Kidney International, Vol. 49 (1996), pp. 1695–1699

Single channel properties of a volume sensitive anion channel: Lessons from noise analysis

PAUL S. JACKSON and KEVIN STRANGE

Critical Care Research Laboratories, Departments of Neurosurgery, Medicine (Nephrology) and Anesthesia, Children's Hospital, Harvard Medical School Boston, Massachusetts, USA

Single channel properties of a volume sensitive anion channel: Lessons from noise analysis. Swelling activated anion channels have recently been recognized to play an important role in not only volume regulatory electrolyte movement, but also in organic osmolyte transport. A swelling-activated, outwardly rectifying anion channel termed VSOAC (volume-sensitive organic osmolyte/anion channel) is a major pathway for swelling-induced loss of organic osmolytes and organic anions from mammalian cells. VSOAC has been described in numerous cell types. Until recently, however, the unitary conductance and gating kinetics of VSOAC were uncertain. Stationary noise analysis and single channel measurements have produced estimates for the unitary conductance of swelling-activated, outwardly rectifying anion channels that vary by > 15-fold. This review describes our current understanding of the single channel properties of VSOAC.

Animal cells must maintain a constant volume in the face of extracellular and intracellular osmotic perturbations. Cells respond to volume changes by activating volume regulatory mechanisms that mediate the net gain or loss of osmotically-active inorganic ions [1] and small organic solutes (amino acids, polyols and methylamines) termed organic osmolytes [2, 3].

Organic osmolytes are lost from cells in response to swelling by a rapid (that is, seconds) increase in passive efflux, which is mediated by a swelling-activated, anion-selective channel [4–7; reviewed in 8]. The channel responsible for organic osmolyte efflux is termed VSOAC (*v*olume-*s*ensitive *o*rganic osmolyte/*a*nion *c*hannel) and has been characterized extensively in rat C6 glioma cells. VSOAC has a broad anion selectivity ($SCN^- > I^- > NO_3^- > Br^- > Cl^- > F^-$ > isethionate > gluconate), is outwardly rectifying and displays voltage-dependent inactivation at positive membrane potentials [6, 9]. Swelling-induced activation of VSOAC requires intracellular nonhydrolytic ATP binding [7] and is modulated by intracellular Cl levels and possibly ionic strength [10–13]. Swelling-activated, whole cell anion conductances with the characteristics of VSOAC have been described in numerous mammalian cell types [reviewed in 8], *Xenopus* oocytes [14], and hepatocytes of the skate *Raja erinacea* [11, 12].

Estimates of unitary conductance

The single channel properties of VSOAC were, until recently, controversial. Stationary noise analysis measurements yielded an estimated unitary conductance at 0 mV of ~1 to 2 pS for the swelling-activated, outwardly rectifying anion channel in chromaffin cells, endothelial cells, neutrophils and T cells [15–18]. Lewis, Ross and Cahalan have termed this channel the 'mini' volume-sensitive anion channel [17]. Measurement of single channel transitions in epithelial cells, however, revealed the presence of a swelling-activated, outwardly rectifying anion channel with a unitary conductance of ~40 to 90 pS at depolarizing voltages [19–21].

One possible interpretation of these results is that different types of channels are responsible for the swelling-activated, outwardly rectifying whole cell currents in different cell types. Our studies in C6 glioma cells, however, have demonstrated that stationary noise analysis significantly underestimates the unitary conductance of VSOAC.

Theoretical basis of noise analysis

The activity of a specific population of channels gives rise to a whole cell or macroscopic current (I). Whole cell current is defined simply as

$$I = NiP_o \qquad \text{(Eq. 1)}$$

where N is the number of channels in the membrane, i is the current flowing through a single channel and P_o is channel open probability. Analysis of whole cell current 'noise' can be used to estimate the unitary conductance and number of specific types of channels in cell membranes [22]. Noise analysis is usually performed under the assumptions: (1) there are N independent and identical channels in the membrane; (2) the channels have two conductance states, open and closed, that obey binomial statistics; and (3) graded changes in macroscopic current are due to graded changes in channel open probability [22, 23]. If these assumptions are correct, then current 'noise' or variance, σ^2, is defined as

$$\sigma^2 = iI - I^2/N \qquad \text{(Eq. 2)}$$

By measuring the variance of the mean whole cell current during different levels of channel activity channel size and density can be estimated.

