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K-Current Fluctuations in Inward-Rectifying Channels of Frog Skeletal Muscle

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Summary. K currents and K-current fluctuations were recorded in inwardly rectifying K channels of frog skeletal muscle under voltage-clamp conditions. External application of 0.2 to 10 mM Cs reduces the inward mean K current but produces a distinct increase of the spectral density of K-current fluctuations. The additional fluctuations arise from the random blocking by Cs ions. From the variance of current fluctuations, the steady-state current and the probability of the open unblocked channel an effective single-channel conductance γ^* was calculated. γ^* strongly depends on the external Cs concentration (7.8 pS at 0.2 mM Cs, 2.1 pS at 10 mM Cs). This dependence is interpreted in terms of a two-step blocking process: (1) a fast exchange of Cs ions between the external solution and a first binding site inside the channel which leads to the Cs-modulated effective single-channel conductance, and (2) a slow Cs binding to a second site deeper in the channel which produces the observed current fluctuations. With this hypothesis we obtained a real single-channel conductance of $\gamma \approx 10$ pS and a real density of $n \approx 4$ inwardly rectifying channels per μm^2 of muscle surface area.

Key words: Skeletal muscle, K channel, inward rectifier, fluctuation analysis, single-channel conductance

Several biological membranes contain K channels that conduct better in the inward direction than outward. These channels are called inward, inward-going or anomalous rectifiers, and were first described in K-depolarized frog skeletal muscle (Katz, 1949). Subsequently, such K channels were also found in heart muscle (Noble & Tsien, 1968), in electric organ (Nakamura, Nakajima & Grundfest, 1965), and in egg cells of starfish (Hagiwara & Takahashi, 1974; Miyazaki, Ohmori & Sasaki, 1975) and tunicates (Takahashi, Miyazaki & Kidakoro, 1971).

Like other K channels in excitable membranes the inward rectifier can be blocked by Cs ions (Hille, 1975; Hagiwara, Miyazaki & Rosenthal, 1976; Gay & Stanfield, 1977; Adelman & French, 1978; Coronado & Miller, 1979; Ohmori, 1980). This block may be explained by a multi-ion single-file pore where Cs ions can bind to a receptor inside the channel but are not able to penetrate the pore (Hille & Schwarz, 1978; Ciani, Krasne & Hagiwara, 1980; Ohmori, 1980). If K and Cs ions cannot pass each other as they move through the pore, the binding of Cs prevents K-ion permeation and thus causes a steep voltage-dependent block.

As an alternative to such a pore model carrier mechanisms (Horowicz, Gage & Eisenberg, 1968; Adrian, 1969) have been discussed to explain inward rectification. The two groups of models may be distinguished by measurements of the single-channel conductance since values of several pS are typical for pores but are orders of magnitude larger than those expected for single-carrier molecules. By this criterion Na and delayed K channels in axon membranes have been identified as pores (Armstrong, 1975). Recently, Ohmori (1978, 1980) estimated a single-channel conductance of about 10 pS for inwardly rectifying K channels in tunicate eggs. Hence, K channels in these cells must also be pores.

This paper describes an estimate of the single-channel conductance for the inward rectifier of frog skeletal muscle by analysis of Cs-induced K-current fluctuations. From our experiments we also derive details about the blockage of the inward rectifier by

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external Cs ions. A short report on part of this work has appeared (Palade, Neumcke & Schwarz, 1980).

Materials and Methods

Fragments of twitch muscle fibers of the frog *Rana esculenta* were prepared and voltage clamped in a manner similar to that described by Hille and Campbell (1976). Currents through the inward rectifier have already been studied using this method (Hestrin, 1980). Fiber dissection was performed in a solution of 80 mM ethylenglycol-*bis*(β-aminoethylether) N,N'-tetraacetic acid (EGTA) neutralized to pH 7.25 with KOH. This solution was used as artificial myoplasm solution in the side pools of the muscle chamber in contact with the cut ends of the fiber. The average dimensions of the fiber preparation were as follows: lengths of the cut ends in the side pools 1 mm, length of the fiber segment in the test pool 80 μm, and fiber diameter 150 μm.

