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An L-type Calcium Channel in Renal Epithelial Cells

M.I.N. Zhang, R.G. O'Neil

Department of Integrative Biology, The University of Texas-Houston Health Science Center, 6431 Fannin, Houston, Texas 77030

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Abstract. A voltage-activated Ca^{++} channel has been identified in the apical membranes of cultured rabbit proximal tubule cells using the patch-clamp technique. With 105 mM $CaCl_2$ solution in the pipette and 180 NaAsp in the bath, the channel had a conductance of 10.4 ± 1.0 pS ($n = 8$) in on-cell patches, and 9.8 ± 1.1 pS ($n = 8$) in inside-out patches. In both on-cell and inside-out patches, the channel is active by membrane depolarization. For this channel, the permeation to Ba^{++} and Ca^{++} is highly selective over Na^+ and K^+ ($P_{Ca(Ba)}$:$P_{Na(K)}$ >200:1). The sensitivity to dihydropyridines is similar to that for L-type channels where the channel was blocked by nifedipine (10 μM), and activated by Bay K 8644 (5 μM). When activated by Bay K 8644, the channel showed subconductance levels. Treatment with forskolin (12.5 μM), phorbol ester (1 μM), or stretching (40 cm water) did not activate this channel. These results indicate that this Ca^{++} channel is mostly regulated by membrane voltage, and appears to be an epithelial class of L-type Ca^{++} channel. As such, it may participate in calcium reabsorption during periods of enhanced sodium reabsorption, or calcium signaling in volume regulation, where membrane depolarization occurs for prolonged periods.

Key words: Epithelia — Ion channel — Patch-clamp — Membrane depolarization — Calcium reabsorption — Apical membrane

Introduction

Renal proximal tubule (PT) cells perform highly efficient reabsorption of filtered plasma. Most free calcium is recovered during the reabsorption in this segment of the renal tubule. On one hand, PT cells efficiently transport calcium across the epithelia. On the other hand, the cells regulate the calcium entry in such a way so that a low intracellular calcium level is maintained for normal physiological function. Although it is known that calcium is reabsorbed as sodium is reabsorbed (*see* Suki, 1979; Wright & Bomsztyk, 1986) and that both passive diffusion and active transport contribute to calcium influx (Wright & Bomsztyk, 1986), a detailed mechanism for calcium reabsorption remains unclear (*see* Bell & Franco, 1990).

A second process of controlled calcium entry by PT cells is evident during calcium signaling where a transient increase in intracellular calcium concentration is observed (Christensen & Hoffmann, 1992; McCarty & O'Neil, 1992; Clapham, 1995), as common for many other cells (*see* Yamaguchi et al., 1989; Suzuki et al., 1990; Wong, Debell & Chase, 1990; Beck et al., 1991). When exposed to hypo-osmotic conditions, PT cells swell and then undergo a regulated volume decrease (RVD). In rabbit PT cells, two phases of Ca^{++} signaling are present: an initial transient phase due to a combined contribution of Ca^{++} release from internal stores and Ca^{++} influx from the extracellular medium, and a subsequent plateau phase due to sustained Ca^{++} entry from the extracellular medium (McCarty & O'Neil, 1991*a*). It is further shown that the initial transient Ca^{++} release from ER was through an IP_3 receptor coupled pathway and the sustained Ca^{++} entry in the second phase was partially through a dihydropyridine (DHP) sensitive Ca^{++} channel (O'Neil & Leng, 1995, 1996; Suzuki et al., 1990).

In either Ca^{++} signaling, or Ca^{++} reabsorption, PT cells must handle the influx of Ca^{++} in an effectively regulated way. In many excitable cells, this is achieved through L-type Ca^{++} channels (Tsien, 1983; Miller, 1992; Kass, 1994; Clapham, 1995). L-type Ca^{++} channels are characterized by activation at depolarizing membrane potential and by the sensitivity to DHP antagonist (e.g., nifedipine) and agonist (e.g., Bay K 8644) (*see*

Correspondence to: M.I.N. Zhang

Hess, Lansman & Tsien, 1984; Kunze & Ritchie, 1990; Dolphin, 1991; Hille, 1992; Barry et al., 1995). By contrast, nonexcitable cells generally depend on a receptor coupled inositol-trisphosphate (IP_3) pathway as their primary mode of regulating Ca^{++} channels to generate Ca^{++} signaling (*see* Berridge, 1993; Putney, 1993; Clapham, 1995; Karin et al., 1996). In this pathway, the activation of phospholipase C (PLC) leads to the production of IP_3 which triggers Ca^{++} release from endoplasmic reticulum (ER). Following the internal Ca^{++} release, the empty ER sends a signal to activate the plasma membrane Ca^{++} channel which is normally voltage-insensitive (for review, *see* Putney, 1993; Clapham, 1995). In this general sketch of the Ca^{++} signaling in nonexcitable cells, the contribution of L-type Ca^{++} channel remains unknown. There has been no report of voltage-regulated Ca^{++} channels in renal PT cells (*see* Bell & Franco, 1990), or in epithelial cells in general, although other types of Ca^{++} permeable channels have been observed (e.g., Filipovic & Sackin, 1991; Matsunaga et al., 1994; Kawahara & Matsuzaki, 1993; Tan & Lau, 1993).

