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A Cation Channel in Frog Lens Epithelia Responsive to Pressure and Calcium

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Summary. Patch-clamp recording from the apical surface of the epithelium of frog lens reveals a cation-selective channel after pressure (about ±30 mm Hg) is applied to the pipette. The open state of this channel has a conductance of some 50 pS near the resting potential (−56.1 ± 2.3 mV) when 107 mM NaCl and 10 HEPES (pH 7.3) is outside the channel. The probability of the channel being open depends strongly on pressure but the current-voltage relation of the open state does not. With minimal Ca^{2+} (55 ± 2 μM) outside the channel, the current-voltage relation is nonlinear even in symmetrical salt solutions, allowing more current to flow into the cell than out. The channel, in minimal Ca^{2+} solution, is selective among the monovalent cations in the following sequence $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$. The conductance depends monotonically on the mole fraction of K^+ when the other ion present is Li^+ or Na^+. The single-channel current is a saturating function of $[K^+]$ when K^+ is the permeant ion, for $[K^+] \leq 214$ mM. When $[Ca^{2+}] = 2$ mM, the current-voltage relation is linearized and the channel cannot distinguish Na^+ and K^+.

Key Words ionic channels · epithelial transport · lens physiology · patch clamp · pressure-activated channels · cataracts

Introduction

Ionic channels have been known in excitable tissues for some time (Hodgkin & Katz, 1949; reviewed recently by Hille, 1984), but the role of channels in inexcitable tissues is just becoming apparent (Van Driessche & Zeiske, 1985). Membranes contain many channel types whose existence was often not suspected—and certainly could not be proven—without direct experimental recordings of currents through physically distinct single channels. Macroscopic data did not and cannot provide enough *independent* information to convincingly identify more than a few of the many channel types imbedded in parallel in most membranes. Identification usually requires recordings from a single type of channel, *in situ* with the patch-clamp technique or reconstituted into artificial membranes.[1]

The membranes of epithelia transport many solutes (and thereby solvent as well) and so one must expect their inexcitable membranes to be particularly complex, containing a variety of transport systems and concomittant channels (Van Driessche & Zeiske, 1985). The ionic channels presumably mediate and control membrane transport much as the properties of enzymes mediate and control metabolism.

The lens of the eye is an unusual epithelium specialized to focus an image on the retina, refracting light with minimal absorption or scattering. The paucity of intracellular organelles, the absence of blood vessels, and the specialized properties of the membranes of lens fibers (Rae & Mathias, 1985) presumably represent evolutionary adaptations to these functional imperatives.

The lens must maintain the integrity of its constituents for the life of the animal despite its large size, absence of vasculature, and limited organelles. Organelles are found in the anterior epithelial layer of the lens and in the outer layers of lens fibers (Hogan et al., 1971) and the products of these organelles must be transported throughout the lens to the sites where they are needed. These 'outer' membranes of the lens contain the moieties of integral membrane proteins called pumps and channels common to other epithelial membranes.

Recently, it has become possible to measure the transporting properties of a few channels at a time

[1] Hodgkin and Huxley (1952) were able to separate the macroscopic current of axon membranes because two quite selective channel types predominante. The larger number of often not very selective channel types in cardiac or epithelial membranes makes such separation unconvincing, if not logically impossible.

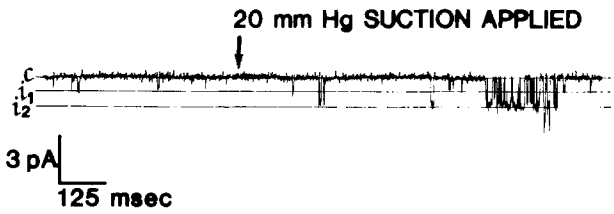

Fig. 1. Typical recording of channel activity from the apical surface of frog lens epithelium before and after application of additional suction. The pipette solution contained 107 mM KCl, 2 $CaCl_2$, 1.5 $MgCl_2$, and 10 HEPES. This record was from an on-cell configuration made at the cell's resting voltage (i.e., with zero applied current) and filtered at 1 kHz. 20 mm Hg of suction was applied at the arrow. The delay in onset of pressure-activated current (i_2) is not significant because suction was applied manually and had a slow uncertain time course. A downward deflection represents a negative current, i.e. positive charge flowing out of the pipette

of the lens epithelium (Rae & Levis, 1984*a*; Jacob et al., 1985) using the patch-clamp technique. Here we further investigate one of the channels described previously (by Rae & Levis, 1984*a*,*b*; and probably by other workers, *see* Discussion) with several convenient and notable properties. It is the predominant channel of the apical membrane of frog lens epithelia when the external solution contains very little calcium ion; thus it can be studied in isolation from the many other channels observed in lens epithelia.

The channel has many interesting properties even though it seems to be occupied by only one ion at a time: when external calcium is minimal, it is a rectifying cation channel which selects weakly among monovalent cations. In the presence of physiological concentrations of external calcium, the channel allows monovalent cations to carry most of the current with little rectification and little selectivity. When calcium is the only external cation, it itself carries considerable current with little rectification. The channel has substantial amounts of open channel noise even at low frequencies and, in minimal calcium, shows few of the rapid closings called flicker. Finally, changes in pressure (either positive or negative) are the *only* way we know to open (or 'activate') the channel: once a gigaseal is established, significant changes in pressure (either positive or negative) noticeably increase the probability of opening of the channel without changing the gigaseal. The pressure sensitivity is similar to that reported by Guharay and Sachs (1984, 1985) and Sigurdson and Morris (1986).

Given the important and unusual osmotic properties of the frog lens (for example, the low osmolarity of the Ringer's solution needed to maintain transparency: Mathias et al., 1979), it is attractive to speculate that this channel may be involved in volume regulation of the tissue in some unknown way. Given the large increase in intra-lenticular sodium and decrease in potassium concentration reported in many types of cataract (Paterson, 1972), it is possible that activation of this channel is involved in cataract formation, even if activation does not occur under normal conditions. An increase in intra-lenticular pressure—*whatever the cause*—might pathologically activate this channel, allowing the further entry of sodium, calcium, and water, the further increase in intra-lenticular pressure (and thus activation of still more channels and still more depolarization), finally leading to general destruction of the tissue. Some of these results have been previously presented in abstract (Cooper et al., 1985).

