CALCIUM CHANNELS OF AMPHIBIAN STOMACH AND MAMMALIAN AORTA SMOOTH MUSCLE CELLS

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ABSTRACT Whole-cell and single-channel calcium currents were studied using single smooth muscle cells enzymatically-isolated from stomach of *Amphiuma tridactylum* and from guinea-pig aorta. These cells have a high specific resistance and can sustain calcium action potentials after suppression of potassium currents. Dialyzed *Amphiuma* smooth muscle cells had calcium currents which were stable for several hours whereas the calcium currents of aortic cells ran down quickly. Single channel calcium currents in cell-attached patches behaved similarly for the two cell types. Calcium channel conductance in 110 mM barium was 12 pS and the mean open time was 1.4 ms at a nominal membrane potential of +10 mV. Exposure of both cell types to BAY K8644 resulted in a dramatic prolongation of the calcium channel open times and a shift in the probability of opening to more negative potentials. Low-threshold calcium channels were not identified in the extensively studied amphibian cells. High-threshold calcium channels therefore appear to be the primary pathway for the calcium influx that produces contraction in these smooth muscle cells.

INTRODUCTION

In smooth muscle cells, voltage-dependent calcium influx plays a central role in excitation-contraction coupling (1, 2). The details of the mechanism regulating the calcium influx are unknown, although the influx is presumed to occur through plasmalemmal calcium channels. We examined the process by recording whole cell and single channel calcium currents in smooth muscle cells isolated from amphibian stomach and mammalian aorta. We found that the pathway for calcium influx was via high threshold calcium channels having many properties similar to those of calcium channels in other excitable membranes. Some of these results have been reported in abstract form (3).

MATERIALS AND METHODS

Single smooth muscle cells were isolated from the stomach muscle of *Amphiuma tridactylum* by the method of Caffrey and Anderson (4). Tissue was minced and incubated for 3–4 h at 31°C in modified amphibian Ringer's solution containing (in millimoles per liter): 110 NaCl, 2 KCl, 10 $CaCl_2$, 10 Hepes (pH 7.3), 10 glucose, 0.5 mg/ml collagenase (Type II; Worthington-Millipore, Freehold, NJ), and 0.5 mg/ml bovine serum albumin (fraction V; Sigma Chemical Co., St. Louis, MO). Only cells which remained relaxed and optically refractile in elevated-calcium Ringer's solution were selected for experimentation.

Single smooth muscle cells were isolated from the descending portion of guinea-pig thoracic aorta by methods similar to those of Benham and Bolton (5). The tissue was cleaned of fat and adventitia and sliced into rings of 2 to 3 mm length. These were incubated at 22°C in modified Tyrode's solution containing (in millimoles per liter) 130 NaCl, 4 KCl 3 $MgCl_2$, 5 Hepes (pH 7.35), 10 glucose, 0.5 mg/ml collagenase (type I; Sigma Chemical Co.) and 0.5 mg/ml soybean trypsin inhibitor (Sigma Chemical Co.). The tissue was gently agitated by oxygenation. After 3 h, the tissue was gently triturated with a Pasteur pipette to release single cells. Cells were resuspended in normal Tyrode's solution (containing 2.5 mM $CaCl_2$ but without enzymes) and were observed to retain morphological and electrical integrity for at least 24 h.

Whole-cell ionic currents were recorded from *Amphiuma* stomach smooth muscle cells using a single-suction-pipette voltage-clamp method similar to that described by Lee et al. (6). The suction pipettes had inner diameters of 10 to 14 μm and resistances of 0.5 to 0.8 MΩ when filled with internal perfusate. Seal resistances of 500 to 1,000 MΩ were considered acceptable for electrophysiological recordings. Sodium currents are absent in these cells (7) and calcium currents were isolated by suppression of potassium currents using a modified Ringer's solution, containing (in mM): 50 $CaCl_2$, 60 TEACl, 2 CsCl, 10 Hepes (pH 7.3), 10 glucose; and by an internal dialysate containing 110 Cs·aspartate, 20 CsCl, 5 EGTA, 5 Hepes (pH 7.3). Calcium currents could be corrected for capacity and leakage currents by digital subtraction of scaled, averaged hyperpolarizing pulses.