Stationary noise analysis of VSOAC

We used two types of noise analysis to estimate the unitary conductance of VSOAC. Figure 1 shows an example of stationary noise analysis of the swelling-activated whole cell anion current in

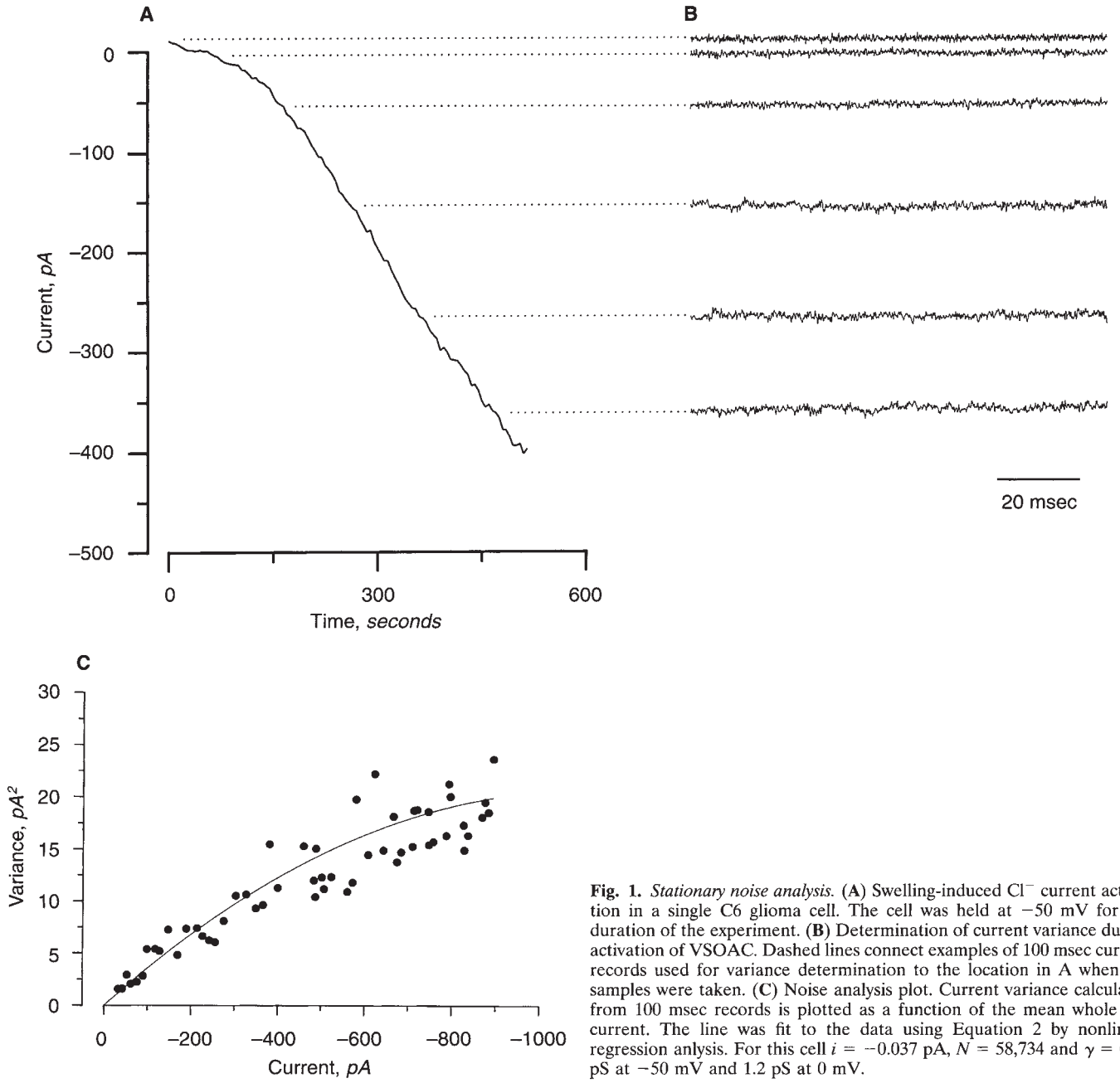

Fig. 1. *Stationary noise analysis.* (**A**) Swelling-induced Cl^- current activation in a single C6 glioma cell. The cell was held at −50 mV for the duration of the experiment. (**B**) Determination of current variance during activation of VSOAC. Dashed lines connect examples of 100 msec current records used for variance determination to the location in A when the samples were taken. (**C**) Noise analysis plot. Current variance calculated from 100 msec records is plotted as a function of the mean whole cell current. The line was fit to the data using Equation 2 by nonlinear regression anlysis. For this cell $i = -0.037$ pA, $N = 58{,}734$ and $\gamma = 0.55$ pS at −50 mV and 1.2 pS at 0 mV.

