A stretch-activated K^+ channel sensitive to cell volume

(osmoregulation/kidney/proximal tubule)

HENRY SACKIN

Department of Physiology and Biophysics, Cornell University Medical College, New York, NY 10021

ABSTRACT   The role of K^+ channels in cell osmoregulation was investigated by using the patch-clamp technique. In cell-attached patches from *Necturus* proximal tubule, the short-open-time K^+ channel at the basolateral membrane could be stretch-activated by pipette suction, where a negative pressure of 6 cm H_2O (588.6 Pa) was sufficient to increase the open probability of the channel by a factor of 4.0 ± 0.8 ($n = 7$ tubules). A 50% reduction in bath osmolarity increased cell volume by $66 \pm 10\%$ and increased the K^+-channel open probability by a factor of 5.8 ± 1.4 ($n = 7$) in the same cell-attached patches that were activated by pipette suction. A kinetic analysis indicates one open state and at least two closed states for this epithelial K^+ channel. Both suction and swelling shorten the longest time constant of the closed-time distribution by a factor of 3, without significant effect on either the mean open time or the shorter closed-state time constant. The similar effect of suction and swelling is consistent with the hypothesis that stretch-activated K^+ channels mediate the increase in macroscopic K^+ conductance that occurs during osmoregulation of amphibian proximal tubules. Calculations based on a simple model indicate that small increments in cell volume could produce statistically significant increases in K^+-channel activity.

Swelling is known to increase K^+ conductance in a variety of cell types, resulting in exit of K^+ down its electrochemical gradient (1–13). This could restore swollen cells to their original volume if K^+ efflux were accompanied by efflux of an anion and followed by osmotic water flow. Involvement of K^+ in proximal tubule osmoregulation is supported by loss of cell K^+ following exposure to hypotonic media (14), increases in basolateral K^+ permeability during swelling (6, 15), and block of volume regulation by Ba^{2+} (10) and quinine (15), two inhibitors of K^+ conductance.

Cell potential (16), cytosolic Ca^{2+} (1, 3, 9, 17–20), pH (21), and the ATP/ADP ratio (22) have all been proposed as mediators of the volume-regulatory increase in K^+ conductance. However, the finding of stretch-activated channels in a variety of cell types (23–26), including the proximal tubule (27), raises the possibility that changes in membrane tension may mediate the increase in K^+ conductance that occurs during cell swelling. The present studies were designed to investigate whether increases in cell volume can directly activate stretch-sensitive K^+ channels at the basolateral membrane of the renal proximal tubule.

MATERIALS AND METHODS

The basement membrane of early segments of *Necturus* renal proximal tubule was removed manually without the use of collagenase or other enzymes (28). The solutions used in the paired patch-clamp experiments are given in Table 1, where

Table 1. Compositions of solutions for paired experiments

	Concentration, mM			
			Bath	
			High K^+	
Component	Patch pipette	Standard Ringer	Normal	Hypotonic
Na^+	80.0	92.0	6.4	6.4
K^+	2.5	2.5	35.0	35.0
Ca^{2+}	3.6	3.6	3.6	3.6
Mg^{2+}	1.0	1.0	1.0	1.0
Cl^-	2.0	103.7	2.0	2.0
Gluconate	89.7	0	48.6	48.6
D-Glucose	0	2.0	2.0	2.0
Sucrose	23.0	0	95.0	0
Hepes	10.0	10.0	10.0	10.0
(Osmolarity, mosM)	(204)	(204)	(203)	(100)

The pH of all solutions was adjusted to 7.5. The concentrations of sucrose added to the pipette and high-K^+ solutions were determined empirically. Although the tubules were unperfused, their short length (≈ 500 μm) and open ends allowed the bath solution free access to both sides of the epithelium.

the normal and the hypotonic high-K^+ solutions have identical electrolyte compositions.