Materials and Methods

The preparation used in most experiments was the isolated anterior epithelium of the frog lens developed by Rae (Rae & Levis, 1984*a*) although several experiments used myocytes cultured from *Xenopus laevis* kindly provided to us by Dr. H.B. Peng (Peng & Nakajima, 1978). In all lens experiments, the eyes of pithed *Rana castebiana* or *pipiens* were removed and an inicision was started just posterior to the corneal limbus and extended circumferentially to allow excision of both the cornea and iris. The lens was removed from the globe by placing a fine lens loop under its posterior surface and gently lifting until it was free of surrounding tissue. After all adherent material had been carefully removed from the lens, the posterior capsule was gently grasped with two pairs of jeweler's forceps. The capsule was then torn into several flaps which were held to a Sylgard® disc with stainless steel insect pins (#000). The mass of lens fibers was lifted carefully from the capsule, exposing the clean apical surface of the lens epithelium, which remained attached to the capsule below. The surface of the lens epithelium was stretched to improve visibility and remove folds, introducing an unknown amount of strain and perhaps some stress, into the resting preparation.

The Sylgard disc with attached capsule and epithelium was pinned down in a Sylgard-lined acrylic plastic chamber filled with amphibian Ringer's solution made of 104.5 mM NaCl, 2.5 KCl, 2 $CaCl_2$, 1.5 $MgCl_2$, and 10 HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) adjusted to pH 7.3 by adding NaOH, typically 4 mmol/(liter of Ringer's). The osmolality of the solution (222 mOsm/[kg H_2O]) was routinely monitored with a high precision osmometer (Model 3MO: Advanced Instruments, Needham MA). Solutions made without added calcium (which we call solutions of 'minimal' Ca^{2+} concentration) were found to contain $[Ca^{2+}] = 55 \pm 2$ μM (mean ± standard error of the mean, 9 measurements) using the pH metric method of Moisescu and Pusch (1975) as described by Lüttgau and Spiecker (1979: Fig. 1), modified for greater sensitivity.

Patch pipettes were made from Corning 7052 glass (outside diameter 1.65 mm, inside diameter 1.15 mm, purchased from Garner Glass, Claremont CA) in a two-stage process, using a vertical Kopf puller (David Kopf, Inc., Tujunga CA) with a home-built circuit providing constant power to the heating coil, independent of variations in coil impedance. The pipettes were coated with Sylgard 184 (Dow Corning, Midland MI) and fire polished immediately before use to a final diameter of 0.5 to 1.0 μm inside the tip. The electrodes, typically filled with 107 mM

KCl and 10 HEPES (adjusted to pH 7.3 by adding KOH, some 4 mmol/[liter of electrode solution]), had resistance in the range of 10 to 30 MΩ. Pressure in the lumen of the pipette was generated and monitored in two ways: in our earlier experiments a pressure transducer (Model P23DB: Gould Electronics, Cleveland OH) monitored the action of a micrometer-actuated syringe; in our later experiments a pneumatic tester (Model DP1: Bio-Tek Instruments, Burlington VT) generated and monitored the pressure. Current collected in the patch pipette was converted to a voltage signal with the circuitry of Sigworth and Neher as implemented in the List patch-clamp amplifier EPC-7 (Medical Systems Corporation, Great Neck, NY). The voltage signals were stored in an analog tape recorder with bandwidth DC-5 kHz (Model Store 4DS, RACAL Recorders, Sarasota FL). Data were digitized every 250 μsec after passing through a low-pass 8-pole Bessel filter, −3 dB at 1 kHz. Some single-channel data were displayed, plotted, and analyzed using the automated pattern recognition program of Sachs et al. (1982) generously made available to us by Dr. F. Sachs. All experiments were done at room temperature of about 22°C.

Gigaseals were made by two different procedures. In preliminary experiments, not documented here, suction was applied by a syringe. Later experiments used the pressure control and monitoring apparatus; care was then taken to follow a stereotyped procedure for making gigaseals and studying pressure activation. Positive pressure of 5 mm Hg was applied to the patch pipette before it was advanced to the preparation. As soon as the pipette touched the cell membrane (judged by the increase in electrode plus convergence resistance and the concomittant visual changes observed at 400× magnification), the positive pressure was removed. In the 'best experiments' (about one-third), a gigaseal formed without further intervention. In the others, slight additional suction (less than 10 mm Hg) was needed to form the gigaseal.

Resting potential was measured in all experiments. After the required data had been recorded, typically 30 min after the gigaseal had been formed, a voltage pulse of some −300 mV was applied to the pipette to remove the impedance of the membrane patch. The voltage control circuitry was turned off, the current through the pipette was set to zero, and the resulting 'open circuit' voltage was measured. This resting potential was stable for at least 15 min, whether the pipette was filled with KCl or NaCl, presumably because of the electrotonic couplings between cells. Liquid junction potentials and offset currents through the gigaseal undoubtedly limited the precision of our estimates.

Results

A wide variety of channels are observed in frog lens epithelia if a pipette containing physiological concentrations of calcium is placed next to the apical surface and small amounts of suction are used to make a gigaseal (Rae & Levis, 1984*a*,*b*; Rae, 1985). If suction (20 to 40 mm Hg) is applied to the pipette *beyond that needed to form the gigaseal,* additional channel openings are consistently seen, if one can use that word to describe a stochastic process. It is those openings we report and study here.

Change in Pressure Opens the Channel

Figure 1 shows the channel openings that occur before and after we start changing the pressure in the pipette (at the arrow) when the pipette contains physiological concentrations of calcium. After this small pressure change, a new channel type appears without an evident change in background activity, either baseline 'noise' or other presumptive channel activity. When the pressure control apparatus was used to form gigaseals with minimal suction, we rarely saw openings of this channel until the pressure was changed further. In early experiments, however, when a syringe was used to produce a gigaseal and calcium was within the pipette, activity was often seen as soon as the seal was formed.