The external test solutions were composed of 90 mM K_2SO_4 and 8 mM $CaSO_4$, or 120 mM KCH_3SO_4 and 1.8 mM $CaCl_2$; in either case the solution also contained 300 nM tetrodotoxin to block Na channels and 5 mM morpholino-propane-sulfonic acid (MOPS) buffer adjusted to pH 7.2. SO_4^{2-} or $CH_3SO_4^-$ were used as anions to minimize contributions of chloride currents, and high K concentrations were applied to avoid depletion or accumulation effects during K-current flow (Almers, 1972*a*, *b*). As blocking ions Cs was added as the sulfate or chloride salt in concentrations of 0.2–10 mM. The membrane holding potential was set to $V=0$ mV which was close to the K reversal potential. At this depolarized membrane potential the delayed-rectifying K channels (Adrian, Chandler & Hodgkin, 1970*a*, *b*) and Ca channels (Sanchez & Stefani, 1978) in skeletal muscle are inactivated; thus, the inward rectifier could be studied separately.

Muscle fibers were voltage clamped at 12 °C using the method described by Nonner (1969). Membrane currents were directly calculated from voltage drops across an external resistor of 470 kΩ in series with the longitudinal resistance of the myoplasm. The currents were measured between 145 and 460 msec after the onset of the test pulse when the mean current and the variance had come to a steady state (*see* Fig. 1). This long period of time after a change in the clamp potential should be sufficient to neglect potential- and time-dependent changes in membrane capacitance; under these conditions also those inwardly rectifying K channels which are located in the transverse tubular system should be under voltage clamp control. Current fluctuations were recorded through a 1.5 kHz low-pass filter at 0.32 msec intervals, and the procedure of trend compensation described by Conti, Neumcke, Nonner and Stämpfli (1980) was used to avoid fluctuation artifacts from slow variations of the mean current. Mean currents were filtered at 100 Hz and samples were taken every 10 msec. From 60 test pulses applied every 3 sec average values of the mean current (I) and one-sided spectral densities (S) were determined. No more than 6 sets of test pulses were performed on one fiber which took about 30–40 min. Differences of currents and spectral densities obtained in the test solution and in an external solution in which all K was replaced by Cs were assumed to be pure K currents (I_K) and spectral densities of K-current fluctuations (S_K), respectively. In some cases (e.g. Fig. 2, curve *a*) difference spectra S_K obtained from negative test pulses and equal-sized, but positive reference pulses were used and gave the same results. Up to 500 Hz the spectral density of the reference spectrum (Fig. 2, curve *b*) measured during a depolarizing pulse of +60 mV is only slightly larger than the spectral density from an equivalent circuit with the estimated passive impedance properties of the fiber (curve *c*).

From spectral densities S_K in the presence of small amounts of Cs a Lorentzian component was extracted which we attribute

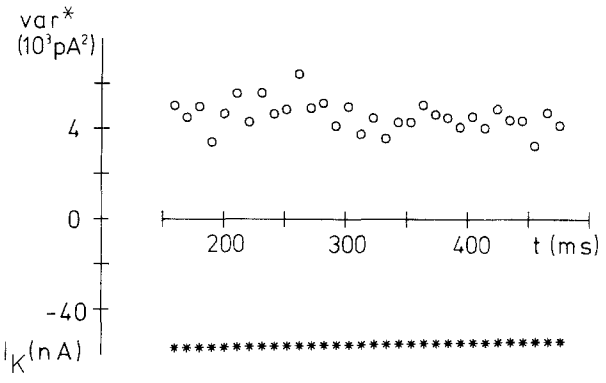

Fig. 1. K current I_K (*) and effective variance of K-current fluctuations *var** (o) between 145 and 460 msec after a hyperpolarizing step to −60 mV; Exp. M44. The external test solution contained 0.4 mM Cs

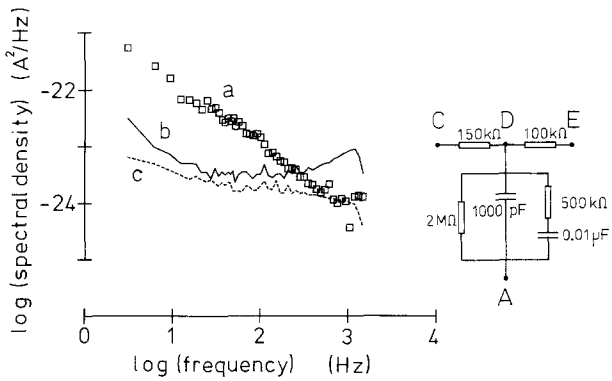

Fig. 2. Spectral densities of current fluctuations at 60 mV; Exp. M11. The fiber was bathed in test solution without Cs. Curve *a* (□): spectral density S_K of K-current fluctuations obtained as difference between densities at −60 and +60 mV. Curve *b* (——): background spectral density at +60 mV. Curve *c* (----): spectral density at +60 mV from a model circuit which was made to imitate the passive impedance properties of fiber M11. The parameters of the model circuit are given in the diagram on the right, and were calculated from standard parameters of Hille and Campbell (1976) and the dimensions of fiber M11. The resistors between *D* and *C*, *D* and *E* are the longitudinal resistances of myoplasm. The tubular membranes are represented by the resistor in series with the capacitor between *D* and *A*, the surface membrane by the two remaining parallel components

