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Voltage-activated and stretch-activated Ba^{2+} conducting channels in an osteoblast-like cell line (UMR 106)

Randall Duncan and Stanley Misler

Departments of Internal Medicine (Jewish Hospital), Cell Biology and Physiology and Electrical Engineering (Program in Biomedical Engineering), Washington University, St. Louis, MO, USA

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Calcium entry may mediate osteoblast activation by calciotropic hormones or changes in electrical fields or mechanical stress in bone. Using cell-attached patch clamping techniques, we have seen two Ba^{2+} conducting channels in UMR-106 osteoblast-like cells: (i) a voltage-dependent, dihydropyridine-sensitive channel resembling L-type Ca^{2+} channels in other cells, and (ii) a stretch-activated non-selective channel resembling those involved in mechanoreception and triggering of volume regulation in other cells.

Patch clamp; Stretch-activated channel; Ca^{2+} channel; (Osteoblast)

1. INTRODUCTION

Osteoblasts, which rest at the surface of bone matrix, are key cells in bone remodelling (see [1] for review). They synthesize bone matrix proteins, including type I collagen and osteonectin, which binds Ca^{2+} to collagen. They participate in bone resorption by both 'priming' the unmineralized surface of bone matrix for targeting by osteoclasts and secreting an osteoclast stimulation resorption factor. Regulation of osteoblast function is complex. It includes control by local and systemic hormones (PTH, vitamin D_3, prostaglandins), local electric fields and mechanical stress. Osteoblast stimulation by hormones often increases intracellular calcium concentration, though the relative contribution of intracellular and extracellular sources remains to be clarified. Other regulatory factors might do likewise. It is possible that osteoblast stimulation might be mediated by the activation of voltage-dependent, receptor-mediated or stretch-activated Ca^{2+} conducting channels in the plasma membrane. Recently, both transient and sustained voltage-activated Ca^{2+} currents have been observed in 'whole-cell' patch-clamp recordings from rat osteoblasts [2].

Clonal UMR-106 cells, derived from a rat osteogenic sarcoma, possess many of the enzymatic properties of normal osteoblasts (including high alkaline phosphatase activity and PTH-stimulated adenyl cyclase activity [3,4]). They produce bone specific type I collagen [5] and have similar resting membrane potentials as the osteoblast. Using cell-attached patch-clamping techniques, we found single voltage-dependent channels and single stretch-activated channels which conduct Ba^{2+} across the plasma membrane of UMR-106 cells. This work has previously been presented in abstract form [6].

Correspondence address: S. Misler, Renal Division, Yalem 713, The Jewish Hospital of St. Louis, 216 S. Kingshighway Boulevard, St. Louis, MO 63110, USA

2. MATERIALS AND METHODS

UMR-106 cells (passages 17–25) were plated from mother cultures onto glass coverslips and were grown in minimal essential medium Eagles with non-essential amino acids and Earle's Salts (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum. These cells were fed twice weekly and maintained in a humidified atmosphere of 95% air/5% CO_2 at 37°C. Individual coverslips were transferred to a 3 ml volume plexiglass

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recording chamber which allows rapid change of bathing solutions with minimal perturbation to the cells. Cells were initially bathed in a mammalian extracellular-type solution (ES) consisting of (in mM): 140 NaCl; 5.5 KCl; 1 $CaCl_2$; 1 $MgCl_2$ and 20 Hepes buffer; titrated to pH 7.3 with NaOH. During the experiment, the bath was often changed to an intracellular-type solution (IS) containing (in mM): 144 KCl; 1 $MgCl_2$; 20 Hepes; titrated to pH 7.3 with KOH with no added Ca^{2+}.