We investigated the effect of a range of positive pressure in a number of experiments under a variety of ionic conditions and found, to our surprise, that positive pressure activated this channel in much the same way as suction (negative pressure). Gigaseals were much harder to maintain with positive pressure (except in the outside-out configuration) and so the records shown were taken with suction.

Figure 1 also illustrates a 12-pS channel unaffected by pressure. The upper construction line indicates the size of its single-channel current i_1; the lower line indicates the size of the current i_2 through a single pressure-activated channel. We think it fortuitous that the 12-pS channel is roughly half the size of the pressure-activated channel under these conditions. We have not studied this smaller channel.

Figure 2 shows a continous long-duration (20 min) recording of channel activity from a patch of membrane on the cell, with the cell at its resting potential, with constant suction, and with a pipette filled with 107 mM KCl, 10 HEPES, and minimal [Ca^{2+}] (upper trace) or [Ca^{2+}] = 2 mM (lower trace). The probability of opening of the channel is frustratingly stochastic. It varies irregularly with time, at

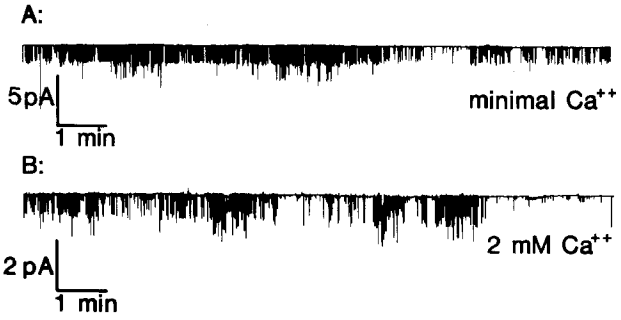

Fig. 2. Long duration (20 min) recordings of channel activity. These records were filtered at 500 Hz and so fast events are attenuated and distorted: the large spikes represent multiple channel events. *A*: The pipette solution contained 107 mM KCl, minimal Ca^{2+}, and 10 HEPES. *B*: The pipette solution contained 107 mM KCl, 2 Ca^{2+}, and 10 HEPES. Note the difference in current scales. These recordings were made at the cell's resting voltage in an on-cell configuration

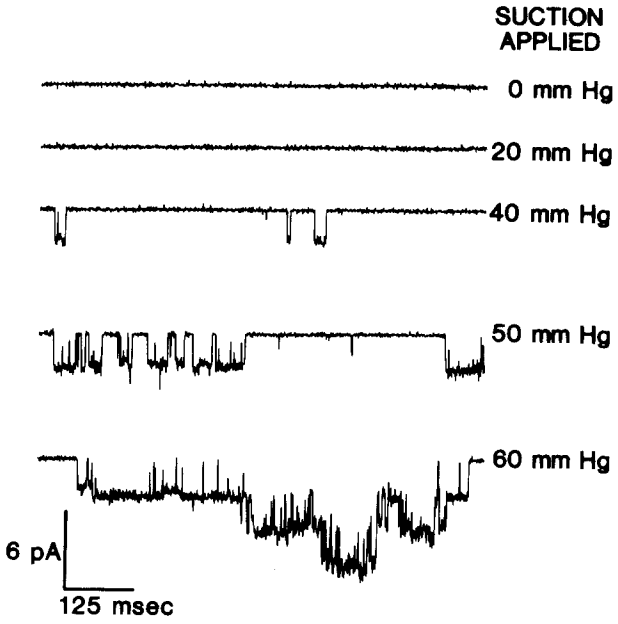

Fig. 3. Suction dependence of the channel opening. The pipette solution contained 107 mM KCl, minimal Ca^{2+}, and 10 HEPES. The recordings were made from an on-cell patch at the cell's resting voltage. These records were filtered at 1 kHz

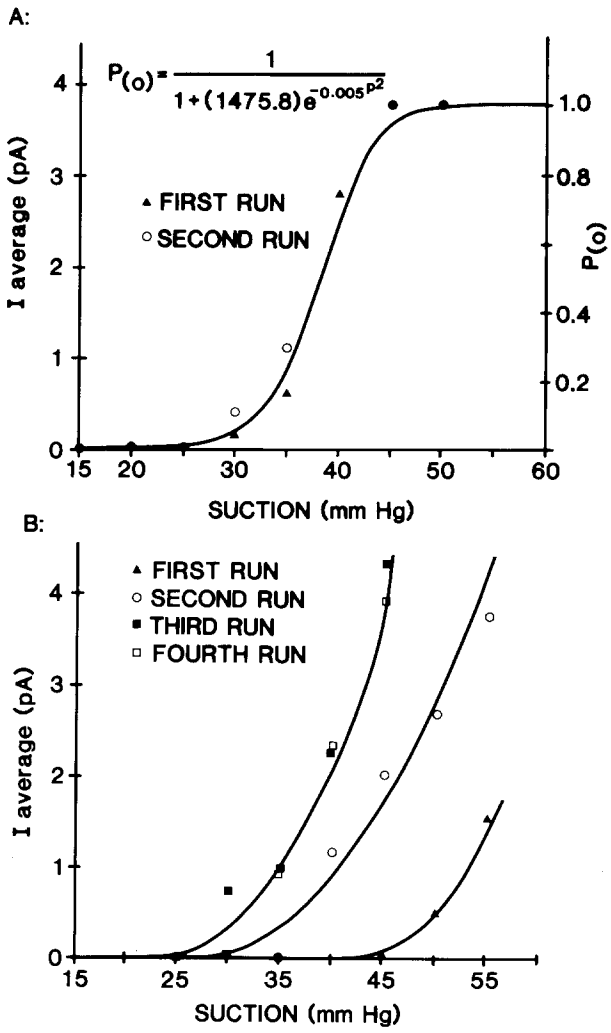

Fig. 4. *A*: Average current and open probability as a function of applied suction from an on-cell patch at the cell's resting voltage. The solid triangles represent an ascending series of suction steps with intervening rest periods. The open circles represent a subsequent descending series. The patch contained only one channel. The solid line represents a fit of the data to a simple two-state open-closed gating scheme (*see text*). The best fit was determined by eye. *B*: This graph is the same as *A* except that there were at least two channels in the patch. Each successive run was made with the same protocol as in the ascending series in *A*. The lines here are for visual effect only. Note the lack of reproducibility in these curves

least on this time scale, as the channel apparently drifts from one kinetic behavior to another, without known cause. This recording may be a glimpse of a stochastic (but stationary) process as it makes low probability transitions among the many states of a complex kinetic regime.[2] The variation may reflect a channel switching between different modes of gating, as proposed by Hess et al. (1984) for a cardiac calcium channel. Or the variation may be caused by a deterministic but unknown and uncontrolled drift in the properties of the channel, caused, for example, by a drift in the mechanical state of the membrane within the pipette or the chemical state of the channel, membrane, or cell.

