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STRETCH-ACTIVATED CHANNELS IN SINGLE EARLY DISTAL TUBULE CELLS OF THE FROG

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SUMMARY

1. Single stretch-activated channels have been studied in cell-attached and excised patches from single early distal tubule (diluting segment) cells of *Rana temporaria*.

2. The channels can be reversibly activated, in both cell-attached and excised patches, by the application of negative pressure to the pipette causing mechanical stretching of the cell membrane. In cell-attached patches, application of 14·8 cmH_2O negative pressure to the patch pipette increased reversibly the open probably from 0·11 to 0·87.

3. The channel conductance in the cell-attached configuration with standard Ringer solution in the pipette is 21·3 pS.

4. The channel is non-specific. In excised inside-out patches ion substitution experiments show that the channel does not discriminate between sodium and potassium ions, nor does it appear to select for cations over anions.

5. The channel is voltage sensitive such that depolarizing the cell opens the channel. The open probability at the resting membrane potential, 0·89, was reduced to 0·26 at a hyperpolarizing potential of 100 mV (holding pressure of $-20·1$ cmH_2O or -206 Pa).

6. The sensitivity of the channel to mechanical stretching suggests that the channel may be involved in cell volume regulation.

INTRODUCTION

In a number of renal epithelia, exposure to hypotonic media causes cell swelling followed by a decrease in cell volume towards the original level, or an increase in cell volume significantly lower than that predicted from the solute content of the cell (Dellasega & Grantham, 1973; Guggino, Oberleithner & Giebisch, 1985; Kirk, DiBona & Schafer, 1987; Lopes & Guggino, 1987; Hebert & Sun, 1988; Volkl & Lang, 1988*a*; Welling & Linshaw, 1988). This volume regulatory decrease (VRD) response is mediated by the concomitant loss of potassium and accompanying anions, followed by osmotic movement of water (Dellasega & Grantham, 1973; Siebens, 1985; Volkl & Lang, 1988*a*; Hoffmann & Simonsen, 1989). The mechanism responsible for the activation of the solute exit pathway remains one for debate (Hoffmann & Simonsen, 1989).

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One possible mechanism is that channels in the cell membrane open when the cell swells allowing calcium to enter the cell, which acts as an intracellular second messenger, eliciting activation of the solute exit pathway (Christensen, 1987). Several cells appear to use this strategy during VRD. In *Necturus* choroid plexus epithelium a cation-selective calcium-permeable channel that opens in response to membrane stretch has been found. The epithelium also contains a calcium- and voltage-activated potassium channel, the activity of which was found to increase in concert with the activity of the stretch-activated channels (Christensen, 1987). Additionally, cultured cells from opossum kidney (OK cells) respond in a similar manner to hypotonic shock; non-selective stretch-activated channels and outwardly rectifying potassium channels are evoked when the osmolarity of the bathing solution is lowered (Ubl, Murer & Kolb, 1988*a*). The potassium channels can also be activated by the addition of the calcium ionophore A23187 to an isotonic bath solution containing calcium (Ubl, Murer & Kolb, 1988*b*), suggesting that a rise in intracellular calcium plays a key role in the activation of the potassium channel.

A more direct linkage between an increase in cell volume and the loss of cellular potassium has been described in *Necturus* proximal tubule where a stretch-activated potassium channel has been found on the basolateral membrane (Sackin, 1987). If cell swelling stretches the basolateral membrane the resulting increase in open probability of this channel would restore cell volume by loss of potassium (and an accompanying anion) followed by osmotic exit of water (Sackin & Palmer, 1987). This mechanism negates the necessity for an intermediate second messenger, at least for potassium loss.