Single channel recordings were performed using standard patch-clamp techniques previously described in more detail [7]. A fire-polished, 3–10 MΩ resistance pipette fabricated from borosilicate glass capillary tubes was lowered to the surface of an isolated cell. A GΩ resistance pipette-to-membrane seal was formed by gentle application of suction to the sideport of the pipette holder. Pipettes were usually filled with a 'Ba^{2+} solution' which contained (in mM): 88 $BaCl_2$ and 20 Hepes; titrated to pH 7.3 with tetraethylammonium hydroxide, although in some experiments, the pipette was filled with ES or IS. The clamping potential (V_c) is defined as the negative of the potential applied to the interior of the pipette with the bath held as ground. This convention seemed particularly useful because with IS in the bath, the resting potential across the cell membrane is 'nulled' and V_c is very nearly the true membrane potential across the patch at any time. Pulses of suction used to activate the stretch-activated channels were generated by a microprocessor controlled on-off valve connected to suction, via a suction regulator (Puritan-Bennett, St. Louis, MO) and applied to the sideport of the pipette. Currents were filtered at 400–500 Hz and sampled at 1 kHz.

In these experiments, capacity transient cancellation was performed as best possible. No attempt was made to perform capacity transient subtractions needed to average current traces as it was often difficult to find sufficient null traces to average at all V_c required. The average number of channels open in a patch during a specified period of time was denoted (by convention) as I/i. I/i is formally equal to the number of channels in the patch (N) multiplied by the open probability of an individual channel (P_o). Operationally, I/i was measured using an interactive graphics based analysis system available in our laboratory which uses level crossings to determine in a segment of record the fraction of time when zero, one or more channels are open.

BAYK 8644, a gift from Dr Alexander Scriabine, Miles Laboratory, was dissolved in 100% ethanol at a concentration of 5 mM and then frozen in small aliquots wrapped in light proof foil.

3. RESULTS

In pilot experiments, cell attached membrane patches of UMR-106 cells, bathed in ES and patched with pipettes filled with Ba^{2+} solution, occasionally displayed unitary inward currents at rest ($V_c = 0$ mV). With depolarization, these channels increased in frequency and decreased in amplitude. Channel frequency increased after addition of the Ca^{2+} channel agonist BAYK 8644 (3 μM) to the bath. These observations suggested the presence of a voltage-activated, dihydropyridine-sensitive, Ba^{2+} conducting channel.

The cell membrane potential was not directly measured in this study and previous reports have shown the membrane potential to fluctuate [8,9]. Therefore, to further identify and characterize this channel, we performed most of our experiments with the cell bathed in IS solution. Fig.1a and b displays sample traces and time courses of average current activity (I/i) seen in the presence of BAYK 8644 at membrane potentials of −20 to 0 mV. This range of membrane potentials is only minimally to moderately depolarized from the average resting membrane potential of −25 to −30 mV reported for UMR-106 cells based on the partitioning of the voltage-sensitive dye, bis-oxynol [10]. Channel activity increased ~6-fold over the range of −20 mV to 0 mV when measured between 150 and 300 ms after pulsing the membrane potential from a holding potential of −70 mV to the given clamping potential (V_c). Time-dependent inactivation of activity was apparent at larger depolarizations, although some activity was maintained throughout the entire 3 s of the depolarizing voltage pulses. Construction of a current-voltage curve of unitary currents seen over a wide range of pulses, shown in fig.1c, yielded a single channel conductance (γ) of 20 pS and a linearly extrapolated zero current or reversal potential (E_{rev}) of at least +55 mV. In other experiments, channels with nearly identical amplitude were recorded at corresponding V_c values prior to addition of BAYK 8644; their activity usually increased after addition of BAYK 8644 (fig.1d). In companion experiments with cells bathed in ES, the normalized activity vs voltage curve and the current vs voltage curve of the 20 pS voltage-dependent channel were shifted to the right by ~25 to 30 mV as predicted if the resting membrane potential is ~ −25 mV to −30 mV. In total, these channels were seen in approx. 60% (22/38) of the cell attached patches.