to the blocking kinetics of Cs ions (*see* Results and Discussion). By integration of this Lorentz spectrum an effective variance *var** of conductance fluctuations was determined. If all K channels are identical, noninteracting, and have only one conducting state, effective single-channel currents i^* and effective numbers N^* of voltage-clamped channels can be calculated from (Conti, Hille, Neumcke, Nonner & Stämpfli, 1976; Sigworth, 1977):

$$var^* = N^* \cdot i^{*2} \cdot p \cdot (1-p) \quad (1)$$

$$I_K = N^* \cdot i^* \cdot p \quad (2)$$

where p is the probability of the open channel not blocked by Cs ions. In the absence of Cs ions and in the potential range investigated, the probability of an open channel is close to one; thus, p was taken as the ratio of mean currents I_K in test solutions with and without added Cs.

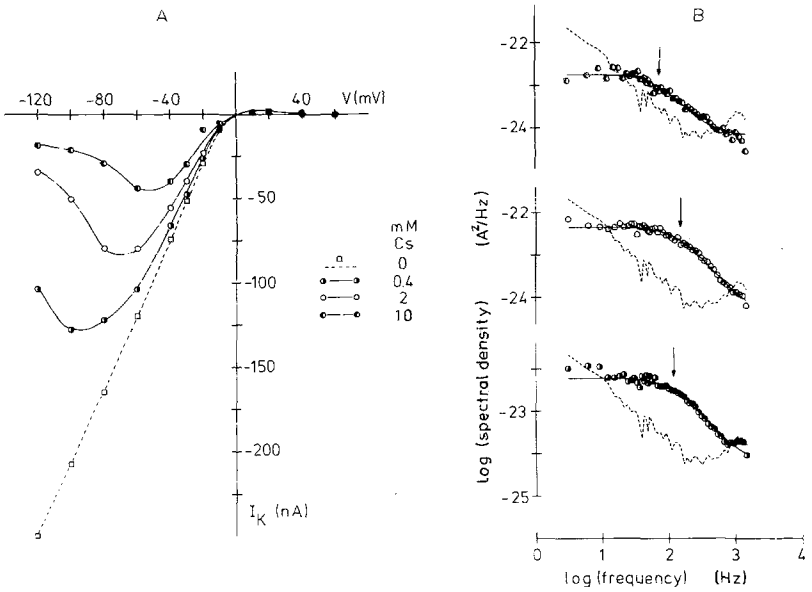

Fig. 3. (*A*) Current-voltage relations of the inward rectifier in Cs-free (squares) test solution and with different concentrations of Cs as blocking ion (circles). Currents were measured 330 msec after the onset of the test pulse; Exp. M 38. (*B*) Spectral densities of K-current fluctuations at a hyperpolarization of −80 mV in Cs-free test solution (- - - -) and in the presence of different concentrations of Cs ions (symbols); Exp. M 38. Spectra are differences of spectral densities in the test solution and in an external solution in which all K was replaced by Cs. The solid lines are fits to the symbols according to Eq. (3); arrows indicate the location of the corner frequencies f_c. The fitted parameters are: for 0.4 mM Cs: $S_1 = 59.8 \times 10^{-24}$ A^2/Hz, $f_c = 753$ Hz, $S_2 = 0.59 \times 10^{-24}$ A^2/Hz; for 2 mM Cs: $S_1 = 45.1 \times 10^{-24}$ A^2/Hz, $f_c = 943$ Hz, $S_2 = 0.42 \times 10^{-24}$ A^2/Hz; for 10 mM Cs: $S_1 = 17.1 \times 10^{-24}$ A^2/Hz, $f_c = 481$ Hz, $S_2 = 0.65 \times 10^{-24}$ A^2/Hz

To convert N^* into the effective number n^* of K channels per surface area we calculated the area of the voltage-clamped section from individual fiber dimensions assuming a cylindrical shape of the muscle fiber.

