°C. An \(-2\) cm section of the left epicardial coronary artery was dissected free of the myocardium, carefully cleaned of adherent tissue, and slit longitudinally. The blood vessel was then placed in a 5-mL vial of calcium-free buffer containing (mg/mL) collagenase 0.6, protease 0.6, and trypsin inhibitor 0.4. The tissue was then gently agitated at 35°C for 20 minutes in a temperature-controlled shaker bath. The digested tissue was then placed in calcium-free buffer containing 1 mg/mL bovine serum albumin and stored at 5°C. Elongated smooth muscle cells were dispersed by gentle trituration with a fire-polished Pasteur pipette. Cells were calcium tolerant and generally remained viable for 6 to 8 hours after isolation.

Human coronary artery myocytes were obtained from two patients. Both had undergone previous bypass surgery. One was from an explanted heart at the time of transplantation; the other was from an organ donor whose heart was not transplantable. The procedure for obtaining these blood vessels was approved by Committee A at the University of Iowa (human subject use). First-order branches of the left anterior descending coronary artery were dissected free of adherent connective tissue and cut into 1-mm-wide longitudinal strips. These strips were digested for 30 minutes at 30°C in an enzyme-containing buffer identical to that used for the rabbit coronary artery. Cells were dispersed by gentle trituration.

Experimental Procedures

The patch-clamp apparatus and technique were similar to those previously described.\(^27,28\) Whole-cell currents were recorded at room temperature with an Axopatch 1C patch-clamp amplifier (Axon Instruments) driven by a Gateway 2000 486/33C computer using pClAMP software (Axon Instruments, version 5.6). Calcium currents were sampled at 2 kHz and chloride currents at 0.7 kHz by using a split-screen protocol because of the slow deactivation of the tail current. A four-pole low-pass Bessel filter was used at a cutoff frequency (~3 dB) of 1 kHz. These data acquisition parameters were selected to allow reliable resolution of the calcium current while still accurately recording the much slower tail current. Acquisition of the tail currents at faster sampling rates did not appreciably alter the amplitude of the current. Pipette resistances were 2 to 4 MΩ when filled with Cs-TEA pipette solution.

Membrane capacitance and series resistance were determined from 10-mV voltage-clamp steps at a holding potential (HP) of ~60 mV.\(^29\) The average membrane capacitance from 21 cells was calculated to be 25.1 ± 0.9 pF. Average series resistance was 6.8 ± 0.8 MΩ (n=21). When a peak chloride current of ~200 pA was used, the membrane voltage error was <1.5 mV. Current recordings have not been compensated by capacity and leak current subtraction. In experiments in which low pipette chloride concentrations were used, the junction potential was nulled before making the pipette-membrane seal. Since intracellular and extracellular chloride concentrations were not subsequently altered in any individual cell, further junction potential corrections were not necessary.

Solutions and Drugs

Standard pipette solution contained (mmol/L) CsCl 120, TEACl 22, MgCl\(_2\) 1, Na\(_2\)ATP 5, NaGTP 0.25, and HEPES 10 (pH 7.2 with CsOH). In some experiments, EGTA (10 mmol/L) was added as a calcium chelator. Reversal potential experiments were performed with an intracellular chloride concentration of 33 mmol/L. This solution contained (mmol/L) CsCl 9, CsOH 111, TEACl 22, MgCl\(_2\) 1, Na\(_2\)ATP 5, NaGTP 0.25, and HEPES 10 (pH titrated to 7.2 with methanesulfonic acid). Cell digestion and storage buffer contained (mmol/L) NaCl 140, KCl 4.5, MgCl\(_2\) 1, HEPES 10, and glucose 5.5 (pH 7.35 with NaOH). CaCl\(_2\) (2.5 mmol/L) was added to this buffer to yield the standard bath solution.