Although most of the Ca^{++} channels found in nonexcitable cells are of non-L-type (e.g., Bear, 1990; Bear & Li, 1991; Filipovic & Sackin, 1991; Matsunaga et al., 1994; Kawahara & Matsuzaki, 1993; Tan & Lau, 1993; Zhang & O'Neil, 1996), an L-type Ca^{++} channel was found in fibroblast (Chen et al., 1988) and osteosarcoma cells (Guggino et al., 1988; Duncan & Misler, 1989; Barry et al., 1995), both of which are nonexcitable cell types. It is thus possible that L-type Ca^{++} channels are present in renal PT cells. It has been shown that DHP-sensitive calcium channels are involved in transepithelial calcium transport in mouse distal convoluted tubule cells (Friedman & Gesek, 1993) and in chick duodena (de Boland, Nemere & Norman, 1990), indicating the possible presence of L-type calcium channels. Is there an L-type calcium channel in the renal PT cells? It is to this interest that the present study was undertaken. Indeed, an L-type calcium channel was found in the apical border of these cells.

Materials and Methods

Cell Culture

Rabbit PT cells were isolated according to the methods by Vinay et al. (1981). Cells were grown to confluence on No. 1 glass coverslips in 60-mm petri dishes (Corning glass works, Corning, NY) in DMEM/F-12 medium supplemented with 10% fetal bovine serum (Life Technologies, Gaithersburg, MD) in a humidified atmosphere of 95% air-5% CO_2 at 37°C. Cells cultured under these conditions maintain cell polarity, brush border, and transport properties (Merot et al., 1989). Cells were replenished with fresh media 18–20 hr before their use. Confluent cells from passages 1–4 were used for patch-clamp experiments.

Patch Clamping

The cell-attached (on-cell patch) and excised (inside-out patch) configuration of the patch-clamp technique was used for single-channel recordings at 24°C, according to the method described by Hamill et al. (1981). Patch-pipettes were fabricated from soda lime glass hematocrit tubes (Fisher Scientific, Pittsburgh, PA) by using a two-stage pull (Narishige pp-83 pull, Narishige, Tokyo). The pipette tip was coated with Sylgard (Dow Corning, Midland, MI) and fire polished (Narishige MF-83, Narishige, Tokyo) immediately before use. Pipettes with tip resistance of 2–4 MΩ were used. For membrane stretching, negative pressures were applied to the patch pipette from the sideport in the patch pipette holder through a 50 ml syringe and a three-way valve, connected through plastic tubing to a 90 cm long U-tube filled with colored water for monitoring pipette pressure.

In all configurations, positive current is defined as cation leaving the cell. In both cell-attached and excised patches, with Ca^{++} leaving the pipette, the negative current is recorded and presented in figures as downward deflections. The pipette potential (V_p) is given as $-V_p$ so that the voltages reflect the polarity of the cell membrane, i.e., negative $-V_p$ reflects hyperpolarization of the cell membrane and positive $-V_p$ reflects depolarization of the cell membrane (in excised patches, membrane potential $v_m = -V_p$). Only patches with 10 GΩ or better seals were selected for further observation of channel activity. The channel currents were recorded with an Axopatch-1D patch-clamp amplifier fitted with a CV-4 head stage (Axon Instruments, Foster City, CA). Real-time display and data acquisition were achieved with the use of a TL-1 DMA Interface and DMA Lab Master motherboard (Axon Instruments) in a Pentium 90 MHZ personal computer (Dell OmniPlex 590, Dell Computer, Austin, TX). The records were filtered at a cutoff frequency of 1 KHz and digitized at a rate of 10 points/msec. The analyses of single-channel data were performed with pClamp software suite, version 6.0.2. (Axon Instruments, Foster City, CA).