The study of volume regulation in early distal cells is far from extensive, with only one report of solute loss from *Amphiuma* early distal cells in response to osmotic swelling after the apical Na^+–K^+–Cl^- co-transporter had been blocked with furosemide. This solute loss in response to osmotic swelling was associated with a reduction in intracellular chloride, and presumably potassium, content of the cell (Guggino *et al.* 1985). However, in the absence of the diuretic furosemide, the volume regulatory mechanisms in these cells is masked by the sustained entry of solute on the apical membrane co-transporter (Guggino *et al.* 1985).

Results from the present study establish the presence of non-selective channels in single early distal tubule cells from the frog. The channels can be activated reversibly by mechanically stretching the cell membrane by applying negative pressure to the back of the pipette. These channels may be involved in the activation of solute exit pathways during changes in cell volume.

METHODS

Single early distal tubule cells were isolated from *Rana temporaria* by the following procedure, which is a modification of the method described by Oberleithner, Schmidt & Dietl (1986). Frogs were killed by decapitation and destruction of the spinal cord. The kidneys, with intact vasculature, were removed. The dorsal aorta was perfused for 30 min with oxygenated calcium-free Ringer solution (see Table 1). Each portal vein was then perfused with approximately 3 ml of standard Ringer solution (see Table 1) containing collagenase (50 mg 100 ml^{-1}, Sigma, type I). The kidneys were cut free from their surrounding vasculature and incubated in collagenase-containing Ringer solution for 20 min at 23 °C in a shaking water bath. The early distal tubules lie just beneath

the kidney capsule on the ventral surface. In order to obtain a preparation rich in early distal tubule cells, thin slices of tissue (~ 1 mm thick) were cut from the ventral surface and complete dissociation of the tissue into single cells was achieved by gently drawing the pieces of kidney into and out of Pasteur pipettes with decreasing tip diameters. Enzymatic digestion of the cells was arrested by two consecutive centrifugations at 14000 *g* for 12 s. After each centrifugation the

TABLE 1. Composition of Ringer solutions (concentrations in mM)

	Ca^{2+} free	Standard	Dilute	High K^+
NaCl	99	97	20	0
KCl	3	3	3	100
$CaCl_2$	0	2	2	2
$MgCl_2$	1	1	1	1
HEPES	10*	10*	10*	10**
Mannitol	—	—	140	—

* Titrated to pH 7·4 with NaOH. ** Titrated to pH 7·4 with KOH.

supernatant was discarded and the cells resuspended in enzyme-free amphibian Ringer solution. The cells were finally resuspended in Liebovitz 15 medium (Sigma, prepared with an osmolality of 204 mosmol kg^{-1} H_2O, pH 7·4) and stored on ice prior to use. Cells used in the following experiments were not more than 6 h old.

Cells were transferred in 25 μl aliquots to a Perspex chamber on the stage of an inverted microscope (Zeiss, FRG). A silver chloride-coated silver wire served as the bath electrode. The microscope, fitted with Nomarski optics, was mounted on a vibration isolation table (Ealing Electro-Optics) and enclosed in a Faraday cage. The amplifier head stage was attached to a micromanipulator (Narishige, Japan) mounted directly onto the microscope stage to minimize vibration of the patch pipette. Attached to the back of the patch pipette via a side arm in the pipette holder was a water manometer which, by means of a three-way tap, could be used to apply positive, negative or atmospheric pressure to the back of the pipette.

Patch pipettes were fabricated from microhaematocrit glass tubes (LIP, Shipley) using a two-stage vertical pipette puller (Narishige, Japan) and coated with Sylgard 184 (Dow Corning, USA). Pipette resistances varied between 5 and 15 MΩ. Gigaohm seals were obtained either spontaneously as the pipette touched the cell or by application of negative pressure to the patch pipette.

Channel currents were recorded using a List EPC-7 (FRG) patch clamp amplifier and stored on videotape following pulse code modulation (Sony PCM 701ES, Japan; modified to extend the frequency response to DC). Currents were analysed by replaying the tape through an 8-pole Bessel filter connected to both a digital storage oscilloscope (Gould) and a microcomputer (IBM, XT 286) with a Tecmar TM40 A/D converter via a Labmaster interface (Axon instruments, Burlingame, USA). The records were sampled into computer memory at 1 kHz at a frequency of 500 Hz on the Bessel filter. Analysis was performed using a single-channel analysis program (SCAP) written 'in-house' in QuickBasic 4·0 (Microsoft, USA).