All components of the buffer, niflumic acid, caffeine, and 4,4'-disothiocy anostilbene-2,2'-disulfonic acid (DIDS), were obtained from Sigma. Nifedipine and Bay K 8644 were obtained from Calbiochem. Niflumic acid, nifedipine, and Bay K 8644 were initially dissolved in ethanol as stock solutions. DIDS was dissolved in dimethyl sulfoxide (DMSO). A minimum dilution of 1:1000 was used to produce final experimental concentrations of all compounds, yielding a final solvent concentration of 0.1%. This concentration of DMSO or ethanol had no measurable effect on calcium or chloride currents. Caffeine was dissolved directly into the buffer solution.

Statistical analysis of group differences were performed by using paired or unpaired Student’s t tests as appropriate. A value of P < 0.05 was considered to be statistically significant. Curve fitting for determination of time constants of current decay was performed by using available curve-fitting routines from pCLAMP, version 6.0.

Results

Effects of Calcium Buffering of the Intracellular Solution

Previous experiments in our laboratory have extensively characterized the voltage-dependent calcium current in the rabbit coronary artery.\(^27\) All of these studies were performed with a pipette solution containing 10 mmol/L EGTA, and no tail currents were seen on repolarization after depolarizing pulses. When EGTA is omitted from the pipette solution, 500-millisecond depolarizing pulses from an HP of ~60 to +10 mV elicit an initial inward calcium current, which is followed by a sustained outward current. An inwardly directed tail current is observed after return to the HP (Fig 1A). Similar current patterns are seen in cells obtained from human coronary artery (Fig 1B). The kinetics of this tail current are much slower than that of the inward calcium current (note change in the time scale at return to HP).
If depolarization is maintained for longer periods, the outward current inactivates over the course of several seconds, with a peak at =2 to 3 seconds. Decay of the current is slow at positive potentials, and an inward tail current is still observable after 6 seconds of depolarization (data not shown).

Dependence on Frequency of Stimulation

The frequency of the chloride current was altered by the frequency of application of depolarizing pulses. Fig 2 shows superimposed tracings of six consecutive pulses from -60 to +10 mV, which were delivered at intervals of 20 seconds (Fig 2A) or 5 seconds (Fig 2B). These tracings were obtained sequentially from the same cell. The first pulse in Fig 2B was delivered 20 seconds after the last pulse in Fig 2A. When the frequency is increased to every 5 seconds, the size of the chloride current is diminished and remains so as long as the pulse frequency is maintained. However, this faster pulse frequency had no significant effect on the magnitude of the calcium current. Lengthening the interval between pulses beyond 20 seconds did not significantly affect the size of the chloride current, and this interval was therefore used routinely in all experimental protocols.

Voltage Dependence of Tail Currents

Fig 3 shows the current-voltage relation for the peak of the L-type calcium current and the relation between the magnitude of the depolarizing pulse and the size of the tail current at the moment of repolarization. These currents are measured as the change in current with reference to holding current. HP was -60 mV, and test potentials ranged from -60 to +90 mV (500-millisecond pulses). The calcium and tail currents have a similar threshold of activation, and there is a parallel relation between the magnitude of the two currents. Although it would appear that significant tail currents are observed after depolarizing pulses to voltages at which no calcium current is seen (+40 and +50 mV), this is likely caused by underestimation of the calcium current that is due to the superimposed outward current. Previous experiments have shown the apparent reversal potential of the calcium current in leak-subtracted cells to be =+70 mV.

Analysis of the time course of decay of tail currents (Fig 4) reveals that these events were best fit by a single exponential equation. The time constant of decay of the tail current (τ) was found to be constant and independent of the size of the tail current when it was measured after depolarizing pulses to different test potentials (Fig 4, top panels; τ, 160.0±20.5 milliseconds [n=5] after depolarization to +10 mV and return to -60 mV). τ was voltage dependent and was significantly faster at more negative potentials (Fig 4, bottom panels; τ, 120.6±4.1 milliseconds at -90 mV, 169.8±8.9 milliseconds at -60 mV, and 262±23 milliseconds at -30 mV after depolarization to +10 mV [n=5]).