Solutions

In these experiments, the patch pipettes were filled with (in mM): 105 $CaCl_2$, 10 HEPES, 10 Mannitol, 10 Glucose, pH 7.4; or 65 Ba_2Cl, 60 N-methyl-D-glucamine (NMDG)-Cl, 10 HEPES, 10 Mannitol, 10 Glucose, pH 7.4. The bath solution in on-cell patches consists of (in mM): 180 NaAspartate (NaAsp), 1 $MgCl_2$, 10 HEPES, 10 Glucose, pH 7.4. In inside-out patches, the bath consists of either 180 NaAsp, or 175 KAsp, with 1 $MgCl_2$, 10 HEPES, 10 Glucose, pH 7.4. Often 1 mM Mg-ATP (Sigma, St. Louis, MO) and 200 μg/100 ml trypsin (Life Technologies) were introduced to the bath before excising on-cell patch to an inside-out patch, to minimize channel rundown (Schmid et al., 1995; You, Pelzer & Pelzer, 1995). All solutions were filtered through 0.2 μm Millipore cellulose disks (Corning Glass Works, Corning, NY).

The drugs used in this experiments were phorbol 12-myristate 13-acetate (PMA) (CalBiochem, La Jolla, CA), forskolin (Sigma), nifedipine (CalBiochem), and Bay K 8644 (CalBiochem). PMA was dissolved in dimethyl sulfoxide (DMSO) with a stock concentration of 4 mM. Forskolin, nifedipine and Bay K 8644 were dissolved in 100% ethanol with a stock concentration of 12.5, 10 and 10 mM, respectively. All drugs were freshly diluted immediately before use and the final diluted solution contained no more than 0.1% DMSO or ethanol.

Statistics

Data are shown as single-channel recordings and as mean values ±SE (n), where n is the number of replicates. Comparison between samples

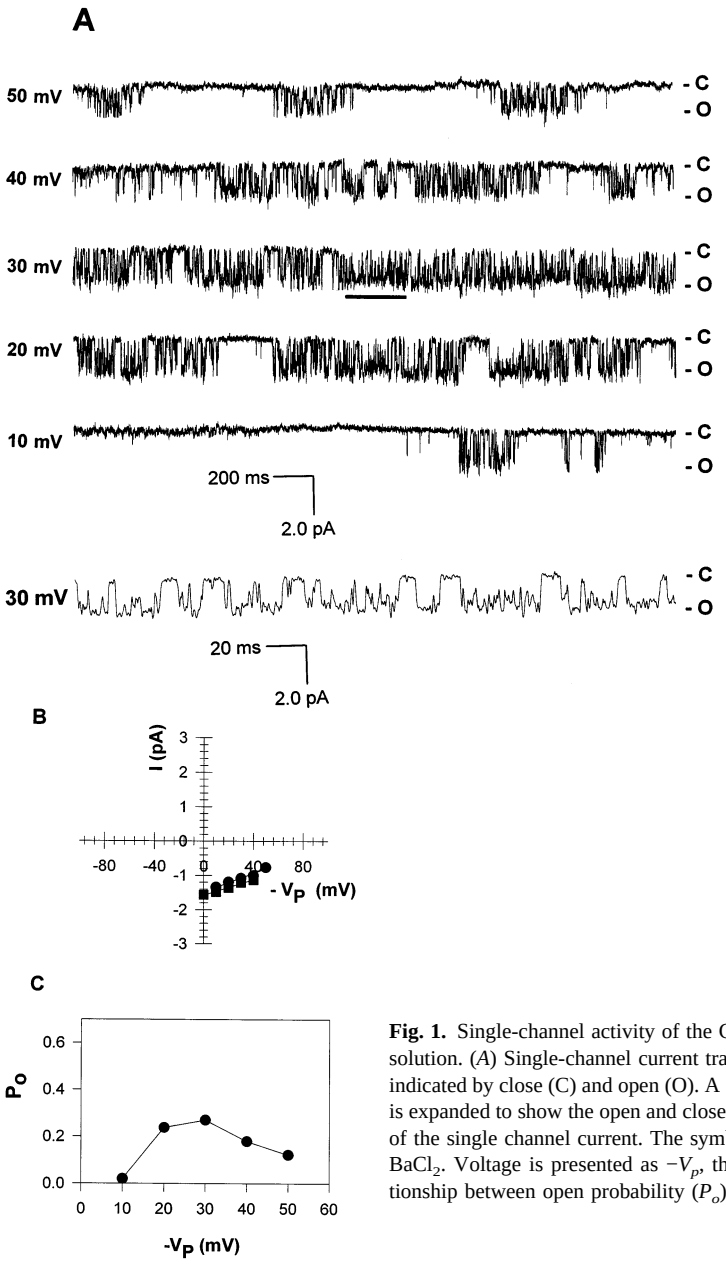

Fig. 1. Single-channel activity of the Ca^{++} channel in on-cell patches, with 105 mm $CaCl_2$ as pipette solution. (*A*) Single-channel current traces at various patch pipette potentials ($-V_p$). Channel status is indicated by close (C) and open (O). A segment of current trace at 30 mV (as indicated by underlining) is expanded to show the open and close (*see* bottom trace). (*B*) Current voltage (*I-V* curve) relationship of the single channel current. The symbol of filled square shows an *I-V* curve for pipette solution 65 $BaCl_2$. Voltage is presented as $-V_p$, thereby reflecting the change in membrane potential. (*C*) Relationship between open probability (P_o) and $-V_p$.

