Current Fluctuations and Oscillations in Smooth Muscle Cells From Hog Carotid Artery
Role of the Sarcoplasmic Reticulum

Michel Désilets, Steven P. Driska, and Clive Marc Baumgarten

Electrical activity of enzymatically isolated, smooth muscle cells from hog carotid arteries was recorded under current clamp and voltage clamp. Under the experimental conditions, membrane potential usually was not stable, and spontaneous hyperpolarizing transients of approximately 100-msec duration were recorded. The amplitude of the transients was markedly voltage dependent and ranged from about 20 mV at a membrane potential of 0 mV to undetectable at membrane potentials negative to -60 mV. Under voltage clamp, transient outward currents displayed a similar voltage dependency. These fluctuations reflect a K+ current; they were abolished by 10 mM tetraethylammonium chloride, a K+ channel blocker, and the current fluctuations reversed direction in high extracellular K+ concentration. Modulators of intracellular Ca2+ concentration also affected electrical activity. Lowering intracellular Ca2+ concentration by addition of 10 mM EGTA to the pipette solution or suppressing sarcoplasmic reticulum function by superfusion with caffeine (10 mM), ryanodine (1 μM), or histamine (3-10 μM) blocked the rapid voltage and current spikes. However, caffeine and histamine induced a much slower hump of outward current before blocking the rapid spikes. This slower transient outward current could be elicited only once after external Ca2+ was removed and is consistent with an activation of K+ channels by Ca2+ released from internal stores. In contrast, removal of external Ca2+ alone failed to abolish the rapid spikes. These results suggest that 1) a Ca2+-dependent K+ conductance can markedly affect the electrical behavior of arterial smooth muscle cells and 2) internal Ca2+ stores, probably the sarcoplasmic reticulum, can support rapid and frequent releases of Ca2+. Exposure to a low concentration of histamine (3 μM) caused synchronization of the irregular, rapid fluctuations giving rise to slow, periodic oscillations of Ca2+-activated K+ conductance with a frequency of 0.1-0.3 Hz. These regular oscillations are reminiscent of periodic Ca2+-induced Ca2+ release, were inhibited by 10 mM caffeine, and point to a modulation of sarcoplasmic reticulum Ca2+ release by histamine. (Circulation Research 1989;65:708-722)
Electrical Recordings

Cells were superfused in a 0.3-mL glass-bottom chamber placed on the stage of an inverted microscope. The superfusion solution had, unless otherwise specified, the following composition (mM): NaCl 150, KCl 5, CaCl₂ 1.8, Na₂HPO₄ 1.75, MgCl₂ 1.2, glucose 10, and N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) 5. The solution was adjusted to pH 7.4 with NaOH and equilibrated with 100% O₂. The bath flow rate was maintained at 4–5 mL/min, and the temperature was kept at 37±0.5°C. Agents were applied by switching to a new superfusate. Ryanodine (Penick Laboratories, Lyndhurst, New Jersey) and histamine dihydrochloride (Sigma, St. Louis, Missouri) were added to the superfusate from prepared stock solutions; caffeine (Sigma), and tetraethylammonium chloride (TEA; Aldrich, Milwaukee, Wisconsin) were added as powders.

Membrane potential was recorded with fine suction pipettes, having a resistance of about 40 MΩ in the superfusion solution. Their filling solution usually contained (mM) K aspartate 95, KCl 20, KH₂PO₄ 1, K₃ATP 5, tris(hydroxymethyl)aminomethane-phosphocreatine 2, MgCl₂ 2.5, tauroine 10, HEPES 5, adjusted to pH 7.3 with KOH (1–3 µM free Ca²⁺), as measured with a Ca²⁺-sensitive electrode (Orion, Cambridge, Massachusetts). In some cases, 10 mM ethylene glycol-bis-(β-aminopropyl ether)-N,N,N',N'-tetraacetic acid (EGTA) was added to reduce the free [Ca²⁺] in the electrode and cell (see "Results"). Based on the change in junction potential of potassium aspartate–filled electrodes on switching between superfusion and potassium aspartate solutions (grounded through a 3 M KCl flowing junction), Eₘ recorded by potassium aspartate–filled electrodes was estimated as 7.8±0.5 mV (n=4) positive to its true value. Results are presented after correcting the measured Eₘ by this estimate. Similar potassium aspartate–filled electrodes were found by a different method to underestimate Eₘ by 9.8 mV in cardiac myocytes. In a few initial experiments, electrodes filled with 150 mM KCl were used. As previously described, these give a value of Eₘ 4.9 mV positive to its true value, and the reported Eₘ are corrected.

Membrane potential was voltage-clamped with the chop-clamp technique (Axoclamp 2A, Axon Instruments, Burlingame, California) using a chopping frequency of 5–10 KHz. The chopping frequency was chosen using a high-gain display to verify that measured Eₘ was within less than 1 mV of its true value. Usually the electrodes were dipped in Dow Corning 200 silicone oil (dimethyldipylsioxane; Dow Corning, Midland, Michigan) just before their use. This prevented creep of solution along the electrode wall and improved their response time. In some cases, a layer of polystyrene (Q-Dope, GC/Thorsen, Rockford, Illinois) was applied instead. The chop-clamp method permits the use of higher resistance electrodes than series resistance compensation methods, and dialysis of the myocytes can be minimized.