It seems wise to postpone kinetic analysis and modelling of any channel until such slow irregular variation is reasonably understood, if not controlled: a study of the faster events that ignores slow irregularities is likely to be irreproducible and misleading.

Figure 3 shows representative samples from a continuous record when calcium was minimal in the solution outside the channel, i.e., in the pipette. The suction was increased in the steps shown from 0 to 60 mm Hg every 10 sec. The 12-pS channel is absent at all pressures and few spontaneous openings of the pressure-activated channel are seen before the pressure is changed, as was usually the case when $[Ca^{2+}]$ was minimal. Increasing suction produces increasing channel activity just as in Fig. 1, when calcium was present; the probability of a channel being open is evidently a monotonically increasing function of pressure. Interestingly, spontaneous closings to baseline (flicker) are reduced in

[2] These transitions are reminiscent of transitions progress of voltage (Schauf & Chuman, 1986) or agonist-activated channels from one inactivated or desensitized state to another (Hille, 1984, pp. 69–71; 134–135).

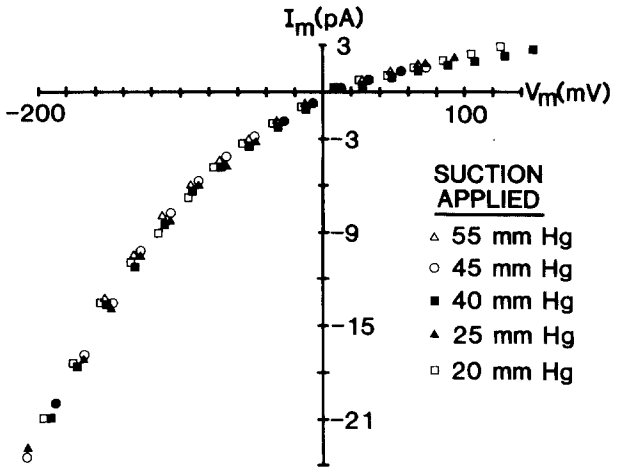

Fig. 5. Open-channel current-voltage relations at different pressures. Data is from four lenses with various pressures across the patch membrane, recorded in an on-cell configuration with a 107 mM KCl, minimal Ca^{2+}, and 10 HEPES solution in the pipette. The curves labeled 25 and 45 mm Hg are from the same patch. V_m is the voltage across the patch membrane, i.e. the cell's resting voltage minus the pipette voltage. I_m is the single-channel current

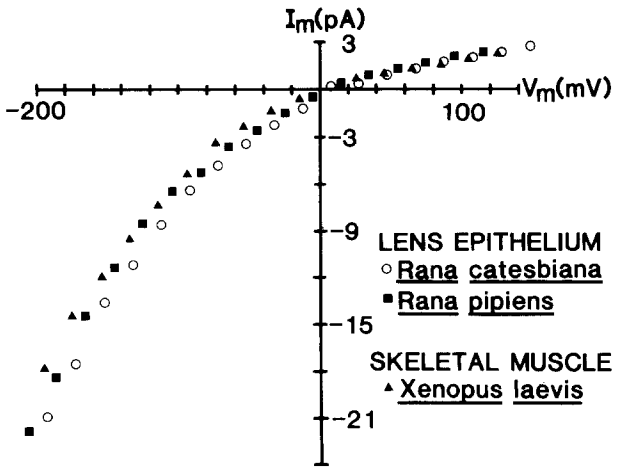

Fig. 6. Open-channel current-voltage relation from a pressure-sensitive channel in various amphibian preparations. Note that these recordings represent both lens epithelium and cultured skeletal muscle. These recordings were done in an on-cell configuration with a 107 mM KCl, minimal Ca^{2+}, and 10 HEPES solution in the pipette

these solutions while the open-channel noise remains large.

Figure 4*A* shows the effect of pressure in a rare but useful experiment (3 out of 210) in which only one channel was present within our pipette, judging by the complete absence of 'double' openings over a period of 2 min at pressures where the channel open probability was nearly unity. Records were taken for 30 sec at each pressure separated by rest periods (with zero pressure) of 2 min, hoping to avoid hysteretic complexities akin to desensitization and slow inactivation.

The relationship of open probability to pressure is surprisingly reproducible in this experiment, given the general instability of kinetic properties, and so we have fit the data by a simple two-state opened-closed kinetic scheme with opening rate constant β and closing rate constant α. In such a model (Lecar & Sachs, 1981) the open probability is

$$P(O) = \frac{\beta}{\beta + \alpha} + \frac{1}{1 + (\alpha/\beta)}. \quad (1)$$

Following the derivation of Guharay and Sachs (1984), we assume β alone to be pressure dependent and of the form

$$\beta = \beta_o e^{\theta p^2} \quad (2)$$

where β_o is the rate constant at zero pressure and θ is a measure of the sensitivity of the rate constant to pressure. Substituting Eq. (1) into Eq. (2) gives

$$P(O) = \frac{1}{1 + (\alpha/\beta_o)e^{\theta p^2}}. \quad (3)$$

Guharay and Sachs (1984) estimate $\theta = 0.66 \pm 0.36$ [cm Hg]$^{-2}$ compared to our estimate of 0.51.