were done with the *t*-test using paired or unpaired analysis as appropriate.

Results

While both Ba^{++} (65 mM $BaCl_2$) and Ca^{++} (105 mM $CaCl_2$) were used in the patch pipette as the current carrier, better quality seals were obtained with $CaCl_2$. In our latest experiments, only $CaCl_2$ was used as the pipette solution. In this paper, only results with $CaCl_2$ pipette solution will be reported except where noted for use of $BaCl_2$ as pipette solution. Here we describe a small Ca^{++} channel that is activated by membrane depolarization. We refer to this channel as an L-type Ca^{++} channel in renal epithelial cells, which is separate from an earlier reported epithelial Ca^{++} channel described as type I (ECaChl) (Zhang & O'Neil, 1996).

On-cell Patches: Channel Regulation

The Ca^{++} channel is observed infrequently occurring in 20 of a total of 1,000 patches (2%). This channel is characterized by an increased open probability (P_o) at depolarized membrane potential. The most active open-

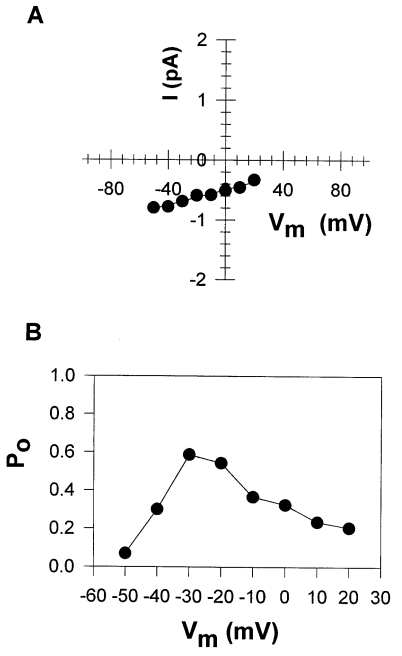

Fig. 2. Single-channel activity of the Ca^{++} channel in inside-out patches, with 105 mM $CaCl_2$ as pipette solution and 180 NaAsp as bath solution. (*A*) Current voltage relationship (*I-V*) of the single channel current. Voltage is presented as the V_m ($= -V_p$), which is the actual membrane potential. (*B*) Relationship between open probability (P_o) and V_m.

ing was found at $-V_p$ of 10 mV to 50 mV (Fig. 1*A* and *C*). As these cells have a resting potential near −40 mV as measured by whole cell current clamp, the 10 to 50 mV depolarizing potential would correspond to an actual membrane potential of −30 to 10 mV. Within this active voltage range, this channel has a conductance of 10.4 ± 0.98 pS ($n = 8$) (*see* Fig. 1*B*). When 65 mM $BaCl_2$ was used as pipette solution, similar *I-V* curve and comparable channel conductance were obtained (*see* Fig. 1*B*).

In current-voltage plots (Fig. 1*B*), a reversal potential of near 100 mV would be approximated if linear extrapolation was used. This indicates that the Ca^{++} channel is highly selective as expected for selective Ca^{++} channels (*see below*).

We have not seen any cases when this channel was turned on by either forskolin (12.5 μM) or PMA (10 μM) ($n = 100$) in the bath, although we have seen another Ca^{++} channel that was activated by PMA under the same condition used in the present study (Zhang & O'Neil, 1996). Stretching through suction up to −40 cm water did not affect this channel either when the channel was closed at hyperpolarizing membrane potentials or when it was open at depolarizing membrane potentials. In more than 100 seals where no Ca^{++} channel was seen, periodic stretching to as much as −40 cm water did not activate this Ca^{++} channel activity (although other channels were activated).

Inside-out Patches: Channel Characteristics

In inside-out patches, the channel activity is similar to that observed for on-cell patches (*see* Fig. 2) except that the channel activity may ''run down'' in 10–15 min. In those cases where channel ''rundown'' was observed, application of ATP (1 mM) and trypsin (200 μg/100 ml) did not recover channel activity ($P_o = 0$, $n = 5$) as has been reported for L-type Ca^{++} channels (Schmid et al., 1995; You et al., 1995).

