Voltage and Temperature Dependence of Single K^+ Channels Isolated from Canine Cardiac Sarcoplasmic Reticulum

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ABSTRACT The temperature and voltage dependence of gating and conductance of sarcoplasmic reticulum K^+ channels (S-R K^+) isolated from adult canine hearts were studied using the reconstituted bilayer technique. Fusion of vesicles from this preparation frequently resulted in the incorporation of a single channel. Only bilayers into which a single S-R K^+ channel had fused were studied. The three conductance states of the channel, fully open (O2), substate conductance (O1), and closed (C) were studied as a function of voltage (−50 to +50 mV) and temperature (16 to 37°C). Permeation through the O1 state showed the same temperature dependence as the O2 state corresponding to an enthalpy of permeation of 4.1–4.2 kcal/mol, which is similar to that for K^+ diffusion through water. As expected, increased temperature increased the frequency of gating transitions and shortened the average dwell time spent in any conductance state. Over the range of 25 to 37°C, the average dwell time spent in the O1, O2, and C states decreased by 44 ± 11, 36 ± 13, and 78 ± 7% (n = 3 to 4 channels), respectively. The ratio of probabilities between the various conductance states was not strongly temperature sensitive. Analysis of the voltage dependence of this channel was carried out at 37°C and revealed that the dwell times of the O1 and O2 states were voltage insensitive and the probability ratio (P_{O2}:P_{O1}) was approximately 7 and was voltage insensitive. Nonlinear least-squares analysis of dwell times revealed that the closed state was biexponential and was thus composed of a fast (C_f) and a slow (C_s) component. τ_{C_f} was voltage insensitive with an average value of 5.9 ms, whereas τ_{C_s} was approximately two orders of magnitude slower and was voltage dependent. The voltage dependence of C_s was described by τ_{C_s} (ms) = exp(−0.025·(V_m (mV) − 250)).

INTRODUCTION

The cardiac sarcoplasmic reticulum K^+ (S-R K^+) channel and the closely related skeletal muscle S-R K^+ channel are interesting models for the analysis of ion channel conduction and gating (1–9) because gating is weakly voltage dependent (4,6) and a prominent substate is routinely observed (4,10–12). In addition, it has been proposed on the basis of pharmacological interventions (13) that the S-R K^+ channel mediates a shunt conductance across the SR membrane, compensating for the charge movement that accompanies Ca^{2+} efflux through ryanodine-sensitive Ca^{2+} channels during excitation-contraction coupling (14, 15). Therefore, further study of S-R K^+ channel gating and permeation characteristics may enhance our understanding of excitation-contraction coupling in the heart.

Early work on the S-R K^+ channel isolated from rabbit skeletal muscle demonstrated selectivity for potassium ions and an enthalpy of permeation to K^+ of 4.6 kcal/mol (1). Gating was described by a two-state (open and closed) model (6). Later studies in which only a single channel had fused with the bilayer revealed that the S-R K^+ channel of frog skeletal muscle also possessed a prominent subconductance state (11). However, the infrequent occurrence of the subconductance state precluded detailed analysis of the voltage and temperature sensitivity of its gating and permeation properties.

Subsequent work revealed that S-R K^+ channels isolated from both the canine myocardium and skeletal muscle exhibited a similar conductance activity relationship for potassium, selectivity sequence for monovalent cations, subconductance level, and voltage dependence of P_{open}:P_{closed} at 22°C (1–3, 16). However, important qualitative differences exist between the S-R K^+ channels isolated from skeletal muscle and myocardium (3, 16). For example, permeation of K^+ through skeletal S-R K^+ channels is well described by a single ion occupancy model (1), whereas permeation through the canine myocardial S-R K^+ channel is a multi-ion process (3). The gating process of canine cardiac S-R K^+ channels also differs from that observed in rabbit skeletal muscle preparations. For example, the myocardial channel shows a more frequently observed substate (4). These differences in biophysical characteristics imply that the canine cardiac S-R K^+ channel expresses more internal ionic binding sites than and a conformational stability different from that of the skeletal muscle channel. This study describes several findings concerning the temperature and voltage sensitivity of the cardiac S-R K^+ channel and discusses how they differed from or were similar to previous findings for skeletal muscle S-R K^+ channels.

Preliminary accounts of some portions of this study have appeared in abstract form (17–19).

MATERIALS AND METHODS

Vesicle preparation

Vesicle isolation procedures were modified from those used by Jones (20) as described by Hill et al. (3) and are briefly summarized here. Left ventricular tissue was isolated, and the endocardium and epicardium were dis-

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sected away. The tissue was minced, and the cells were disrupted physically. Intracellular proteins were removed via a series of extractions in saline solutions. Remaining cellular membranes were vesiculated and separated by differential density centrifugation.

Reconstitution and electrical measurements

Bilayer construction, channel incorporation, and electrical recording techniques have all been reported previously (3, 4). Briefly, decane solubilized 1:1 phosphatidylethanolamine:phosphatidylserine lipids were "painted" over a small hole in a lucite chamber forming a bilayer of approximately 200 pF. Channels were incorporated by stirring in the presence of Ca^{2+} and an osmotic gradient across the bilayer. Upon channel incorporation, Ca^{2+} was removed by the addition of EGTA, the osmotic gradient was abolished, and stirring was discontinued. Electrical measurements were obtained from a custom-made head stage (3) and recorded on a video cassette recorder–pulse code modulator system.