Another channel capable of conducting inward Ba^{2+} current was also seen in most cell attached patches. Although this channel was spontaneously active, its activity was increased by application of pulses of suction (≥5 mmHg) to the interior of the pipette. Fig.2a demonstrates this channel's activity at several V_c values in a cell-attached membrane patch formed with IS in the pipette and ES in the bath. Over repeated pulses, little voltage

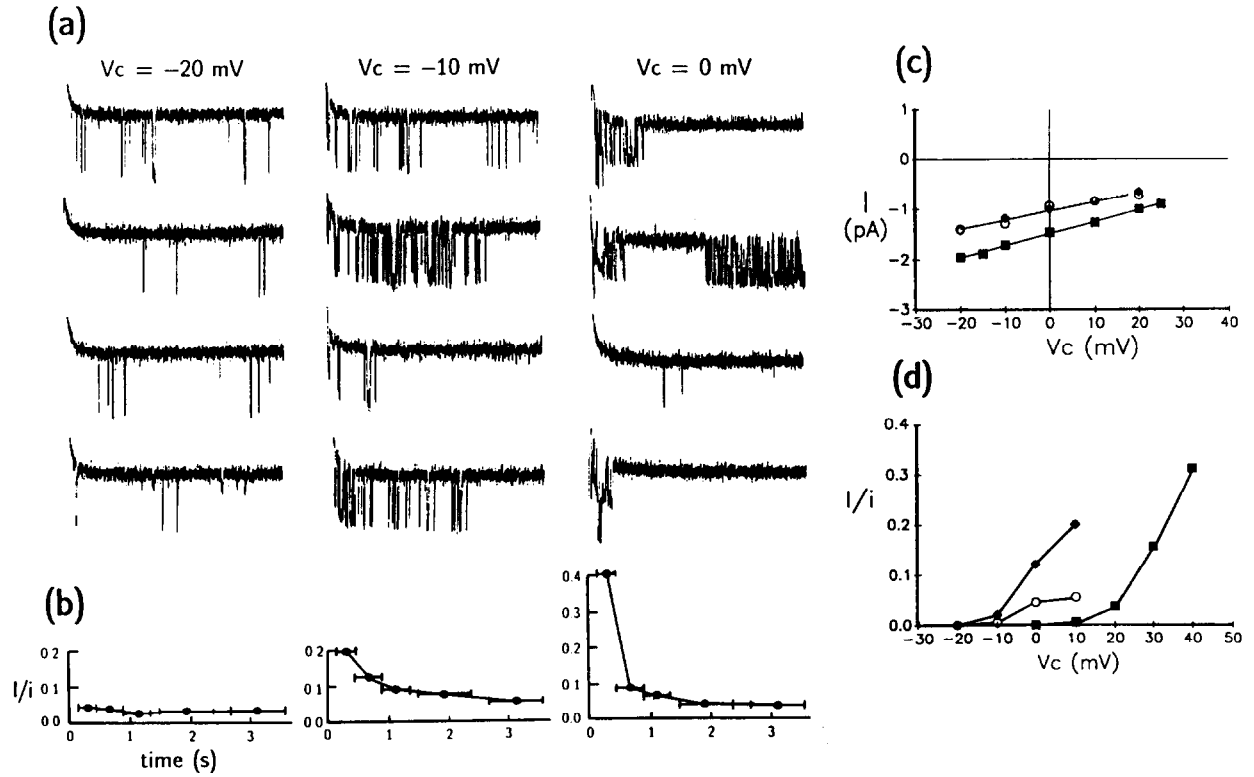

Fig.1. Identification of 'L-type' Ba^{2+} conducting channels. (a) Sample traces showing voltage-dependent activation after steps of depolarization from a holding potential of −70 mV to test potentials of −20, −10, and 0 mV. The pipette contained a 'Ba^{2+} solution' defined in the text. Cells were bathed in IS with 3 μM BAYK 8644. (b) Time courses of channel activity (I/i) averaged over total of 26 runs at each potential for the experiment shown in (a). (c) Current-voltage curve for the channel shown with cell bathed in ES (■) or IS (○). The conductance of the channel was not affected when 3 μM BAYK 8644 (◆) was added to the IS bath. (d) Curve of average channel activity (I/i) vs voltage demonstrating increases in activity with increasing depolarizations in both ES (■) and IS (○). Addition of BAYK 8644 to IS substantially increased channel activity (◇).