Single Ca^{++} currents were measured by the patch clamp method of Hamill et al. (8) using a 10 GΩ feedback resistor in the cell-attached recording mode with Ba^{++} (110 mM) and 10 mM Hepes titrated to pH 7.4 with CsOH in the pipette. The patch current traces were filtered at 1–5 kHz and recorded on FM tape (Racal Recorders Inc., Irvine, CA) for further analysis. Selected experiments were digitized (100–200 μs/point) on a PDP 11/73 minicomputer (Digital Equip. Corp., Marlboro, MA). Current traces were corrected for capacity and leakage currents by subtraction of an average of those traces displaying no single-channel transitions (i.e., "failures"). Single-channel transitions were detected using a threshold scheme in which an interactive process determined the half-maximum amplitude of unitary events (9; Josephson and Brown, 1986. Manuscript submitted for publication.). The background rms of current noise at 1 kHz bandwidth was 0.05–1.0 pA. Histograms were fit using the method of maximum likelihood applied to sums of exponentials for open-time distributions and sums of Gaussian functions for amplitude distributions (9).

RESULTS

Photomicrographs in Fig. 1 show isolated smooth muscle cells from *Amphiuma* stomach (*upper*) and from guinea

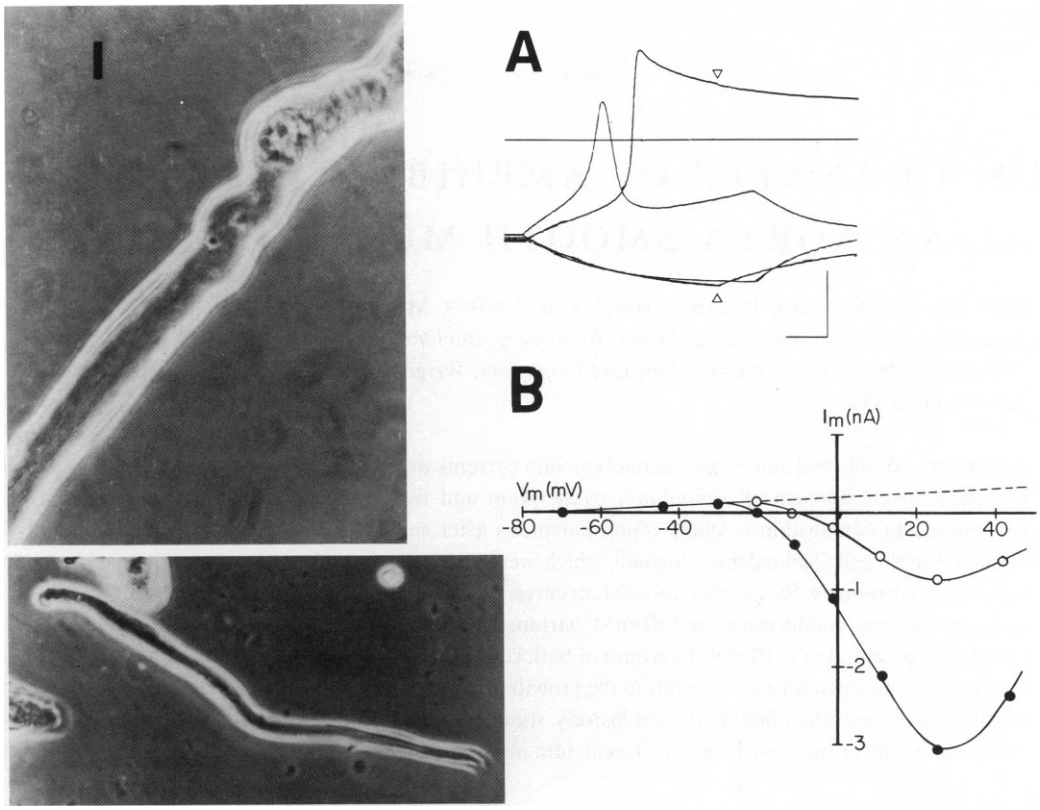

FIGURE 1 Photomicrographs of isolated smooth muscle cells from *Amphiuma* stomach (*upper left*) and a guinea-pig aorta (*lower left*). Calibration bar is 5 μm. The overall cell lengths were of 870 and 80 μm, respectively. *Panel A* shows action potentials elicited from an *Amphiuma* smooth muscle cell in 50 mM Ca^{++}/60 mM TEA^+-Ringer (current pulse duration 300 ms) and after superfusion of 50 mM Ba^{++}/60 mM TEA^+-Ringer's solution (current pulse duration 250 ms). Vertical calibration: 30 mV. Horizontal calibration: 50 ms. The corresponding hyperpolarizing responses are shown below. Changing from Ca^{++} to Ba^{++} increased the maximum rate of rise of the action potential from 2.1 V/s to 7.6 V/s. The prolonged Ba action potential repolarized some 30 s after the cessation of polarizing current (Δ). *Panel B* shows current-voltage relations of peak inward voltage clamp current in 50 mM Ca^{++}/60 mM TEA (○) and 50 mM Ba^{++}/60 mM TEA-Ringer's solution. The peak inward currents were proportional to the $(dV/dt)(max)$; of the action potentials obtained in current clamp. The dashed line shows extrapolation of the linear component of leakage current obtained from hyperpolarizing voltage steps. Holding potential was −70 mV.

pig aorta (*lower*). Cells of the amphibian preparation typically have lengths varying between 800 and 1,200 μm, diameters between 10 and 15 μm and membrane surface areas between 3×10^4 and 10^5 μm^2 (average value is $5.15 \pm 1.75 \times 10^4$, $n = 115$), the latter based on measurements of membrane capacitance (7). Cells of the aortic smooth muscle preparation have lengths between 80 and 160 μm and diameters between 3 and 5 μm.