Temperature control was achieved by use of a copper-jacketed circulating water bath. The bilayer chamber temperature was monitored continuously during the experiment. During most of the experimental measurements the circulating water was turned off to minimize vibrational noise. The water bath had sufficient thermal mass to maintain a relatively constant temperature (within 2°C of desired) for several minutes. Longer periods of elevated temperature were achieved with an infrared spotlight, which directed radiant heat onto the system and was controlled manually to balance passive heat loss.

Analysis techniques

Data analysis was performed using a data acquisition and control program written by H. Affolter, described elsewhere (3). Briefly, data were filtered at 100 Hz using an eight-pole Bessel filter and digitized at a rate of 500 samples/s using an analog-to-digital converter (Keithley, Series 500) interfaced to an IBM AT computer. Only bilayers in which a single channel had fused were analyzed. The entire digitized record was visualized prior to analysis. Current amplitude histograms were generated to estimate subconductance, fully open and closed state probabilities P_{O1}, P_{O2}, and P_C, respectively.

Event analysis was accomplished by defining discriminators of transitions midway between the means of O1 and O2, and O1 and C. An event was described as O2 if it had four or more consecutive points above the O1, O2 discriminator, as O1 if four or more points fell between the O1, O2 discriminator and the O1,C discriminator, and as C when four points fell below the O1,C discriminator. Dwell time histograms were generated with exponentially increasing variable bin widths and histogram height normalized by bin width (21). Bin widths were also constrained to be integer multiples of sampling rate in order to minimize curve fitting errors at small bin widths (22). The minimum bin width was the sample rate (2 ms). Events of shorter duration than the four sample point (8 ms) criteria were recorded but not included in the analysis. Customized programs were used to analyze the data for mean dwell time, generate histograms of dwell times, and perform nonlinear least-squares fit of one and two exponentials to dwell time histograms.

The valence, z, and the chemical potential $\Delta G_{0\ mV}$ of equilibrium gating reaction at zero mV was estimated by fitting a Boltzmann distribution to data from one channel. The resulting estimates of z from all channels were averaged and reported as mean ± SEM. Because $\Delta G_{0\ mV}$ might come from a bimodal distribution, values were estimated similarly, but averages are from groups as noted in the text.

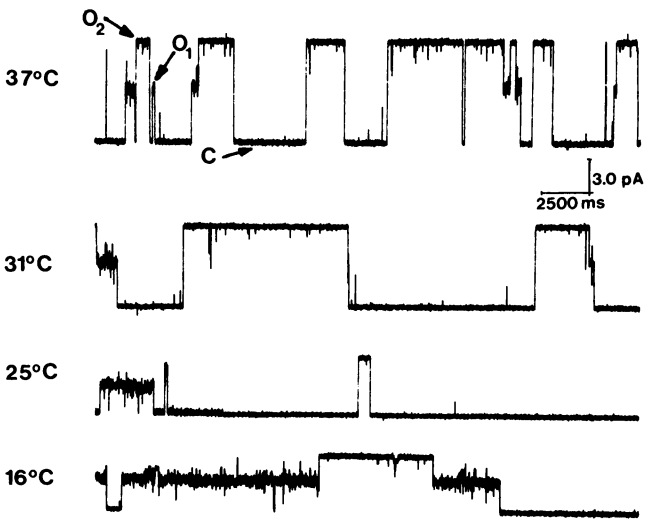

FIGURE 1 Current traces from a reconstituted S-R K^+ channel displaying the O2, O1, and closed conductance states at four different temperatures. Symmetrical K^+ acetate (500 mM), −30 mV.

Experimental solutions

Bilayer experiments were performed with symmetrical 500 mM potassium acetate solutions with a 10 mM histidine buffer adjusted to pH 7.1.

RESULTS

Fig. 1 illustrates the characteristic gating behavior of the S-R K^+ channel at different temperatures with clear transitions between a closed state (C) and two distinct levels of open current (O1 and O2). Compared with the S-R K^+ channel of rabbit skeletal muscle, the cardiac channel displays a more frequently observed substate, which made it more amenable to detailed analysis. Each conductance state (C, O1, and O2) can undergo a direct transition randomly to the other two, as has been reported for S-R K^+ channels from other species and tissues (2, 4, 11, 12).

Temperature dependence of conductance

Typically the permeation process for ionic diffusion through the open channel pore is relatively temperature insensitive, with a Q_{10} in the range of 1.4 (23). The mechanism underlying the lower conductance of the O1 state is not known. One hypothesis is that the O1 conductance resulted from the filtering of rapid transitions between a short-lived fully open state with a conductance equal to the O2 state and a short-lived closed state. If this was the case, the observed conductance level was an average of the equilibria between these states due to the finite bandwidth of the measuring apparatus (for a review see Ref. 24). Fig. 1 also shows that the O1 substate was observed at a variety of temperatures (16 to 37°C). Cooling to 16°C, or even to 10°C (data not shown), did not slow the noisy flickering of the O1 conductance state sufficiently to resolve the discrete transitions that are putatively responsible for the substantial "noise" of the substate conductance. Over the range of voltages studied (−40 to +50 mV) the conductance relationship of both the O1 and O2 states remained linear (Fig. 2, *A* and *B*). In addition, the ratio of the O1 to O2 conductance remained constant at approximately 60% (Table 1), indicating an enthalpy of permeation for both states of approximately 4.2 kcal/mol (Fig. 2*C*), which is similar to the enthalpy of diffusion of K^+ ions in water (25).