Command potentials (V_c) are given with respect to the pipette (ground), such that a V_c of -40 mV corresponds to hyperpolarization of the patch by 40 mV. In the case of excised inside-out patches V_c is equivalent to the membrane (patch) potential. The current–voltage (I–V) relationships for cell-attached and excised patches were constructed from the average height of transitions between open and closed channel current levels at each applied potential. Single-channel conductance was determined from linear regression of such I–V relationships. Records showing pressure activation of the channels were obtained at a command potential of -40 mV so that discrete changes between open and closed channel current levels could be observed easily. The voltage dependence was assessed by changing the holding potential at a constant negative pressure.

Single-channel open probability (P_o) in multichannel patches was calculated as:

$$P_o = (\Sigma n t_n)/N,$$

where n represents the state of the channels, 0 = closed, 1 = one open channel, etc. and t_n is the

length of time in state n. N is the number of channels in the patch, which we took to be the maximum number of channels seen to be open simultaneously during the recording. P_o was calculated from data segments of at least 10 s duration.

Groups of paired data were analysed using Student's t test and presented as means ± S.E.M. with the number of observations (n). More than two groups of data were analysed by analysis of variance.

RESULTS

Effect of membrane stretch in cell-attached and excised patches

Figure 1 illustrates a typical current recording from a cell-attached patch from an isolated frog early distal tubule cell. The control part of the trace was obtained with zero suction applied to the pipette. Application of 10·3 cmH_2O negative pressure to the rear of the pipette produced a large increase in channel open probability. In the illustrated experiment the single-channel open probability, P_o, increased from 0·1 to 0·9 when suction was applied and fell to 0·1 on removal of the applied pressure.

Figure 2 shows mean data from five cell-attached patches ($V_c = -40$ mV). Standard Ringer solution bathed the cells and the pipette was filled with a high-K^+ solution. Single-channel open probability was calculated before, during and after the application of negative pressure. With zero pressure applied to the pipette P_o was 0·11 ± 0·049 ($n = 5$). On applying a negative pressure of 14·8 ± 0·34 cmH_2O to the pipette P_o increased significantly ($P < 0{\cdot}001$) to 0·87 ± 0·034 ($n = 5$). Releasing the applied pressure caused a significant fall in P_o ($P < 0{\cdot}01$) to 0·19 ± 0·038 ($n = 4$).

Repeating the same experiments as above only with standard Ringer solution as the pipette solution gave similar results. With zero pressure applied to the pipette P_o was 0·026 ± 0·023 ($n = 5$). On application of 17·2 ± 0·95 cmH_2O negative pressure to the patch pipette P_o increased significantly ($P < 0{\cdot}01$) to 0·69 ± 0·11 ($n = 5$).

Increases in channel activity upon the application of negative pressure to the pipette could also be elicited in excised patches. The inset in Fig. 1 shows channel activation upon application of negative pressure to the pipette in an excised inside-out patch. In the experiment shown, P_o was zero with no applied pressure. On application of 18·0 cmH_2O negative pressure P_o increased to 0·8 and fell back to zero on removal of the pressure. Mean data from excised patches with high-K^+ as the pipette solution and standard Ringer solution as the bathing solution show an increase in P_o from zero to 0·67 ± 0·098 ($n = 3$) on application of negative pipette pressure.

Figure 3 shows the relationship between open probability and applied negative pressure with high-K^+ solution as the pipette solution and standard Ringer solution as the bathing solution. From the graph it is apparent that single-channel P_o increases linearly with negative pressure applied to the pipette. The sensitivity of this relationship is such that a twofold increase in P_o results from an increase in negative pressure of 5·0 cmH_2O.