The size of *Amphiuma* smooth muscle cells permits utilization of the suction pipette method of Lee et al. (6) for voltage clamp and internal dialysis. The suction pipettes have an area that is 100 times that of a patch clamp pipette so that exchange of intracellular ion content is greatly facilitated. Reliable isolation of I_{Ca} and I_{Ba} required dialysis with solutions in which K^+ was replaced by Cs^+ or *N*-methyl-d-glucamine$^+$. Extracellular TEA^+ was used in some experiments as an additional measure for K^+ blockade and also as a substitute for Na^+. The effectiveness of the dialysis could be followed by the timecourse of block of the outward currents in the modified Ringer's solution. This required from 3 to 5 min. Stable calcium or barium currents could then be recorded for up to 2 h. This should be contrasted with the more rapid "rundown" within minutes of calcium currents that has been reported in many preparations (10, 11) including the mammalian aortic cells studied presently.

Previous analyses of cable properties (7) indicate that temporal and spatial voltage control is adequate for study of slow inward currents such as I_{Ca} or I_{Ba}. Resting specific membrane resistivity measured with microelectrodes is high in normal Ringer's solution (143 ± 92 kΩcm^2, $n = 50$, reference 7), and is increased three- to fivefold in solutions used to isolate I_{Ca}; thus the resting length constant is >5 mm. This is a minimum value since membrane leakage currents are largest with microelectrodes. Steady state voltages at the ends of the cell deviate by <0.1% from those of the point of voltage control at the center of the cell. Action potentials elicited in the presence of K^+ channel blockers showed maximum rates of rise proportional to peak inward current values recorded subsequently in volt-

age clamp. The currents were calculated according to the relation $I_p = C_m (dV/dt)$ (max). Rates of rise average 2 ± 1 V/s and 7 ± 2 V/s (n = 20 and 35, respectively) for Ca^{++}- and Ba^{++}-dependent action potentials, respectively. The peak inward currents underlying them have maximum values of 1 ± 0.5 nA and 3 ± 1 nA (n = 35 and 65, respectively) (Fig. 1 *A* and *B*). Maximal conductances underlying currents of this magnitude will produce voltage deviations of <5% along the length of the cell.

Activation of Ca^{++} and Ba^{++} currents was rapid ($t_{1/2}$ ~ 3.5 ms) and inactivation was comparatively slow ($t_{1/2}$ ~ 200 ms). Ba^{++} current was inactivated more slowly ($t_{1/2}$ ~ 2 s). Thresholds for activation of I_{Ca} and I_{Ba} were typically −20 mV and −25 mV, respectively. Maximum inward current occurred at membrane potentials of +30 and +40 for I_{Ba} and I_{Ca}. Peak current-voltage relations were nonlinear as V_m approaches E_{Ca} (or E_{Ba}). The calcium currents were blocked by La^{3+} (100 μM), Mn^{2+} (2 mM) and the organic calcium antagonist nitrendipine (100 μM). The block by nitrendipine depended upon holding potential and its potency was significantly increased at depolarized potentials (Caffrey, Yatani, and Brown; manuscript in preparation). Low threshold calcium channels (2–5) were not identified. To this point we have not recorded whole-cell calcium currents in adult guinea pig aortic cells reliably because rundown occurs within 5 min of penetration after establishing the gigaseal.