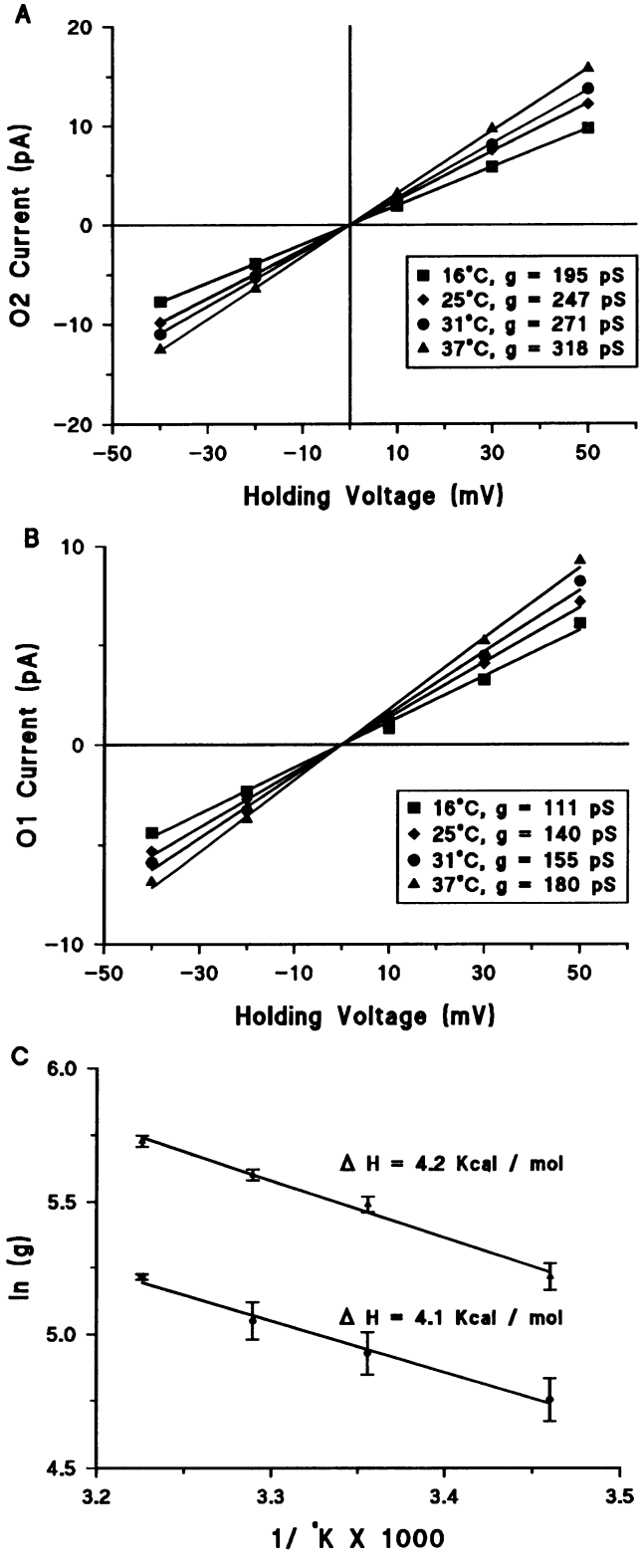

FIGURE 2 Temperature dependence of open channel conductance through (*A*) the O2 state and (*B*) the O1 state from an individual experiment. Note that the voltage dependence remained linear throughout the range of potentials employed. (*C*) The temperature dependence of the O2 (*top trace*) and O1 (*bottom trace*) displayed as an Arrhenius plot. The ratio of conductances remained constant and gave a calculated enthalpy for conduction of 4.2 kcal/mol for O2 and 4.1 kcal/mol for O1 (data plotted ± SEM, n = 4 to 6).

TABLE 1 Temperature dependence of conductance

State	Temperature (°K)			
	289	298	304	310
O1 (pS)	116 ± 11	138 ± 13	156 ± 12	184 ± 3
O2 (pS)	184 ± 10	242 ± 8	271 ± 6	307 ± 8
O1/O2 (%)	63 (n = 4)	57 (n = 6)	58 (n = 6)	60 (n = 5)

O2, open state; O1, substate. Data are expressed as mean ± SE. All experiments were performed with symmetric 500 nM potassium acetate.

Temperature dependence of gating

In contrast to permeation, gating is often a strongly temperature-dependent phenomenon (23). The dwell times for all states (C, O1, and O2) for the cardiac S-R K^+ channel become shorter as temperature increases. The temperature dependence of dwell times was analyzed in four bilayers containing single S-R K^+ channels and for which the temperature was varied. Because subsequent analysis revealed that the closed state dwell time of the channel was voltage sensitive while the O1 and O2 states were voltage insensitive, the data for the closed-state dwell time were analyzed at a single potential (+20 mV), and the data for the temperature dependence of O1 and O2 were averaged over all potentials. The dwell times of the C, O1, and O2 states decreased by 78 ± 7, 44 ± 11, and 36 ± 13%, respectively, as the temperature increased from 25°C to 37°C. Increasing temperature can also affect the equilibria between states in channels. However, Fig. 3 shows no convincing evidence for a shift in the probability of opening over the range of temperatures studied (25, 31, 37°C) for our bilayer configuration.