Signals were displayed on an oscilloscope and a chart recorder (model 2400S, Gould, Cleveland, Ohio) and were simultaneously stored on FM tape (HP 3964, Hewlett-Packard, Palo Alto, California). Data subsequently were reproduced directly on the chart recorder or digitized for computer analysis. The sampling rate in the latter case depended on the signal to be analyzed, but the data were always low-pass filtered (8-pole Butterworth) at a corner frequency less than or equal to one half of the signal frequency.
sampling rate. Data acquisition, mathematical analysis, and digital plotting were performed on an IBM PC-AT by an integrated software package (ASYST, ASTI, Rochester, New York).

Results

Spontaneous Voltage and Current Fluctuations

The initial events on recording from arterial myocytes were similar to those described in visceral smooth muscle cells. $E_m$ was transiently 10–20 mV more negative than the basal value attained within a few seconds. Access to the cell interior also invariably induced a contraction that was either localized around the site of impalement and caused shortening of the cell by less than 10% or that was more widespread and caused shortening of the cell by approximately 50%. The initial depolarization was not paralleled by cell elongation, however (compare with reference 9).

$E_m$ was not constant under these conditions; large, rapid voltage fluctuations were usually observed. A typical example is shown in Figure 1. The fluctuations consisted of hyperpolarizing spikes with a duration of about 100 msec and an amplitude of up to 15 mV at this level of $E_m$. These rapid spikes are referred to as transient hyperpolarizations (TH). The basal $E_m$ from which THs arose varied from cell to cell and ranged from about -10 to -30 mV when measured with potassium aspartate-filled pipettes. Somewhat more negative $E_m$, up to -45 mV after correction for junction potential, were obtained from pipettes filled with 150 mM KCl. This difference may be related to a greater diffusion of K+ from KCl-filled electrodes than from those filled with potassium aspartate. The low mobility of aspartate and consequent junction potential should restrain K+ diffusion from the pipette to the sarcoplasm (compare with Reference 16). Figure 1 also shows the recording from a second suction pipette applied to the same cell. The voltage fluctuations followed each other very closely. Such simultaneous recordings indicate that THs were not an artifact arising at the electrode tip and demonstrate the spatial uniformity of $E_m$.

Rapid, spontaneous spikes of outward current, $I_{to}$, which are related to the THs in voltage recordings, were observed under voltage clamp. A comparison between the voltage and current transients is presented in Figure 2. First, THs were recorded in the absence of current injection (Figure 2A). Switching to voltage-clamp mode (Figure 2B) revealed an $I_m$ with a maximum amplitude of about 150 pA. The shape of the current spikes was similar to that of the voltage transients. Plots of the respective power spectral densities are shown in Figure 2C. Most of the power in the current spectrum (lower panel) was distributed below 20 Hz, maxima were present near 2 and 6 Hz, and the spectral density decreased at frequencies less than 2 Hz. The voltage spectrum was generally similar; some frequency shifts are expected due to the complex impedance of the membrane. As elegantly discussed by Kass and Tsien, this type of analysis leads to important conclusions regarding the mechanism underlying the fluctuations. First, the presence of $I_{to}$ at a constant voltage rules out the involvement of $E_m$-dependent conductances as a critical step in generating the fluctuations. Second, a maximum in the current power spectrum at frequencies different from 0 Hz indicates that the fluctuations are not due to simple random opening and closing of ion channels because this behavior would generate a Lorentzian spectral density function (or a sum of Lorentzians) with a maximum at 0 Hz.

K+ Conductance Underlies the Fluctuations

The experiment illustrated in Figure 3 demonstrates the voltage dependence of $I_m$ and its sensitivity to TEA, a K+ channel blocker. $E_m$ was held at -48 mV, and 3-second test pulses were applied every 10 seconds. Under control conditions (Figure 3A), $I_m$ was detectable at potentials positive to -40 mV, sharply increasing in amplitude and frequency as the test potential was made more positive (also see Figure 4). The current spikes were virtually abolished by 10 mM TEA (Figure 3B); this occurrence suggests that $I_m$ reflects changes in K+ conductance. TEA also reduced the outward-going rectification of the steady-state current (as measured at 3 seconds) and appeared to inhibit a slowly decaying outward current component that was more clearly revealed in TEA at potentials positive to -10 mV. However, TEA had virtually no effect on...
the holding current near the "resting" potential. This is consistent with the failure of low concentrations of TEA to depolarize several arterial preparations. Very little inward current was elicited by the depolarizing pulses, although a net inward current was detectable when TEA was added (e.g., for the depolarization pulse to $-8 \text{ mV}$). Finally, the effects of TEA were reversible (Figure 3C), and the amplitude of $I_o$ sometimes was greater after wash-out than during the control period.

As expected, the main characteristics observed under voltage clamp were reproduced as voltage changes during constant current injections (not shown). The amplitude of the TH sharply increased with depolarization positive to $-40 \text{ mV}$; THs were reversibly inhibited by 10 mM TEA, and a slow depolarization occurred at $E_m$ positive to $-10 \text{ mV}$, but the basal $E_m$ was unchanged.