Single Ca^{++} Channel Currents

Amphimua Stomach Smooth Muscle Cells. The cell-attached patch clamp technique (8) was employed to examine the elementary currents that contribute to the whole-cell Ca^{++} currents in these smooth muscle cells. Barium ion was chosen over calcium ion as the charge carrying species since it was found to be useful in blocking

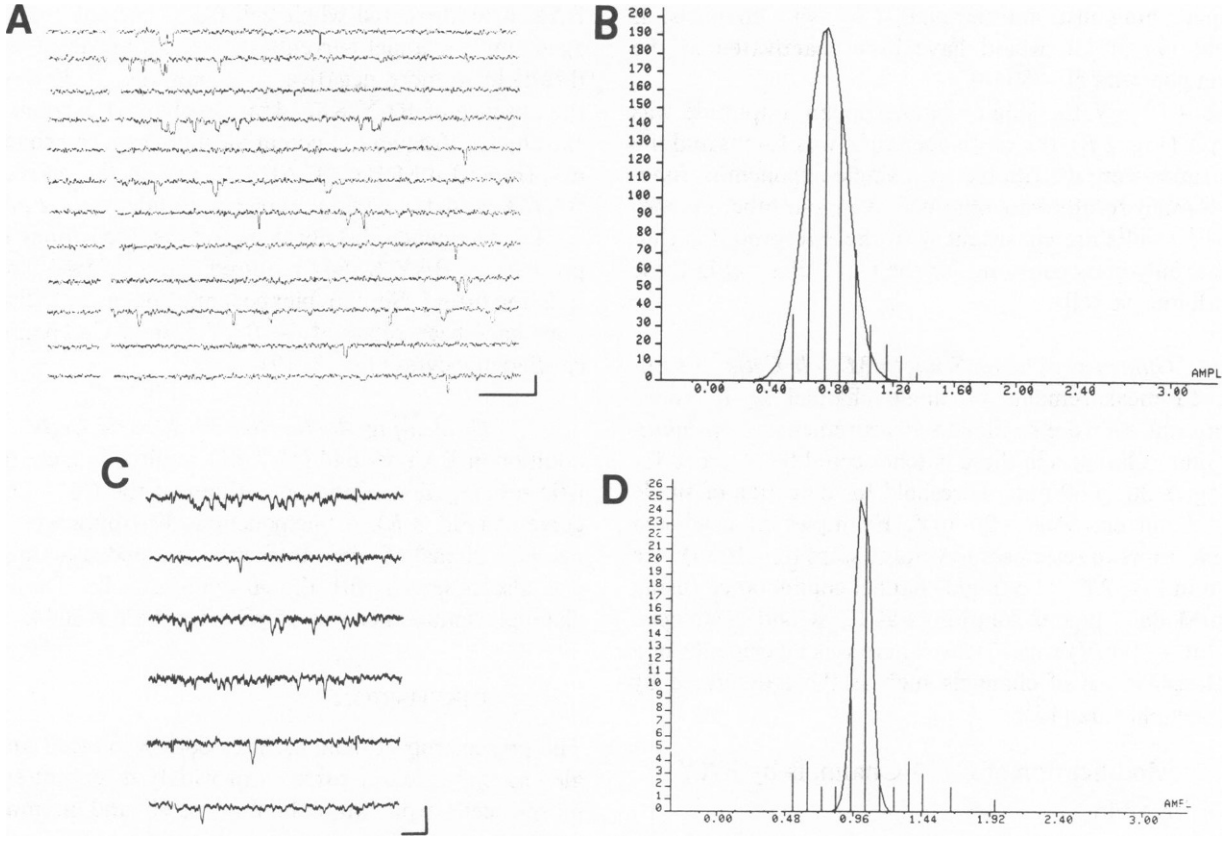

FIGURE 2 (*A*) Examples of single Ca^{++} channel currents recorded from a cell-attached patch on an isolated *Amphiuma* smooth muscle cell bathed in amphibian Ringer's solution using a pipette filled with 110 mM $BaCl_2$ (pH 7.4). The patch potential was stepped from the resting potential (about −50 mV) to a test potential of +10 mV at a frequency of 0.2 Hz. The gaps in the current traces result from an incomplete correction of the capacity current and serve to mark the time of the onset of the voltage step. The current and time calibrations are 1 pA and 20 ms. Filter frequency was 1 kHz. (*B*) Frequency distributions of amplitudes for single *Amphiuma* Ca^{++} channel currents recorded at +10 mV. These data were fit by a Gaussian curve with a mean of 0.76 pA. (*C*) Six representative single Ca^{++} channel current traces recorded from a guinea pig aorta muscle cell (corrected for capacity and leakage currents). The holding potential of the patch was set at 30 mV more negative than the resting potential and stepped by 50 mV at a frequency of 0.3 Hz. The cell was bathed in normal Tyrode's solution and had an estimated resting potential of −30 mV; the test potential was approximately −10 mV. Calibrations: 1 pA, 20 ms. (*D*) Frequency distributions of amplitudes for single guinea pig aorta smooth muscle cells recorded at +10 mV. These data were fit by a Gaussian curve with a mean of 1.0 pA.

single-channel K^+ currents (5) (which may be activated by Ca^{++}), and because Ba^{++} has been shown to be more permeant than Ca^{++} through high threshold Ca^{++} channels (17). Fig. 2 *A* shows examples of single Ca^{++} channel current traces. During the voltage step brief, inward current pulses of <1 pA were recorded. The events occurred randomly and were distributed throughout the trace. Evidence that suggests that these events are Ca^{++} channel currents is that: (*a*) Ba^{++} ion was the only charge carrier for inward current in the pipette solution and blocks K^+ channels; (*b*) the threshold potential for the appearance of the single-channel currents was around −10 mV, which is similar to the threshold for activation