Results

Fig. 2 shows spectral densities of current fluctuations recorded at a hyperpolarized membrane potential of −60 mV. The difference spectrum (curve *a*) was obtained as the difference between the spectral densities recorded in Cs-free test solution at −60 and +60 mV, and the reference spectral density (curve *b*) was measured at +60 mV. The higher spectral densities below 500 Hz of curve *a* compared with curve *b* indicate that the flow of K ions through the inward rectifiers produces substantial current fluctuations. However, there is no pronounced relaxation component visible in the spectrum of curve *a* that could be correlated to open-close kinetics of the channel. One possible reason might be that at a hyperpolarization of −60 mV most inward rectifiers are in the open state (i.e. $p \approx 1$). Since the variance of current fluctuation is proportional to $p(1-p)$ [*see* Eq. (1)] a large spectral component due to channel gating would then not be expected.

Fig. 3*A* shows the current-voltage relation of the inward rectifier without blocking Cs ions (broken line with square symbols). Indeed, at potentials below −30 mV the curve is already nearly linear, indicating a probability p close to 1. One way to reduce the probability of the open-channel state would be to apply still smaller test potentials. Significantly smaller values of p are only obtained for voltages close to 0 mV or for slightly positive potentials. However, this procedure is not practical, because in this voltage range the steady-state current itself becomes too small for an accurate measurement. Therefore, we used another method to reduce p, namely the introduction of an artificial gating particle in the form of a blocking ion. The solid lines with the circular symbols in Fig. 3*A* show current-voltage relations of inward-rectifying K currents in the presence of 0.4, 2 and 10 mM Cs in the external solution and demonstrate the typical voltage-dependent block by Cs (e.g. Hille, 1975; Gay & Stanfield, 1977). At $V = -80$ mV the p values are now 0.74, 0.49 and 0.18 for 0.4, 2 and 10 mM Cs, respectively. In Fig. 3*B* the symbols represent the corresponding spectral densities which are above 10 Hz obviously larger than the spectral densities without Cs (broken lines). In addition, a marked hump appears in the Cs-induced spectra (indicated by the arrows), and the data can be fitted by the sum of a Lorentz spectrum and a plateau S_2 (solid lines in Fig. 3*B*):

$$S(f) = S_1/(1 + (f/f_c)^2) + S_2. \quad (3)$$

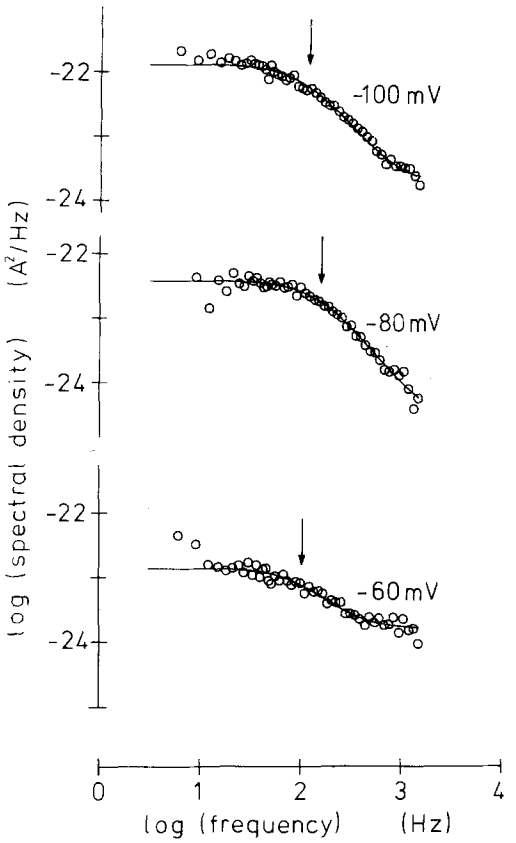

Fig. 4. Spectral densities of K-current fluctuations in the presence of 0.4 mM external Cs at three different hyperpolarizing test potentials (circles); Exp. M 44. Spectra are differences of spectral densities in the test solution and in an external solution in which all K was replaced by Cs. Solid lines are fits to the data according to Eq. (3); arrows indicate the locations of the corner frequencies f_c. The fitted parameters are: for -60 mV: $S_1 = 12.1 \times 10^{-24}$ A²/Hz, $f_c = 640$ Hz, $S_2 = 1.58 \times 10^{-24}$ A²/Hz; for -80 mV: $S_1 = 37.4 \times 10^{-24}$ A²/Hz, $f_c = 945$ Hz, $S_2 = 0.23 \times 10^{-24}$ A²/Hz; for -100 mV: $S_1 = 127 \times 10^{-24}$ A²/Hz, $f_c = 665$ Hz, $S_2 = 1.78 \times 10^{-24}$ A²/Hz

S_1 and S_2 are constant spectral densities and f_c is the corner frequency of the Lorentz spectrum (indicated by the arrows in the Figures). This relaxation spectrum can be attributed to the blocking of K channels by Cs ions (*see* Discussion). The plateau S_2 is mainly due to passive impedances of the preparation that remained uncompensated by the method of difference spectra (Neumcke, Schwarz & Stämpfli, 1980).