a C6 glioma cell. The cell was patch clamped in the whole cell mode and membrane potential was held at −50 mV. Cell swelling was induced by reducing extracellular osmolality. At various times after induction of the current, 100 msec long current records were sampled. During the 100 msec sampling period, there is little change in the magnitude of the whole cell current. Therefore, the whole cell current is 'stationary.'

The mean current and current variance of these records were quantified. Figure 1C shows a plot of the current variance against the amplitude of the mean whole cell current. The data were fitted with Equation 2 and yielded an estimated unitary conductance of 1.2 pS at 0 mV. The number of channels required to account for the whole cell current was 59,000. These results are similar to those obtained with neutrophils, T-cells, chromaffin cells and endothelial cells [15–18].

Nonstationary noise analysis of VSOAC

In an effort to corroborate these findings, we also performed nonstationary noise analysis of whole cell currents. Nonstationary noise analysis relies on the same assumptions and statistical treatment as described above for stationary noise analysis. The key difference, however, is that nonstationary noise analysis measures current variance continuously during experimentally-induced changes in current amplitude. Nonstationary noise analysis is typically carried out during voltage-induced changes in channel activity [24, 25].

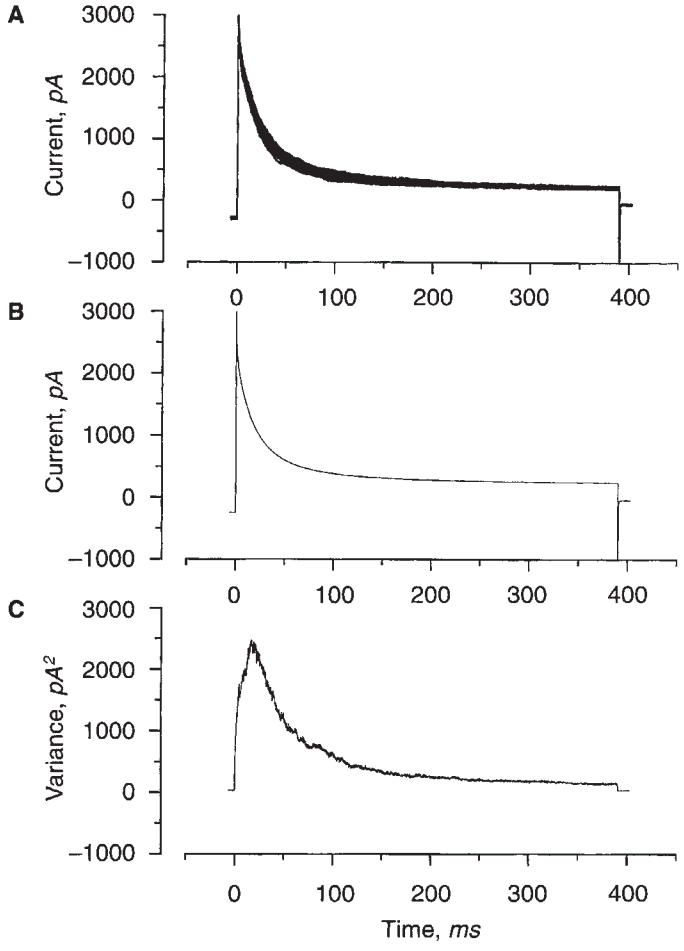

Fig. 2. *Voltage-dependent inactivation of VSOAC current.* (**A**) Membrane potential was stepped to +120 from −60 mV. Potential was held at +120 mV for 390 msec and then returned to −60 mV. The pulse sequence was repeated once every five seconds. Whole cell current is inactivated by ~90% by membrane depolarization [9]. Figure shows 25 of the 350 current inactivation records obtained from this cell. (**B**) Ensemble average of 350 current inactivation records. (**C**) Current variance at each time point of the ensemble average obtained by the method of successive differences [25].