of the whole-cell Ba^{++} current; (*c*) the amplitude of the single-channel currents decreased with increasing depolarization, as would be expected for an ionic current with a positive equilibrium potential; (*d*) the probability of channel opening (P_o) increased with increasing depolarization, consistent with our whole-cell current measurements; and (*e*) the single channel currents were blocked by the selective Ca channel antagonist, nitrendipine; (*f*) Na^+ channels, if present (7) at all, would have been inactivated at the resting potential of −50 mV.

At +10 mV the single channel mean amplitude was 0.76 pA (Fig. 2 *B*), the mean open time was 1.4 ms and the open times were distributed as a single exponential function. Similar results were obtained from four other patches and the results are consistent with the interpretation that there is only one open state for the Ca^{++} channel in these smooth muscle cells.

Guinea-pig Aortic Smooth Muscle Cells. Cell-attached measurements of single channel i_{Ba} in aortic smooth muscle were similar to measurements in *Amphiuma*. Single channels in these patches could be recorded for as long as 30 to 60 min. Threshold for detection of single channel current was −20 mV. Examples of corrected current traces in responses to voltage steps to −10 mV are shown in Fig. 2 *C*. The single channel conductance (using 110 mM Ba^{++} pipette solution) was 12 ps, and mean open time (at −10 mV) was 2.0 ms. There was no evidence of a low threshold set of channels such as those described by Carbone and Lux (12).

Modification of Ca^{++} Channels by BAY K8644

Amphiuma Stomach Smooth Muscle Cells. Exposure of *Amphiuma* smooth muscle cells to the dihydropyridine Ca^{++} channel agonist BAY K8644 (10^{-7} M) produced an augmentation of Ca^{++} current within 2 min (Fig. 3 *A* and *B*). Upon termination of the depolarizing voltage step, a larger and more prolonged tail current was observed in the presence of BAY K8644 than in control solutions. Similar effects were observed when external Ca^{++} was replaced with Ba^{++} ion. More complete current-voltage relationships (*I-V*) for control whole-cell Ca^{++} currents and after exposure to two concentrations of BAY K8644 are shown in Fig. 2 *C*. The most prominent effect is the dose-dependent enhancement of the Ca^{++} current; increases of 240% and 320% were measured at the peaks of the *I-V*s at 50 and 500 nM BAY K8644, respectively. Also of interest is the shift of the *I-V* to more negative potentials in the presence of BAY K8644.