Calcium Dependence of Tail Currents

Evidence for the calcium dependence of this current is provided by the parallel nature of the current-voltage plots for the calcium current and the tail current and by the effects on the tail current seen with blockade or enhancement of the calcium current. Nifedipine (10^-6 mol/L)
Lamb et al  Calcium-Activated Chloride Current in Coronary Artery Myocytes

Fig 3. Current-voltage plot for inward calcium currents and inward tail currents. Holding potential is -60 mV, and all depolarizing pulses are 500 milliseconds. Points represent the peak inward current during depolarization and inward current at -60 mV immediately after repolarization (n=5). On the right are typical tracings from one cell at test potentials of -10, 0, +10, and +50 mV.

inhibits both the calcium current and the subsequent tail current elicited by repeated pulses from -60 to +10 mV at 20-second intervals (Fig 5). Calcium current was completely blocked by nifedipine (before, -148±2.2 pA; after, 0.0 pA; *P<.05 [n=4]). Subsequent tail currents were also suppressed (before, -83.4±13.0 pA; after, -10.1±1.2 pA;

Fig 4. The time constant of decay of the tail current (tau) does not vary with the amplitude of the current (upper bar graph and current tracings). However, tau is altered by changing the voltage at which the tail current is recorded. Tau is longer at more depolarized potentials (lower bar graph and current tracings). I indicates current. *P<.05.
Fig 5. Tracings showing the effect of altering the calcium current on the inward tail current. A. Current evoked by pulses from a holding potential of −60 to +10 mV for 500 milliseconds before and after the addition of $10^{-6}$ mol/L nifedipine to the cell chamber. Nifedipine dramatically inhibits both the calcium current and the tail current. B. Similar protocol pulsing before and after the addition of $10^{-6}$ mol/L Bay K 8644. Calcium currents and tail currents are both potentiated.

$P<.05$ [n=4]). Conversely, application of the dihydropyridine calcium channel agonist Bay K 8644 ($10^{-6}$ mol/L) enhances both currents (current current: before, −11.7±3.8 pA; after, −31.1±3.6 pA; $P<.05$; tail current: before, −91.1±19.3 pA; after, −230.4±22.9 pA; $P<.05$ [n=3]).

Barium can substitute for calcium as the charge carrier through the dihydropyridine-sensitive calcium channel.27 After isosmolar replacement of calcium with barium, the initial inward current in response to depolarization to +10 mV from HP of −60 mV is larger, and the kinetics of inactivation are slower. Fig 6 shows this effect of barium substitution for extracellular calcium on both the initial inward current and the tail current. Under these conditions, tail currents are completely absent (n=4).

 Charge Carrier of the Tail Current

In the presence of CsCl and TEACl as major intracellular ions, outward currents through potassium channels, including the calcium-activated potassium current, are absent. We felt that chloride was the best candidate for the charge carrier of the tail current. Fig 7 shows representative current tracings estimating the reversal potential of the tail current. Methanesulfonic acid was used as an anion substitute for chloride in the pipette solution in order to set the chloride reversal potential ($E_C$) to be either −1.3 mV (extracellular chloride, 151.5 mmol/L; intracellular chloride, 144 mmol/L) or −39.7 mV (extracellular chloride, 151.5 mmol/L; intracellular chloride, 33 mmol/L). In these experiments, cells were initially depolarized to +10 mV for 500 milliseconds, followed by repolarization to various potentials on either side of the calculated $E_C$ (5-mV steps at $E_C=-1.3$ mV, 3-mV steps at $E_C=-39.7$ mV). Zero current levels were estimated visually for each individual cell, and these values were averaged. Tail currents were determined to have a zero current level of $0.9±0.56$ mV (n=5) at $E_C=-1.3$ mV and of $-33.1±0.85$ mV (n=7) at $E_C=-39.7$ mV. The observed negative shift in measured reversal potential produced by substitution of intracellular chloride with methanesulfonic acid represents 89% of the shift that would be predicted based on calculations from the Nernst equation.

Fig 6. Tracing showing the effect of replacing extracellular calcium (2.5 mmol/L) with barium (2.5 mmol/L). Current is elicited by 500-millisecond pulses from a holding potential of −60 to +10 mV with return to −60 mV. Barium cannot substitute for calcium in the production of inward tails.