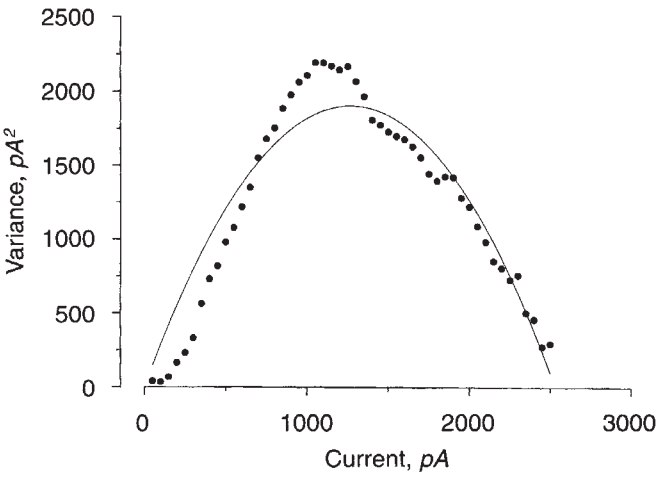

Fig. 3. *Variance versus current amplitude plot of data derived from the experiment shown in Figure 2.* The line was fit to the data using Equation 2 by nonlinear regression analysis. For this cell i = 3.0 pA, N = 843 and γ = 39.2 pS at +120 mV and 10.8 pS at 0 mV.

Figure 2 shows an experiment in which nonstationary noise analysis was used to estimate the unitary conductance of VSOAC. A C6 cell was patch clamped in the whole cell mode and membrane potential was held at −60 mV. The cell was swollen and the whole cell current was monitored until it was fully activated and stable. Membrane potential was then stepped to +120 mV for 390 msec once every five seconds. During this time, whole cell current is inactivated by ~90% (Fig. 2A) [9]. This inactivation procedure was repeated 350 times and the individual inactivation traces averaged to yield an ensemble average (Fig. 2B) and variance of the mean current (Fig. 2C).

Figure 3 shows a plot of the current variance against the amplitude of the mean whole cell current. The data were fitted with Equation 2. In this experiment, the predicted single channel conductance was 39.2 pS at +120 mV. This value is similar to that obtained by investigators measuring single channel transitions in epithelial cells [19–21] and is ~15-fold greater than the estimate produced by stationary noise analysis.

Single channel measurements

To resolve the discrepancy between the stationary and nonstationary noise analysis results, we examined isolated membrane patches for single channel events. Membrane patch currents with pharmacological and voltage-dependent characteristics identical to those of VSOAC were observed regularly in outside-out patches. The current-to-voltage relationship for these patches was outwardly rectifying and the currents were inhibited by known inhibitors of the whole cell VSOAC current [26].

Single channel transitions were not observed when outside-out patches were held at negative holding potentials. When the patch was depolarized, the current inactivated with a time constant similar to that of the whole cell current. Inactivation occurred in discrete steps, which represent the closure of single channels (Fig. 4). Measurement of these channel closures yielded single channel conductances of 40 to 50 pS at +120 mV. These values are in excellent agreement with the results of nonstationary noise analysis.

Single channel closures were also observed in whole cell currents during depolarization-induced inactivation [26]. For these experiments, membrane potential was held at −60 mV. Immediately and 30 and 60 seconds after cell swelling was induced, the potential was depolarized to +120 mV. When observed at high amplifier gain, whole cell currents inactivated in discrete steps. Measurement of these channel closures again yielded single channel conductances of 40 to 50 pS at +120 mV. We conclude that VSOAS is an intermediate conductance channel and that stationary noise analysis underestimates the unitary conductance of VSOAC by ~15-fold.

Accounting for the underestimation of channel conductance by stationary noise analysis

Changes in whole cell current amplitude can be brought about changes in channel P_o, single channel conductance and/or channel