We tested the effects of BAY K8644 on single Ca^{++} channel currents, to examine the mechanism of its action at the microscopic level. Exposure of a patch containing active Ca^{++} channels to BAY K8644 at concentrations of 10^{-6} to 10^{-5} M (which produce maximal enhancement of the whole-cell Ca^{++} currents) resulted in dramatic changes in the single channel properties as shown in Fig. 3 *D*. The most striking difference from the control records was the great increase in opening probability, P_o, due to marked prolongation of the open times. This is associated with the occurrence of simultaneous openings of two and three channels at the higher potentials. Consistent with the BAY K8644-treated whole-cell Ca^{++} currents, the averaged single channel currents showed an apparent shift in threshold to more negative potentials (i.e., −30 mV). In the absence of BAY K8644 single channel currents were extremely infrequent at potentials more negative than −10 mV (in 110 mM Ba^{++}). At +10 mV in the presence of BAY K8644, the mean unitary amplitude was 1.2 pA.

The frequency distributions for the open times in the presence of BAY K8644 required a sum of two exponentials for fitting. Similar biexponential open time distributions have been reported for BAY K8644 Ca channels in cardiac myocytes (17, 18, 19).

Guinea-pig Aortic Smooth Muscle Cells. The addition of BAY K8644 (10^{-5} M) resulted in a characteristic prolongation of the open times of the Ca^{++} channel currents (Fig. 3 *E*). A biexponential distribution (τ_1 = 2.0 ms, τ_2 = 20 ms) of open states was obtained; a feature that was also observed with the *Amphiuma* cells. The single-channel conductance was 15 pS after BAY K8644.

DISCUSSION

The present report demonstrates both whole-cell and single-channel calcium currents in widely divergent smooth muscle cells from amphibian stomach and mammalian aorta. An important result from this comparative approach was the relative stability of the calcium currents in dialyzed stomach cells. The ionic composition of the cytoplasm was clearly being modified since the outward potassium current was blocked within a few minutes of perfusion with intracellular cesium. The mammalian aortic cells, on the other hand, showed rundown which has been attributed to the loss of cytoplasmic substances necessary for calcium channel function (10, 11). Rundown has been described in a wide variety of excitable cells although some