Fig 7. Tracings showing the reversal potential of tail current changes with alterations in the chloride gradient. A. Under conditions of approximately balanced chloride (chloride reversal potential [$E_C$] = −1.3 mV) tail currents had a measured zero current level at $0.9±0.56$ mV (n=5). B. When $E_C$ was shifted to −39.7 mV with methanesulfonic acid substitution for chloride in the pipette, zero current level for the tail current shifted to −33.1±0.85 mV (n=7).
Tail currents were blocked by DIDS and by niflumic acid, both of which have been shown to block calcium-activated chloride channels in other cell systems.\textsuperscript{30,31} DIDS (10\textsuperscript{-4} mol/L) produced a 74\% inhibition (before, \(-190\pm52\) pA; after, \(-50\pm7\) pA; \(P<.05\) \([n=6]\)); niflumic acid inhibited them by 79\% (control, \(-200\pm15\) pA; niflumic acid, \(-58\pm6\) pA; \(P<.05\) \([n=3]\)). This increase in the inward current observed immediately after depolarization is consistent with an unmasking of calcium current that is due to blockade of a concomitant outward current. The fact that the peak of the calcium current is augmented suggests that the onset of the outward current is very rapid (<50 milliseconds). Niflumic acid (10\textsuperscript{-5} mol/L) also inhibited tail currents (before, \(-200\pm15\) pA; after, \(-58\pm6\) pA; \(P<.05\) \([n=3]\)) while having a marked effect on the kinetics of the current. The time course of decay of the current appeared to slow dramatically. The onset of the blocking effect of niflumic acid was much slower than that of DIDS, requiring several minutes to achieve a stable effect. Because of this slow time course and our inability to control for possible rundown of the calcium current, it was impossible to accurately estimate the effect of niflumic acid on the initial inward (calcium) current. To avoid overestimation of the inhibitory effect of niflumic acid that was due to rundown of the calcium current, pre- and post-niflumic acid tail current measurements were made from current tracings that were acquired 5 minutes apart and that had associated calcium currents of similar size. This may, in fact, have led to underestimation of the efficacy of niflumic acid.

On the basis of these reversal potential and channel blocker experiments, the major charge carrier for the tail current appears to be chloride. We will therefore subsequently refer to it as a chloride current.

**Dependence on Intracellular Calcium Release**

The role of intracellular calcium stores in producing the chloride current was tested by exposing the cells to 10 mmol/L caffeine in order to unload these stores. With the cell held continuously at \(-60\) mV, the application of caffeine (10 mmol/L) elicited an inward current that decayed over a period of several seconds (Fig 9). When the cells were pretreated with DIDS (10\textsuperscript{-4} mol/L), this current was markedly inhibited (control, \(-23\pm4.7\) pA \([n=7]\); DIDS, \(-3.9\pm2.3\) pA \([n=4]\); \(P<.05\)) suggesting that this represents a chloride current activated by intracellular calcium release. When a depolarizing pulse to +10 mV was delivered during exposure to caffeine (Fig 10A), chloride currents were nearly completely inhibited (control, \(-140.1\pm43.9\) pA; caffeine, \(-17.4\pm6.9\) pA; \(P<.05\) \([n=5]\)) despite the persistence of a significant calcium current (control, \(-21.4\pm2.0\) pA; caffeine, \(-12.4\pm2.3\) pA; \(P<.05\) \([n=5]\)). This suppression of chloride current seen after the caffeine-induced current was reversible. The calcium current itself was most likely diminished because of the elevated levels of intracellular calcium.\textsuperscript{32} If Bay K 8644 was used to augment the calcium current (to levels larger than control), there was an observable increase in the chloride current; however, it did not approach control levels (Fig 10B). These results suggest that the chloride current is highly dependent on intracellular calcium release but that the inward calcium current can elevate intracellular calcium at the level of the plasma membrane sufficiently to activate it to some degree.
portal vein and guinea pig mesenteric vein, spontaneous depolarizations have been observed in intact tissues and are felt to be related to calcium-activated chloride currents. These depolarizations are not associated with contraction themselves but can lead to contraction when threshold is reached for calcium-driven action potentials. Alterations in the chloride gradient have also been shown to affect action potential duration in portal vein. Finally, spontaneous transient inward currents have been extensively characterized in isolated cells and appear to represent intermittent activation of a calcium-activated chloride current due to quantal release of calcium from intracellular stores in isolated single vascular smooth muscle cells. The importance of these currents to normal cell function remains to be determined.