Conductance

In the cell-attached mode with standard Ringer solution bathing the cells the channel conductance is 21·6 ± 1·17 pS ($n = 12$) with high-K^+ solution in the pipette and 21·3 ± 1·67 pS ($n = 6$) with standard Ringer solution in the pipette. Figure 4 shows the I–V relation in six excised inside-out patches with Ringer solution in the

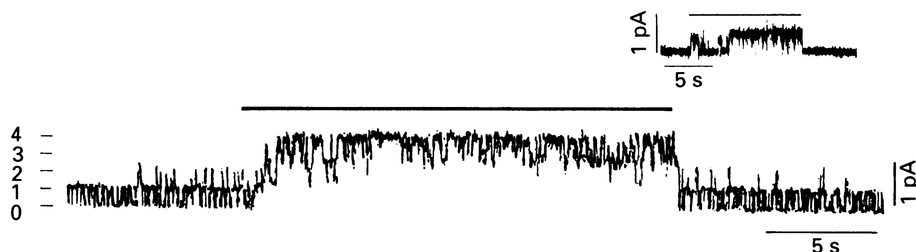

Fig. 1. Typical recording of stretch-activated channels in a cell-attached patch. Inset, recording showing stretch activation of a channel in an excised patch. In both traces currents were recorded with $V_c = -40$ mV. High-K^+ solution in the pipette, standard Ringer solution in the bath (see Table 1). Upward deflections from closed to open state denote current flow from the pipette. The bar shows the duration of applied pressure.

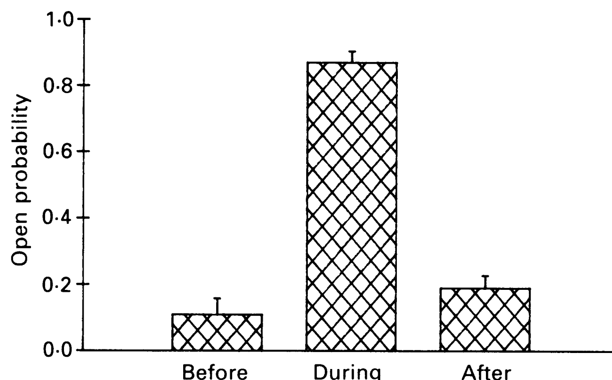

Fig. 2. Mean data of single-channel open probability before, during and after application of negative pressure. In all experiments V_c was -40 mV. High-K^+ solution in the pipette, standard Ringer solution in the bath (see Table 1). Single-channel open probability increased significantly ($P < 0·001$, paired t test, before *versus* during) from $0·11 \pm 0·049$ (mean ± S.E.M., $n = 5$) to $0·87 \pm 0·034$ ($n = 5$) on applying a mean negative pressure of $14·8 \pm 0·34$ cmH_2O and fell significantly ($P < 0·01$, paired t test during *versus* after) to $0·19 \pm 0·038$ ($n = 4$) when the pressure was released.

bath. The open and filled symbols indicate the pipette solutions (high-K^+ and standard Ringer, respectively). The single-channel slope conductance is $19·2 \pm 1·1$ pS ($n = 6$, $r = 0·98$) and shown as the continuous line on the graph.

Comparison of the conductance measurements in all three of the above cases, cell-attached with Ringer solution or high-K^+ pipette solution or in excised patches, by analysis of variance, indicates that they are not significantly different from each other ($F_{2,21} = 0·76$).

Selectivity

The channel does not discriminate between sodium and potassium ions. The reversal potential of the channel in excised inside-out patches is not significantly different from zero ($0·41 \pm 0·8$, $n = 6$, $t = 0·5$) with standard Ringer solution on one side of the patch and high-K^+ solution on the other (Fig. 4).

