COUPLING OF WATER AND ION FLUXES IN A K⁺-SELECTIVE CHANNEL OF SARCOPLASMIC RETICULUM

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ABSTRACT   Streaming potentials arising across a K⁺-selective channel from fragmented sarcoplasmic reticulum were measured by incorporating the channel into planar bilayer membranes and imposing osmotic gradients across the membranes by addition of sorbitol or urea to only one side. Single-channel zero-current potentials were determined, and dilution artifacts were corrected for by addition of valinomycin to the bilayer. The streaming potentials were found to be unusually small, 1.1 mV per osmolal. The potentials were linearly related to the osmotic gradient across the bilayer, and were identical for sorbitol and urea. The results imply that the channel cannot be envisioned as a long tube, like gramicidin, but rather as a short constriction of < 10 Å in length opening out into wider mouths on either side of the membrane.

INTRODUCTION

The study of the function of ionic channels has very naturally been concerned with the rates of ion movement through these membrane-spanning proteins. Far less experimental effort has been expended upon the rates of water movements through channels, and the coupling of ion and water movements. Recently, Rosenberg and Finkelstein (1978) and Levitt et al. (1978) have shown that measurement of water flux and ion-water flux coupling can be related to the known structure of the gramicidin A channel. (Also see Finkelstein and Andersen, 1981.) Specifically, applying the assumption that inside of the narrow gramicidin A channel ions and water are constrained to diffuse in a single file, these authors estimated that 6–9 water molecules are obligatorily coupled to the diffusion of an ion through the channel. Such a picture can be easily accommodated into the known conducting structure of the channel, a β_6-helix, with a hydrophilic pore 3 Å in diameter and ~25 Å in length (Urry, 1971).

Many channels from animal cell membranes display ionic selectivity superior to that of gramicidin, and some of these channels at the same time conduct ions at rates several times higher than maximal rates in gramicidin. For example, the K⁺ channel from mammalian sarcoplasmic reticulum (SR), which has been under detailed study in this laboratory (Miller, 1978; Miller and Rosenberg, 1979; Labarca et al., 1980; Coronado et al., 1980; Coronado and Miller, 1982; Miller, 1982), has a maximum K⁺-conductance more than five times that of gramicidin, although it is also far better able to discriminate among monovalent cations. This fact runs against the intuition suggesting that the higher conductance a channel has, the less ion-selective it ought to be.

One possible escape from the dilemma caused by this counterintuitive result is to suggest that perhaps not all channels are "long tubes" like gramicidin. Instead, more complicated channels may have rather short constrictions at which the rate-limiting steps for ion movement occur; ions may gain access to these constrictions through wider "mouths" facing the aqueous solutions on either side of the membrane. Models such as this have been suggested for the K⁺ channel of squid axon (Armstrong, 1975; Swenson, 1981; French and Shoukimas, 1981), for the acetylcholine-activated channel of muscle end plate (Horn and Stevens, 1980), and, indeed, for the SR K⁺ channel studied here (Coronado and Miller, 1982; Miller, 1982).

In this report, I address the possibility of using an electrokinetic measurement—that of streaming potential—to estimate the length of the ion-selectivity region of the K⁺ channel from SR. Water activity gradients are placed across membranes into which K⁺ channels have been inserted, and the tendency of K⁺ to be dragged through the channel against its own electrochemical gradient is measured. The ion-water coupling is found to be remarkably small, when compared to that determined previously for gramicidin. Based on the present data, I estimate that at most three water molecules move in single file with a K⁺ ion through this channel, and this leads to a picture of the selectivity region of the channel as a short tube of <10 Å in length.

METHODS

The methods for preparing SR vesicles and phospholipids have been described earlier (Miller and Rosenberg, 1979; Labarca et al., 1980), as has the procedure for fusing SR vesicles into planar bilayers (Miller, 1978). For these experiments, bilayers were cast onto a 0.2-mm diameter hole in a 3-ml polystyrene cup seated in a 6-ml dish, using decane

solutions of rabbit muscle phosphatidylethanolamine (30 mM), rabbit muscle phosphatidylcholine (10 mM), and beef brain phosphatidylserine (10 mM). The aqueous phase of the system was K_2SO_4 (125 mM):MOPS (morpholino propane sulfonic acid, 10 mM):EDTA (0.1 mM), adjusted to pH 7.0 with KOH. In all experiments, SR vesicles were added to the "*cis*" chamber; the opposite chamber, called "*trans*," was defined as zero voltage. In all experiments, temperature was controlled to 25 ± 0.5°C.