Increases in chloride conductance can produce depolarization, because intracellular chloride concentrations in smooth muscle exceed that which would be predicted on the basis of passive distribution. Estimates of chloride equilibrium potential range from −11 to −47 mV; all of these values are significantly more positive than resting potential. Transport mechanisms play a major role in maintaining this gradient. Rabbit aortic smooth muscle has been shown to possess both a chloride-bicarbonate exchanger, which may be involved in regulation of pH, and a furosemide-sensitive Na-K-Cl cotransporter, which appears to be primarily responsible for maintaining the elevated intracellular chloride concentration. Addition of 1 mmol/L furosemide reduced intracellular chloride of rabbit aorta from 32.6 to 13.5 mmol/L as determined by uptake studies with 36Cl. Furthermore, furosemide incubation (1 mmol/L, 40 to 90 minutes) was shown to hyperpolarize rabbit pulmonary artery smooth muscle from −61.6 to −67.1 mV. This suggests that not only does activation of a chloride current have the potential to mediate depolarization, chloride conductance may also contribute to resting membrane potential.

It is important to note that although the absolute magnitude of the chloride currents observed in the present study is small, vascular smooth muscle cells have been documented to have very high input resistances. Previous studies in our laboratory have yielded an estimate of input resistance in the coronary artery cells of 3.79±0.58 GΩ. When this estimate and an arbitrary membrane potential of −50 mV are used, only 13 pA of net current need flow to maintain this potential. Thus, small inward currents can produce significant depolarizations. In addition, vascular smooth muscle cells have been shown to have a large resting electrogenic contribution to resting membrane potential (=10 mV) due to activity of the Na+,K+-ATPase. This contribution is dependent on maintenance of a high-input resistance. Even small increases in inward current such as we have observed could short-circuit this current and produce further depolarization.

The calcium-activated chloride current in rabbit coronary artery myocytes can be activated by the calcium that enters the cell in response to a depolarizing pulse. However, the majority of the chloride current activation appears to be related to calcium-induced calcium release from the sarcoplasmic reticulum. The chloride current is abolished by caffeine and is not seen when barium is substituted for calcium. This effect of barium

**Discussion**

The present study was undertaken to characterize what appears to be a calcium-activated chloride current in smooth muscle myocytes from the rabbit coronary artery. Preliminary data show that a similar current can also be found in the human coronary artery. This current is calcium dependent (related to the magnitude of the calcium current, absent when barium is substituted as the charge carrier), reverses at test potentials consistent with the chloride equilibrium potential, and is blocked by compounds (DIDS and niflumic acid) that have been shown to inhibit chloride currents in other systems. In addition, the calcium-activated chloride current is elicited by both inward calcium current and by release of calcium from intracellular stores (calcium-induced calcium release or caffeine-induced current).

A calcium-activated chloride current has been implicated in a number of physiological conditions in vascular smooth muscle. It has been shown to mediate depolarization in response to norepinephrine in cells from rat portal vein and rabbit carotid artery and in response to endothelin in porcine coronary and human mesenteric arteries. These responses are mediated by agonist-induced release of intracellular calcium stores, presumably through production of inositol trisphosphates. In electrically active venous tissues such as the rabbit