Figure 4*B* shows the typical case, when several channels are within the pipette, and the function relating the total number of open channels to pressure is not so reproducible. While this unstable behavior might be the result of an irreproducible stress-strain relation of the patch of membrane within the pipette, producing capricious recruitment of open channels as pressure increased, as suggested by Yang et al. (1986), it cannot be distinguished from the generally unstable behavior of the opening of the channel, even when constant pressure is applied.

Current-Voltage Relations

Despite the complexities of the opening-closing process (i.e., 'gating'), the properites of the open channel, particularly its current-voltage relation, are quite reproducible and independent of pressure (Fig. 5) at least at pressures ranging from −20 to −55 mm Hg. Figure 6 demonstrates that the current-voltage relation of the pressure-activated channel is surprisingly independent of its source. The channel properties are also independent of the po-

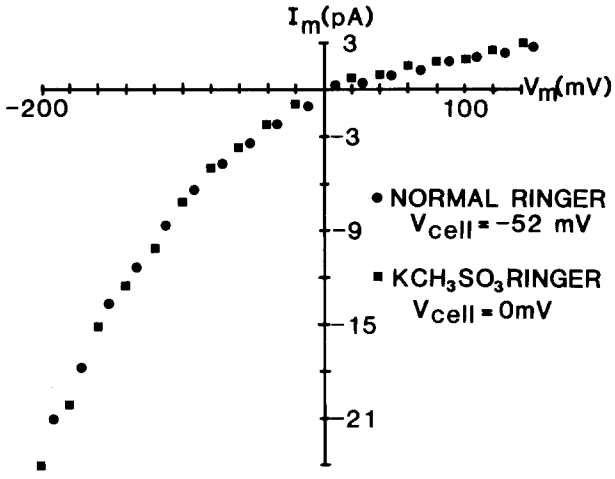

Fig. 7. Open-channel current-voltage relation at different resting voltages. These recordings were made in an on-cell configuration with a 107 mM KCl, minimal Ca^{2+}, and 10 HEPES solution in the pipette. The cells were depolarized by placing them in a 107 mM KCH_3SO_3 (potassium methane-sulfonate) solution

tential across the membrane of the cell *outside* the pipette (Fig. 7): we see no sign of a voltage-dependent action of the preparation on the channel, as might be expected if it answered to a chemical message sent by the membrane potential outside the patch.[3]

Table 1 shows the reversal potential and single-channel conductance measured with a number of different solutions outside the channel, i.e. inside the pipette. The single-channel current $I(V)$ is zero at the reversal potential E_{rev} (i.e., $I(E_{rev}) = 0$). The open-channel conductance at voltages near the resting potential of the preparation was determined from the slope of a straight line fit to the measurements of single-channel current in the voltage range from −20 to −80 mV. Because the current-voltage relation is linear in this region, measurements of the slope $[dI/dV]$, or the ratios $I(V_r)/V_r$ or $\{I(-60) - I(-40)\} \div \{-20 \text{ mV}\}$, are statistically identical, where V_r is the resting potential, −56.1 ± 2.3 mV in these 30 cells, indistinguishable among groups A–D. The slopes and intercepts, and the standard deviations of these parameters (usually called 'standard errors') were determined by classical regression analysis (Draper & Smith, 1966; Lotus, 1985: pp. 155–157).

The single-channel conductance, at voltages near the cell's resting potential, when the outside of the channel is exposed to physiological solution (D in the Table) is some 28 pS, close to the value of 35

[3] Given that the cells of the epithelium are electrically coupled (e.g., Rae & Kuszak, 1983).

Table 1. Single-channel conductance[a]

Pipette solution (mM)	Number of cells	Reversal potential (mV)	Conductance (pS)
A: 107 KCl	15	−1.58	62.8
		(2.9)	(1.6/0.987)
B: 107 NaCl	5	−1.83	49.9
		(2.5)	(2.2/0.989)
C: 107 KCl	5	5.13	30.4
2 Ca/1.5 Mg		(2.8)	(1.3/0.990)
D: 107 NaCl	5	6.11	28.2
2 Ca/1.5 Mg		(2.6)	(1.2/0.989)

[a] Conductances were calculated as described in text. Parentheses surround standard deviation of the parameter followed by correlation coefficient, when applicable.

pS reported by Guharay and Sachs (1985) working with similar solutions. With this concentration of divalents, the conductance in K^+-rich solution (C) is much the same. In solutions with minimal divalents, however, the conductance depends significantly on the predominant monovalent cation species. In K^+-rich solutions (A), it is some 63 pS; in Na^+-rich solutions (B), it is nearly 50 pS as reported in our preliminary publication (Cooper et al., 1985). To ease communication, we named the pressure-sensitive channel CAT-50 (for *CAT*ion specific *50* pS conductance channel). The name suffers from a certain embarrassing ambiguity, however, given the sensitivity of the channel to divalent ions and potential.

Selectivity

Table 2 demonstrates the cation selectivity of the channel. The reversal potential E_{rev} as just defined was measured from current-voltage relations measured with the indicated concentration of KCl outside the channel, i.e., inside the pipette. The difference between E_{rev} and E_K is hardly ever significant.

Figure 8 shows the current-voltage relationship of the pore of CAT-50 (i.e., the open channel) recorded with different ions outside the channel, i.e., in the pipette. When no current flows through the channel, the 'equilibrium' selectivity is rather small. When current flows inward from the pipette to the cell interior, the selectivity sequence $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$ (Eisenman's sequence IV, Eisenman and Horn, 1983) is maintained. When outward current flows, selectivity does not depend much on the external monovalent cation present, presumably because the current carrier is mostly determined by the (constant) ionic composition of

Table 2. Cation selectivity[a]

[KCl] (mM)	Reversal potential (mV)	E_K (mV)	E_{Cl} (mV)
6.7	−71.9	−69.8	12.5
13.375	−61.3	−52.4	−4.9
26.75	−40.9	−34.9	−22.4
53.5	−20.0	−17.5	−39.8
107	−1.6	0	−57.3
160.5	19.6	10.2	−67.4
214	43	17.4	−74.8

[a] E_K, E_{Cl} are equilibrium potentials computed with $[K_i]$ = 107 mM and $[Cl_i]$ = 11 mM (Mathias, 1985).