The experimental procedure for measuring streaming potential through the channel is as follows. A bilayer was formed, and $CaCl_2$ (1 mM) and SR vesicles (1–5 μg/ml) were added to the *cis* chamber, with stirring. Conductance was continuously monitored at 25 mV. Immediately after the first fusion event was observed (as indicated by an abrupt increase in bilayer conductance), 1.2 mM EDTA was added to stop further fusion. The stirrer was turned off, and after ~1 min single-channel current fluctuations were recorded at voltages in the range −10 to +10 mV, to generate a control current-voltage (*I-V*) curve. Then, the *cis* chamber was extensively perfused with 125 mM K_2SO_4 buffer, also containing a suitable concentration (0.5–2 M) of a nonelectrolyte, such as urea or sorbitol. Introduction of this new solution had the dual purpose of removing excess SR vesicles and at the same time establishing a water activity gradient across the membrane. Again, single channel fluctuations were observed between −10 and +10 mV to generate the single-channel *I-V* curve in the presence of water flow towards the *cis* side of the membrane. After these records had been collected, 10 nM valinomycin was added to the system, with 10 s of vigorous stirring, so that the membrane conductance increased at least 1,000-fold. After the stirrer was stopped, the zero-current voltage was seen to increase by 1–2 mV over ~1 min. This increase is due to the re-establishment of the unstirred layers (Rosenberg and Finkelstein, 1978). In each experiment it was ensured that the zero-current voltage had stabilized to within 0.1 mV before it was recorded. In several experiments, it was checked that similar values of streaming potential were observed when a constant rate of stirring was maintained throughout the entire experiment (although with stirring the precision of the measurement was reduced because of noise on the channel fluctuations).

This procedure was used for several reasons. First, we want to know the streaming potential arising only from K^+-water coupling through the channel itself, uncomplicated by the "leak" conductances also present in the system. Thus, it is necessary to determine the zero-current potential of only the single-channel *I-V* curve. Second, we need to realize that artifactual shifts in zero-current potential can arise from addition of nonelectrolyte to the *cis* solution. These shifts can come about by either of two mechanisms: (*a*) a reduction of K^+ activity coefficient in the *cis* chamber, and (*b*) a local dilution of *cis* K^+ and concentration of *trans* K^+ near the membrane surface, caused by the flow of water through the bulk lipid bilayer. Both of these effects would tend to give a positive zero-current potential to the single-channel *I-V* curve. The measurement of the valinomycin-mediated zero-current potential corrects for both of these effects, as discussed by Rosenberg and Finkelstein (1978). The streaming potential of the channel itself, ψ_o, is given by the difference between the zero-current potential of the channel, V_c, and that in the presence of valinomycin, V_v:

$$\psi_o = V_c - V_v. \tag{1}$$

The corrections for dilution artifacts by the calculations of Levitt et al. (1978) were not used, because they are theory dependent, whereas the valinomycin correction depends only on the assumption (which Levitt's analysis does not accept) that the K^+-valinomycin complex does not couple to water movement. For further discussion of this point, see Rosenberg and Finkelstein (1978).

RESULTS

The single-channel currents to be described are rather small (<1 pA) because the experiments were carried out at low voltages, and so it is useful to demonstrate that these
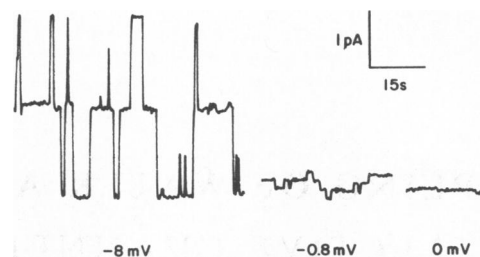

FIGURE 1 K^+ channel fluctuations at low voltage. Fusion of a single SR vesicle with a bilayer was brought about as described in Methods, and single-channel recordings were taken at several voltages, indicated on the figure.

currents can be measured reliably. Fig. 1 shows traces of single-channel recordings under fully symmetrical conditions. It can be seen that even at ≪1 pA, the channel sizes remain well-defined and easily measurable, as has been demonstrated earlier at higher voltages (Miller, 1978; Labarca et al., 1980; Coronado et al., 1980). Perfusion with solution containing high concentrations of nonelectrolyte, when done carefully, does not introduce any additional noise into these low-voltage records. It is necessary to measure these small currents accurately to be able to determine the streaming potential to within 0.2–0.3 mV, and this can be readily accomplished in this system.