The effectiveness of TEA in inhibiting $I_o$ and THs strongly suggests involvement of a $K^+$ conductance. Consequently, modification of extracellular $K^+$ concentration ($[K^+]_o$) should alter the fluctuations. An example of the effect of increasing $[K^+]_o$ from 5 to 155 mM by equimolar replacement of Na$^+$ is shown in Figure 4. In 5 mM $[K^+]_o$, the $I_o$ spikes grew in amplitude monotonically at voltages positive to $-40 \text{ mV}$ and were not detectable at more negative potentials. Increasing $[K^+]_o$ to 155 mM induced cell shortening and complicated the voltage-dependence of the spikes. $I_o$ was still outward at positive voltages, but now it reversed near $+7 \text{ mV}$. The amplitude of the inward $I_o$ was maximum at about $-30 \text{ mV}$ and decreased at more hyperpolarized potentials. This pattern can be accounted for by a strong outward-going rectification of the $K^+$ conductance (due to either voltage dependence of channel gating or unitary conductance) and explains the absence of a current reversal at negative potentials (i.e., near $-80 \text{ mV}$) in 5 mM $[K^+]_o$. The voltage-dependence of the transients is quantified in Figure 4B where the variance of the current is plotted as a function of test potential. To avoid the effect of the initial decaying outward phase of the background current, variances were calculated from the last 1.5 seconds of each pulse. The current variance in 5 mM $[K^+]_o$ rose steeply at potentials positive to $-40 \text{ mV}$. In 155 mM $[K^+]_o$, the variance interpolated to a minimum at $+10 \text{ mV}$, the reversal potential of $I_o$. Similar results were obtained in five cells.

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Voltage and current fluctuations and spectral densities. Panel A: Voltage fluctuations (upper tracing) recorded without current injection. Basal membrane potential was $-27 \text{ mV}$, and typical spontaneous hyperpolarizing transients are shown. Bottom tracing is expanded portion of upper trace (marked by bar) showing spikes with a duration of 60–90 msec. Panel B: Current tracing recorded less than 1 minute later, after clamping membrane potential to $-29 \text{ mV}$ (upper tracing). Spontaneous outward current fluctuations of up to 150 pA were elicited (middle tracing); their shape and duration were similar to those of voltage spikes. Panel C: Spectral densities determined for voltage (upper graph) and current (lower graph) by fast Fourier transform analysis (ASYST) of 4096 points obtained at 3-msec intervals (8-pole Butterworth, low-pass filtered at 100 Hz). Spectral density was distributed mainly below 20 Hz, and maxima were between 2 and 6 Hz. Decrease in spectral density at less than 2 Hz is more obvious in current data.
FIGURE 3. Effect of membrane potential ($E_m$) and tetraethylammonium chloride (TEA) on current fluctuations. Currents were obtained before (panel A), during (panel B), and 5 minutes after washout of 10 mM TEA (panel C). Holding potential ($E_h$) was $-48 \text{ mV}$. $E_m$ was varied by 3-second pulses to potentials between $-88 \text{ mV}$ and $+12 \text{ mV}$, denoted by $E_p$, at 10-second intervals. $E_p$ and zero current levels (panel A, bars) apply to all panels. Analogous to voltage fluctuations, spontaneous current spikes were voltage dependent, with amplitude and frequency increasing with depolarization. TEA reversibly abolished current transients. TEA also reduced both outward rectification of steady-state current and a slowly decaying phase of current that was evident at $+12 \text{ mV}$. Some capacitive transients were off scale.

To consider the voltage range over which conductance fluctuates, the variance of the conductance ($\sigma_g^2$) was calculated from the variance of the current ($\sigma_I^2$) by:

$$\sigma_g^2 = \sigma_I^2 / (E_m - E_{rev})^2$$

where $E_{rev}$ is the reversal potential. Taking $E_{rev}$ as $+10 \text{ mV}$ in 155 mM $[K^+]_o$, the voltage-dependence of $\sigma_g^2$ calculated from the data in Figure 4B is shown in Figure 5. The $\sigma_g^2$ increased beginning at about $-60 \text{ mV}$ and appeared to plateau near $+25 \text{ mV}$. This type of analysis also was performed for the curve obtained in 5 mM $[K^+]_o$. Assuming that the channels are perfectly selective for $K^+$ and that intracellular $K^+$ concentration ([K$^+$]) did not change, the $E_{rev}$ in 5 mM $[K^+]_o$ can be calculated as $-82 \text{ mV}$ from the measured $E_{rev}$ in 155 mM $[K^+]_o$. When this value is used, $\sigma_g^2$ in 5 mM $[K^+]_o$ increased at potentials positive to $-40 \text{ mV}$ and failed to plateau by $+37 \text{ mV}$. Similar results were obtained in five cells.

The Role of Intracellular Ca$^{2+}$

The ubiquitous occurrence of Ca$^{2+}$-activated K$^+$ channels in smooth muscle$^{2-10}$ and the contraction observed on impaling the myocytes suggested that elevated intracellular Ca$^{2+}$ might play a role in $I_{to}$ and the TH. This hypothesis was tested simply by adding 10 mM EGTA to the filling solution of the suction pipette. Diffusion of EGTA into the cell should lower the basal [Ca$^{2+}$], and reduce or abolish [Ca$^{2+}$], fluctuations that might arise. In five of five cells studied with EGTA-containing pipettes, $I_{to}$ and TH were never observed after 5 minutes. In contrast, spontaneous fluctuations were observed and well maintained in more than 80% of cells (n=58) when EGTA was not included. These results strongly implicate increased [Ca$^{2+}$] in the genesis of the fluctuations and, together with the effects of TEA and [K$^+$], suggest that a Ca$^{2+}$-activated K$^+$ conductance underlies the phenomenon.