Voltage dependence of gating at 37°C

Because channel gating transition rates were more rapid at 37°C than at 25°C, and channel probability distributions

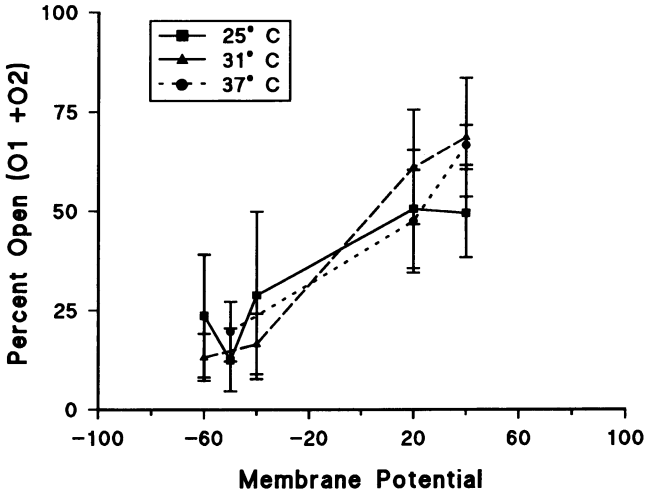

FIGURE 3 Temperature dependence of P_{open} versus voltage for 37°C (●), 31°C (▲), and 25°C (■). P_{open} was calculated as the sum of $P_{O1} + P_{O2}$; each point is the average of from 3 to 5 bilayers, with error bars representing the SE. No significant changes with temperature were observed.

were more evenly distributed between O1, O2, and C at 500 mM than for 100 mM K^+ concentration (4,26) we examined the voltage dependence of gating more closely at 37°C and 500 mM K^+. What follows is an analysis of four different bilayers into which a single S-R K^+ channel was incorporated. Fig. 4*A* demonstrates that depolarization favors steady-state open probability ($P_{O1} + P_{O2}$) as described previously (4). While these channels consistently exhibited relatively weak voltage sensitivity for all bilayers studied, three of the four channels in Fig. 4*A* showed a fairly high probability for the combined open state, and one channel showed a much lower probability of channel opening. The source of this variability is unknown, but similar variability has been observed in situ for the S-R K^+ channel of lobster muscle (12).

Depolarization increased the percentage of time that the channel spent in the open states relative to the closed state, but the ratio of $P_{O2}:P_{O1}$ was independent of voltage with an average value of approximately 7:1. From this ratio, we calculated a value of 1.2 kcal/mol for ΔG between O1 and O2. The voltage insensitivity suggests that there was very little transmembrane charge movement associated with transitions between the O1 and O2 states and that the mechanism underlying the voltage dependence of the equilibrium between open and closed states did not affect the equilibrium between O1 and O2.

While the voltage dependence of $P_{open}:P_C$ was qualitatively consistent with that observed in rabbit skeletal muscle channels, how the voltage dependence is distributed between the various states in cardiac S-R K^+ channels remains unaddressed. The average dwell times of the O2, O1, and C states are summarized in Fig. 4 (*B–D*). The dotted lines are the individual regressions of the logarithmically linearized data from a single experiment, while the solid line is the regression line obtained by averaging the parameters from the individual experiments. O2 and O1 dwell times were voltage insensitive. Surprisingly, all of the voltage dependence of channel gating was attributable to changes in closed-state dwell time (Fig. 4*D*). The voltage dependence of the closed state was described by

$$\tau_C = \exp\{-0.025[V_m\,(\text{mV}) - 247]\} \qquad (1)$$

This preponderance of voltage-dependent behavior due to the closed state was distinctly different from the findings in S-R K^+ channels from rabbit skeletal muscle, where both open- and closed-state dwell times were found to be voltage dependent (11).

Previous studies on the S-R K^+ channel from canine cardiac cells (4) reported a mean closed-state dwell time that was markedly different from the mean value as calculated from exponential fits to the data histograms, suggesting that the dwell times of the closed conductance state were not single-exponentially distributed. Examination of the closed-state dwell time histograms obtained at 37°C revealed a probability distribution that was consistently biexponential. An example is shown in Fig. 5*C*. Indeed, examination of the O2 and O1 states revealed that they were also consistently biexponential in nature (Fig. 5, *A* and *B*). This finding was also different from the results reported in rabbit skeletal muscle, in which open and closed dwell times were found to be single-exponentially distributed.

We examined the least-squares estimated time constants from a biexponential fit to the data as a function of potential. Fig. 4*E* summarizes the data and shows that the slow component of dwell time distribution decreased as a function of membrane potential, whereas the rapid component showed no voltage dependence. The voltage dependence of C_s could be described by

$$\tau_{C_s}\,(\text{ms}) = \exp\{-0.025 * [V_m\,(\text{mV}) - 250]\} \qquad (2)$$

This voltage dependence of a single component of the closed-state dwell time histogram was in contrast to the behavior of the biexponential distributions for the O1 and O2 dwell times from the same experiments, which showed no voltage dependence.

The relative frequency of each component of closed-state dwell time can be analyzed by examining the ratio of areas as determined by the biexponential fit. The results of this process are shown in Fig. 4*F*. The area comprising each component should correspond to the number of slow and fast events. The voltage insensitivity of this ratio indicates that there was no voltage dependence favoring either slow or fast events. This allowed us to conclude that voltage dependence of gating of the cardiac S-R K^+ channel was not mediated by changes in the relative ratio of fast to slow events but by changes in the dwell time of long-lived closed events.

DISCUSSION

The S-R K^+ channel has been postulated to play a role in the sequence of events surrounding calcium release from the SR during excitation contraction coupling (13) by facilitating K^+ current to neutralize the influence of Ca^{2+} flow through the ryanodine-sensitive channel (14). This hypothesis was based largely on the use of pharmacological agents that block the S-R K^+ channel (13). Because the action and affinity of such compounds are frequently sensitive to channel opening, it is important to understand the gating behavior of this channel at physiological temperatures.