A streaming potential determination made in 1.9 mol/kg urea is shown in Fig. 2. Here we see the I-V curve for the control channels, before application of a water activity gradient. This curve is clearly shifted to positive potentials by decreasing the activity of water in the *cis* chamber by adding urea. The arrow in the figure marks the zero-current potential after addition of valinomycin. This valinomycin potential is positive, as expected for the artifactual effects mentioned above. The single-channel reversal potential, however, is substantially more positive than this, and the difference between the two is the channel streaming potential, ~2.3 mV in this membrane.
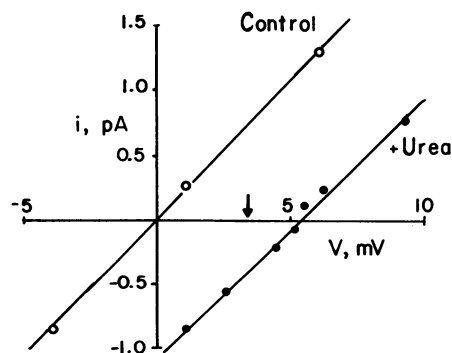

FIGURE 2   Streaming potential in 1.9 mol/kg urea. Determination of single-channel *I-V* curves was done as described in Methods. Open points, control *I-V* curve under symmetrical conditions. Closed points, *I-V* curve after perfusion with solution containing 1.9 mol/kg urea. The arrow marks the zero-current potential after addition of valinomycin. For this membrane, the streaming potential was 2.3 ± 0.2 mV (range of uncertainty on measurement).
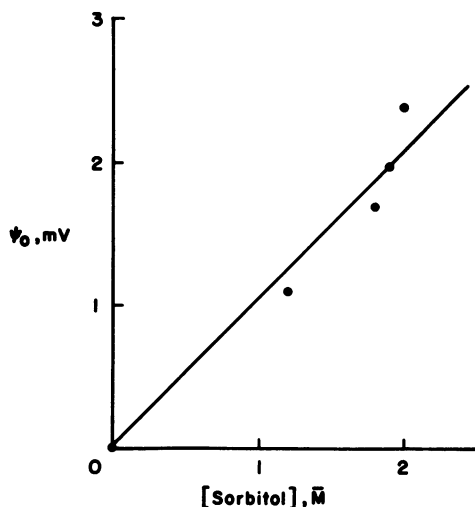
FIGURE 3 Dependence of streaming potential on osmolality. Streaming potentials were determined as in Fig. 2, using sorbitol to induce the osmotic gradient, added to the *cis* solution to the molality indicated on the abscissa. Straight line is drawn by eye with a slope of 1.1 mV/mol · kg⁻¹; here $\tilde{M}$ represents moles per kilogram water.

The streaming potential depends linearly upon the molality of added nonelectrolyte, as shown in Fig. 3. From compiled data using sorbitol, a slope of 1.1 mV/mol · kg⁻¹ is found. This value is not significantly different from the slope found when using urea to establish the osmotic gradient.

DISCUSSION

The single experimental result of this report is that the SR K⁺ channel displays ion-water flux coupling, as measured by a streaming potential of 1.1 mV per molal osmotic gradient across the membrane. There is no unequivocal way to interpret this result quantitatively. We can, however, state that this amount of flux coupling is remarkably small when compared to those of gramicidin A, which gives streaming potentials of ~3–6 mV/mol · kg⁻¹ (Rosenberg and Finkelstein, 1978; Levitt et al., 1978), or amphotericin B, which gives ~3 mV/mol · kg⁻¹ (Coronado and Alvarez, unpublished). It is notable that potentials induced by a large molecule, sorbitol, are not significantly different from those induced by urea, a molecule that might be small enough to permeate the channel. The equal effectiveness of these two nonelectrolytes argues that the urea permeability through the channel is much smaller than that of water (Rosenberg and Finkelstein, 1978).