Ca$^{2+}$ From Intracellular Stores Activates the K$^+$ Conductance

The Ca$^{2+}$ responsible for activating the K$^+$ conductance may come from internal stores or from outside the cell. To differentiate between these possibilities, we examined the effect of removal of extracellular Ca$^{2+}$ on the transient spikes. Figure 6 shows the effect of switching from 1.8 mM extracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]) to a nominally Ca$^{2+}$-free bathing media for nearly five minutes and then returning to 1.8 mM [Ca$^{2+}$]. The amplitude and frequency of $I_{to}$ diminished gradually in Ca$^{2+}$-free solution and recovered gradually. Since transmembrane entry of Ca$^{2+}$ should have been virtually
FIGURE 4. Effect of extracellular \( K^+ \) concentration \([K^+]_o\) on voltage-dependent current fluctuations. Replacing 150 mM of bathing solution Na\(^+\) with K\(^+\) caused a reversal of current spikes. Panel A: Currents in a cell exposed to 5 mM \([K^+]_o\) (left column) and then to 155 mM \([K^+]_o\) (right column). Membrane potential was clamped from holding potential \((E_h = -53 \text{ mV})\) to pulse potentials \((E_p)\) between \(-113 \text{ mV} \) and \(+27 \text{ mV}\) for 3 seconds at 12-second intervals. In 155 mM \([K^+]_o\), reversal potential of current fluctuations was near \(+7 \text{ mV}\), but a reversal potential could not be defined in 5 mM \([K^+]_o\). Panel B: Plot of the variance of the current as a function of \(E_p\). Current variance was calculated from last 1.5 seconds of each pulse (same cell as panel A). Variance in 5 mM \([K^+]_o\) (•) increased monotonically with depolarization, while 155 mM \([K^+]_o\) (▲) induced a biphasic relation with a minimum value, representing the reversal potential, interpolated to \(+10 \text{ mV}\). Ordinate was broken to include variance at \(+27 \text{ mV}\) in 5 mM \([K^+]_o\).

abolished in a matter of seconds (solution changes were complete in less than 10 seconds), these data suggest that extracellular \(Ca^{2+}\) is not primarily responsible for activating the \(K^+\) conductance. On the other hand, the slow changes are consistent with the progressive depletion of intracellular \(Ca^{2+}\) stores expected under these conditions. Depression of the transients tended to be greater after longer duration exposure to \(Ca^{2+}\)-free solution and varied from cell to cell.

The effects of caffeine\(^{12,13,21}\) and ryanodine\(^{14}\) suggest that the SR is involved in triggering \(I_{ca}\) and TH. Records from a typical experiment are shown in Figure 7. Exposure to 10 mM caffeine quickly and reversibly inhibited \(I_{ca}\). Ryanodine (1 \(\mu M\)) was as efficacious as caffeine in blocking the current spikes although the effect developed more slowly, taking 5–10 minutes for full inhibition. Further, inhibition by ryanodine appeared irreversible; washout for more than 30 minutes never produced a recovery of \(I_{ca}\). Both caffeine and ryanodine diminished outward-going rectification of the background current as well as its early decaying phase. The latter component was more prominent in the presence of ryanodine, however, (compare with Figure 7B and 7D). This difference was particularly evident in the experiment illustrated and may in part reflect changes of cell properties with time rather than differential effects of ryanodine and caffeine.

Another observation consistent with the idea that \(Ca^{2+}\) from the SR activated \(K^+\) conductance was that caffeine but not ryanodine induced a large, slow, transient hyperpolarization before blocking the rapid spikes. This effect is depicted in Figure 8. Addition of caffeine (10 mM, upper trace) caused a nearly 20-mV hyperpolarization that decayed in about 10 seconds. The effect of caffeine was rapidly reversible (lower trace). Practically complete recovery of the TH was observed after about 10 seconds of washout (excluding the 10-second period needed to clear the dead space in the superfusion system). Corresponding caffeine-induced outward currents were present under voltage-clamp conditions, as illustrated in Figure 9A. The inhibition of \(I_{ca}\) spikes was preceded by a slow, transient, outward current (current hump) that lasted about 10 seconds. In a manner similar to \(I_{ca}\), the current hump was voltage-sensitive and completely disappeared at holding potentials negative to \(-50 \text{ mV}\). Independent of holding potential, cell shortening usually
Addition of caffeine 6 minutes after superfusion with about 0.1 \( \mu \)M free-Ca\(^{2+}\) solution (1 mM CaCl\(_2\) with 2 mM EGTA) induced a current hump. In contrast to findings in 1.8 mM [Ca\(^{2+}\)]\(_o\), reexposure to caffeine in low [Ca\(^{2+}\)]\(_o\) solution failed to produce a second transient. Similarly, \( I_{\text{to}} \) did not resume after caffeine washout. This behavior was expected inasmuch as removal of external Ca\(^{2+}\) should prevent reloading of depleted intracellular stores. Return to 1.8 mM [Ca\(^{2+}\)]\(_o\), presumably by reloading intracellular stores, rekindled both \( I_{\text{to}} \) and the ability of caffeine to induce an outward current hump although the amplitudes of the transients often remained depressed.