The voltage dependency of the Ca^{++} channel in excised patches was similar to that observed for the channel in on-cell patches. With stepwise voltage holding, the channel was active within the membrane potential (V_m) range of −40 to 0 mV (*see* Fig. 2*B*), similar to that observed for on-cell patches. Within this active range, the channel conductance was 9.8 ± 1.1 pS ($n = 8$) (*see* Fig. 2*A*).

Linear extrapolation of the current-voltage relation of the channel would indicate a reversal potential near 80 mV (Fig. 2*A*) or more in most cases. From this reversal potential, the estimated P_{Ca}:P_{Na} was larger than 200:1. When 180 mM NaAsp bath was replaced by 175 mM KAsp, there was no change in channel conductance or the extrapolated reversal potential. Similarly, replacement of 105 mM $CaCl_2$ by 65 mM $BaCl_2$ in pipette solution did not alter channel conductance or reversal potential. Therefore $P_{Ca} \simeq P_{Ba} \gg P_{Na} \simeq P_K$, with $P_{Ca(Ba)}$; $P_{Na(K)} > 200{:}1$. This is, therefore, a highly selective Ca^{++} channel.

In this study, inside-out patches were used to test the channel sensitivity to DHP. When the patches were exposed to Bay K 8644 (5 μM) at the cytosolic side, the channel was more active than the control level. By contrast, when the patches were subjected to nifedipine (10 μM), the Ca^{++} channel was completely blocked (Fig. 3*B*). Before treatment with Bay K 8644, the channel open time and close time are both fit well by a single exponential component, with an open time constant and close time constant of 1.49 msec and 0.87 msec, respectively. After Bay K treatment, the channel open time distribution consists of two exponential components: a shorter constant of 0.80 msec and a longer constant of 10.6 msec, although the close time distribution is still fit well by one exponential component (Fig. 3*C*). The Bay K 8644 increased open probability is therefore partially due to increased open time.

In some patches, Bay K 8644 activation of the Ca^{++} channel was occasionally accompanied by the appearance of two opening levels (Fig. 4). These two levels switched back and forth but only one open level was present at one time. Even though both levels were active, there was no occasion for both levels to be present at the same time (Fig. 4). It is likely therefore, that the two levels represent two open states (substates) of the same channel. The substate properties remain to be evaluated in detail in future studies.