K^+-channel currents were amplified with a Dagan model 8900 patch clamp (50-GΩ probe), digitized with a pulse code modulation processor (Sony 501 ES), stored on video tape (Panasonic 1820 VCR), and later sampled at 5 kHz with a PDP 11/23 and 8-pole Bessel filter set at 1 kHz. In cell-attached patches, the intracellular potential was estimated as the initial potential under zero-current clamp conditions in a whole-cell recording that was obtained by breaking the patch at the end of each experiment. The transpatch potential was determined as the difference between the intracellular potential and the pipette holding potential. The level of K^+-channel activity was quantitated by defining the mean number of open channels (NP_o) as $\sum_{n=1}^{N} nP_n$, where P_n is the probability that n identical channels are open simultaneously, N is the apparent number of channels in the patch, and P_o is the open probability of the channel (28).

Given the low open probability of this channel type, simultaneous events were extremely rare, and the open/closed-time kinetics were analyzed as if each patch contained a single channel. Three sets of open/closed-time histograms were constructed for each tubule, corresponding to the three conditions: control, pipette suction, and swelling. Data from presuction and postsuction control periods were pooled to increase the number of events for kinetic analysis. The histograms were fit with a sum of up to two exponentials, determined by a regression of the log(events) vs. time interval distribution. The relatively small number of total events for each condition prevented consideration of more than a two-exponential fit to the data. Differences in mean values $\pm$ standard errors were analyzed for significance by using Student's t test.

RESULTS

The short-open-time (τ_{open} = 0.8 ± 0.1 msec) K^+ channel at the basolateral membrane of *Necturus* renal proximal tubule is activated by both pipette suction and cell swelling. With each patch as its own control, K^+-channel records were compared for the four conditions: control, suction, control, and hypotonic bath. Fig. 1 illustrates typical current records from one of seven cell-attached patches. Upward deflections from the closed state (horizontal bars) denote outward current from cell to pipette. In Fig. 1 the transpatch potential is +20 mV (cell − pipette), as determined from a pipette holding potential of −40 mV and an average cell membrane potential of −20 ± 2 mV (with respect to bath). The P_K/P_{Na} selectivity (>20), single-channel conductance (42pS, cell-attached), and kinetics identify this as the short-open-time basolateral K^+ channel previously described (27, 28).

Although the results of pipette suction were always reversible, it was not possible to return swollen cells to normal osmolarity without breaking the GΩ seal between the membrane and the pipette. After about 5 min of hypotonic bath (95 mosM less than normal), the mean increase in cell volume was 66 ± 10%, as estimated with an eyepiece micrometer in seven tubules. This was associated with consistently significant increases in the value of NP_o (Figs. 1 and 2). On the other hand, reliable increases in channel activity could not be detected for cell swelling of less than 30%. Random variations in channel activity over short intervals prevented a more complete description of the time course of activation during swelling.

Use of a high-K^+ bathing solution (35 mM K^+, 6.4 mM Na^+) was remarkably effective in maintaining both cell K^+ and membrane potential within narrow limits. This is indicated by the relatively constant single-channel current amplitude in the four conditions of Fig. 1. Since the current–voltage relation of this channel is approximately linear (28), changes in membrane potential or cell K^+ during the swelling
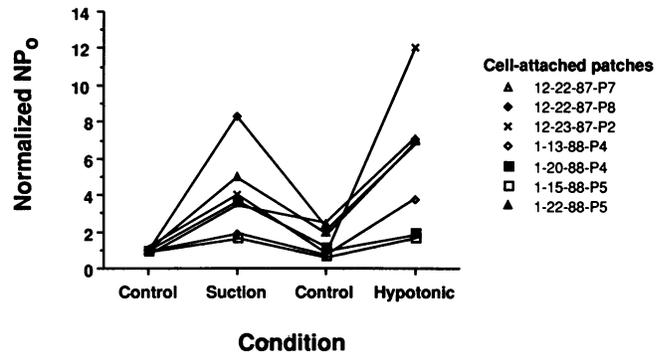

Fig. 2. Effect of pipette suction and cell swelling on seven cell-attached basolateral patches from seven isolated *Necturus* proximal tubules. Control: no applied pipette suction, normal bath osmolarity (204 mosM). Suction: negative pressure (−6 cm H_2O) applied to pipette. Hypotonic: 95 mosM sucrose removed from the bath with no change in electrolytes.

process should have produced a noticeable change in the single-channel current.