Another effect of caffeine is obvious in Figure 9. While caffeine blocked \( I_{\text{to}} \), it consistently induced a low amplitude background noise. This noise was more apparent in low [Ca\(^{2+}\)]\(_o\) and was abolished by washout of caffeine (Figures 9B and 9C).

**Effect of Histamine**

Histamine is reported to modify intracellular Ca\(^{2+}\) homeostasis, and its effects on the voltage and current fluctuations were analogous to those of caffeine. As illustrated in Figure 10, 10 \( \mu \)M histamine reversibly inhibited TH and \( I_{\text{to}} \), and blockade was preceded by a slow hyperpolarization and corresponding outward current hump. Usually the cell was exposed to histamine for only 40–60 second periods. Prolonged exposure often resulted in only partial recovery after washout and caused desensitization. An interesting characteristic of histamine washout was the pattern of recovery of the transients. Both TH and \( I_{\text{to}} \) initially returned as bursts of 2–10 spikes that eventually fused together to give the usual random appearance.

Experiments in Ca\(^{2+}\)-free solution tested whether histamine directly affects intracellular Ca\(^{2+}\) stores or transmembrane Ca\(^{2+}\) fluxes. The results, shown in Figure 11, were very similar to those obtained with caffeine (compare with Figure 9). Histamine added 2 minutes after superfusion with nominally Ca\(^{2+}\)-free solution induced an outward current hump followed by inhibition of \( I_{\text{to}} \) (Figure 11A). The time course of these events was slower than that observed with caffeine, however.
FIGURE 7. Effect of caffeine and ryanodine on current fluctuations. Panels show sequential exposure of a cell to control solution, 1.8 mM [Ca²⁺],, (panel A); 10 mM caffeine (panel B); 2 minutes after washout of caffeine (panel C); and 6 minutes after exposure to 1 μM ryanodine (panel D). Holding potential (Ező), -48 mV, and pulse potentials (Ep) were the same in each panel (bar indicates 0 current). Both caffeine and ryanodine blocked voltage-dependent current spikes and also reduced outward rectification of steady-state current. In this example, slow decaying current elicited at +2 mV appeared to increase with time and was more evident after washout of caffeine and in ryanodine.

the whole process took about 1 minute compared with about 10 seconds with caffeine. With no added extracellular Ca²⁺, Ilo did not resume after histamine washout, and a second exposure to histamine did not produce a current hump (Figure 11B). Reperfusion with 1.8 mM [Ca²⁺], reinitiated the Ilo and restored the effectiveness of histamine (Figure 11C). The outward current humps induced by histamine were generally smaller than those with caffeine. However, a transient increase in Ilo frequency often occurred during the hump. This feature was especially evident in the absence of extracellular Ca²⁺ (Figure 11A). Overall, these experiments are consistent with the idea that, like caffeine, 10 μM histamine depleted Ca²⁺ from intracellular stores. However, the timing and characteristics of the action of histamine may reflect slower or less synchronous release of Ca²⁺.

The occurrence of bursts of spikes during washout of 10 μM histamine (see Figure 10) suggested that lower concentrations of histamine may induce synchronous behavior. Examples of stable, voltage and current oscillations initiated by 3 μM histamine are shown in Figure 12. The oscillations appeared as fused bursts of spikes with a regular period ranging from 3 to 10 seconds in different myocytes and developed within 2–5 minutes after exposure. Conversely, spikes were suppressed between oscillations. In a fashion similar to Ilo, the oscillation amplitude increased on depolarization, and they were never detected negative to -40 mV. The central role played by intracellular Ca²⁺ stores in the generation of these oscillations was demonstrated with caffeine. An example is depicted in Figure 13. After initiating oscillations with 3 μM histamine, 10 mM caffeine was added. Caffeine caused the typical outward current hump followed by complete inhibition of the histamine-induced oscillations (again, a low amplitude background current noise became apparent). The oscillations reappeared within 2 minutes of caffeine washout.

Discussion

Spontaneous Fluctuations of Ca²⁺-Activated K⁺ Conductance in Smooth Muscle Cells

Large THs and corresponding Ilo under voltage clamp were prominent features of recordings from
Caffeine-induced transient hyperpolarization. Basal membrane potential was maintained at —6 mV by constant current injection. Addition of 10 mM caffeine induced a transient hyperpolarization of about 20 mV that lasted 10 seconds. Rapid hyperpolarizing spikes were not present during the relaxation phase of this transient. The lower trace, recorded 1 minute later, demonstrates rapid recovery of voltage spikes after washout of caffeine. Bar (left) denotes 0 mV.

FIGURE 8.

single, hog carotid artery, smooth muscle cells. These phenomena appear to reflect transient activation of a K+ conductance by [Ca2+]j fluctuations. As expected for a Ca2+-activated K+ conductance, the fluctuations were blocked by TEA, progressively inhibited by hyperpolarizing potentials, and reversed by increasing [K+]o. 3,5,7 Suppression of the transients when 10 mM EGTA was added to the recording pipette directly implies involvement of [Ca2+]j. Several experiments argue that Ca2+ release from intracellular stores, presumably the SR, underlies the Ca2+ fluctuations: 1) The transients were resistant to Ca2+-free media, indicating that the proximate source of Ca2+ was intracellular. 2) In contrast, the transients were abolished by caffeine, histamine, and ryanodine, agents that act on the SR by different mechanisms. 3) Consistent with involvement of intracellular stores, fluctuations failed to resume after washout of caffeine and histamine in Ca2+-free media and only gradually resumed after reexposure to extracellular Ca2+ allowed refilling of intracellular stores.