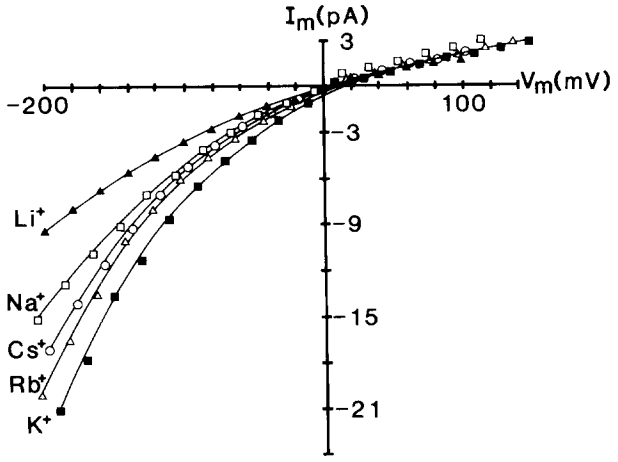

Fig. 8. Open-channel current-voltage relation with various monovalent cations outside the channel, i.e. in the pipette. These data were measured in the on-cell configuration with 107 mM of the indicated monovalent cation, minimal Ca^{2+}, and 10 HEPES in the pipette. The channel transports the ions preferentially in the sequence $K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$ (i.e. Eisenman's sequence IV). The lines are for visual effect only and were drawn to coincide at far positive patch membrane voltages

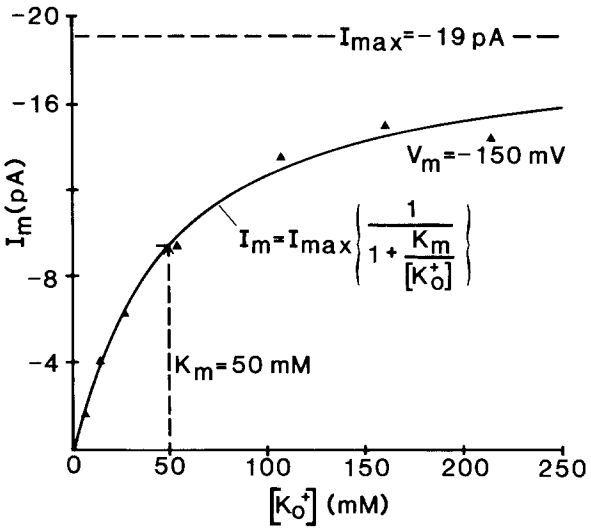

Fig. 9. Current saturation with increasing concentration of permeant ion. These data were recorded in an on-cell configuration with −150 mV across the patch. The solid line represetns a fit of the data to a simple Michaelis-Menten saturation function. The best fit was determined by eye. The K_m is 50 mM and I_{max} is −19 pA. The pipette solution contained minimal Ca^{2+} with the indicated [KCl]

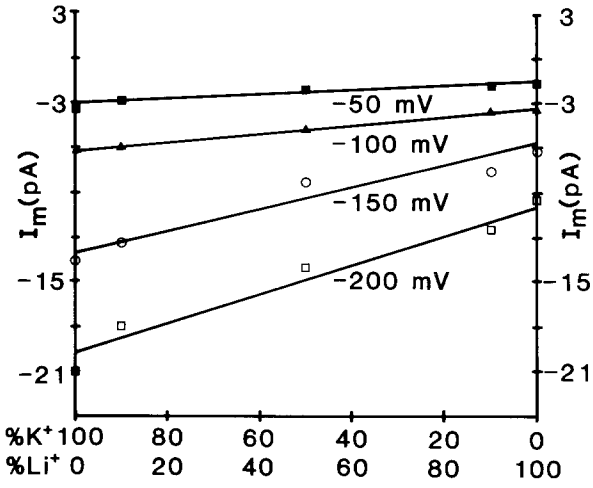

Fig. 10. Li^+/K^+ mole fraction experiment showing no evidence of anomalous behavior. These data were recorded in an on-cell configuration at the indicated patch membrane voltages. The lines are for visual effect only

the epithelial cell, not that of the external solution.

The concentration dependence of the open-channel current is simple (Fig. 9), what one would expect from a channel occupied by one ion at a time (Läuger, 1973; Hladky & Haydon, 1984). The decline in the point at the highest concentration was not convincing in this experiment or reproducible in others. These results would, of course, be more conclusive if they were done over a wider concentration range or with identical solutions on both sides of an excised patch. Such experiments will be needed to build a model of CAT-50.

Experiments were also performed with mixtures of ions outside the channel, i.e., in the pipette (Fig. 10). When Li^+ was substituted for varying fractions of K^+, or Na^+ was substituted for K^+ (not illustrated but similar), the current through the open channel varied in a simple monotonic—seemingly linear—manner at many potentials, as would be expected from a channel occupied by just one ion (Urban & Hladky, 1979).

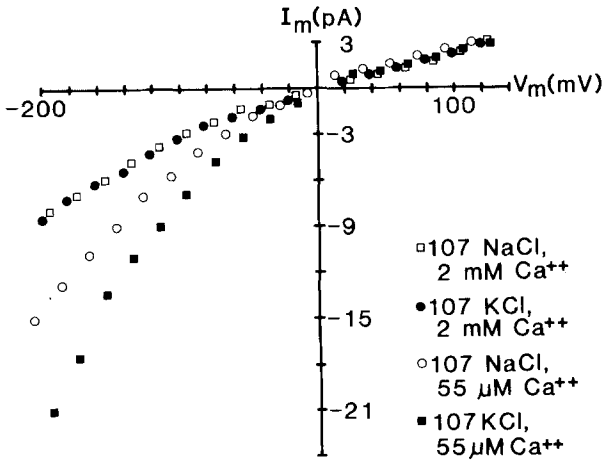

Fig. 11. Ca^{2+} effect on monovalent permeation. Note that physiological $[Ca^{2+}]$ significantly alters the shape of the current-voltage relations and makes them indistinguishable. These data were recorded in the on-cell configuration with the indicated solutions outside the channel, i.e. in the pipette