We also tested other blocking ions like Ba^{++}, Rb^+ and tetraethylammonium; but with none of them did we obtain reproducible spectra that contained a Lorentz-like spectral component. Usually the spectra showed a $1/f$ component superimposed on a frequency-independent background.

Another set of Cs-induced spectral densities is shown in Fig. 4 for different test-pulse potentials. The fluctuations were recorded in the presence of

Table 1. Cs and voltage dependence of the probability p of the open unblocked channel, the time constant $\tau_c = 1/(2\pi f_c)$, the effective value of the single-channel conductance γ^*, and the surface density n^* of K channels[a]

Cs (mM)	V (mV)		p	τ_c (msec)	γ^* (pS)	n^* (μm^{-2})
0.2	− 80	(1)	0.84	1.0	6.6	2.3
	−120	(1)	0.71	1.4	8.9	1.4
mean ± SEM				1.2 ± 0.2	7.8 ± 1.2	1.9 ± 0.5
0.4	− 60	(4)	0.89 ± 0.02	1.3 ± 0.2	8.2 ± 2.5	4.8 ± 1.4
	− 80	(10)	0.76 ± 0.02	1.2 ± 0.1	6.3 ± 0.4	5.2 ± 0.6
	−100	(7)	0.47 ± 0.02	1.5 ± 0.2	5.9 ± 0.4	6.0 ± 1.0
	−120	(1)	0.23	1.3	3.8	15.3
mean ± SEM		(22)		1.3 ± 0.1	6.3 ± 0.5	5.8 ± 0.7
1	− 60	(2)	0.87 ± 0.04	0.7 ± 0.1	6.3 ± 1.3	4.6 ± 0.6
	−100	(3)	0.58 ± 0.10	1.2 ± 0.2	5.6 ± 1.5	4.3 ± 1.1
mean ± SEM		(5)		1.0 ± 0.2	5.9 ± 1.0	4.4 ± 0.6
2	− 60	(4)	0.68 ± 0.02	1.0 ± 0.2	5.2 ± 0.4	7.6 ± 1.8
	− 80	(6)	0.45 ± 0.03	1.2 ± 0.2	4.0 ± 0.5	8.8 ± 1.7
	−100	(4)	0.13 ± 0.04	2.3 ± 0.4	3.7 ± 0.3	13.4 ± 5.0
mean ± SEM		(14)		1.5 ± 0.2	4.2 ± 0.3	10.0 ± 1.5
5	− 60	(1)	0.46	1.3	3.8	4.1
10	− 80	(3)	0.15 ± 0.02	2.7 ± 0.7	2.1 ± 0.8	22.0 ± 9.0

[a] Number of averaged values are given in parentheses. Data are mean values ± SEM.

0.4 mM Cs at $V = -60$, -80 and -100 mV; the corresponding p values are 0.84, 0.68 and 0.37, respectively. Again, the sum of a Lorentz spectrum and a plateau (solid lines) can be fitted to the data (symbols). At higher hyperpolarizations with smaller p values the Lorentz spectrum becomes particularly prominent. As in Fig. 3*B* the arrows in Fig. 4 indicate the location of the corner frequencies f_c. Table 1 gives mean values of the related time constants $\tau_c = 1/(2\pi f_c)$ for different Cs concentrations and potentials. Neither the Cs concentration nor the potential has a pronounced effect on τ_c.

According to Eq. (1) the effective single-channel currents were calculated from the effective variance $var^* = \frac{\pi}{2} S_1 f_c$ of the Lorentz component:

$$i^* = var^*/(I_K(1-p)). \tag{4}$$

The effective single-channel conductances are then given by:

$$\gamma^* = i^*/(V - V_K) \tag{5}$$

with V being the test potential and V_K the K reversal potential which was assumed to be $V_K = 0$ mV. Table 1 contains average values of γ^* for various Cs concentrations and membrane potentials. There is only a small dependence of γ^* on potential in the

range of -60 to -120 mV. However, with increasing Cs concentration γ^* is dramatically reduced. For this reason we use the word "effective" single-channel conductance to characterize the calculated γ^* values. A detailed discussion of the origin of the Cs dependence of γ^* will be presented later.

