brief communication

Streaming potential measurements in Ca^{2+}-activated K^+ channels from skeletal and smooth muscle

Coupling of ion and water fluxes

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ABSTRACT Streaming potentials arising across large-conductance Ca^{2+}-activated K^+ channels incorporated into planar lipid bilayers were measured. Ca^{2+}-activated channels obtained either from skeletal muscle or from smooth muscle membranes were used. Streaming potentials were extracted from the current–voltage relationship for the open channel obtained in the presence of an osmotic gradient. The osmotic gradient was established by adding glucose to one side of the membrane. At 300 mM KCl, the average streaming potential was 0.72 mV/osmol per kg for t-tubule channels and 0.83 mV/osmol per kg for smooth muscle channels. Streaming potential values depend on KCl concentration, they decrease as KCl concentration increases, and the value obtained by extrapolation to zero KCl concentration is 0.85 mV/osmol per kg. Assuming that water and ions cannot pass each other, at least in a region of the channel, the streaming potential values obtained indicate that this region contains a minimum of two and a maximum of four water molecules. It is concluded that the channel has a narrow region with a length of 0.6–1.2 nm.

INTRODUCTION

In narrow ion channels, ions and water cannot pass each other, so the flow of these two species is coupled. If a membrane containing channels of this type separates two solutions of different osmolalities, a streaming potential is generated. The sign and magnitude of this potential depends on the ion selectivity of the channel, the number of ions, and the number of water molecules contained in the channel (Levitt, 1984; Finkelstein, 1987). Gramicidin A forms cationic channels that, at low salt concentration, are occupied by at most one ion, and water and ions move by a single-file mechanism. Thus, ion and water cannot pass each other (no-pass condition). In this channel the streaming potential has been related to the number of water molecules contained in the channel (Rosenberg and Finkelstein, 1978; Levitt et al., 1978). Streaming potential measurements indicate that the gramicidin A channel can contain up to seven water molecules in the no-pass region (Rosenberg and Finkelstein, 1978). On the other hand, structural studies indicate that gramicidin A pore is a 0.4-nm diam cylinder with a length of 2.5 nm (Urry et al., 1975). The length of a row of seven water molecules is 2.2 nm, a number in good agreement with pore length obtained from the Urry et al. (1975) model. Therefore, streaming potential measurements can be used to estimate the length of the no-pass region of channels for which the detailed molecular structure is unknown (Miller, 1982; Cecchi et al., 1982).

The Ca^{2+}-activated K^+ channel from skeletal muscle is unique because it has a large conductance and high cation selectivity. To reconcile these two apparently contradictory properties of this channel, Latorre and Miller (1983) proposed a structure consisting of two large vestibules connected by a short and narrow tunnel at which the rate-limiting steps for ion conduction occur. More recently, Jordan (1987) has proposed that to attain such a large conductance, the Ca^{2+}-activated K^+ channel has a fixed negative charge near the constriction entrance. Such a model may be a common motif for most ion channels in biological membranes and has been previously proposed for the squid axon potassium channel (Armstrong, 1975; French and Shoukimas, 1981; Swenson, 1981), for the acetylcholine receptor channel (Horn and Stevens, 1980; Dani and Eisenman, 1987), and for the sarcoplasmic reticulum K^+ channel (Miller, 1982).

The existence of the large vestibules in the Ca^{2+}-activated K^+ channel is suggested by the work of Vergara et al. (1984) who found two different tetraethylammonium (TEA) receptors located in the external and the cytoplasmic side of the channel. Therefore, the channel mouths are wide enough to accommodate TEA. In the present work we measured streaming potentials arising across the Ca^{2+}-activated K^+ channel to estimate the length of the short and narrow region of its ion conduction system. We found that this region contains two to four water molecules, which implies a length of 0.6–1.1 nm.

METHODS

Channel incorporation and bilayer formation

Single-channel currents were recorded by incorporating purified rat skeletal muscle or rabbit intestinal muscle membrane vesicles into

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planar bilayers made from a solution of brain phosphatidylcholine (Avanti Polar Lipids, Inc., Birmingham, AL) in decane. The phospholipid was dissolved in decane to a final concentration of 15 mg/ml. The preparations of skeletal muscle or smooth muscle membranes have been described in detail previously (Moczydlowski and Latorre, 1983; Cecchi et al., 1986). Membrane vesicles were added to one side of the bilayer only, and the voltage of this compartment was controlled externally. The opposite compartment is defined as zero voltage. In general, it is more difficult to incorporate smooth muscle than skeletal muscle channels. In the former case, to favor channel incorporation, the KCl concentration of the side to which the vesicles were added was made fivefold more concentrated than the opposite side. Aqueous solutions were composed of KCl (with or without the non-electrolyte) at the desired concentrations plus 5 mM 2-(*N*-morpholino)propane sulfonic acid, pH 7. Calcium concentration was adjusted to have the channels open near zero voltage, and it never was higher than 100 μM. Osmolality was measured with a water vapor pressure osmometer (model 5100B; Wescor Inc., Logan, UT).