One possible interpretation of the streaming potential of the SR K⁺ channel can be made in terms of single-file diffusion. If a constriction in the channel exists such that water and K⁺ ion are unable to pass one another, then a particularly simple situation arises that may be treated by equilibrium thermodynamics. Rosenberg and Finkelstein (1978) showed that in such a single-filing situation, the streaming potential, V_f, may be related simply to the

molality difference across the membrane, $\Delta\tilde{M}$, and the number of water molecules constrained to move in single file with a conducting ion, N:

$$V_f = (NRT/F)\,(\Delta\tilde{M}/\tilde{M}_w),\qquad(2)$$

where $\tilde{M}_w$ is the molality of pure water, 55.6 mol/kg. This equation allows an immediate calculation of N, the number of water molecules per ion in the single-filing region of the channel. For the data presented here, we calculate that there are 2.3 ± 0.2 water molecules per K⁺; because this channel has previously been shown to be able to accommodate at most a single K⁺ ion at a time (Coronado et al., 1980), we can, on the basis of this single-filing model, assert that 2–3 water molecules and a K⁺ ion are constrained to diffuse in single file in some region of the channel.

Unfortunately, this simple analysis is equivocal. In the case of gramicidin it is known, to begin with, that the channel's dimensions are such that single-filing must take place along most of the length of the pore (Finkelstein and Andersen, 1981). With the SR channel, we have no such structural information, and there might be other models of ion-water flux coupling that could give the same value of streaming potential. For instance, it might be the case that the channel is a wide, cation-selective pore, and that water flowing through the channel can frictionally drag K⁺ along with it. In such a case, no a priori calculation of the streaming potential can be made, as we do not know values for the several irreversible thermodynamic parameters that apply (Levitt et al., 1978; Katchalsky and Curran, 1967).

The wide-pore picture above, however, does not apply to the SR K⁺ channel, at least not along its entire length. The channel is ideally selective for monovalent cations, and shows substantial selectivity among alkali metal cations; K⁺, for example, is >50 times more conductive than is Cs⁺, although the two ions differ by only 0.2 Å in unhydrated diameter (Coronado et al., 1980). Furthermore, the size of the narrowest part of this channel has been determined to be ~4 × 5 Å (McKinley and Meissner, 1978; Coronado and Miller, 1982). Therefore, we can say that there is at least one place within this channel at which single-file diffusion must occur. In other words, we can picture the channel as containing, in general, a single-filing region in series with a region at which water and K⁺ ions can pass one another.

To address this more complicated two-region picture of the channel, we need only consult the general irreversible thermodynamic treatment of streaming potential of Levitt et al. (1978). As it applies to the case here (that of a channel ideally selective to monovalent cations), the streaming potential, ψ_o, is given by

$$\psi_o = \frac{N'RT}{F}\,(\Delta\tilde{M}/\tilde{M}_w),\qquad(3)$$

where

$$N' = \left(\frac{J_w}{J_+}\right)_{\Delta\mu_w - 0.} \qquad (4)$$

Here, all parameters are as in Eq. 2, except that N' is the electro-osmotic coupling ratio, i.e., the ratio of the flux of water, J_w, to the flux of cations, J_+, when current is passed through the channel with no water activity difference across the membrane. For the data here, $N' = 2.3 \pm 0.2$.

The parameter N' cannot by itself provide any structural information about the channel. But in terms of the two-region model of the channel, we can say that N' must be an upper limit on N, the number of water molecules in the single-filing region. Thus, we conclude that the single-filing constriction of the SR K^+ channel can accommodate at most three water molecules and a K^+ ion. Consequently, this constriction cannot be much longer than 10 Å.

Thus, the picture of the channel emerges not as a long tube, like gramicidin, but rather as a short constriction opening out into wider mouths on one or both ends. This picture is appealing for several reasons. First, it would help explain why the channel can conduct ions at higher rates than gramicidin while maintaining a higher ionic selectivity; the access radius, or convergence permeability, of the SR channel would be much larger than that of gramicidin (Läuger, 1973; Andersen and Procopio, 1980). Second, this view would permit the entire business of ionic selectivity to be placed in a rather small region in space, an "active site," of the channel. Finally, this estimate is consistent with an independent estimate (Miller, 1982) of the length of the channel's constriction, 10–12 Å, made on the basis of the voltage dependence of bis-quaternary ammonium blockers.