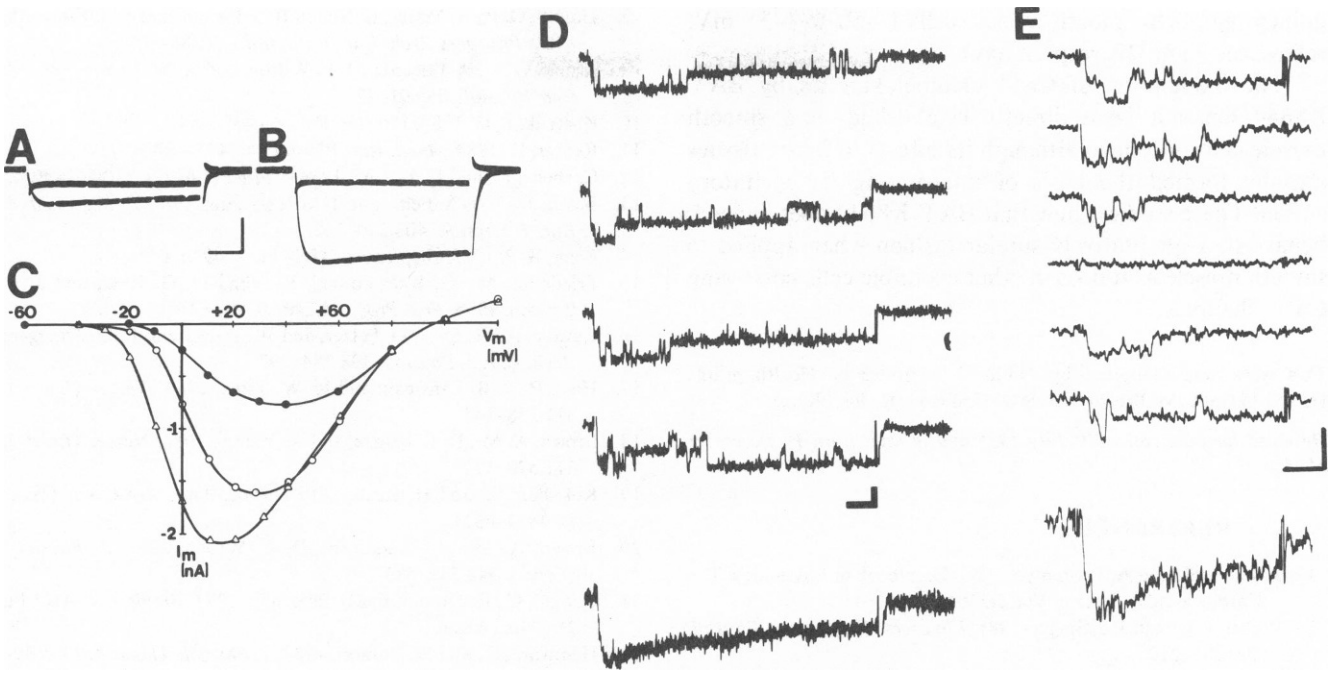

FIGURE 3 *Panels A and B* show whole-cell Ca^{++} currents recorded from an isolated *Amphiuma* smooth muscle cell produced by voltage steps to +20 mV from a holding potential of −60 mV, before (*A*) and after (*B*) exposure to 10^{-7} M BAY K8644. Leakage currents produced by voltage steps to −140 mV are superimposed and are seen to be unaffected by BAY K8644. Voltage steps were delivered at a frequency of 0.2 Hz. Current signals were filtered at 2 kHz and photographed directly from the oscilloscope display. Vertical calibration: 500 pA; horizontal calibration: 20 ms. *Panel C* shows current-voltage relations for peak I_{Ca} in control (●) and in the presence of 50 μM (○) and 500 μM (△) BAY K8644. Data were corrected for the linear compound of leakage current. *Panel D* shows single channel i_{Ba} (110 mM Ba^{++} in the pipette) recorded from an *Amphiuma* smooth muscle cell after addition of 10^{-5} M BAY K8644 at a test potential of +10 mV. Voltage steps were delivered at a frequency of 0.2 Hz. Samples were filtered at 500 Hz for display. Vertical calibration: 1 pA; Horizontal calibration: 50 ms. *Panel E* shows single channel current traces (i_{Ba}, 110 mM Ba^{++} pipette) recorded from guinea pig aortic smooth muscle cell after addition of 10^{-5} M BAY K8644. Vertical calibration 1 pA; horizontal calibration: 20 ms. Records were filtered at 1 kHz. Voltage steps were delivered at 0.2 Hz. Averages of 100 and 53 traces from cells shown in *D* and *E* are illustrated at the bottom of each column.

molluscan neurons (20, 21) have calcium currents that are similarly resistant to rundown following cytoplasmic exchange. In this regard it is interesting to note that, for cell-attached single channel recordings, there was no distinct experimental advantage for the amphibian cells. The stability of whole cell currents is clearly advantageous for the pharmacological experiments described here and for searching for the presence of low-threshold channels. The *Amphiuma* cells have a very high specific membrane resistance and this, combined with a low density of voltage-activated channels (7), provides good spatial control of membrane potential. The density of calcium channels in *Amphiuma* cells is sufficient, however, to produce an active membrane response when potassium currents are blocked.

The single Ca^{++} channel conductance of 12 pS reported here for smooth muscle cells using 110 mM Ba^{++} as the charge carrier is <18 pS found in cardiac myocytes (19), but is >7–8 pS in GH3 cells (22). At a membrane potential of +10 mV, macroscopic I_{Ba} between 1.5 and 2.5 nA, and a single channel i_{Ba} of 0.76 pA (where the probability of opening is ~0.1), we estimate that there are between 20,000 and 30,000 channels per cell. An average surface area of 5×10^4 μm^2 gives a channel density of between 0.1 and 1.0 per μm^2.

We found that voltage-dependent Ca^{++} influx in these cells is produced by a set of Ca^{++} channels essentially similar to the "high-threshold" Ca^{++} channels found in other cell membranes (16–18, 22–26), with respect to voltage-dependence, kinetics and pharmacology, both at the macroscopic and microscopic levels. For all these reasons we believe that *Amphiuma* stomach is a useful preparation for the study of calcium currents in smooth muscle cells.