Lipid bilayers were formed in a small hole (≈300-μm diam) in a Teflon partition (25-μm thick) that separates the two chamber compartments. Membrane capacitance varied from 250 to 400 pF. The current passing through the membrane was measured with a two electrode voltage clamp. Connections were made through silver/silver chloride 1 M KCl electrodes with 1 M KCl agar bridges. One compartment was connected to a waveform generator and the other to a current-to-voltage converter (model OPA 101 operational amplifier; Burr-Brown Corp., Tucson, AZ) with a feedback resistor of 1 G Ω.

Streaming potential measurements

Current–voltage relationship for the open channel was determined in the voltage range of ±12 mV in 2-mV steps. The current amplitude at each voltage was determined from single-channel current records (see Fig. 1). Once the channel was incorporated current fluctuations were recorded in order to generate the control single-channel *I-V* relationship. An osmotic gradient was then established by perfusing the internal compartment (total volumen 3 ml) with 30 ml of a solution containing KCl (at the same concentration as in the control) and glucose (E. Merck, Darmstadt, FRG). Under these conditions a new *I-V* curve is taken. The reversal potential was determined by linear regression of the *I-V* curve. Valinomycin to a final concentration of 10 nM was added to both compartments. The reversal potential for the valinomycin-induced current (V_v) was determined by measuring the potential at which this current is zero. The valinomycin-induced currents were several thousand-fold larger than the current passing through a single Ca^{2+}-activated channel. The streaming potential arising only from K^+–water coupling (V_s) is the difference between the reversal potential for the channel obtained in the presence of glucose (V_c) and the reversal potential obtained with valinomycin (V_v). The measurement of the reversal potential in the presence of valinomycin corrects for differences of electric potential generated by reductions in the activity coefficient due to the presence of the nonelectrolyte, and the differences in local salt concentration in unstirred layers, caused by water flow through the lipid bilayer (Rosenberg and Finkelstein, 1978). Therefore

$$V_s = V_c - V_v. \quad (1)$$

It is assumed here that the K^+ flux induced by valinomycin is not coupled to water transport. For a different point of view see Levitt et al., 1978.

RESULTS

Fig. 1 shows channel current records obtained in the presence of an internal solution containing 2 osmol/kg glucose and 100 mM KCl and an external solution containing 100 mM KCl. Due to the large channel conductance the channel current is clearly resolvable, even at very low voltages, and single channel currents can be measured in a reliable manner. This is very important since channel reversal potentials under these conditions are quite small. The current is zero at +4 mV, meaning that the water flow, resulting from the glucose osmotic gradient, drives a K^+ current.

Current–voltage relationships in the absence and in the presence of glucose in the internal compartment are shown in Fig. 2. When an osmotic gradient across a lipid bilayer containing one Ca^{2+}-activated channel is established, the reversal potential obtained, V_c, is the sum of the dilution potential plus the streaming potential. Following Rosenberg and Finkelstein (1978), we obtained the dilution potential by determining the reversal poten-

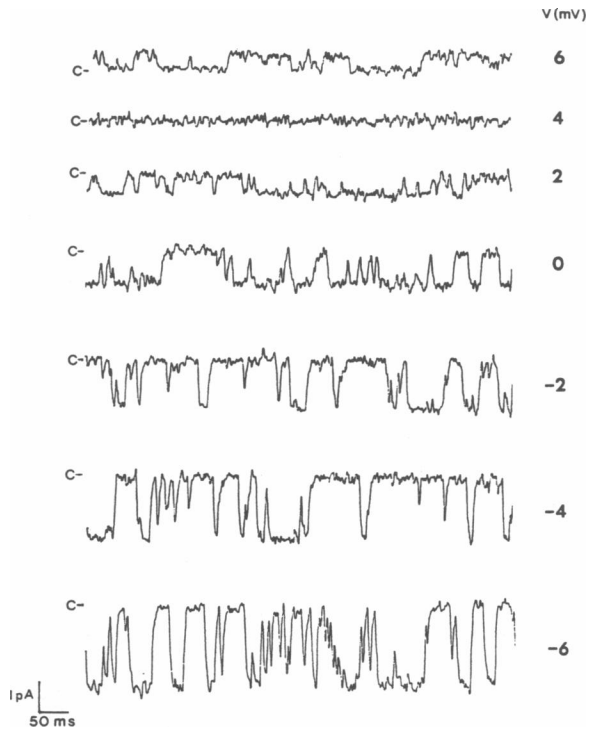

FIGURE 1 Single-channel record of the Ca^{2+}-activated K^+ channel from T-tubule membranes, incorporated to a bilayer separating a 100 KCl solution and a 100 mM KCl plus 2 osmol/kg glucose solution. The applied potential is shown on the right side of each record, and the closed state is indicated on the left side. A measurable current is recorded at 0 mV, which is driven by the osmotic water flow.