Spontaneous transients with a similar duration and voltage dependence were observed in cultured mouse and bullfrog ganglion cells several years ago and in myocytes from rabbit jejunum and ear artery and rabbit ileum recently. Benham and Bolton concluded, as we have, that the fluctuations reflect activation of Ca2+-dependent K+ current (I_{K,[Ca2+]}) after spontaneous release of Ca2+ from intracellular stores. They provided a similar line of evidence that included the following: the fluctuations were blocked by TEA, Ba2+, and internal dialysis with EGTA; reduction of Ca2+ influx by

FIGURE 9. Effect of caffeine on current fluctuations. Trace at left show effect of 10 mM caffeine (horizontal bars) and trace at right show steady-state current recorded after washout of caffeine for 2 minutes; holding potential was —8 mV. Panel A: Hump of outward current was induced by caffeine in presence of 1.8 mM extracellular Ca2+ concentration ([Ca2+]o). Panel B: Superfusion with very low [Ca2+]o (≈0.1 μM; 2 mM EGTA +1 mM CaCl2 for 6 minutes substantially decreased frequency of spikes, but caffeine still induced a large hump of outward current. Note increased basal current noise in caffeine and absence of outward spikes after washout. Panel C: Cell was reexposed to caffeine 5 minutes later. Outward current hump could not be elicited a second time in low [Ca2+]o media (panel C). Panel A was obtained 8 minutes after washout of low [Ca2+]o media (panel C). Recovery of spike amplitude was incomplete.
Cd2+ or removal of extracellular Ca2+ (for vascular but not visceral myocytes) failed to abolish the spikes; and caffeine induced a rapid discharge followed by inhibition of the transients. The frequency of transients reported by Benham and Bolton2 appeared to be significantly lower than in the present study. This difference is most likely explained by their use of room temperature rather than 37° C. Nevertheless, the striking similarity of the present results and those of Benham and Bolton2 suggests that these phenomena may be common in vascular smooth muscle.

Several types of Ca2+-activated K+ channels have been identified: a high conductance maxi channel,4-7,28-30 a low conductance channel,5,29,31 and, in portal vein, an intermediate conductance channel that is sensitive to [Ca2+]o and [Ca2+]i > 10 μM and is insensitive to voltage.32 Which type of Ca2+-activated K+ channel is responsible for the behavior observed here? The intermediate conductance channel can be eliminated immediately because neither its voltage- nor [Ca2+]o-dependence correspond to the present results. Block of fluctuations by 10 mM TEA (see Figure 3) suggests that

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**Figure 10.** Effect of histamine on voltage and current fluctuations. Panel A: Effect of 10 μM histamine on spontaneous hyperpolarizing spikes. Basal membrane potential (E_m) was maintained at −5 mV by constant current injection. Histamine abolished voltage spikes after inducing a small transient hyperpolarization. Panel B: In the same cell clamped at −8 mV, histamine inhibited current spikes after inducing a small outward current hump. During washout of histamine, both current spikes and E_m hyperpolarizations initially occurred as bursts separated by silent periods. I_m, membrane current.

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**Figure 11.** Effect of histamine on current fluctuations in low extracellular Ca2+ concentration ([Ca2+]o). Panel A: After 2-minute superfusion in nominally Ca2+-free solution, 10 μM histamine was introduced. An outward current hump preceded inhibition of spikes. As with caffeine, spontaneous spikes showed very little recovery after histamine washout in 0 [Ca2+]o. Panel B: Reexposure to histamine 4 minutes later failed to cause detectable changes in membrane current. Panel C: After reperfusion with 1.8 mM [Ca2+]o for 10 minutes, histamine again elicited an outward current hump and inhibited the rapid spikes. Holding potential was −8 mV.
maxi channels are responsible. Maxi channels are known to be sensitive to low concentrations of TEA,3,5,30,33 whereas low conductance channels are quite resistant to TEA.5,34 The involvement of maxi channels was confirmed with apamin, a blocker of low conductance but not maxi $I_{K(Ca)}$ channels.34 Superfusion with 50 or 500 nM apamin for 15 minutes (n=2) had no effect on the fluctuations (authors' unpublished observations); 10-minute exposure to 10 nM apamin is sufficient to block the low conductance channel.34

**Voltage- and Ca$^{2+}$-Dependence of Transient Currents**

A large increase in amplitude with depolarization and an apparent absence of a reversal at negative potentials when superfusing with 5 mM [K+]o are notable in the voltage-dependence of $I_{to}$. This behavior reflects both the voltage- and Ca$^{2+}$-dependence of $I_{K(Ca)}$ in smooth muscle4,5,7 and other tissues.35

The probability of channel opening ($P_{open}$) is a sigmoidal function of $E_m$, which becomes negligible at negative potentials. Increasing Ca$^{2+}$ at the internal face of excised membrane patches shifts the $P_{open}$-voltage relation to more negative potentials by 30–60 mV/10-fold increment.4,28–30,35 The absence of an $I_{to}$ reversal in 5 mM [K+]o indicates that the underlying channels fail to open near the reversal potential at the prevailing [Ca$^{2+}$]. In contrast, the reversal potential in 155 [K+]o was within the voltage range of channel activity.