Accordingly, effective numbers N^* of voltage-clamped potassium channels can be calculated from Eq. (2) to give

$$N^* = I_K/(i^* p) \tag{6}$$

which can be converted into an effective surface density n^* of channels by dividing by the surface area (*see* Materials and Methods). The obtained n^* values exhibit relatively little voltage dependence (*see* Table 1). Though there is a large scatter of the data, an increase of n^* with Cs concentration is apparent ($n^* = 5.8\,\mu m^{-2}$ for 0.4 mM, $n^* = 10\,\mu m^{-2}$ for 2 mM). The average value over all potentials and concentrations is $n^* = 7.54 \pm 0.96$ (mean $\pm$ SEM, $n = 46$) channels per μm^2 of surface area.

Discussion

The flow of K ions through open inward-rectifying K channels of skeletal muscle is accompanied by current fluctuations which, however, are not produced by a simple first-order relaxation process (curve *a* in Fig. 2, broken lines in Fig. 3). In agreement with the results of Ohmori (1978) for inward rectifiers of tunicate eggs at best a $1/f$ component is visible in the spectral density of K-current fluctuations in the absence of blocking ions. To produce additional current fluctuations we added small amounts of Cs to the extracellular solution which caused an increase of the spectral density in the frequency range of 10 to 1000 Hz though the mean current decreased (*see* Fig. 3). This demonstrates that the relaxation components visible in the spectra of Fig. 3 are produced by Cs ions which may block the inward rectifier by binding via a first-order reaction to a receptor in the channel. The random Cs binding and release is then the origin of the additional current fluctuations visible in Fig. 3*B*. Since the block of inward rectifiers by Cs is strongly voltage-dependent (compare Fig. 3*A*), the Cs binding site must be located within the channel. Similar conclusions were deduced by Ohmori (1978, 1980) who investigated the effects of various external blocking ions like Cs^+, Na^+, hydrazinium and Sr^{++} on K-current fluctuations in inward rectifiers of tunicate eggs. In his experiments, the corner frequency of the relaxation component could be related to the relaxation time constant of the blocking kinetics of K currents. Unfortunately, we were not able to analyze the macroscopic blocking kinetics of the inward rectifier in skeletal muscle for two reasons: (1) Voltage clamp of the channels located in the tubular membranes follows the onset of the test pulse with a delay which could be larger than the blocking time constants. (2) In skeletal muscle the time constants of capacitive currents and of the blocking reaction are of the same order, and thus, capacity and ionic currents are difficult to separate. With Ba^{++} as blocking ion one can observe macroscopic current relaxations, but in this case no relaxation spectra could be obtained (*see* Results).

If the relaxation spectra shown in Figs. 3*B* and 4 are produced by the random binding of Cs ions to a single site within the channel, the derived conductance of a single open channel should be independent of the Cs concentration. However, the γ^* values in Table 1 are clearly lower at higher Cs concentrations. The simplest possibility to explain this Cs dependence would be a two-step block of inward rectifiers by external Cs ions. In previous experimental and theoretical investigations on K channels the existence of several cation binding sites within the channel has been deduced (Hodgkin & Keynes, 1955; Hille, 1975; Ciani, Hagiwara & Miyazaki, 1977; Adelman & French, 1978; Hille & Schwarz, 1978; Ciani et al., 1980). Thus, there could be a fast exchange of Cs ions between the extracellular solution and a first blocking site, and a slow transition between a Cs ion at this site and a second reaction center deeper within the channel. If the fast exchange occurred with time constants shorter than 1 msec the corresponding spectral components will be suppressed by the 1.5 kHz low -pass filter. Therefore, the variance of current fluctuations determined in the frequency range up to 1.5 kHz will not resemble the total variance of all channel transitions but will only cover the slow reactions between the first and second Cs-binding site. In this case the measured variance and all related quantities would be "effective" parameters which would depend on the external Cs concentration. Alternatively, a Cs-dependent single-channel conductance could arise if Cs ions did not block completely but had a low but finite probability to pass the channel. However, a significant Cs permeability can only be expected at hyperpolarizations exceeding -120 mV (Gay & Stanfield, 1977; Standen & Stanfield, 1980) and not at the smaller potentials applied in our experiments. Therefore, we prefer the simple two-step blocking model, which is analyzed in detail in the Appendix and in which formulae are derived to extrapolate the real single-channel conductance γ of the inward rectifier from the dependence of effective γ^* values on

Cs concentration. Our result $\gamma = 9.8$ pS is comparable to the values of 6.5–9.3 pS reported by Ohmori (1980) for the inward rectifier in tunicate eggs. Since a similar blocking process may be responsible for the current fluctuations in tunicate eggs, Ohmori's conductance values may need to be increased accordingly. Hence, inward rectifiers in tunicate eggs and frog skeletal muscle have approximately the same conductance of about 10 pS and both must be pores.