dependence of channel activity was seen. Increasing suction at a given V_c, increased channel activity without affecting single channel amplitude. The current-voltage curve for this channel demonstrated a maximum slope conductance of 18 pS (fig.2b). Outward currents were seen at positive V_c values. Channels with a similar γ and E_{rev} were seen with IS solution in the pipette, suggesting that the channel may be a non-selective cation channel. (This hypothesis was confirmed by excising some patches in the inside-out configuration into ES or IS supplemented with 0.25–1.0 μM $CaCl_2$. Under these conditions, the channels which opened spontaneously had an E_{rev} of 0 mV. Diluting ES or IS by half with water or isotonic sucrose (320 mM) shifted E_{rev} by +17 mV as expected for an overwhelming cation-selective channel which did not discriminate between Na^+ and K^+.)

Fig.2c and d demonstrate that a channel with similar stretch sensitivity and E_{rev} was also seen in patches made with 'Ba^{2+} solution' in the pipette. Here, however, γ was 13 pS (fig.2b). Fig.2d shows that, although the channel demonstrated spontaneous activity, activity increased approximately 5-fold with 15 mmHg suction. However, with Ba^{2+} in the pipette, successive pulses of suction at negative V_c values sometimes made the channel insensitive to stretch for several seconds, suggesting that the channel had been thrown into a long closed state. Note that E_{rev} is not detectably altered by substituting ES or IS pipette solution with the Ba^{2+} solution. Using a modified form of the Goldman-Hodgkin-Katz equation, which allows consideration of divalent as well as monovalent cations (see eqn 1 of [11]), and assuming an activity coefficient for Ba^{2+} of 0.25 and an activity coefficient for Na^+