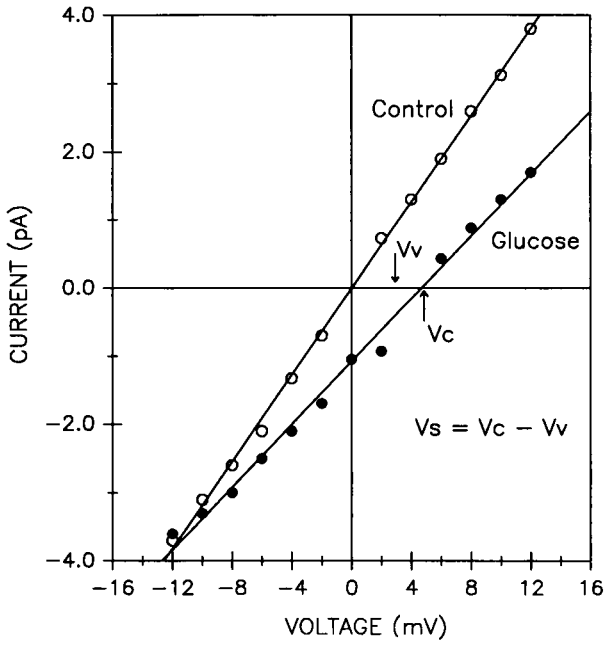

FIGURE 2 Current–voltage relationships in 100 mM KCl, in the absence (*open circles*) and presence of 2 osmol/kg glucose (*solid circles*) on one side of the membrane. According to the linear regression analysis, the reversal potential for the glucose *I-V* curve (V_c) is 4.66 ± 0.23 mV (standard error). The reversal potential for valinomycin (V_v) is 2.8 ± 0.2 mV, and the streaming potential (V_s) is 1.86 ± 0.30 mV.

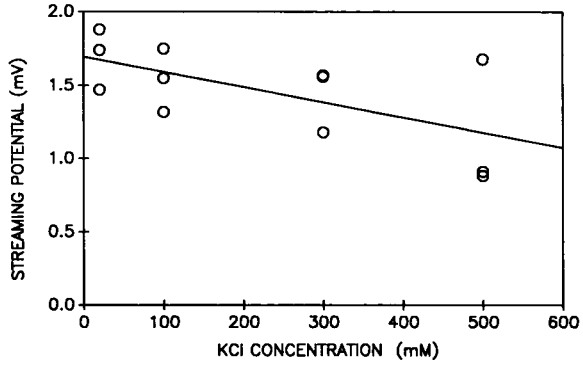

FIGURE 3 Streaming potentials measured at different KCl concentrations. Each point is the result of a different membrane, and all measurements are in 2 osmol/kg glucose. The regression line is V_c = (1.7 ± 0.1) − (0.0010 ± 0.0004) [KCl]. Dispersion values are the standard error of the parameters. The slope is statistically different from zero according to the single-tailed Student's *t* statistics at a level of confidence of 99% (t = 2.5, 10 degrees of freedom).

tial in the presence of valinomycin. This corresponds to V_v in Fig. 2. The values determined for V_c and V_v from the straight line obtained by least square fitting of the experimental points are V_c = 4.66 mV and V_v = 2.8 mV, therefore the streaming potential V_s is 1.86 mV. In the presence of glucose the channel conductance is smaller (230 pS) than in the control situation (318 pS). For all the KCl concentrations tested the decrease in conductance amounts to a 27%. An increase in the convergence resistance (Andersen and Procopio, 1980), a decrease in solution conductivity in the vestibules, and a glucose blockade can, in principle, produce such a decrease. The current–voltage relationships were also determined at different KCl concentrations and keeping the glucose concentration at 2 osmol/kg (Fig. 3). Linear regression analysis demonstrates that the streaming potential decreases as KCl concentration increases. The slope of the straight line is −1.0 mV/mM, and the standard error of the slope is 0.4 mV/mM. The slope is different from zero, according to the single-tailed Student's *t* statistics, at the 99% confidence level. The limiting value of the streaming potential is 1.7 ± 0.1 mV, for an osmotic pressure difference of 2 osmol/kg.