Increasing Cs concentration as well as increasing hyperpolarization produce stronger block of the inward rectifiers (*see* Fig. 3*A*); therefore, the effective conductance γ^* should also be a function of the test potential. The data listed in Table 1 indeed exhibit a tendency toward decreasing γ^* values with increasing hyperpolarization, but the scatter for one potential is about as large as the standard deviations of the mean values averaged over all potentials.

Corresponding to Eq. (2) the real number of single channels is determined by $I_K = N \cdot i \cdot p$. Since the channel numbers were calculated from the effective single-channel currents i^* [*see* Eq. (6)], the effective channel number N^* should increase with increasing Cs concentration ($N \cdot i = N^* \cdot i^*$). The data of Table 1 fulfill this prediction, and the products $n^* \cdot \gamma^*$ at various Cs concentrations give a nearly constant total K conductance of 30–40 pS/μm^2. From this value and the real single-channel conductance of 10 pS the real surface density of K channels can be calculated to be $n = 3$–4 channels/μm^2.

The parameter τ_c was derived from the corner frequency of the Lorentz spectrum and reflects the time constant of the slow transition of Cs ions between the first and final blocking site within the channel. The τ_c values of Table 1 lie in the range of 0.7 to 2.7 msec and exhibit only a small voltage and Cs dependence. This suggests a prevailing voltage dependence of the fast Cs exchange between the extracellular solution and the first binding site. The kinetics of the final Cs binding in the interior of the channels might then hardly be affected by the membrane potential and consequently not much influenced by the external Cs concentration.

In conclusion, this paper presents evidence that the inward rectifiers in skeletal muscle are pores, and that the blocking of these pores by external Cs ions can be understood in terms of a two-step binding process. Further analysis of the fast and slow steps of this block would help to obtain more information on the locations and the heights of the proposed energy barriers within the pore.

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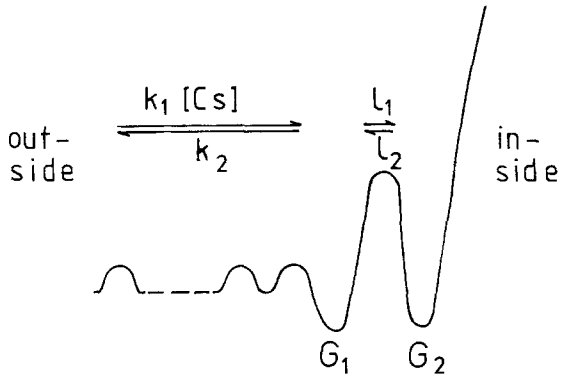

Fig. 5. A possible model energy profile for Cs-ion movement in the inward rectifier pore. G_1 and G_2 represent binding sites for Cs ions within the pore

Appendix

In this section we describe the two-step blocking model and derive formulae used to interpret our data. The K channel is assumed to be a single-file pore (*see* Fig. 5) where the ions can easily cross several low energy barriers to move into or out of the external channel mouth. A further additional transition over a high energy barrier leads to the final Cs binding within the channel.

Since the fast Cs transition over the low energy barriers cannot be resolved by our methods, the transitions may be treated as a quasi-equilibrium in front of the main high energy barrier. Thus, we can deal with the following reaction scheme:

$$A \underset{k_2}{\overset{k_1[\mathrm{Cs}]}{\rightleftharpoons}} B \underset{l_2}{\overset{l_1}{\rightleftharpoons}} C \tag{A1}$$

in which A, B and C represent the states with sites G_1 and G_2 unoccupied, site G_1 occupied, and site G_2 occupied by Cs, respectively. In general, a further state with sites G_1 and G_2 both occupied should be introduced, but the simple scheme (A1) already describes the essential features of a two-step blocking mechanism. All rate constants k_1, k_2, l_1 and l_2 may depend on membrane potential but it is not necessary to specify their voltage dependence in the following.