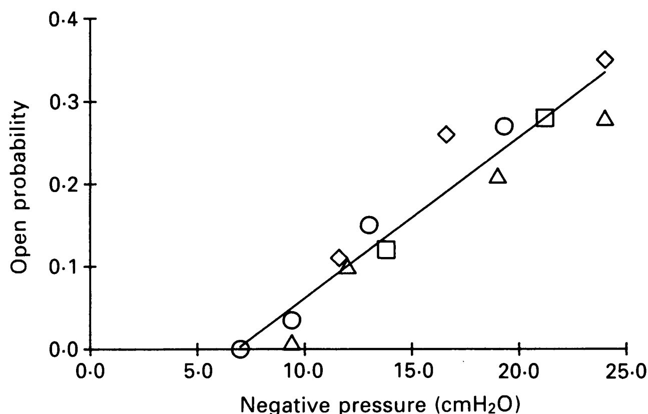

Fig. 3. Relationship between P_o and negative pressure. Different symbols are from different patches with high-K^+ as the pipette solution and standard Ringer solution in the bath. V_c was held at either −40 or −60 mV as the applied pressure was varied. The continuous line is a linear least-squares best fit of the data ($r = 0{\cdot}96$). A twofold change in P_o results from 5·0 cmH_2O change in negative pressure.

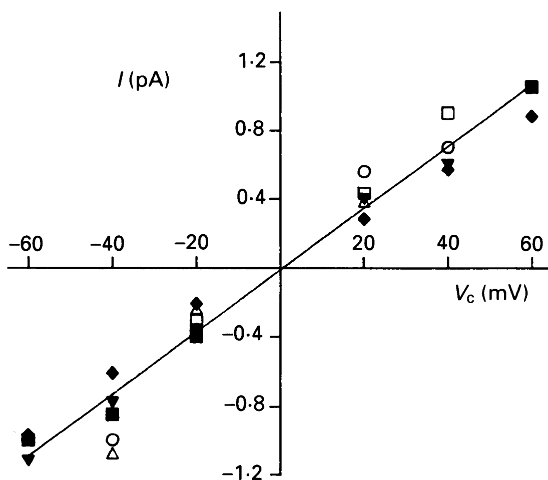

Fig. 4. I–V relation for six excised inside-out patches. The open symbols are data from patches with high-K^+ solution in the pipette and standard Ringer solution in the bath; filled symbols are data from patches with standard Ringer solution in both the pipette and the bath. V_c is the command potential applied to the cell with respect to the pipette. The continuous line is a linear least-squares best fit of the data ($r = 0{\cdot}98$) and corresponds to a single-channel slope conductance of $19{\cdot}17 \pm 1{\cdot}09$ pS (mean ± S.E.M., $n = 6$). The reversal potential is $0{\cdot}41 \pm 0{\cdot}8$ mV ($n = 6$).

The channel also does not discriminate between cations and anions (Fig. 5). If both the sodium and chloride concentrations of the bath solution are reduced (dilute Ringer solution; see Table 1) with respect to the pipette solution (standard Ringer solution) then anion- and cation-selective channels should behave differently. If the channel is cation selective, with a dilute bath solution, inward current fluctuations would be observed at 0 mV pipette potential as cations flow from the pipette down

their electrochemical gradient into the bath. The converse would be true if the channel is anion selective since, with a dilute bath solution at 0 mV pipette potential, chloride would flow down its electrochemical gradient from the pipette to the bath. Taking care to correct for the Nernstian change in electrode potential at the bath