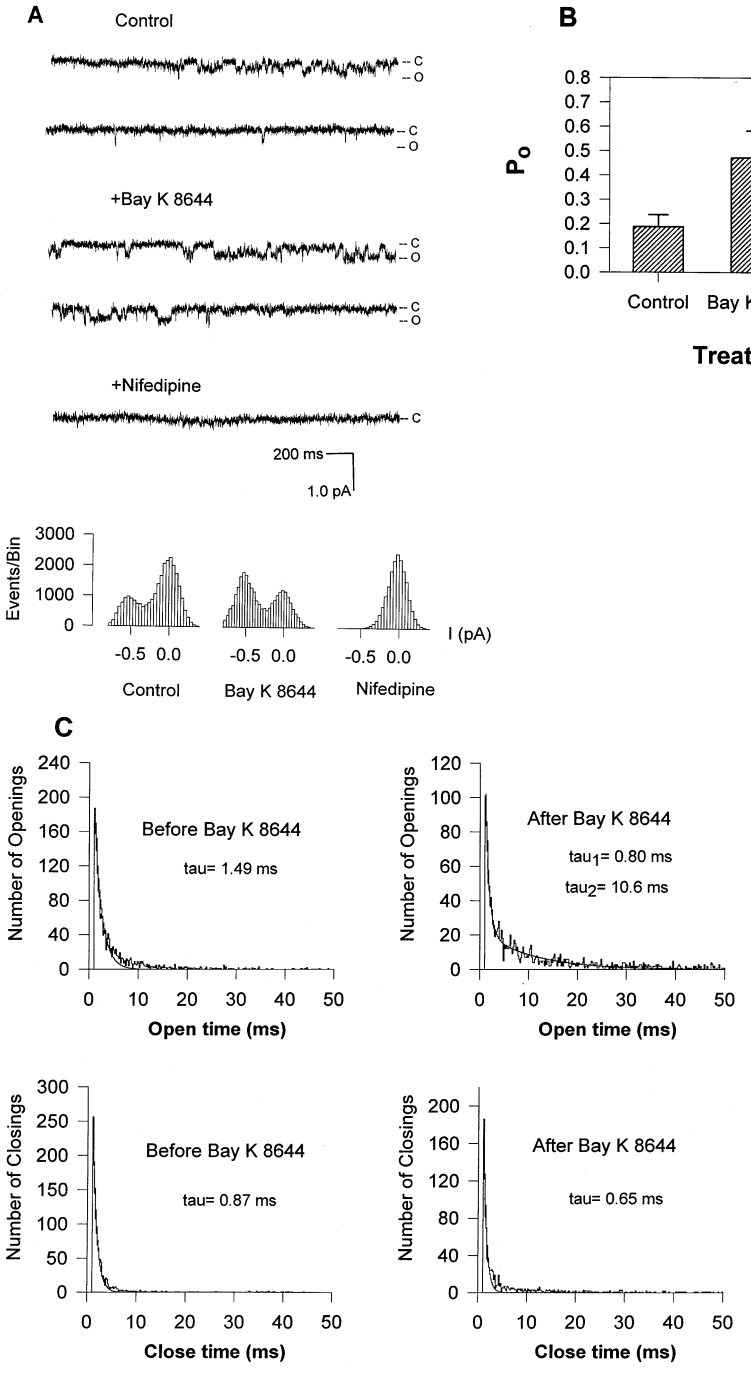

Fig. 3. Dihydropyridines (DHP) sensitivity of the Ca^{++} channel in inside-out patches. Pipette solution is 105 mM $CaCl_2$ and bath solution is 180 NaAsp. Channel state is indicated by close (C) and open (O). (*A*) Top: single channel current traces for Control, +Bay K (5 μM), +Nifedipine (10 μM). Bottom: representative histograms of top current traces. (*B*) Effect of nifedipine (10 μM) and Bay K (5 μM) on Ca^{++} channel open probability (P_o) (at V_m = 0 mV) (n = 4). *P < 0.05 compared with control level of P_o. (*C*) Channel open time constant and close time constant as affected by application of Bay K 8644 (5 μM).

Discussion

The present study found a Ca^{++} channel in the apical membrane of rabbit PT cells that is DHP-sensitive and activated by membrane depolarization. This channel is mostly voltage regulated, as it did not show measurable response to protein kinase C (PKC) activation (PMA treatment), protein kinase A (PKA) activation (forskolin treatment), or membrane stretching. Since DHP-sensitivity (nifedipine blocking, Bay K 8644 activation and Bay K 8644 increased open time constant) and depolarization activation are typical of L-type Ca^{++} channels (*see* Hess et al., 1984; Chen et al., 1988; Kunze & Ritchie, 1990; Dolphin, 1991; Hille, 1992; Barry et al., 1995), the present Ca^{++} channel would appear to be an epithelial class of L-type Ca^{++} channel. Compared with a typical L-type calcium channel in excitable cells, this channel shows slightly different properties. Firstly, it is relatively small, 10 pS for both on-cell and inside-out patches, whereas a typical L-type Ca^{++} channel would

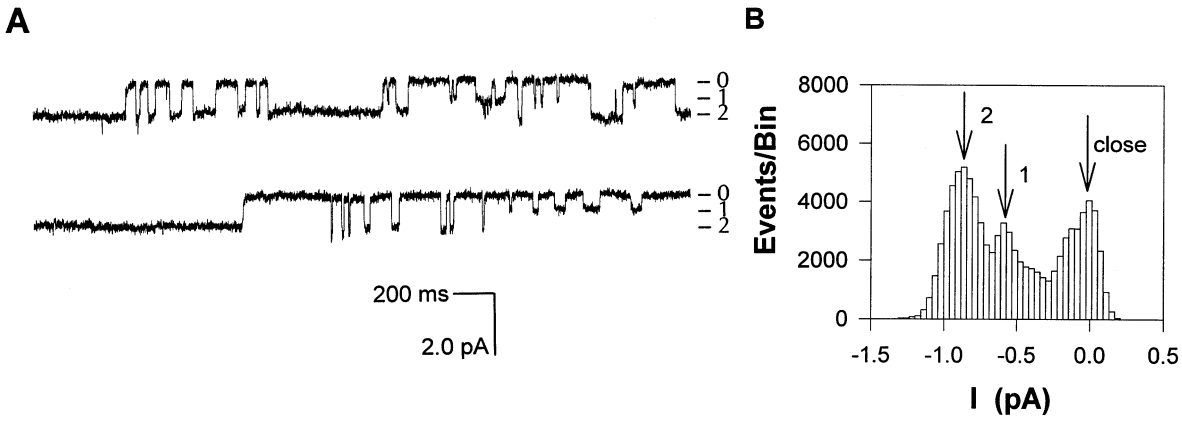

Fig. 4. Subconductance levels of the Ca^{++} channel induced by Bay K 8644 in 180 NaAsp bath solution. (*A*) An example of single channel current trace in an inside-out patch with 5 μM Bay K 8644 in bath. Channel status is indicated by close (0), open1 (1) and open2 (2). (*B*) A histogram showing the two distinctive subconductance levels (indicated as 1 and 2).