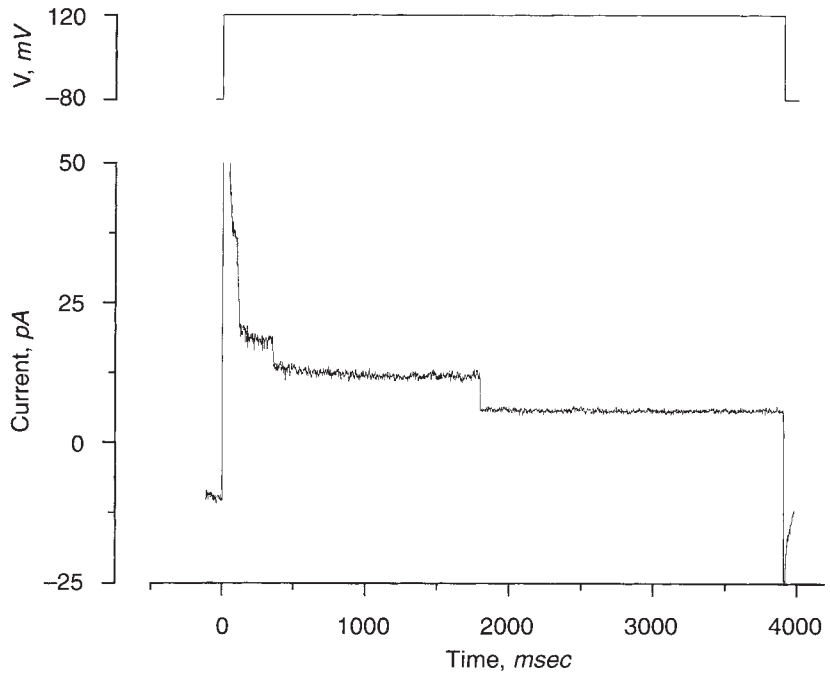

Fig. 4. *VSOAC single channels revealed by voltage dependent inactivation.* The outside-out patch was pulled from a swollen C6 glioma cell. A voltage pulse to +120 mV induced current inactivation that occurred in discrete steps. The mean step size in this patch was 5.4 pA at +120 mV.

number. For our stationary noise analysis studies, we made the conventional assumptions [22, 23] that swelling-induced increases in VSOAC whole cell current were due to increases in the P_o of a fixed number of channels with a fixed unitary conductance. Previous studies using stationary noise analysis to characterize swelling-activated whole cell anion currents have made similar assumptions [15–18].

Why does stationary noise analysis underestimate the unitary conductance of the channel by at least 15-fold? One or more of the assumptions made about the mechanism of VSOAC activation must be wrong. Which assumptions are invalid? The unitary conductance of VSOAC is fixed throughout activation as we assumed initially [26]. This means that whole cell current does not increase by graded increases in the P_o of a fixed number of channels. Instead, swelling-induced activation of the outwardly rectifying anion current appears to occur by abrupt switching of individual channels from an *OFF* state, where channel P_o is zero, to an *ON* state, where P_o is near unity [20, 26]. In other words, cell swelling increases the number of active channels, *N*, in the membrane. Once a channel is turned *ON*, its P_o is effectively fixed and spontaneous channel closures are very brief [26].

Conclusions

The findings discussed in this review have helped to clarify and simplify our understanding of volume sensitive anion channels. It is now clear that the outwardly rectifying, volume sensitive, anion channel VSOAC is an intermediate conductance channel with a unitary conductance of ~50 pS at +120 mV. Although stationary noise analysis accurately captures the current fluctuations generated during current activation, the underlying assumptions which allow calculation of single channel parameters, namely that current activation is the result of the graded and uniform increase in the open probability of a constant number of channel, is not valid. The channel has a novel mechanism of activation with individual channels switching one at a time from a completely *OFF* state into an *ON* state with very high open probability. The next problem will be to relate the kinetic states observed during VSOAC activation to particular molecular states of individual protein molecules. This work is still in its infancy, however, a molecular candidate for the VSOAC ion channel has been identified [8]. This discovery may open the door for a variety of new approaches to understanding VSOAC channel activation and gating in the near future.

Acknowledgments

Investigations described in this review were funded by NIH grants NS30591 and DK45628 to K. Strange. P. Jackson was supported by the Boston Neurosurgical Foundation and by NIH training grant T32EY07110. K. Strange is an Established Investigator of the American Heart Association.