Fig. 2 summarizes the results of seven separate experiments similar to the one of Fig. 1, where paired data from each patch are connected by straight lines and all values are normalized to the initial control state. Application of about 6 cm H_2O pipette suction increased NP_o by a factor of 4.0 ± 0.8, and cell swelling increased NP_o by a factor of 5.8 ± 1.4. Both these values were significantly different from their respective controls ($P < 0.02$, paired t test) but not significantly different from each other ($P > 0.2$, paired t test). Hence, those patches that responded strongly to suction also responded strongly to cell swelling. Furthermore, the effects of either maneuver on NP_o seem equally sensitive to intertubule variability.

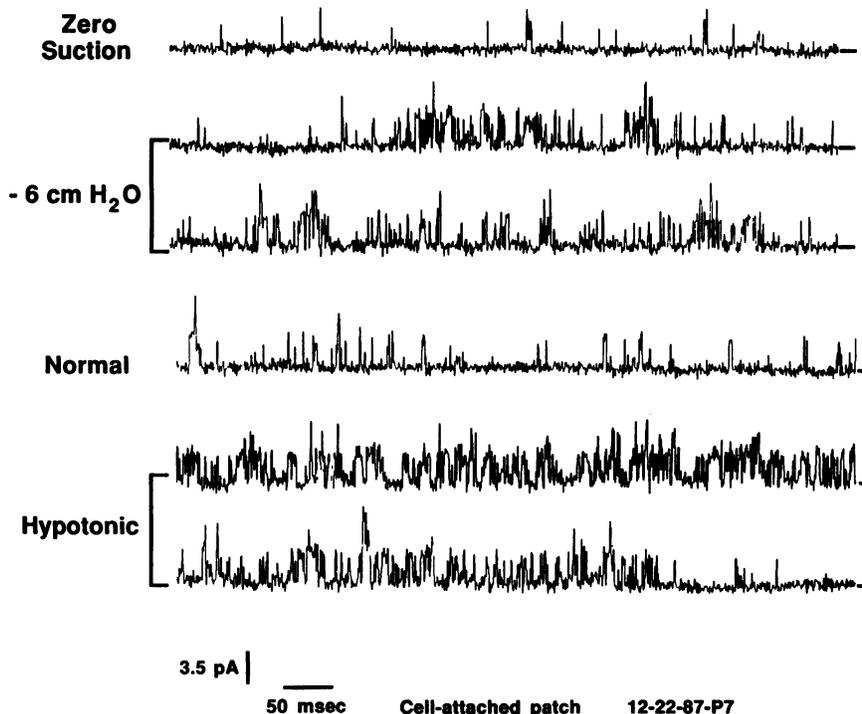Open and closed times were constructed for five tubules that contained a sufficient number of events and few enough

Fig. 1. Effect of pipette suction and hypotonic medium on K^+ currents in a cell-attached patch from basolateral membrane of *Necturus* proximal tubule. All records in this figure were obtained from the same patch (Exp. 12-22-87-P7), using the solutions of Table 1. Upward deflections from closed state (horizontal bars) denote outward currents from cell to pipette.