Assuming, as was shown for astrocytoma $I_{K(Ca)}$ channels,28 that Ca$^{2+}$ sensitivity is not altered by excision of a patch, the voltage dependence of the fluctuations allows approximation of the [Ca$^{2+}$]i transients in the carotid artery myocytes. In excised patches from guinea pig mesenteric artery myocytes, $P_{open}$ increases from near 0 at −40 mV to 0.5 at +40 mV in 0.2 μM [Ca$^{2+}$], and was nearly constant ($P_{open}=0.6–0.7$) between −60 and +40 mV.

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**Figure 12.** Rhythmic oscillations of voltage and current induced by 3 μM histamine. Panel A: Voltage oscillations after a 15-minute exposure to histamine. Bar at right indicates 0 mV. Panel B: Current oscillations 3 minutes after addition of histamine (different cell than in panel A). Holding potential ($E_h$) was switched for 1-minute periods to potentials between −53 and +7 mV in a random order. The amplitude of the slow oscillations was strongly voltage dependent, but the periodicity was little affected by membrane potential. Bar at right indicates 0 pA.

**Figure 13.** Effect of caffeine on histamine-induced current oscillations. Oscillations were induced by 3 μM histamine at holding potential of +7 mV. After 8 minutes, 10 mM caffeine (upper trace) caused an outward current hump followed by inhibition of oscillations. Discernible current oscillations reappeared 1.5 minutes after caffeine washout (bottom trace).
in 1 μM [Ca\textsuperscript{2+}]. These data can be compared with the voltage range for Ca\textsuperscript{2+}-activated K\textsuperscript{+} channel activity shown in Figures 4 and 5. The threshold for activating I\textsubscript{K(CO)} channels was positive to -40 and -60 mV in 5 and 155 mM [K\textsuperscript{+}], respectively. Although outward-going rectification of the single channel conductance is likely to have contributed to the voltage-dependence of the transients in 5 mM [K\textsuperscript{+}], the unitary current-voltage relation for I\textsubscript{K(CO)} is linear over a wide voltage range in symmetrical K\textsuperscript{+} solutions. Consequently, the voltage dependence of the current fluctuations in 155 mM [K\textsuperscript{+}] should reflect variations in P\textsubscript{open}, and fluctuations at a constant voltage should reflect variation of [Ca\textsuperscript{2+}]. If the I\textsubscript{K(CO)} channels in the carotid are like those in mesenteric artery, the threshold of the fluctuations suggests that [Ca\textsuperscript{2+}] may be less than 0.2 μM during quiescence, and the voltage dependence suggests that [Ca\textsuperscript{2+}] was less than 1 μM at the peak of the transients.

These estimates of the [Ca\textsuperscript{2+}] transient imply that current fluctuations are likely to occur physiologically. [Ca\textsuperscript{2+}] can rise to micromolar levels during contraction of smooth muscle and should activate outward current via I\textsubscript{K(CO)} channels. The subsequent hyperpolarization will limit Ca\textsuperscript{2+} entry and modulate the contractile state of the vessel. Such inferences must be regarded as quite speculative, however. The sensitivity of I\textsubscript{K(CO)} to [Ca\textsuperscript{2+}] varies greatly in different cell types. For example, P\textsubscript{open} for I\textsubscript{K(CO)} channels in toad stomach muscle ranges from 0 at -50 mV to 0.8 at +40 mV in 1 μM [Ca\textsuperscript{2+}]. I\textsubscript{K(CO)} channels in some secretory cells are greater than 10 times more sensitive to [Ca\textsuperscript{2+}] (compare with reference 30), while those in skeletal muscle are greater than 10 times less sensitive. Furthermore, it is likely that [Ca\textsuperscript{2+}] varies with voltage.

**Does Cyclic Release of Ca\textsuperscript{2+} From the SR Trigger Current Fluctuations?**

The inhibitory effect of both caffeine and ryanodine provided pharmacological evidence of the involvement of intracellular Ca\textsuperscript{2+} stores in the generation of spontaneous voltage and current fluctuations. Caffeine is well known to release Ca\textsuperscript{2+} from internal stores. Vascular contractions elicited upon exposure to histamine have a phasic component that has been attributed to intracellular
Ca\textsuperscript{2+} release.\textsuperscript{12,22} As with caffeine, histamine-induced contractile transients can be evoked in the absence of [Ca\textsuperscript{2+}]\textsubscript{o} but are not repeatable with subsequent additions of the drug.\textsuperscript{12} A histamine-induced release of Ca\textsuperscript{2+} from intracellular stores was directly demonstrated recently by fluorescent determination of [Ca\textsuperscript{2+}], in cultured aortic myocytes bathed in Ca\textsuperscript{2+}-free medium.\textsuperscript{25} Our results are consistent with these observations and indicate that histaminergic receptors remain functional in isolated arterial cells.