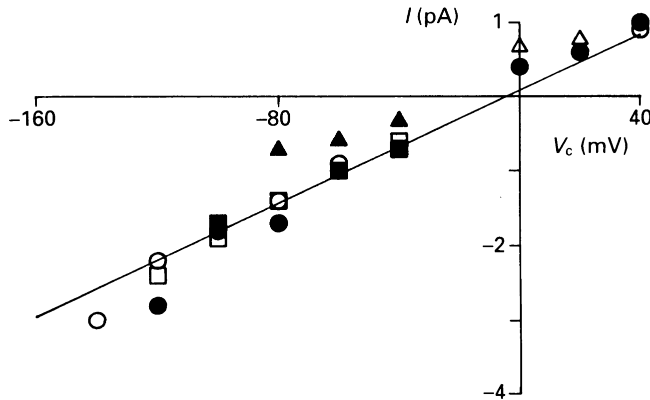

Fig. 5. I–V relation for six excised inside-out patches with standard Ringer solution as the pipette solution and dilute Ringer solution as the bath solution. The reversal potential of the channels was $-4{\cdot}5 \pm 2{\cdot}9$ mV ($n = 6$). The continuous line was calculated from Ohm's law ($I = GV$), where the single-channel conductance was taken as 19·17 pS (see legend to Fig. 3) and V was the command potential plus the measured reversal potential ($+4{\cdot}5$ mV). Different symbols represent separate experiments.

electrode and given the dilution factor of 5 used in the present study, we would predict a reversal potential of +40 mV for a cation-selective channel and −40 mV for an anion-selective channel.

Figure 5 shows the I–V relation from six excised patches with standard Ringer solution as the pipette solution and dilute Ringer solution as the bathing solution. The reversal potential of the channel was $-4{\cdot}5 \pm 2{\cdot}9$ mV ($n = 6$). This value is not significantly different ($P = 0{\cdot}22$) from zero. This indicates that the channel does not discriminate between cations and anions.

Voltage dependence

The channel is voltage dependent such that hyperpolarizing the cell closes the channel. Figure 6 shows the relationship between open probability and command potential. The data were taken from six cell-attached patches, with a high-K^+ solution in the pipette and standard Ringer solution in the bath, where the holding potential was altered at constant negative holding pressures ranging from 16·0 to 22·8 cmH_2O (mean pressure = $20{\cdot}1 \pm 0{\cdot}9$ cmH_2O). Making the assumption that the channel is fluctuating between one open and one closed state the best fit of the data (using the Simplex algorithm) to the Boltzmann distribution was determined from:

$$P_o = (1 + \exp(-nF(V - V_o)/RT))^{-1},$$

where F, R and T have their usual meanings, V is the test potential, V_o is the potential at which the open probability is 0·5 and n is the equivalent number of charges moving

across the transmembrane potential during an open–closed transition (Hille, 1984). The value of n determines the slope of the relationship between P_0 and voltage; larger values of n give a steeper dependence of open probability on membrane potential. From the above equation $V_0 = -82$ mV and $n = 1{\cdot}1$.

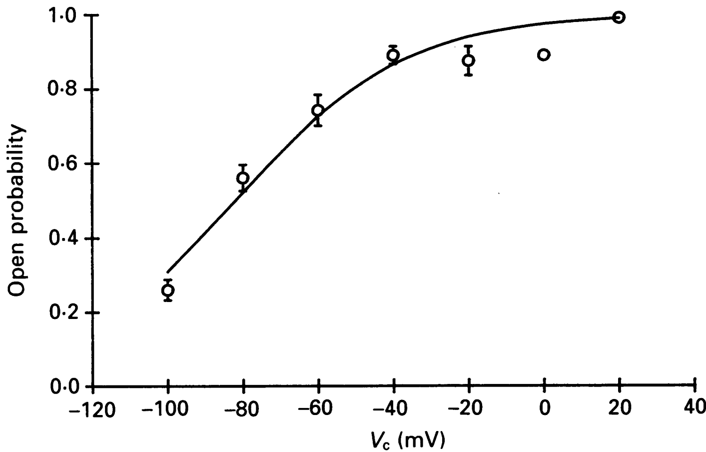

Fig. 6. Relationship between open probability and command potential (V_c). Data are from six cell-attached patches with a high-K^+ pipette solution and standard Ringer solution in the bath. The applied negative pressure was $20{\cdot}1 \pm 0{\cdot}9$ cmH_2O (mean ± S.E.M., $n = 6$). The continuous line is calculated from the Boltzmann equation and is the best fit to the data ($r = 0{\cdot}98$). The best-fit parameters are $V_0 = -82$ mV and $n = 1{\cdot}1$.