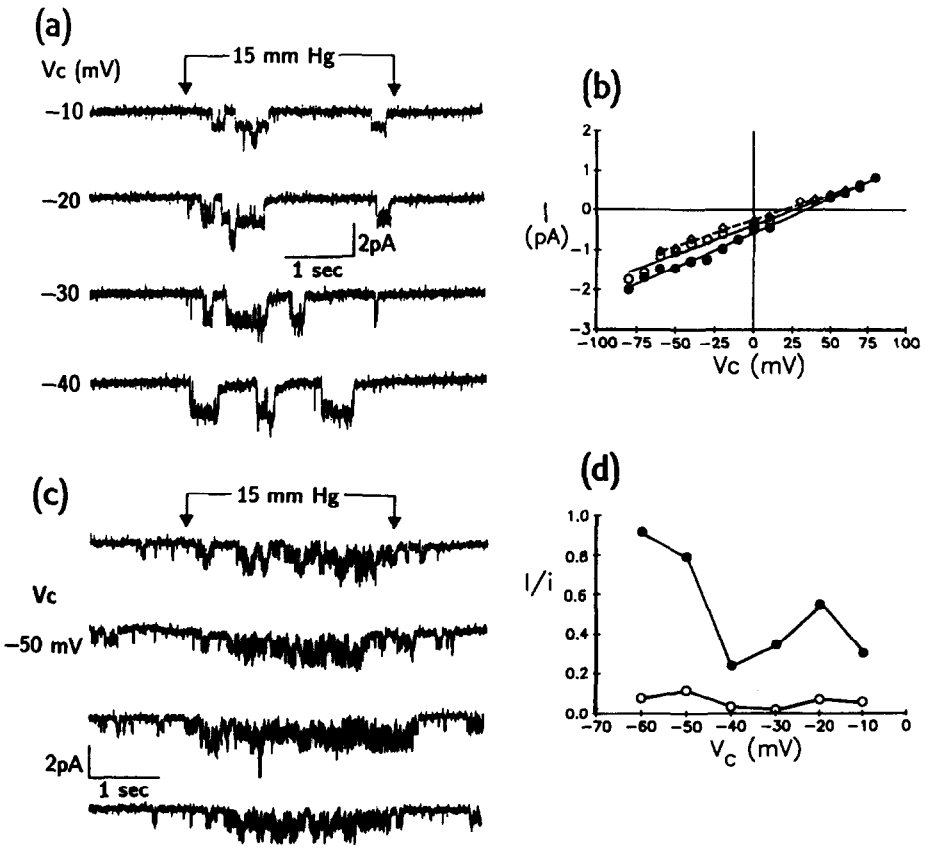

Fig.2. Identification of stretch-activated cation channel which conducts Ba^{2+}. (a and c) Activation of ion channel in cell-attached patch by application of 15 mmHg suction (marked by arrows). Cells were bathed in ES with either IS in the pipette (a) or with Ba^{2+} solution in the pipette (c). (b) Current-voltage curves for this stretch-activated channel with IS (●), ES (○), or 'Ba^{2+} solution' (◇) in the pipette, and ES in the bath. (d) Average channel activity measured over a range of voltages in the absence (○) and presence (●) of suction (15 mmHg). 'Ba^{2+} solution' in the pipette, ES in the bath.

or K^+ of 0.80, the relative permeability of the channel to Ba^{2+} vs Na^+ and K^+ was estimated as ~1.3 to 1.

4. DISCUSSION

Using cell-attached patch-clamp recording, we have presented evidence for two ionic channels in UMR-106 osteogenic sarcoma cells which are capable of carrying inward Ba^{2+} currents at membrane potentials near to or moderately depolarized from the resting membrane potential of the cell. These channels would be expected to carry Ca^{2+} currents as well. The first type of channel is active at membrane voltages positive to −30 mV, slowly inactivates, has a 20 pS conductance with isotonic $BaCl_2$ in the pipette and shows enhanced activity in the presence of the Ca^{2+} entry agonist BAYK 8644. These features strongly resemble those of 'L-type' Ca^{2+} channels found in many excitable cells [12]; the voltage-dependent activity of this channel is also similar to that displayed by whole cell 'S-type' Ca^{2+} channel currents described in normal osteoblasts [2]. This channel appears quite distinct from a 3–5 pS, voltage-dependent, phenylalkylamine-sensitive divalent cation conducting channel reported in ROS 17/2.8 osteoblast-like cells [13]. The second channel is voltage-insensitive, but 'stretch-activated'. At a given V_c, this channel carries inward currents of similar magnitude when physiological saline, near isotonic KCl or near isotonic $BaCl_2$ are present at the extracellular sur-

face, suggesting that it is poorly selective among cations. The amount of suction needed to activate the channel is equivalent to the pressure gradient imposed on the membrane by a transmembrane osmolar gradient of 1–2 mosm/l. This channel resembles the stretch-activated, cation-selective channel C^+(SA) of lens epithelia, endothelial cells, choroid plexus epithelia and neuroblastoma [14–17]. In choroid plexus epithelia and neuroblastoma cells, these channels are activated during cell swelling in hypotonic solution [16,17].

It is enticing to speculate that these channels might be easily recruitable as pathways for Ca^{2+} entry during stimulus-induced triggering of osteoblast function, including secretion. For example, the voltage-dependent Ba^{2+} conducting channel, like similar L-type channels in other cell types, may be a target for enhanced activation by cAMP-dependent protein kinase and thus be affected by PTH via stimulation of adenyl cyclase. Recently, Guggino et al. [18] have found that secretion of bone matrix proteins by osteoblasts and osteoblast-like cells is enhanced by BAYK 8644 and is suppressed by phenylalkylamine Ca^{2+} channel antagonists. Enhanced activity of the C^+(SA) type channel might be physiologically triggered by local deformation or stress of the cell membrane or by cell swelling provoked by osteoblast activating growth factors, like EGF, which in other cells promote alkalinization and KCl uptake. Recent evidence [19] suggests that platelet derived growth factors activate a non-selective cation channel of similar conductance in fibroblasts.

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