Reprint requests to Dr. Kevin Strange, Critical Care Research Laboratories, Children's Hospital, Enders 12, 320 Longwood Ave., Boston, Massachusetts 02115, USA.
E-mail: strange_k@a1.tch.harvard.edu

References

1. Hallows KR, Knauf PA: Principles of cell volume regulation, in *Cellular and Molecular Physiology of Cell Volume Regulation*, edited by Strange K, Boca Raton, CRC Press, 1994, p 3
2. Garcia-Perez A, Burg MB: Renal medullary organic osmolytes. *Physiol Rev* 71:1081–1115, 1991
3. Yancey, PH: Compatible and counteracting solutes, in *Cellular and Molecular Physiology of Cell Volume Regulation*, edited by Strange K, Boca Raton, CRC Press, 1994, p 81
4. Banderali U, Roy G: Anion channels for amino acids in MDCK cells. *Am J Physiol* 263:C1200–C1207, 1992
5. Kirk K, Ellory JC, Young JD: Transport of organic substrates via a volume-activated channel. *J Biol Chem* 267:23475–23478, 1992

6. JACKSON PS, STRANGE K: Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. *Am J Physiol* 265:C1489–C1500, 1993
7. JACKSON PS, MORRISON R, STRANGE K: The volume-sensitive organic osmolyte-anion channel VSOAC is regulated by non-hydrolytic ATP binding *Am J Physiol* 267:C1203–C1209, 1994
8. STRANGE K, JACKSON PS: Swelling-activated organic osmolyte efflux: A new role for anion channels. *Kidney Int* 48:994–1003, 1995
9. JACKSON PS, STRANGE K: Characterization of the voltage-dependent properties of a volume-sensitive anion conductance. *J Gen Physiol* 105:661–677, 1995
10. STRANGE K: Are all cell volume changes the same? *News Physiol Sci* 9:223–228, 1994
11. STRANGE K, CHURCHWELL K, BALLATORI N, BOYER JL, JACKSON PS: Properties of volume-sensitive organic osmolyte/anion channels (VSOAC) in hepatocytes of the marine skate *Raja erinacea.* (abstract) *FASEB J* 9:A636, 1995
12. JACKSON PS, CURCHWELL, K, BALLATORI N, BOYER JL, STRANGE K: Swelling-activated anion conductance in skate hepatocytes: Regulation by cell Cl^- and ATP. *Am J Physiol* 270:C57–C66, 1996
13. EMMA F, MCMANUS M, STRANGE K: Intracellular electrolyte levels modulate volume regulatory organic osmolyte (OO) efflux. (abstract) *J Am Soc Nephrol* 6:360, 1995
14. ACKERMAN MJ, WICKMAN KD, CLAPHAM DE: Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J Gen Physiol* 103:153–179, 1994
15. DOROSHENKO P, NEHER E: Volume-sensitive chloride conductance in bovine chromaffin cell membrane. *J Physiol* 449:197–218, 1992
16. STODDARD JS, STEINBACH JH, SIMCHOWITZ L: Whole cell Cl^- currents in human neutrophils induced by cell swelling. *Am J Physiol* 265:C156–C165, 1993
17. LEWIS RS, ROSS PE, CAHALAN MD: Chloride channels activated by osmotic stress in T lymphocytes. *J Gen Physiol* 101:801–826, 1993
18. NILIUS B, OIKE M, ZAHRADNIK I, DROOGMANS G: Activation of a Cl^- current by hypotonic volume increase in human endothelial cells. *J Gen Physiol* 103:787–805, 1994
19. WORRELL RT, BUTT AG, CLIFF WH, FRIZZELL RA: A volume-sensitive chloride conductance in human colonic cell line T84. *Am J Physiol* 256:C1111–C1119, 1989
20. SOLC CK, WINE JJ: Swelling-induced and depolarization-induced Cl^- channels in normal and cystic fibrosis epithelial cells. *Am J Physiol* 261:C658–C674, 1991
21. OKADA Y, PETERSEN CCH, KUBO M, MORISHIMA S, TOMINAGA M: Osmotic swelling activates intermediate-conductance Cl^- channels in human intestinal epithelial cells. *Jpn J Physiol* 44:403–409, 1994
22. HILLE B: *Ionic Channels of Excitable Membranes* (2nd ed). Sunderland, Sunderland Associates, Inc, 1992, p 312
23. NEHER E, STEVENS CF: Conductance fluctuations and ionic pores in membranes. *Annu Rev Biophys Bioeng* 6:345–381, 1977
24. SIGWORTH FJ: The variance of sodium current fluctuations at the node of Ranvier. *J Physiol* 307:97–129, 1980
25. HEINEMANN SH, CONTI F: Nonstationary noise analysis and application to patch clamp recordings. *Meth Enzymol* 207:131–148, 1992
26. JACKSON PS, STRANGE K: Single channel properties of a volume-sensitive anion conductance: Current activation occurs by abrupt switching of closed channels to an open state. *J Gen Physiol* 105:643–660, 1995