The temperature dependence of K^+ permeation through the fully open (O2) state of the cardiac S-R K^+ channel was consistent with that observed for the fully open state of the skeletal muscle channel (1). The enthalpy of permeation through the open channel as determined by the temperature dependence of conductance was very similar to that for the free diffusion of K^+ in aqueous solutions (25). The measured enthalpy of permeation was qualitatively in agreement with the maximum free-energy change of permeation proposed for this channel by Hill et al. (3) in their two-binding-site, three-energy-barrier model for the ionic permeation of K^+. By comparing the calculated free energy of activation incorporated in the model (6.58 kcal/mol) to the experimentally determined activation enthalpy (4.2 kcal/mol), we speculate that the entropic contribution to permeation was at most

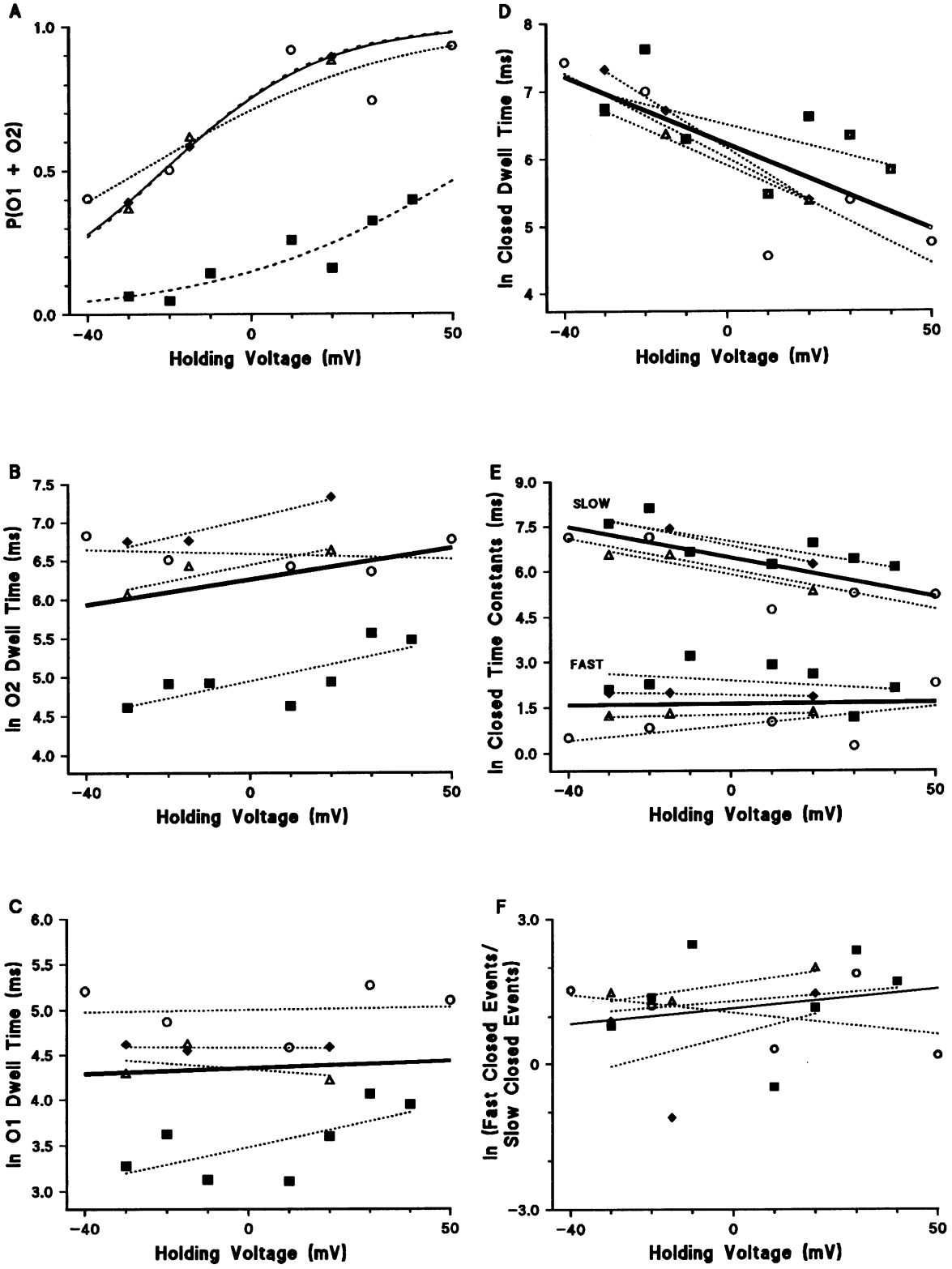

FIGURE 4 (*A*) Voltage dependence of $P_{O2} + P_{O1}$ as a function of potential at 37°C for four separate channels. The open probability for the channel displayed the characteristic weak voltage dependence of gating associated with the S-R K^+ channel. The lines (*dotted, dashed,* and *solid*) represent fits of the Boltzmann equation to individual data sets to obtain estimates of $\Delta G_{0\,mV}$ and z. All four data sets showed a voltage dependence that indicated a consistent z for all channels. The observed $\Delta G_{0\,mV}$ was consistent between 3 of the data sets (○, △, ◆) but was very different for one channel (■). This type of variability has also been reported in situ for the sarcoplasmic reticulum K^+ channel of split lobster muscle fibers (12). Voltage dependence of ln(dwell times) for the O2 state (*B*), the O1 state (*C*), and the closed state (*D*). (*E*) Extracted fast and slow closed state time constants as a function of membrane potential. (*F*) Ratio of areas under the slow versus fast components of closed state dwell times as a function of potential. Each symbol type represents data from an individual bilayer. ·····, regression of points from one individual bilayer; ——, average of individual regression curves.