Physiological Sciences: Sackin

Proc. Natl. Acad. Sci. USA 86 (1989) 1733

Open Time Histogram

$N = 51^*exp(-1.5^*t)$

$\tau_{open} = 0.7$ msec

Closed time Histogram

$N = 66exp(-0.6^*t) + 5exp(-.02^*t)$

$\tau_1 = 1.7$ msec $\tau_2 = 43$ msec
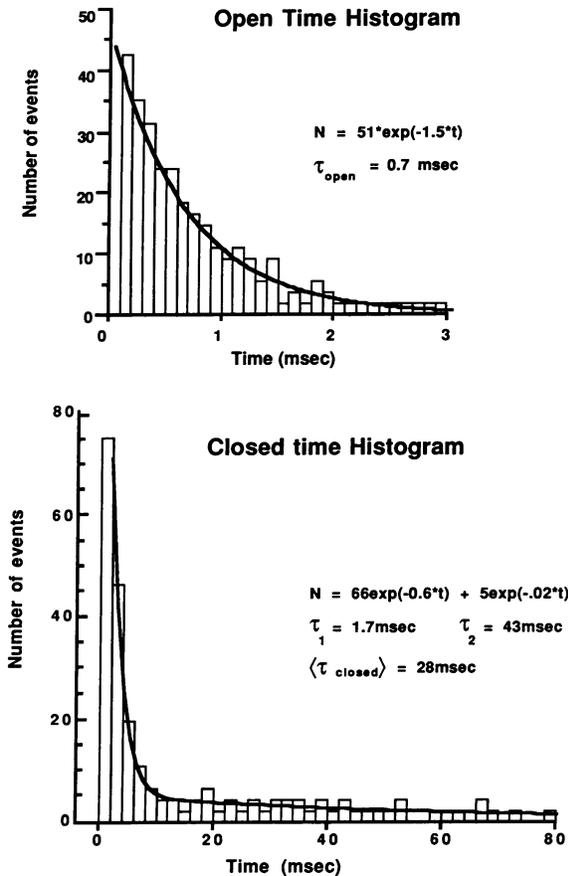
$\langle \tau_{closed} \rangle = 28$ msec

FIG. 3. Open- and closed-time histograms for the basolateral stretch-activated channel of *Necturus* proximal tubule under control conditions with high-K^+ in the bath. All open-time histograms could be fit by one exponential with mean open time τ_{open}, whereas all closed-time histograms required fitting by at least two exponentials with closed-times τ_1 and τ_2, respectively. The symbol $\langle \tau_{closed} \rangle$ denotes the mean closed time of the distribution function as defined by Eq. **1**.

multiple openings to be analyzed as single-channel patches. Fig. 3 illustrates control-state open- and closed-time distributions for one such tubule. In all three conditions (control, suction, and swelling), the open-time distributions could be adequately fit by a single exponential. On the other hand, the closed-time distributions for all three conditions were fit best by a probability density function of the form $A \exp(-t/\tau_1) + B \exp(-t/\tau_2)$, where τ_1 and τ_2 are the closed-time constants and the effective closed time, $\langle \tau_{closed} \rangle$, is defined by Eq. **1**, based on equation 38 of ref. 29:

$$\langle \tau_{closed} \rangle = (A\tau_1^2 + B\tau_2^2)/(A\tau_1 + B\tau_2). \qquad [1]$$

The shorter time constant (τ_1) probably reflects flickery closures of the channel during bursting events, whereas the longer time constant (τ_2) is related to the average time interval between bursts of channel activity. The total number of events were insufficient to consider fitting by more than two exponentials or to consider a burst analysis of the data.

Table 2 summarizes the average time constants of the open- and closed-time distributions for the three conditions: control, suction, and swelling. Neither pipette suction nor cell swelling produced a significant change in either the mean open time ($\tau_{open} = 0.8 \pm 0.1$ msec) or the shorter time constant of the closed-time distribution ($\tau_1 = 2.0 \pm 0.4$ msec). However, both suction and cell swelling produced a similar, $\approx 68\%$ decrease in the longer time constant ($\tau_2 = 49 \pm 8$ msec) of the closed-time distribution. This decrease in τ_2 appears to be almost completely responsible for the decrease in $\langle \tau_{closed} \rangle$ and the 4-fold increase in NP_o during suction and cell swelling.

DISCUSSION

The results of the present study indicate that basolateral K^+ channels of amphibian proximal tubule can be similarly activated by both membrane stretch and cell swelling. Although the magnitude of the change in NP_o was different in each of the seven experiments in Fig. 2, those patches that responded to the effects of suction also responded to cell swelling.

A kinetic analysis of the current records implies that the stretch-activated K^+ channel of amphibian proximal tubule basolateral membrane has at least one open state and two closed states. Stretch-activation of the channel is almost entirely the result of a decrease in the closed time between short bursts of channel openings.

Perhaps the most important result of the kinetic analysis is that both cell swelling and pipette suction decrease the longer closed-time constant (τ_2) by a similar factor, without significant change in τ_{open} or τ_1. This suggests that suction and swelling may activate K^+ channels by a common mechanism.