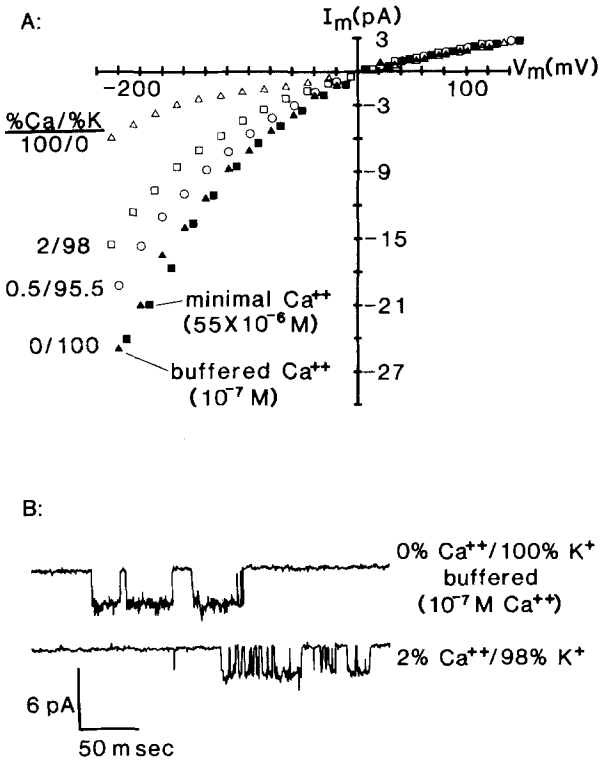

Fig. 12. *A*: Ca^{2+} permeation experiment. These data were recorded in an on-cell configuration. Note Ca^{2+} was able to carry significant current through the channel when $[K^+]$ in the pipette was zero. In these experiments the total concentration of charge was constant, 100% K^+ being [KCl] = 107 mM and 100% Ca^{2+} being $[CaCl_2]$ = 53.5 mM. *B*: Sample records from this set of experiments showing an increase in channel flicker and a decrease in current with physiological $[Ca^{2+}]$ present. These records were taken with −60 mV across the patch and with a filter setting of 1 kHz

Calcium Dependence

The effects of raising calcium suggest that CAT-50 is far more complex: Figure 11 shows, for example, that external calcium has substantial effects on selectivity and conduction. In the presence of 2 mM Ca^{2+} outside, the current-voltage relations for pipettes filled with Na^+ and K^+ are indistinguishable and rather linear; in the absence of calcium they are quite different and nonlinear, as shown before (Fig. 8). Figure 12 shows that Ca^{2+} can flow inward through the channel (open triangles) with conductance more or less independent of voltage, if K^+ is not present in the extracellular solution. Indeed, the simplest interpretation of the data in Figs. 11 and 12 is that calcium can carry current even in the presence of monovalent cations. The figure also justifies our routine use of pipette solutions with minimal $[Ca^{2+}]$, to which Ca^{2+} had not been added, instead of solutions buffered by EGTA: it shows that a solution with minimal calcium, some 50 μM, has the same action as a solution with negligible calcium, buffered by EGTA to $[Ca^{2+}]$ = 100 nM. The inset to Fig. 12 shows records taken with varying amounts of calcium, and potassium outside the channel. The current flow through the pore of CAT-50 depends strikingly on the external solution. Other experiments show that barium carries somewhat more current than calcium, as in calcium channels (Fatt & Ginsborg, 1958; Hille, 1984, p. 221).

The unexpected sensitivity of CAT-50 to calcium was discovered in channels in excised patches. These had a linear current-voltage relation but similar sensitivity to pressure as on-cell channels, directly contradicting our expectations in both regards. Evidently, when calcium is present in physiological concentrations, the current-voltage relation is rather linear and selectivity among monovalent cations minimal, as documented already in Fig. 11. When calcium is minimal on both sides, selectivity and nonlinearity are evident. The entire effect of excision can be explained this way.

Discussion

Possible Artifacts

We hope, of course, that the currents reported here flow through a distinct membrane protein involved in functionally significant membrane transport. But the fact that CAT-50 is activated by pressure (and by nothing else we know) while the membrane and

tissue in which it is embedded has no known functional response to pressure means we must consider the possibility of mechanical artifact.

It seems possible that the pressure-activated current flow through the lipid of the membrane, not through an integral membrane protein. But the current is almost certainly not flowing through the gigaseal. One would expect such shunt currents to have a wide dispersion of sizes and an exponential time course, reflecting the distributed impedance of the membrane-gigaseal-glass complex. Our records show an irresolvably rapid opening to a definite unitary conductance. The possibility remains (as with most single-channel measurements) that an agonist (in our case pressure) forms pores with the requisitely specific properties in the dome of lipid within the pipette (Corcia & Babila, 1985).

More likely is the possibility that the pressure-activated current flows through a pressure-induced pore of an integral membrane protein *not* normally involved in ion movement. After all, the anatomy of a number of soluble proteins includes a central pore (e.g., triose phosphate isomerase and pyruvate kinase, as illustrated in Richardson, 1981); and the polypeptides hemocyanin (Cecchi et al., 1984) and gramicidin (Hladky & Haydon, 1984) form well-defined (and very well-studied) channels, even though neither is known to have a physiological function involving membranes or ion transport. Lens membranes contain no shortage of integral membrane proteins (Alcala & Maisel, 1985), some invovled in membrane transport, but others—presumably *not* normally involved in membrane transport—that anchor the extensive cytoskeleton. The possibility that CAT-50 arises in a protein normally anchoring the cytoskeleton seems reasonable, although hard to test directly.

Even more likely is the possibility that CAT-50 is a true ionic channel normally activated by some other, presently unknown, agonist. The failure to find such an agonist does not guarantee its nonexistence; the physiological activator may just be waiting to be discovered!