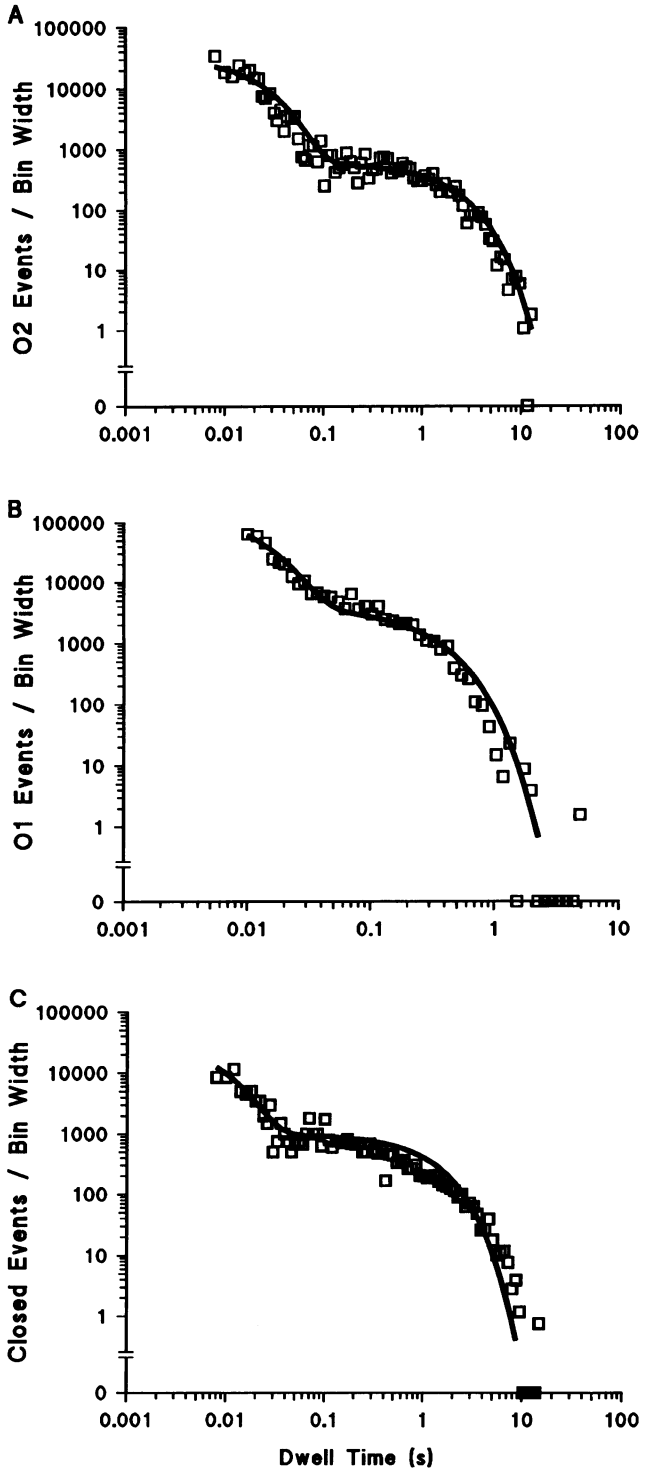

FIGURE 5 Demonstration of biexponential nature of channel states as measured at 37°C and +15 mV. (*A*) O2; (*B*) O1; (*C*) closed. Note that all states were clearly composed of more than one exponential component.

8 cal/mol °K, which is consistent with the estimates obtained for the skeletal muscle S-R K^+ channel (1).

In spite of the fact that the O1 channel substate conductance was approximately 60% of the O2 state, the same enthalpy (4.1 versus 4.2 kcal/mol) of permeation was observed. Since O1 has been hypothesized to arise from rapid unresolved gating transitions, and components of gating transitions are often highly temperature sensitive, it seemed almost peculiar that the O1 conductance did not display a temperature sensitivity different from that of O2. However, if the free energy difference, $\Delta G_{0\ mV}$, between a putative short-lived open state with a conductance equivalent to the O2 state and a short-lived closed state remained constant as a function of temperature then Boltzmann equilibrium theory predicts that a change in O1/O2 of only ~1% would occur over the range of temperatures studied. Furthermore, cooling did not slow down the hypothesized substate kinetic rates sufficiently to allow the resolution of discrete transitions. Indeed, we were unable to discern any systematic changes in the noise of the O1 state as a function of temperature. Thus, whether the subconductance represents a "flickering" state or a pathway of lower ionic mobility for K^+ remains unresolved.

The dwell time of the channel conductance states showed a mild temperature dependence, becoming faster with increasing temperature. The temperature dependence of gating was not as pronounced as is typically observed for ionic channels (23). The reason for this lesser temperature sensitivity was unclear. One possible explanation may lie in the biexponential nature of the dwell-time probability distribution of canine cardiac S-R K^+ channels—an increase in temperature might decrease the mean dwell-time of both the rapid and slow components. However, the rapid component of the dwell time histogram was fairly close to the filter cut-off frequency. Consequently, at higher temperatures, fewer of the rapid events may have been included, proportionately, in the calculation of mean dwell time. Specifically, a higher temperature may have resulted in more of the rapid events being discarded as noise, thus increasing the weighting given to the slow component in the calculation of mean dwell time.