REFERENCES

Andersen, O. S., and J. Procopio. 1980. Ion movement through gramicidin A channels. On the importance of the aqueous diffusion resistance and ion-water interactions. *Acta Physiol. Scand. Suppl.* 481:27–42.

Armstrong, C. M. 1975. Potassium pores of nerve and muscle membranes. *In* Membranes—A Series of Advances. G. Eisenman, editor. Marcel Dekker, Inc., New York. 3:325–348.

Coronado, R., R. L. Rosenberg, and C. Miller. 1980. Ionic selectivity, saturation, and block in a K^+-selective channel from sarcoplasmic reticulum. *J. Gen. Physiol.* 76:425–446.

Coronado, R., and C. Miller. 1980. Decamethonium and hexamethonium block sarcoplasmic reticulum K channels. *Nature (Lond.).* 288:495–497.

Coronado, R., and C. Miller. 1982. Conduction and block by organic cations in a K^+-selective channel from sarcoplasmic reticulum incorporated into planar phospholipid bilayers. *J. Gen. Physiol.* 79:529–548.

Finkelstein, A., and O. S. Andersen. 1981. The gramicidin A channel: a review of its permeability characteristics with special reference to the single-file aspect of transport. *J. Membr. Biol.* 59:155–172.

French, R. J., and J. J. Shoukimas. 1981. Blockage of squid axon potassium conductance by internal tetra-n-alkylammonium ions of various sizes. *Biophys. J.* 34:271–291.

Horn, R., and C. F. Stevens. 1980. Relation between structure and function of ion channels. *Comm. Mol. Cell. Biophys.* 1:57–68.

Katchalsky, A., and P. F. Curran. 1967. Nonequilibrium Thermodynamics in Biophysics. Harvard University Press, Cambridge, Mass.

Labarca, P., and C. Miller. 1981. A K^+-selective, three-state channel from fragmented sarcoplasmic reticulum of frog leg muscle. *J. Membr. Biol.* 61:31–38.

Labarca, P., R. Coronado, and C. Miller. 1980. Thermodynamic and kinetic studies of the gating behavior of a K^+-selective channel from the sarcoplasmic reticulum membrane. *J. Gen. Physiol.* 76:397–424.

Läuger, P. 1973. Ion transport through pores: a rate theory analysis. *Biochim. Biophys. Acta.* 311:423–441.

Levitt, D. G., S. R. Elias, and J. M. Hautman. 1978. Number of water molecules coupled to the transport of sodium, potassium, and hydrogen ions via gramicidin, nonactin, or valinomycin. *Biochim. Biophys. Acta.* 512:436–451.

McKinley, D., and G. Meissner. 1978. Evidence for a K, Na permeable channel in sarcoplasmic reticulum. *J. Membr. Biol.* 44:159–186.

Miller, C. 1978. Voltage-gated cation conductance channel from fragmented sarcoplasmic reticulum: steady-state electrical properties. *J. Membr. Biol.* 40:1–23.

Miller, C. 1982. Bis-quaternary ammonium blockers as structural probes of the sarcoplasmic reticulum K^+ channel. *J. Gen. Physiol.* In press.

Miller, C., and R. L. Rosenberg. 1979. A voltage-gated cation conductance channel from fragmented sarcoplasmic reticulum. Effects of transition metal ions. *Biochemistry.* 18:1138–1145.

Rosenberg, P. A., and A. Finkelstein. 1978. Interaction of ions and water in gramicidin A channels. Streaming potentials across lipid bilayer membranes. *J. Gen. Physiol.* 72:327–340.

Swenson, R. P. 1981. Inactivation of potassium current in squid axon by a variety of quaternary ammonium ions. *J. Gen. Physiol.* 77:255–271.

Urry, D. W. 1971. The gramicidin A transmembrane channel: a proposed $\pi_{L,D}$ helix. *Proc. Natl. Acad. Sci. U. S. A.* 68:672–676.