Smooth muscle membranes contain a Ca^{2+}-activated K^+ channel with characteristics similar to the one found in skeletal muscle membranes (Cecchi et al., 1986). We measured the streaming potentials for these channels. At 300 mM KCl, and 2 osmol/kg, the average streaming potential is 1.66 ± 0.10 mV (n = 3). For T-tubule channels this average is 1.44 ± 0.18 mV (n = 3), and is not significantly different from the value found for smooth muscle channels. Streaming potential values not significantly different from those obtained using glucose were found using sorbitol to establish the osmotic gradient.

DISCUSSION

In the results we showed that, in the limit of low KCl concentration, the streaming potential in lipid bilayers containing skeletal muscle Ca^{2+}-activated channels is 0.85 mV/osmol per kg. This streaming potential is smaller than the one obtained for the gramicidin A channel (≈3 mV/osmol per kg; Rosenberg and Finkelstein, 1978). Small streaming potential have been found for the sarcoplasmic reticulum K^+ channel (1.1 mV/osmol per kg; Miller, 1982), for the hemocyanin channel (1.2 mV/osmol per kg; Cecchi et al., 1982), and for the acetylcholine receptor channel (Dani, 1987).

Rosenberg and Finkelstein (1978) and Levitt et al. (1978) have shown that for a univalent cation-permselective channel that never contains more than one ion, and in which the single-filling situation applies the streaming potential, V_s, is given by the relationship

$$V_s = (NRT/F)\,\Gamma\,(n_s/n_w), \qquad (2)$$

where N is the number of water molecules that are coupled to each ion transported across the narrow region of the channel when there is no osmotic pressure difference, Γ is the molal osmotic coefficient for the nonelectrolyte, n_s and n_w are the number of moles of nonelectrolyte and water, respectively, at the side where the nonelectrolyte is added, and R, T, and F have their usual meanings. At 20°C and with a 1 osM solution, Eq. 2 reduces to $V_s = N \times 0.46$. Using the value for the streaming potential obtained for low KCl concentration, $N \approx 2$ for the Ca^{2+}-activated K^+ channel and means that 2 water molecules are transported per potassium ion. As pointed out by Levitt (1984) this is only valid if one considers that the ion enters the channel by pushing the end water molecule ahead of it. However, if the ion enters and leaves the channel by exchanging with a water molecule, the actual number of water molecules in the narrow region of the channel can be increased to 4.

For multi-ion channels N represents the average number of water molecules between ions (Rosenberg and Finkelstein, 1978; Levitt et al., 1978; for reviews see Finkelstein, 1987; Levitt, 1984). Therefore, it is important to determine the ion occupancy state of a given channel in order to interpret N, inasmuch as the number of water molecules per ion decreases as ion occupancy of the channel increases. Actually, Levitt (1984) showed that in potassium solutions the number of water molecules coupled to the K^+ transport through the gramicidin A channel decreases from 7 at low K^+ activity to 5 at high K^+ activity. There is strong evidence that the Ca^{2+}-activated K^+ channel from muscle membranes behaves as a multi-ion channel (Cecchi et al., 1986; Eisenman et al., 1986; Latorre, 1986). We found a significant decrease in the streaming potentials when KCl concentration was varied in the range betwene 20 and 500 mM. This result suggests that the channel occupancy by K^+ ions increases in this concentration range. Inasmuch as it is unlikely that the channel contains more than one ion at 20 mM, we suggest that the number of water molecules we have determined corresponds to the channel occupied by only one ion.

In the lack of structural information about the architecture of the Ca^{2+}-activated K^+ channel we are assuming here that this contains a single-filling region connecting the two vestibules. We think this assumption is reasonable since the channel is ideally selective for monovalent cations and shows exquisite selectivity towards K^+ ($P_{Na}/P_K < 0.01$; Blatz and Magleby, 1984; Naranjo, D., D. Wolff, and R. Latorre, unpublished results). Furthermore, ions like Cs^+, hydroxylammonium, and methylammonium are not measurably permeant both from conductance and bi-ionic potential measurements (Cecchi et al., 1987; Villarroel, A., and R. Latorre, unpublished results). Hille (1975) has suggested that a pore with these ion-selective characteristic should have at the selectivity filter a diameter of ~0.3 nm. Even if the pore is twice this diameter the no-pass condition would be still met. We conclude, therefore, that Ca^{2+}-activated K^+ channels contain in their conduction system a region in which ions and water are forced to move in single file. Taking a lower limit of 2 and an upper limit of 4 for the number of water molecules contained in the single-filling region this constriction cannot be much shorter than 0.6 nm and cannot be much longer than 1.1 nm. The combination of large conductance and high selectivity is consistent with a structure of two wide mouths connected with a short narrow region.

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