Measurement of changes in the equilibrium between P_{O1}, P_{O2}, and P_C also failed to demonstrate a strong temperature dependence for the cardiac S-R K^+ channel. Because of the variability associated with sampling from biexponential distributions and the limited amount of time for which the reconstituted S-R K^+ channel is stable, equilibrium may still have some temperature dependence, which was beyond the resolution of our measurements for a single channel. In contrast, the change in the internal free energy of opening for the skeletal muscle S-R K^+ channel converts to a ~20-mV shift in the equilibrium relation (6) for the temperature changes employed in this study. However, the different temperature sensitivities may have been due to differences in the composition of the lipid environment. Lipid composition can influence the magnitude and even the direction of the temperature sensitivity of gating equilibrium (6).

Combining the O2 state with the O1 state allowed us to calculate an approximate value of z for the channel opening for the purposes of comparison with the findings of Hill et al. (4) and Labarca et al. (6). $\Delta G_{0\ mV}$ was also estimated, but because of the apparent bimodal nature of P_C the values are reported three ways: (*a*) The average value for $\Delta G_{0\ mV}$ for

all channels was 0.2 ± 0.4 kcal/mol; (*b*) the average value for the three closely grouped channels was 0.64 ± 0.04 kcal/mol; and (*c*) the $\Delta G_{0\ mV}$ for the one outlier was −1.1 kcal/mol. The S-R K^+ channel from rabbit skeletal muscle was reported to have a temperature-sensitive $\Delta G_{0\ mV}$ of 1.5 kcal/mol and a temperature-insensitive z of −1.1 (6). At 22°C in symmetric 100 mM potassium acetate the gating of the S-R K^+ channel from dog ventricle has been reported to be described by a $\Delta G_{0\ mV}$ of 1.9 kcal/mol and a z of −1.2. Examination of the potential dependence of gating at 37°C in symmetric 500 mM potassium acetate reveals a z of −1.1 ± 0.15 taken as the average of four independent observations. Thus, the valence of gating charge movement remained constant when compared with results obtained previously at 22°C and 100 mM potassium acetate (4).

Although a paired experiment was not attempted in this study, the probability of the channel being open in 500 mM potassium acetate was higher at all temperatures than that observed under 100 mM potassium acetate conditions (4). This was qualitatively consistent with the concentration dependence of gating of the S-R K^+ channel as reported by Campos-de-Carvalho (26). We were unable to find any evidence of coupling between gating transition sequences and an electrochemical driving force of permeant ions of the sort described for *Torpedo* electroplax Cl^- channels (27). As mentioned earlier, the valence of the voltage dependence of gating was unaffected by ionic concentration. This suggests that the S-R K^+ channel may be well suited as a model system for studying the relationship between molecular structure and gating, since the valence of gating transitions seems to be a distinct property of the channel.

Quantitative analysis of channel gating showed that the O1, O2, and closed states were all multiexponential, in direct contrast to the findings of Labarca et al. (6), who found that skeletal muscle S-R K^+ channel O2 and closed states were well described as single exponential processes. A further difference observed between the skeletal muscle and cardiac S-R K^+ channels was that the voltage dependence of gating of the cardiac S-R K^+ channel was due entirely to changes in closed-state dwell time; voltage dependence was found in both the open- and closed-state dwell times for the skeletal muscle channel. Furthermore, this voltage dependence was localized to the slow component of the closed-state dwell time. Thus, the valence of gating calculated from the probability curves was probably not a strictly correct interpretation of the gating reaction. The actual valence of the slow closed-state gating reaction was probably slightly greater than calculated due to the voltage insensitivity of the fast component. However, the similarity between the parameters in Eqs. 1 and 2 indicates that this systematic error may be small relative to the error inherent in making the least-squares estimate.

A more precise interpretation of the biexponential nature of the two putative closed states depends upon a determination of how each of the closed states communicates with the O1 and O2 states. Evaluation of the communication between states requires experiments using very large numbers of transitions and a long and sophisticated analysis (for a review see Ref. 21). Preliminary results from one particularly long-lived experiment have been published in abstract form (19). Conditional probability analysis of this channel indicated that a short-lived closed state communicated preferentially with the O1 state, whereas a long-lived closed state communicated with both O1 and O2. While this preliminary result was encouraging, large numbers of transitions are required to confirm the reproducibility of this finding and to allocate the voltage dependence of the various transitions in a Markov model of channel behavior.

As the primary, secondary, and tertiary structure of potassium channels becomes progressively better understood (for reviews see Refs. 28 and 29), detailed biophysical analysis will become essential in elucidating the relationship between structure and function. In a sense, examining the gating properties of similar channels as expressed in different species and organs provides a natural form of mutagenesis experiment. Some of the biophysical differences found between the canine cardiac S-R K^+ channel and the rabbit skeletal muscle channel represent very important physical differences. These physical differences will correspond to variations in the amino acid sequence for the channel. Thus, as the protein constituting this channel is purified (30,31) and ultimately sequenced in various tissues, studies such as this will form a starting point in providing clues for site-directed mutagenesis experiments aimed at resolving structure-function relationships.