In proximal tubule, hypotonic solutions probably increase cell volume initially at the expense of membrane infolding, without a real change in cell surface area. However, above a critical cell volume, membrane tension should increase uniformly. Although tension was not measured in these experiments, there is evidence that osmotic swelling increases both hydrostatic pressure and membrane tension in frog and mouse oocytes (30). Increased membrane tension in amphibian proximal tubule cells would increase the open probability of the basolateral stretch-activated K^+ channels. Subsequent exit of K^+ down its electrochemical gradient, together with an accompanying anion, would reduce cell volume if these ion fluxes were accompanied by osmotic water flow.

Although *Necturus* proximal tubules do volume regulate in hypotonic solutions (6), the presence of high K^+ in the bath and the absence of an intact basement membrane apparently prevented the changes in cell composition required for

Table 2. Kinetics of the proximal tubule stretch-activated K^+ channel

	Mean open time (τ_{open}), msec	Mean closed times, msec		
		τ_1	τ_2	$\langle \tau_{closed} \rangle$
Control*	0.8 ± 0.1	2.0 ± 0.4	49.0 ± 8.0	35.6 ± 7.6
Suction				
(-6 cm H_2O)	0.8 ± 0.1	1.9 ± 0.2	$15.7 \pm 2.2^\dagger$	$9.9 \pm 1.6^\dagger$
Cell swelling				
(95-mosM hypotonic)	0.9 ± 0.1	1.9 ± 0.2	$16.0 \pm 3.0^\dagger$	$11.0 \pm 2.3^\dagger$

Values are averages of 5 tubules, each subjected to the same three conditions: control, suction, and cell swelling. All values of τ_{open} are not significantly different from one another, and all values of τ_1 are not significantly different from one another.
*Includes control data both prior to suction and prior to swelling.
†Significantly different from the respective control value ($P < 0.02$).

normal osmoregulation. Nonetheless, the observed increase in NP_o during swelling could mediate volume regulation under normal conditions. Although cell pH and metabolic factors may still play a role in regulation of K^+ conductance, it is unlikely that changes in cytosolic Ca^{2+} could account for the increase in K^+-channel activity, since (in excised patches) basolateral K^+ channels are essentially unaffected by Ca^{2+} (28).

The rationale for a high-K^+ bath solution was to clamp both cell K^+ and membrane potential within narrow limits, as indicated by the constant single-channel current amplitude in all four records of Fig. 1. The use of high-K^+ solutions throughout the paired experiments probably resulted in higher than normal initial cell volumes. Under these "prestretched" conditions, the membranes would be less flaccid than normal and the channels would be easier to activate by suction and/or swelling. All tubules were preincubated in a high-K^+ bath, and no attempt was made to quantitate the effect of high K^+ on cell volume.

The reason that large (>60%) increases in cell volume were required to elicit consistent increases in K^+-channel activity can be understood as follows. Since the patch pipette is kept in place throughout the four experimental conditions, it serves to clamp the membrane patch mechanically as well as electrically. Cell swelling presumably increases tension in both the bulk membrane of the cell (T_c) and the membrane patch held by the pipette (T_p) according to Laplace's law:

$$T_c = (d_c/d_p)T_p,   [2]$$

where the patch pipette diameter (d_p) is $\approx 1/60$th the diameter of an amphibian proximal tubule cell $(d_c = 30\ \mu m)$. The pipette suction (P_{patch}) of -6 cm H_2O required to elicit a significant change in K^+-channel activity in cell-attached patches corresponds to a patch tension of

$$T_p = d_p \cdot P_{patch}/4 = 0.07\ dyne/cm   [3]$$

or a uniform cell tension $T_c = 4.3$ dyne/cm (from Eqs. 2 and 3; 1 dyne $= 10\ \mu N$).

Although the effect of osmotic swelling on membrane geometry is not known for this preparation, it is possible to estimate the minimum percent volume change $(\Delta V/V)$ required to elicit a noticeable increase in single-channel activity. For the special case of a spherical cell, $\Delta V/V$ is related to cell membrane tension (T_c) and the area elasticity constant (K_A) according to equations A1 and A4 of ref. 27:

$$\Delta V/V = (1 + T_c/K_A)^{3/2} - 1.   [4]$$

It should be emphasized that Eq. 4 represents a minimum value, since cell swelling would not necessarily produce an immediate increase in membrane tension, and the spherical cell assumption is an obvious idealization.