normally have a 20–30 pS channel size (*see* Hille, 1992; Dolphin, 1991). Secondly, this channel shows occasional subconductance levels in response to Bay K 8644 activation. The presence of multiple Ca^{++} conductance levels for L-type Ca^{++} channels were rarely observed in native membranes (Kunze & Richie, 1990) but have been found for Ca^{++} channels reconstituted into lipid bilayers (Ma & Coronado, 1988; Erlich et al., 1986; Smith et al., 1987; Talvenheimo, Worley & Nelson, 1987; Hymel et al., 1988). It is generally thought that a larger subconductance level is produced by opening of multiple smaller subconductance levels in a concerted fashion (Ma & Coronado, 1988; Hymel et al., 1988; Kunze & Richie, 1990). A similar pattern of activation of subconductance states may be present in this L-type Ca^{++} channel.

PT cells do not normally experience major membrane depolarization. One notorious case with prolonged membrane depolarization was found during RVD upon hypo-osmotic treatment (Welling & O'Neil, 1990) and that was accompanied by a sustained Ca^{++} entry through a DHP-sensitive Ca^{++} channel (McCarty & O'Neil, 1990, 1991*a*,*b*; O'Neil & Leng, 1995). It is likely that this L-type Ca^{++} channel is activated during swelling induced membrane depolarization and thus contributes to Ca^{++} signaling as necessary for RVD. The suggestion that this L-type Ca^{++} channel plays a role in Ca^{++} signaling does not, however, exclude the possibility that other Ca^{++} channels may also contribute to Ca^{++} signaling in RVD. There is at least one other Ca^{++}-selective channel, the epithelia Ca^{++} channel type-I (ECaChl), in the same cells (Zhang & O'Neil, 1996). ECaChl does not respond to voltage activation (although it requires hyperpolarized membrane potential as an activation condition), but it is activated by phorbol ester/PKC and membrane stretch (Zhang & O'Neil, 1996). The drastic differences in channel properties between ECaChl and this L-type Ca^{++} channel suggests that these two Ca^{++} channels may function at different phases in RVD. It is likely that ECaChl functions during the initial transient Ca^{++} elevation phase at hyperpolarized membrane potential, and this L-type Ca^{++} channel participates in the plateau phase with sustained Ca^{++} entry at depolarized membrane potential.

Another case for PT cells to experience membrane depolarization is during enhanced sodium entry across the apical membrane, in which the electrochemical gradient for sodium is decreased. It is proposed that both passive diffusion and active transport contribute to calcium entry through the apical side (Wright & Bomsztyk, 1986), and the resultant elevation in intracellular calcium would slow down sodium entry through calcium inhibited sodium channel (*see* Bell & Franco, 1990) if they were present. The finding of the present L-type Ca^{++} channel seems to indicate that voltage-regulated calcium entry is another way to stimulate calcium reabsorption particularly during periods of enhanced sodium reabsorption. Studies of trans-epithelia Ca^{++} transport (Friedman & Gesek, 1993; de Boland et al., 1990) showed that DHP-sensitive Ca^{++} channels are involved. In chick duodena, the Ca^{++} channels was proposed to be located on the basolateral side as Bay K 8644 activated more Ca^{++} transport when it was applied on the basolateral side (de Boland et al., 1990). It is possible that the renal PT cells may also have L-type Ca^{++} channel, possibly even at a higher density, on the basolateral side. This would contribute to the finding of the low density of L-type Ca^{++} channels in the apical side of the PT cells. On the other hand, L-type Ca^{++} channels on the basolateral side may be more important in Ca^{++} signaling than in direct contribution to Ca^{++} reabsorption.

Whether the L-type Ca^{++} channel serves as Ca^{++} signaling in volume regulation or Ca^{++} reabsorption during enhanced sodium reabsorption, a question remains on

how effective this channel is in achieving these functions. Would it be sufficient for such a low density channel on the apical membrane side to act alone to elevate intracellular calcium to a significant level? A calculation with information based on current experimental results indicate there should be approximately two L-type Ca^{++} channels per cell which would be sufficient to significantly contribute to calcium influx during membrane depolarization.[1] Hence this channel is of high enough density to play an important physiological role in calcium signaling or calcium reabsorption.