These data show no evidence for "low-threshold" (activated at −40 to −50 mV), rapidly inactivating calcium channels such as have been found in neurons (12, 13), more recently in myocytes (14) and in the A10 clonal cell line derived from embryonic rat aorta (15). Both macroscopic currents and averaged single channel currents show a "high-threshold" (activated above −20 mV), slowly inactivating current, and hyperpolarization of the membrane potential does not reveal currents with more transient kinetics. It is unclear what role such channels would play even if present, because they would be inactivated at the normal resting potential of both *Amphiuma* stomach and

guinea-pig aorta smooth muscle cells (−50 to −55 mV, references 7 and 27, respectively).

The modulation of Ca^{++} channel kinetics by BAY K8644 has not been directly established in a smooth muscle cell until now, although its effects in intact tissues initially formed the basis of interpreting its excitatory action. These studies show that BAY K8644 does, indeed, behave in a qualitatively similar fashion when applied to smooth muscle as it does in other excitable cells possessing Ca^{++} channels.

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REFERENCES

1. Johnson, B., and A. P. Somlyo. 1980. Handbook of Physiology. The Cardiovascular System. Vol. II. 301–324.
2. Walsh, J. V., and J. J. Singer. 1981. *Pfluegers Arch. Eur. J. Physiol.* 390:207–210.
3. Caffrey, J. M., I. R. Josephson, and A. M. Brown. 1985. *Biophys. J.* (2, Pt. 2):264*a*. (Abstr.)
4. Caffrey, J. M., and N. Anderson. 1978. *Fed. Proc.* 38:2240*a*. (Abstr.)
5. Benham, C. D., and T. B. Bolton. 1983. *J. Physiol.* (*Lond.*). 340:469–486.
6. Lee, K. S., N. Akaike, and A. M. Brown. 1980. *J. Neurosci. Meth.* 2:51–57.
7. Caffrey, J. M. 1982. Ph.D. Thesis. Duke University, Durham, NC.
8. Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. T. Sigworth. 1981. *Pfluegers Arch. Eur. J. Physiol.* 391:85–100.
9. Kunze, D. L., A. Lacerda, D. L. Wilson, and A. M. Brown. 1985. *J. Gen. Physiol.* 86:691–720.
10. Kostyuk, P. G. 1984. *Physiol. Rev.* 64:435–454.
11. Reuter, H. 1984. *Annu. Rev. Physiol.* 46:473–484.
12. Carbone, E., and H. D. Lux. 1984. *Nature* (*Lond.*). 310:501–502.
13. Bossu, J. L., A. A. Feltz, and J. M. Thomann. 1985. *Pfluegers Arch. Eur. J. Physiol.* 403:360–368.
14. Bean, B. P. 1985. *Biophys. J.* 47(2, Pt. 2):497*a*. (Abstr.)
15. Friedman, M., G. Kaczorowski, R. Vandlen, G. Katz, and J. P. Reuben. 1985. *Fed. Proc.* 44:246*a*. (Abstr.)
16. Cavalie, A., R. Ochi, D. Pelzer, and W. Trautwein. 1983. *Pfluegers Arch. Eur. J. Physiol.* 398:284–297.
17. Hess, P., J. B. Lansman, and R. W. Tsien. 1984. *Nature* (*Lond.*). 311:538–544.
18. Brown, A. M., D. L. Kunze, and A. Yatani. 1984. *Nature* (*Lond.*). 311:570–572.
19. Kokubun, S., and H. Reuter. 1984. *Proc. Natl. Acad. Sci. USA.* 81:4824–4827.
20. Brown, A. M., Y. Tsuda, and D. L. Wilson. 1983. *J. Physiol.* (*Lond.*). 344:549–583.
21. Caputo, C., R. DiPolo, and F. Bezanilla. 1983. *Biophys. J.* 41(2 Pt. 2):294*a*. (Abstr.)
22. Hagiwara, S., and H. Ohmori. 1983. *J. Physiol.* (*Lond.*). 336:649–661.
23. Cachelin, A. B., J. E. de Peyer, S. Kokubun, and H. Reuter. 1984. *Nature* (*Lond.*). 311:538–544.
24. Fenwick, E. M., A. Marty, and E. Neher. 1982. *J. Physiol.* (*Lond.*). 331:599–635.
25. Lux, H. D., and A. M. Brown. 1984. *J. Gen. Physiol.* 83:727–750.
26. Brown, A. M., H. Camerer, D. L. Kunze, and H. D. Lux. 1982. *Nature* (*Lond.*). 299:156–158.
27. Kajiwara, M. 1982. *Pfluegers Arch. Eur. J. Physiol.* 393:109–117.