DISCUSSION

The present study establishes the presence of stretch-activated channels in the early distal tubule cells of the frog. The lack of selectivity of the channels and similarity to channels in other epithelial cells suggests that the channels probably act as transducers, signalling that a change in cell volume has occurred.

Location and activation of channels

Epithelial cells are characterized by the differing permeabilities of their apical and basolateral membranes. However, following isolation, the cells appear to be homogenous spheres and the apical and basolateral membranes are indistinguishable from each other. We have recently performed experiments with isolated everted early distal tubules and have recorded stretch-activated channel activity in apical membrane patches of the tubule (unpublished observations). Thus we can say that the channels are present in the apical membranes of the cells but this does not preclude their presence in the basolateral membrane.

Dilution of the basolateral perfusate of microperfused early distal tubules of *Amphiuma* causes cell swelling. This increase in volume is brought about by expansion of the apical membrane into the tubule lumen as the movement of the basolateral surface is restricted by the basement membrane (Guggino *et al.* 1985). Thus stretch-activated channels located on the apical surface of the cells would indeed be exposed to an increase in membrane tension upon hypotonic shock.

The maximum mean pressure applied to the membrane patch was 20·1 cmH_2O (206 Pa) or $2{\cdot}05 \times 10^4$ dyn cm^{-2} (Guharay & Sachs, 1984; Sackin, 1987). If an approximately spherical deformation of the membrane patch occurs when negative pressure is applied to the patch pipette then membrane tension (T) can be estimated from Laplace's law (Guharay & Sachs, 1984):

$$T = Pd/4,$$

where P is the applied pressure and d is the diameter of the membrane patch. If we assume that the patch forms a flat membrane across the tip of the patch pipette ($\sim 1\ \mu m$; Sakmann & Neher, 1983) a pressure of 20 cmH_2O will produce a tension of 0·5 dyn cm^{-2}. This relatively small increase in tension might be produced by cell swelling. Using equation (A 4) from Sackin (1987) and an area elasticity constant of between 130 and 340 dyn cm^{-2} (Guharay & Sachs, 1984) it is possible to estimate that an increase in cell volume of only 0·6 and 0·2% respectively would produce an increase in membrane tension of 0·5 dyn cm^{-2}. From previously reported data much larger percentage increases in cell volume have been reported during cell swelling in hypotonic media. Increases in cell volume of around 30% were observed in perfused *Amphiuma* early distal tubules following a reduction in the osmolality of the bath (basolateral) fluid (Guggino *et al.* 1985). In *Necturus* proximal tubule a 15% increase in cell volume is reported (Lopes & Guggino, 1987), whereas in proximal straight tubules from mice (Volkl & Lang, 1988*b*) and from rabbits (Kirk *et al.* 1987) cell volume increased by 16 and 28%, respectively, on exposure to hypotonic media. From the above, it seems feasible that the stretch-sensitive channels in frog early distal cells would be activated even by the most moderate changes in cell volume.

Contribution to cell conductance

The contribution of the channels to the whole-cell (macroscopic) conductance (G) can be estimated from:

$$G = gnP_o/A,$$

where g is the single-channel conductance (21·3 pS), n the mean number of channels per patch (1·9), P_o the open probability with no applied pressure (0·11) and A is the area of a patch (1·57 μm^2). This gives a macroscopic conductance of 0·28 mS cm^{-2}. This value is much lower than that of the apical or basolateral membranes (1·8 and 4·6 mS cm^{-2}; Oberleithner, Guggino & Giebisch, 1985). The ratio of the apical-to-basolateral surface area is 0·1 (Stanton, Biemesderfer, Stetson, Kashgarian & Giebisch, 1984) giving an estimated macroscopic cellular conductance of 4·3 mS cm^{-2}. Thus the channels may contribute around 6% of the cellular conductance. On the other hand, if the channels are confined to the apical membrane then they could represent as much as 16% of the apical membrane conductance.