The present experimental data on the L-type Ca^{++} channel may expand our view on Ca^{++} channel properties and function in nonexcitable cells. Currently, it has been generally accepted that plasma membrane Ca^{++} channels are insensitive to membrane voltage change and that it is normally activated internally by ER calcium depletion (*see* Putney, 1993; Clapham, 1995). The finding of this L-type Ca^{++} channel in the rabbit renal PT, as well as findings of L-type Ca^{++} channels in other nonexcitable cells (Chen et al., 1988; Guggino et al., 1988; Duncan & Misler, 1989; Barry et al., 1995), suggest that nonexcitable cells may also possess voltage activated Ca^{++} channels. Recent studies of excitable cells showed that cyclic adenosine diphosphate-ribose (cADPR) is a second messenger activating the ryanodine receptor similar to the activation of IP_3 receptors in nonexcitable cells (*see* Galione, 1992, 1994; Lee, 1993; Meszaros, Bak & Chiu, 1993). It this seems that the Ca^{++} signaling between excitable cells and nonexcitable cells has more similarity in their signaling pathway than previously known.

In summary, we have identified a low density Ca^{++} channel on the apical membrane side of the renal PT cells. This channel is activated by membrane depolarization and it is dihydropyridine sensitive. This is the equivalent of an L-type Ca^{++} channel in the PT cells. It is anticipated that as the cell membrane depolarizes in cell volume regulation or in enhanced sodium reabsorption, this Ca^{++} channel will likely function in controlling calcium entry.

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[1] Estimate of calcium influx through L-type Ca^{++} channel: Assuming a 10 μm cubic cell dimension, a 1 mM extracellular Ca^{++}, 100 nM intracellular Ca^{++}, a channel size of 10 pS, an average depolarization of the membrane potential by −20 mV, an average open probability (P_o) of 20%, an approximation of the physiological calcium influx may be derived.

(i) *Physiological reversal potential for calcium* (E_{rev}^{Ca}):

A reversal potential of 118 mV is obtained by $E_{rev}^{Ca} = (RT/2F)\ In\ ([Ca^{++}]_o/[Ca^{++}]_i)$.

(ii) *Membrane conductance of PT cell apical membrane* ($g_{Ca}/cell$):

Given that the patch area is 1 μm^2 and each patch contains 0.02 channel (2% patches contained channels), each cell would on average have 2 L-type Ca^{++} channels, and thus the total conductance of this channel for a single cell is: $g_{Ca}/\text{cell} = 10\ \text{pS} \times 2 = 20\ \text{pS}$.

(iii) *Apical membrane current and calcium flux at membrane depolarization:*

The maximal current (at 100% P_o) of the apical membrane follows the relation $I(\text{pA}) = 20\ (\text{pS})\ (E_{rev}^{Ca} - V_m)\ (V)$, which is 20 (118 − (−20))/1000 = 2.76 pA. Conversion of membrane current to calcium flux in mole gives:

$2.76\ \text{pA} = 2.76 \times 10^{-12}\ \text{A} = 2.76 \times 10^{-12}$ C/s (coulomb/sec), which equates to 1.38×10^{-11} μmole Ca^{++}/sec.

Hence, at 20% P_o, the estimated calcium flux is 2.76×10^{-12} μmole Ca^{++}/sec.

(iv) *Transform calcium flux to intracellular calcium concentration change:*

Cell volume: $(10\ \mu m)^3 = 1 \times 10^{-9}\ cm^3 = 1 \times 10^{-12}$ liter.

Calcium flux in molar concentration of intracellular calcium is therefore $(2.76 \times 10^{-12}$ μmole/sec)/$(1 \times 10^{-12}$ liter) = 2.76 μM/sec

2.76 μM/sec is the initial calcium influx rate. As calcium entry changes the reversal potential for calcium (E_{rev}^{Ca}) (shifts E_{rev}^{Ca} toward the negative direction), the influx rate will be 1.89 μM/s at the end of 1 sec. Even though, this is an enormous amount of calcium influx. Therefore, under these conditions, with two channels per cell, the intracellular concentration could reach μM level in a fraction of second, if calcium is not effectively buffered and removed from the basolateral side by active transport. If the proximal tubule cells have intracellular calcium buffering similarly to those of squid axons (*see* Brinley, 1980), the calcium influx, from 40% to nearly all, will be buffered, depending on whether the intracellular calcium is in submicromolar or micromolar concentration. With such a high capacity calcium buffering system, a maximum calcium influx of 2.76 μM/sec may be maintained, particularly during highly efficient transepithelia calcium transport.

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