**Fig 10.** A. Effect of 10 mmol/L caffeine on currents produced by depolarizing pulses from −60 to +10 mV is shown. Initially, the calcium current is diminished and the chloride current is nearly abolished. Calcium current begins to recover within ~1 minute of starting washout of caffeine, while recovery of the tail current lags 20 to 40 seconds behind. Sample tracings are from 0 seconds after onset of caffeine effect and 2 minutes after starting washout. B. After inhibition of both inward currents by caffeine, chloride current is augmented by Bay K 8644 (10−6 mol/L) to greater than control levels. Despite this larger calcium influx from extracellular sources, the chloride tail current does not return to control levels.
may be due to an inability of barium to elicit calcium release, or alternatively, the sarcoplasmic reticulum may become depleted in the absence of calcium. The ability of inward calcium current to induce calcium release and thereby produce an additional depolarizing chloride current sets up a positive-feedback loop. Release of intracellular calcium in response to an agonist results in an inward chloride current. The resulting depolarization can then stimulate calcium entry through L-type calcium channels. This calcium entry is then capable of both further stimulating the chloride current directly or may produce further release of intracellular calcium. This loop could be broken by the activation of other currents, such as a potassium current, that are also calcium dependent. These observations are in contrast to what has been observed in the portal vein by Baron et al. These investigators found that caffeine produced a dramatic increase (twofold) in the chloride current elicited in response to a depolarizing pulse. This increase was transient (1 to 2 minutes), and subsequent chloride currents in response to depolarizing pulses returned to control levels; they were not inhibited as we have observed. This suggests that in the portal vein very little calcium-induced calcium release is occurring in response to depolarizing pulses and that the chloride tail currents are primarily dependent on the transmembrane movement of calcium.

The precise role of the calcium-activated chloride current in activation of “tonic” vascular smooth muscle such as the coronary artery is unclear. The time course of channel activation under physiological conditions may be brief and parallel the initial intracellular calcium transient or it may be more sustained. This is likely to depend largely on the calcium and voltage sensitivity of the channel. Recently, Klöckner has published the first single-channel recordings of calcium-activated chloride channels from human mesenteric artery. The single-channel conductance was quite small (2 to 3 picosiemens), and the open probability increased dramatically as pCa was changed from 8 to 5. The channel was also voltage dependent with a higher open probability at more positive potentials. Pacao et al have made an attempt to estimate the calcium sensitivity of calcium-activated chloride channels by measuring whole-cell current in freshly isolated rat portal vein cells. Gradual elevations of intracellular calcium concentration were induced by depleting intracellular ATP with amytal and carbonyl cyanide m-chlorophenylhydrazone. The calcium concentration threshold for activation of chloride current ( indo 1 fluorescence) was 180 nmol/L with full activation at 600 nmol/L. The calcium dependence of channel activation was not altered by norepinephrine or membrane potential, and the authors concluded that the channel was mainly controlled by intracellular calcium. Consideration of the calcium sensitivity of the channel in relation to mean concentration of cytosolic calcium may be somewhat misleading. There is now abundant evidence that a functional barrier to calcium diffusion exists where the sarcoplasmic reticulum of vascular smooth muscle is closely approximated to the plasma membrane. Recent evidence suggests that localized micromolar calcium concentrations may be achieved in this space within several milliseconds of cell activation, before significant changes in concentration occur in the rest of the cell.

In our cells, it remains to be determined what the time course of activation of this current is under physiological conditions and how it may contribute to the initiation and maintenance of contraction. As previously discussed, multiple studies have shown transient inward currents in response to vasoconstrictor agonists. In rat aorta, norepinephrine induced an increase in chloride efflux with a calculated increase in permeability that exceeded that for potassium. This increase in efflux displayed an early peak (<1 minute) and a plateau phase. Removal of extracellular calcium 10 minutes before norepinephrine treatment blocked 50% of the early response and totally abolished the plateau phase. This suggests that a chloride conductance may play a role not only in the initial phase of contraction, when intracellular calcium is being released, but also during the maintenance phase, when intracellular calcium levels have stabilized. The rate of decay of the tail current measured in the present study is clearly voltage dependent. It remains to be seen to what extent, if any, the decay of the current is related to the rate of decline of intracellular calcium concentration after a depolarizing pulse. In the presence of an agonist, the chloride current may persist for an extended period of time and act to maintain depolarization, or it may indeed be a transient phenomena and contribute only to the initial phase of vascular smooth muscle cell activation.

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F S Lamb, K A Volk and E F Shibata

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