Voltage dependence

The channels are seen to open with depolarization (Fig. 6). The voltage dependence is similar to that of stretch-activated channels in OK cells (Ubl *et al.* 1988*a*) and in the basolateral membrane of frog proximal tubule cells (Hunter, 1990). In the latter case, the gating charge was estimated at 0·78, similar to that of 1·1 reported in the present study. This voltage dependence is the opposite to that of stretch-activated

channels in the basolateral membrane of *Necturus* proximal tubule (Sackin & Palmer, 1987), underlining the fact that the channels are different between these two species.

Upon cell swelling the basolateral membrane potential of perfused early distal tubules depolarizes transiently by some 30–40 mV, followed by a repolarization to the resting membrane potential (Guggino *et al.* 1985). A similar transient depolarization of cell membrane potential upon hypotonic shock has recently been reported in OK cells (Ubl, Murer & Kolb, 1989). This depolarization could be due to the expected influx of cations upon opening of the stretch-activated channels. This depolarization would itself tend to increase channel open probability, thus there may be an amplification of the initial response to cell swelling analogous to that seen with sodium channels during the upstroke of the action potential.

Role of the channel

At the resting membrane potential inward currents are observed when the membrane is stretched. This current could be carried either by cation entry to or anion exit from the cell. The channel is non-selective, thus the relative fluxes of ions through the channel will depend on the respective prevailing electrochemical gradients. Chloride is maintained above electrochemical equilibrium within the cell whereas sodium is maintained far below equilibrium. Given a resting membrane potential of −51·8 mV (Oberleithner, Weigt, Westphale & Wang, 1987) and intracellular chloride and sodium activities of 11·5 and 12·2 mM, respectively (Oberleithner, Lang, Wang & Giebisch, 1982*b*; Oberleithner, Guggino & Giebisch, 1982*a*) the outward gradient for chloride is 2·3 mV *versus* an inward gradient for sodium of 99 mV. Thus the current would be expected to be carried largely by the inward movement of sodium.

If the channels allow sodium to enter the cell then they cannot play a direct role in voiding solute from the cell during cell swelling. If this were the case, following an increase in cell volume, opening of the stretch-activated channels would be expected to cause further cell swelling, since sodium would flood into the cell down its electrochemical gradient, causing entry to a positive feedback loop. This seems unlikely. In other cell types it is thought that at least a fraction of the inward cation current through the stretch-activated channels is carried by calcium (Christensen, 1987; Ubl *et al.* 1988*b*). The subsequent rise in intracellular calcium concentration acts as a second messenger through which a potassium and accompanying anion exit pathway is triggered (Guggino *et al.* 1985; Ubl *et al.* 1988*a*). Thus the channels are seen as transducers, responding to an increase in cell volume by increasing their open probability, resulting in an increase in cytosolic calcium activity which elicits a VRD response.

Appreciation of the expected relative changes in intracellular sodium and calcium may be gained by considering intracellular levels of sodium and calcium, calculated ionic permeabilities (Schultz, 1980) and calculated ionic fluxes (Hille, 1984) of sodium and calcium. For a hypothetical tenfold increase in intracellular calcium concentration, over the same time course, we calculate an insignificant rise in intracellular sodium concentration of 3 μM. Thus, due to the enormous differences in free cation concentrations inside the cell, a channel that is non-selective can cause an apparent preferential change in the activity of one ion species over another.

The non-selective nature of the stretch-activated channels in frog early distal tubule precludes their role in the removal of osmotically active solute during VRD. The most likely role for the channels is that of stretch transducers, responding to an increase in cell volume by an increase in open probability. The expected subsequent increase in intracellular calcium may be involved in eliciting cell volume regulatory responses.

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