In states B and C the channel is occupied by Cs ions and thus blocked. Therefore, the probability p of the open channel is equal to the steady-state probability of state A:

$$p = \frac{1}{1 + (k_1[\mathrm{Cs}]/k_2)(1 + l_1/l_2)}. \tag{A2}$$

Since p can be determined from the ratio of mean K currents with and without added Cs (*see* Materials and Methods), Eq. (A2) gives a first relation for the unknown rate constants.

The variance of current fluctuations produced by all Cs transitions of scheme (A1) would be:

$$var = N \cdot i^2 \cdot p(1-p) \tag{A3}$$

where N and i are the channel number and the current through one open channel, respectively. If the reaction $A \rightleftharpoons B$ is so fast that only fluctuations from the slow transition $B \rightleftharpoons C$ can be observed, the measured effective variance var^* will be smaller than var and given by

$$var^* = N \langle i \rangle^2 p_{AB}(1 - p_{AB}). \tag{A4}$$

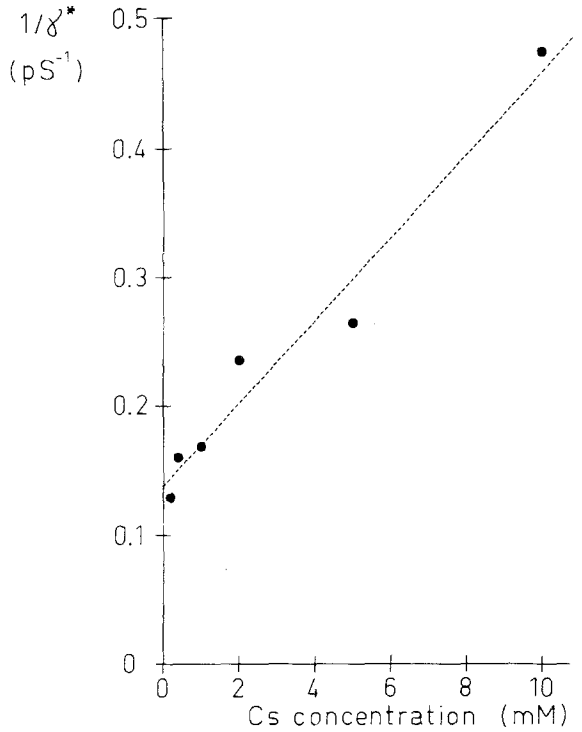

Fig. 6. Dependence of the inverse of the effective single-channel conductance γ^* on Cs concentration. The data are average values over all potentials taken from Table 1. The slope of the straight line [*see* Eq. (A6)] is $0.032\,pS^{-1}\,mM^{-1}$ and the intersection with the ordinate $0.138\,pS^{-1}$

Eq. (A4) follows from (A3) by assuming quasi-equilibrium of the reaction $A \rightleftharpoons B$. Thus, the probability p of the open channel state in Eq. (A3) has to be replaced by the probability p_{AB} of being in state A or B, and the real single-channel current i must be exchanged for the mean single-channel current $\langle i \rangle = (p/p_{AB}) \cdot i$ observed during the fast blocking reaction $A \rightleftharpoons B$. Combining Eqs. (1) and (A4) with the relation $I_K = N i p = N^* \cdot i^* \cdot p$ for the mean K current yields

$$\frac{i}{i^*} = \frac{p_{AB}(1-p)}{p(1-p_{AB})} \tag{A5}$$

or after expressing the probabilities by the rate constants of scheme (A1):

$$\frac{i}{i^*} = \frac{\gamma}{\gamma^*} = (1 + k_1[\mathrm{Cs}]/k_2) \cdot (1 + l_2/l_1). \tag{A6}$$

This relation predicts a proportionality between the inverse $1/\gamma^*$ of the effective single-channel conductance γ^* and the Cs concentration [Cs]. Fig. 6 shows that this linearity is approximately fulfilled. The $1/\gamma^*$ values in the Figure are the reciprocals of the effective conductances γ^* averaged over all voltages (*see* Table 1). The voltage dependence of the model parameters could not be studied because the deviations of γ^* for one potential are as large as the scatter of the average values. The slope of the fitted line in Fig. 6, its intersection with the ordinate and Eq. (A2) provide three relations to determine the ratios k_1/k_2, l_1/l_2 of the rate constants and the real single-channel conductance γ. The results are: $k_1/k_2 = 0.23\,mM^{-1}$, $l_1/l_2 = 2.8$ and $\gamma = 9.8\,pS$.

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