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REFERENCES

1. 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.
2. Cukierman, S., G. Yellen, and C. Miller. 1985. The K^+ channel of sarcoplasmic reticulum. *Biophys. J.* 48:477–484.
3. Hill, J. A., Jr., R. Coronado, and H. C. Strauss. 1989. Potassium channel of cardiac sarcoplasmic reticulum is a multi-ion channel. *Biophys. J.* 55:35–46.
4. Hill, J. A., R. Coronado, and H. C. Strauss. 1990. Open-channel subconductance state of K^+ sarcoplasmic reticulum. *Am. J. Physiol.* 258: H159–H164.
5. Hirachima, N., H. Ishibashi, and Y. Kirino. 1991. Comparative electrophysiological study of reconstituted giant vesicle preparations of the rabbit skeletal muscle sarcoplasmic reticulum K^+ channel. *Biochim. Biophys. Acta.* 1067:235–240.
6. 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.
7. Liu, Q-Y., and H. C. Strauss. 1991. Blockade of cardiac sarcoplasmic reticulum K^+ channel by Ca^{++}: two-binding site model. *Biophys. J.* 60:198–203.
8. Tomlins, B., and A. J. Williams. 1986. Solubilization and reconstitution of the rabbit skeletal muscle sarcoplasmic reticulum K^+ channel into liposomes suitable for patch clamp studies. *Pfluegers Arch. Eur. J. Physiol.* 407:341–347.
9. Uehara, A., M. Yasukohchi, S. Ogata, and I. Imanaga. 1991. Activation by intracellular calcium of a potassium channel in cardiac sarcoplasmic reticulum. *Pfluegers Arch. Eur. J. Physiol.* 417:651–653.

10. Bezprozvanny, I. B., D. S. Benevolensky, and A. P. Naumov. 1991. Potassium channels in aortic microsomes: conductance, selectivity, barium-induced blockage and subconductance states. *Biochim. Biophys. Acta.* 1064:75–80.
11. 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.
12. Tang, J. M., J. Wang, and R. S. Eisenberg. 1989. K^+-selective channel from sarcoplasmic reticulum of split lobster muscle fibers. *J. Gen. Physiol.* 94:261–278.
13. Abramacheck, C. W., and P. M. Best. 1989. Physiological role and selectivity of the in situ potassium channel of the sarcoplasmic reticulum in skinned bullfrog skeletal muscle fibers. *J. Gen. Physiol.* 76: 425–446.
14. Caillé, J., M. Ildefonse, and O. Rougier. 1985. Excitation-contraction coupling in skeletal muscle. *Prog. Biophys. Mol. Biol.* 46:185–239.
15. Oetliker, H. 1982. An appraisal of the evidence for a sarcoplasmic reticulum membrane potential and its relation to calcium release in skeletal muscle. *J. Muscle Res. Cell Motil.* 3:247–272.
16. Shen, W. K., J. A. Hill, Jr., R. L. Rasmusson, and H. C. Strauss. 1990. Reconstitution of cardiac S-R K^+ channel. *In* Potassium Channels: Basic Function and Therapeutic Aspects. Vol. 334. T. Colatsky, editor. Alan R. Liss Publishers, New York. 205–230.
17. Shen, W. K., J. A. Hill, Jr., R. L. Rasmusson, and H. C. Strauss. 1988. Temperature-dependent conductance of a canine cardiac sarcoplasmic reticulum K^+ channel (Abstract). *Biophys. J.* 53:147a.
18. Shen, W. K., R. L. Rasmusson, and H. C. Strauss. 1988. Gating kinetics of a canine cardiac sarcoplasmic reticulum potassium (SR-K) channel (Abstract). *Biophys. J.* 53:147a.
19. Shen, W. K., R. L. Rasmusson, D. N. Jensen, and H. C. Strauss. 1988. Closed state gating kinetics of a canine cardiac sarcoplasmic reticulum potassium (SR-K) channel. *Circulation.* 78:II-410. (Abstr.)
20. Jones, L. R. 1988. Rapid preparation of canine cardiac sarcolemmal vesicles by sucrose floatation. *Methods Enzymol.* 157:85–91.
21. Colquhoun, D., and F. J. Sigworth. 1983. Fitting and statistical analysis of single-channel records. *In* Single Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 191–263.
22. Starmer, C. F., M. A. Dietz, and A. O. Grant. 1986. Signal discretization: a source of error in histograms of ion channel events. *IEEE Trans. Biomed. Eng.* 33:70–73.
23. Hille, B. 1991. Ionic Channels of Excitable Membranes. Sinauer Associates, Sunderland, MA.
24. Fox, J. A. 1987. Ionic channel subconductance states. *J. Membr. Biol.* 97:1–8.
25. Robinson, R. A., and R. H. Stokes. 1955. Electrolyte Solutions. Academic Press, New York.
26. Campos-de-Carvalho, A. C. 1989. Potassium concentration influences the gating of K^+ channels from sarcoplasmic reticulum. *Braz. J. Med. Biol. Res.* 22:107–109.
27. Richard, E. A., and C. Miller. 1991. Steady-state coupling of ion-channel conformations to a transmembrane ion gradient. *Science (Washington DC).* 247:1208–1210.
28. Jan, L. Y., and Y. N. Jan. 1992. Structural elements involved in specific K^+ channel functions. *Annu. Rev. Physiol.* 54:537–555.
29. Miller, C. 1991. Annus mirabilis of potassium channels. *Science (Washington DC).* 252:1092–1096.
30. Ide, T., T. Morita, T. Kawasaki, T. Taguchi, and M. Kasai. 1991. Purification of a K^+ channel protein of the sarcoplasmic reticulum by assaying the channel activity in the planar bilayer system. *Biochim. Biophys. Acta.* 1067:213–220.
31. Liu, Q-Y., A. F. Lai, W. K. Shen, G. Meissner, and H. C. Strauss. 1991. Reconstitution of the solubilized cardiac sarcoplasmic reticulum potassium channel: identification of a putative M_r ~80 kDa polypeptide constituent. *FEBS Lett.* 291:13–16.