If bilayer lysis occurs after a 3% increase in area (23, 31), and basolateral proximal tubule patches of diameter 0.5 μm can withstand (on the average) only $P^* = 30$ cm H_2O, then for proximal tubule

$$K_A = (d_p \cdot P^*)/(4 \cdot 0.03) = 12.2\ dyne/cm.   [5]$$

Using the above values of T_c and K_A, Eq. 4 predicts that a 60% increase in cell volume would be required to elicit a measurable increase in K^+-channel activity in the cell-attached patch. This estimate compares reasonably well with the increase in cell volume of 66 ± 10% observed after about 5 min of hypotonic solution.

Cell swelling of less than 30% failed to increase NP_o in any of patches studied. However, this does not mean that K^+ channels are activated only by extensive cell swelling. Large

volume changes were required to detect channel activity in these experiments because of a mismatch between the pipette diameter and the cell diameter. If the patch pipette were the same diameter as the cell, it might be possible to record statistically significant increases in NP_o after a cell volume increase of only 1% (see Eqs. 2 and 4 with $d_c = d_p$). Even if the estimate of T_c/K_A were 10 times larger, a cell volume increase of only 10% would be required to produce a statistically significant increase in NP_o (Eq. 4 with $d_c = d_p$).

Comparison with other stretch-activated channels indicates that the basolateral stretch-activated K^+ channel of amphibian proximal tubule closely resembles the stretch-activated K^+ channel found in molluscan heart cells (26), although the open-channel probability is much lower in proximal tubule than in heart. Both channel types exhibit fast, flickery transitions to the open state, often occurring in bursts. Both channels have similar single-channel conductances (between 30 pS and 45 pS) and demonstrate a clear preference for K^+ over other cations. This is in contrast to the less selective stretch-activated channels described in chick skeletal muscle (23), frog lens (32), *Escherichia coli* (33), oocytes (34), and plant cells (35). A channel recently described in cultured opossum kidney cells is activated by both pipette suction and osmotic swelling, although it does not appear to discriminate among Na^+, K^+, and Cl^- (36).

Stretch-activated K^+ channels may be important in electrolyte homeostasis as well as in osmoregulation. The high rates of Na^+ and water reabsorption in renal proximal tubule require homocellular mechanisms for maintaining a constant intracellular environment (37). Increments in Na^+–substrate cotransport are followed by parallel increases in transepithelial Na^+ transport and basolateral K^+ conductance (38, 39). The increase in K^+ conductance presumably permits enough K^+ to leave the cell down its electrochemical gradient to balance the increase in K^+ uptake across the basolateral cell membrane via the Na^+,K^+-ATPase. Since Na^+–substrate cotransport produces a significant cell swelling (5, 40), both electrolyte and volume homeostasis may be mediated by changes in cell volume.

In summary, the results of the present study suggest that cell swelling increases K^+ conductance by increasing the open probability of a stretch-activated K^+ channel. Exit of K^+ down its electrochemical gradient, together with bicarbonate (or chloride) and water, would then restore cells to their original volume. Consequently, stretch-activation of K^+ channels could certainly mediate osmoregulation in the physiological range. In addition, stretch-activated K^+ channels may also mediate electrolyte homeostasis during periods of increased transepithelial transport if Na^+-coupled uptake of organic substrates increases cell volume by at least 1%.

1. Chase, H. & Wong, S. (1986) *Am. J. Physiol.* **250,** C841–C852.
2. Germann, W., Ernst, S. & Dawson, D. (1986) *J. Gen. Physiol.* **88,** 253–274.
3. Grinstein, S., Dupre, A. & Rothstein, A. (1982) *J. Gen. Physiol.* **79,** 849–868.
4. Kregenow, F. (1981) *Annu. Rev. Physiol.* **43,** 493–505.
5. Lau, K., Hudson, R. & Schultz, S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 3591–3594.
6. Lopes, A. & Guggino, W. (1987) *J. Membr. Biol.* **97,** 117–125.
7. Richards, N. & Dawson, D. (1986) *Am. J. Physiol.* **251,** C85–C89.
8. Larson, M. & Spring, K. R. (1984) *J. Membr. Biol.* **81,** 219–232.
9. Sarkadi, B., Mack, E. & Rothstein, R. (1984) *J. Gen. Physiol.* **83,** 497–512.