Interestingly, lower concentrations of histamine (3 \textmu M) not only caused an inhibition of the rapid current spikes, but also induced stable slow oscillations of holding current or corresponding hyperpolarizations. Furthermore, a transient synchronization of the current bursts occurred during washout of 10 \textmu M histamine. These oscillations may be involved in histamine-induced rhythmic contractions of carotid artery.\textsuperscript{45} Although the present results do not address the mechanism of synchronization, the voltage dependence of the oscillations and the reversible inhibitory effect of caffeine point to a modulation of cyclic Ca\textsuperscript{2+} release from SR by histamine. A similar but transient oscillatory response to histamine by Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels in HeLa cells has been reported recently.\textsuperscript{46} Furthermore, slow rhythmic increases of K\textsuperscript{+} conductance can be induced by caffeine in bullfrog sympathetic ganglion cells and were attributed to caffeine-induced cyclic release of Ca\textsuperscript{2+} from internal stores.\textsuperscript{47} Cultured sympathetic ganglion cells from bullfrog also exhibit spontaneous rapid fluctuations of I\textsubscript{K(Ca)}.\textsuperscript{27} In view of these findings, it appears likely that spontaneous fast transients and slow oscillations in arterial smooth muscle may reflect differing kinetic properties of cyclic release of Ca\textsuperscript{2+} from internal stores under varied conditions, as opposed to the oscillations reflecting an intrinsic property of the histaminergic system.

The actions of histamine are more complex than was demonstrated in the present experiments. Histamine usually causes tonic contraction of large arteries including the hog carotid, but under some conditions, rhythmic contractions are observed.\textsuperscript{45} Although Ca\textsuperscript{2+} influx is thought to be augmented by histamine, part of the tonic contraction persists in the absence of external Ca\textsuperscript{2+}.\textsuperscript{12} In studies on multicellular preparations, histamine depolarized smooth muscle from rabbit ear\textsuperscript{22} and pulmonary\textsuperscript{48} artery but hyperpolarized dog coronary artery.\textsuperscript{24} The latter effect was blocked by TEA and Mn\textsuperscript{2+}, leading the author to conclude that the hyperpolarization was due to an increased Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductance. These results indicate that the net effect of histamine may depend on the relative contribution of Ca\textsuperscript{2+} and Ca\textsuperscript{2+}-activated K\textsuperscript{+} currents and, therefore, may vary in different tissues and species. Overlap of a histamine-induced inward calcium current (I\textsubscript{Ca}) could account for the relatively small magnitude of the slow outward current hump observed by histamine (Figures 10 and 11) and could explain, at least partially, the larger outward current hump observed when external Ca\textsuperscript{2+} was removed (Figure 11).

### Additional Manifestations of Ca\textsuperscript{2+}-Activated K\textsuperscript{+} Conductance

Although spontaneous fluctuations of K\textsuperscript{+} conductance were by far the most striking feature of the voltage-clamp recordings, depolarizing pulses unveiled other forms of this current (see Figures 3, 4, and 7). First, the current at the end of the pulses showed outward-going rectification that was greatly reduced in the presence of TEA, caffeine, or ryanodine. This suggests that a background I\textsubscript{K(Ca)} is present. That caffeine and ryanodine block this current may appear surprising since their effects are presumably specific to the SR. However, the continuous occurrence of release and reuptake of Ca\textsuperscript{2+} by the SR would cause cytoplasmic Ca\textsuperscript{2+} to deviate from a steady state. Blocking the cyclic release of Ca\textsuperscript{2+} from internal stores would cause a diminution of the average level of intracellular free Ca\textsuperscript{2+} and, consequently, would decrease the Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductance.

Another current with similar properties appeared as an early decaying outward component. This early outward current was discernible only at positive pulse potentials and was partially inhibited by TEA, caffeine, and ryanodine. Decaying outward current induced by depolarization is a common manifestation of Ca\textsuperscript{2+}-activated K\textsuperscript{+} current; one explanation is that depolarization initiates an I\textsubscript{K} that transiently augments [Ca\textsuperscript{2+}].\textsuperscript{6,8,27} I\textsubscript{K} was extremely small in our isolated myocytes, however. Although a small I\textsubscript{K} is consistent with the observation that some arteries exhibit very little, if any, spontaneous electrical activity, the small magnitude of I\textsubscript{K} raises the possibility that it would not be sufficient to directly trigger the early outward current. Several alternative explanations can be proposed. First, partial block of the early outward current by caffeine and ryanodine may indicate the presence of a Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release from the SR that would act as an amplifying mechanism. Second, the possibility that depolarization directly causes release of Ca\textsuperscript{2+} from intracellular stores\textsuperscript{49} cannot be excluded. Finally, a slow transient outward phase persisted in the presence of 10 mM TEA (Figure 3), as did the related delayed depolarization measured under current clamp. This is somewhat surprising if one considers that TEA was very efficient in inhibiting spontaneous spikes of much larger amplitude. Such differential effects of TEA indicate that the early outward current must represent, at least partially, inactivation of a TEA-insensitive I\textsubscript{K(Ca)}\textsuperscript{-49} or, conversely, a slowly activating inward current. A similar early outward current may also be present in smooth muscle cells from toad stomach.\textsuperscript{10}

In conclusion, the present results suggest the presence of a Ca\textsuperscript{2+}-activated K\textsuperscript{+} conductance in...
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Current Fluctuations in Isolated Smooth Muscle Cells

smooth muscle cells of hog carotid artery and the capability of their internal stores to release Ca\(^{2+}\) cyclically. Although Ca\(^{2+}\) loading during impedance may have predisposed hog carotid arterial myocytes to exhibit this behavior, histamine-induced oscillations of tension have been observed in hog carotid artery strips.\(^4\) The physiological significance of these phenomena remains to be determined in intact tissue, but it is possible that similar processes occur during arterial constriction.

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