A pressure-activated channel rather similar to CAT-50 has now been reported in several other preparations. The unitary current we study is at worst a widespread artifact, at best a widespread channel, needing study, even in the former case, if only to prevent further confusion.

Relationship to Other Channels

Brehm et al. (1984), Guharay and Sachs (1984, 1985), Sakmann et al. (1985), Yang et al. (1986), and Sigurdson and Morris (1986) have reported a channel in skeletal muscle, myotubes, oocytes, dorsal root ganglion cells, and snail heart, respectively, activated by suction with selectivity similar to that reported here. They too report that most patches contain more than one channel. The difference in the reported single-channel conductance may just reflect the restricted range of potentials available to those investigators: their current-voltage curves are indistinguishable from ours in that restricted voltage range (*see* Table 1) and the pressure sensitivity is quite similar. It is also possible that these channels from different preparations are different proteins.

The channel we report is likely the same as that described by Jacob et al. (1985), also in frog lens epithelium. Jacob et al. (1985) primarily studied the gating kinetics of the channel but did not discover, apparently, that these kinetics are strongly influenced by pressure. We have not studied kinetics because of the difficulties documented earlier, also visible in Fig. 11 of Jacob et al. (1985). The calcium dependence was studied, however, in both laboratories and is similar. Pharmacological properties apparently differ: we find no action of 50 μM amiloride, although such is reported by Jacobs et al. They report a marked reduction in channel activity when the pipette was perfused with an amiloride-containing solution. Without control or knowledge of pressure effects, the authors naturally attributed the effects of perfusion to the drug. Since we have seen no such action of the drug when it is applied without increment in pressure (as monitored and controlled by the apparatus previously described), but do see a profound effect of pressure itself, one wonders whether the effects reported by Jacob et al. might be caused by a pressure change during perfusion. This seems particularly likely since the 'amiloride effect' reported occurs much faster than the drug can be expected to diffuse from injection cannula to membrane patch.

CAT-50 is clearly distinct from the other nonselective cation channels of ocular epithelia found by Rae (*personal communication*) which are insensitive to pressure and have distinctly different open-channel conductance, kinetics, and/or dependence on Ca^{2+}. Exploratory experiments show a weak pharmacological relationship between CAT-50 and the acetylcholine channel: 60 μM D-tubocurarine produced a long-lived subconductance state in CAT-50, once it is opened by pressure. An agonist of the acetylcholine channel (suberyldicholine), ethanol, lidocaine, tetraethylammonium, and tetramethylammonium have no obvious effects.

The physical argument has been made (Guharay & Sachs, 1984) that pressure sensitivity of the magnitude seen here must reflect strain over a wide region of the cell, far beyond the confines of the channel protein, at least if we accept the assump-

tion the cell does not use chemical energy to nonlinearly amplify its response to pressure. The pressure dependence (i.e., agaonist sensitivity) of CAT-50 would arise then not from its primary structure but covalent links between the channel polypeptide and the cytoskeleton on the inside of the cell or between the channel and basal lamina, elastin, and proteoglycans outside of cells.

Properties of CAT-50

Several properties reported here are of general interest. Apparent nonstationarity of the type we report seems the rule, not the exception in channels of ocular epithelia (Rae, *personal communication*) and is a serious impediment to kinetic analysis. One would like to see documentation of stability or analysis of the effects of instability in studies of channel kinetics following the example of Horn and Vandenberg (1984) and Magleby and Pallotta (1983).

The effects of Ca^{2+} on CAT-50 are interesting but too little studied at this time to interpret in detail. The profound effects on selectivity, flickering, and current-voltage relations are reminiscent of the effects of Ca^{2+} on the calcium channel where removal of external calcium changes the 'nature' of the channel. In many channels, it seems that calcium has large effects on the conformation of the energy barriers which define pores, either directly by binding to a site within the pore (as reviewed recently in McCleskey and Almers, 1985), or indirectly by modifying the conformation of the protein surrounding the pore. Perhaps the latter possibility should receive more attention, since one might expect a profound change in conformation whenever a channel or enzyme is deprived of a natural ligand, particularly a substrate or allosteric effector[4] unless the protein is remarkably rigid (Läuger, 1985).

Functional Role of CAT-50

The nonselectivity of CAT-50 and its activation by both pressure and suction make a *direct* role in volume regulation unlikely. These characteristics do not provide the negative feedback, the stabilizing control of volume, one would expect in the osmoregulatory system of an epithelium, particularly one which cannot be repaired or replaced biologically. Such characteristics resemble those of the stretch-activated channel of mechanoreceptors (reviewed in Ottoson, 1983, pp. 133–140) and it is possible that CAT-50 acts as a pressure sensor, indirectly controlling cell volume through its action on voltage, internal calcium concentration, or an unknown messenger system. One must be concerned, however, about the significance of *any* putative pressure-sensitive channel reported in tissues not known to have a physiological response to pressure.

Finally, we turn to the not-so-remote possibility that CAT-50, whatever its normal function, plays an important role in disease, in cataractogenesis, if in fact it is present in the lens of mammals. A reasonable scenario would be this: the aging process, or an environmental insult, might decrease the secretion of Na^+ by lens epithelia that is required to maintain cell volume in face of the Na^+ influx across the extensive membranes of lens fibers. The resulting *net* Na^+ entry, not necessarily flowing through pressure-sensitive channels, would depolarize the lens; and Cl^- and water would move into the cells, through a variety of pathways. The resulting increase in the hydrostatic pressure within the lens would activate CAT-50 channels, allowing further Na^+ entry through their nonselective pores, further increasing osmolarity then hydrostatic pressure, eventually overwhelming the active transport systems, leading to irreversible swelling, cell death, and cataract.

Examination of channels in precataractous cells of a mammalian lens would be of more than physiological interest.

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[4] Besides the cited action of Ca^{2+} on the calcium channel, consider the action of $[K^+]$ on the inward rectifying K^+ channel of skeletal muscle (Spalding et al., 1981; Hille, 1984, pp. 334–336) and the multifaceted action of substrates and transported species on the sodium pump (Kaplan, 1985).

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