Physiological Sciences: Sackin

Proc. Natl. Acad. Sci. USA 86 (1989) 1735

10. Welling, P., Linshaw, M. & Sullivan, L. (1985) *Am. J. Physiol.* **249**, F20–F27.
11. Hoffmann, E. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 2513–2519.
12. Gagnon, J., Ouimet, D., Nguyen, H., Laprade, R., LeGrimellec, C., Carriere, S. & Cardinal, J. (1982) *Am. J. Physiol.* **243**, F408–F415.
13. Dellasega, M. & Grantham, J. (1973) *Am. J. Physiol.* **224**, 1288–1294.
14. Grantham, J., Lowe, C., Dellasega, M. & Cole, B. (1977) *Am. J. Physiol.* **232**, F42–F49.
15. Kirk, K., Dibona, D. & Schafer, J. (1987) *Am. J. Physiol.* **252**, F933–F942.
16. Welling, P. & O'Neil, R. (1987) *Kidney Int.* **31**, 452 (abstr.).
17. Christensen, O. (1987) *Nature (London)* **330**, 67–68.
18. Davis, C. & Finn, A. (1982) *Science* **216**, 525–527.
19. Foskett, J. & Spring, K. (1985) *Am. J. Physiol.* **248**, C27–C36.
20. Grinstein, S., Rothstein, A., Sarkadi, B. & Gelfand, E. (1984) *Am. J. Physiol.* **246**, C204–C215.
21. Steels, P. & Boulpaep, E. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 465 (abstr.).
22. Romero, P. (1978) *Biochim. Biophys. Acta* **507**, 178–181.
23. Guharay, F. & Sachs, F. (1984) *J. Physiol. (London)* **352**, 685–701.
24. Hamill, O. (1983) in *Single-Channel Recording*, eds. Sakmann, B. & Neher, E. (Plenum, New York), pp. 451–471.
25. Sachs, F. (1987) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **46**, 12–16.
26. Sigurdson, W., Morris, C., Brezden, B. & Gardner, D. (1987) *J. Exp. Biol.* **127**, 191–209.
27. Sackin, H. (1987) *Am. J. Physiol.* **253**, F1253–F1262.
28. Sackin, H. & Palmer, L. (1987) *Am. J. Physiol.* **253**, F476–F487.
29. Colquhoun, D. & Sigworth, F. (1983) in *Single-Channel Recording*, eds. Sakmann, B. & Neher, E. (Plenum, New York), pp. 191–263.
30. Kelly, S. & Macklem, P. (1988) *FASEB J.* **2**, A318 (abstr.).
31. Evans, E., Waugh, R. & Melnik, L. (1976) *Biophys. J.* **16**, 585–595.
32. Cooper, K., Tang, J., Rae, J. & Eisenberg, R. (1986) *J. Membr. Biol.* **93**, 259–269.
33. Martinac, B., Buechner, M., Delcour, A., Adler, J. & Kung, C. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 2297–2301.
34. Methfessel, C., Weitzmann, V., Takahashi, T., Mishina, M., Numa, S. & Sakmann, B. (1986) *Pflügers Arch.* **407**, 577–588.
35. Falk, L., Edwards, K., Misler, S. & Pickard, B. (1986) *Plant Physiol.*, Suppl., **80**, 9.
36. Ubl, J., Murer, H. & Kolb, H.-A. (1988) *J. Membr. Biol.* **104**, 223–232.
37. Schultz, S. (1981) *Am. J. Physiol.* **241**, F579–F590.
38. Grasset, E., Gunter-Smith, P. & Schultz, S. (1983) *J. Membr. Biol.* **71**, 89–94.
39. Messner, G., Oberleithner, H. & Lang, F. (1985) *Pflügers Arch.* **404**, 128–144.
40. Hudson, R. & Schultz, S. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 279–283.