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Inward Rectification in Skeletal Muscle: A Blocking Particle Model

N. B. Standen and P. R. Stanfield

University of Leicester, Department of Physiology, University Rd, Leicester LE1 7RH, Great Britain

Abstract. Inwardly rectifying potassium currents were measured in resting frog skeletal muscle in different $[K]_o$. A model is presented for inward rectification which supposes that the potassium conductance depends on the K^+ concentration within a channel and is reduced by a blocking particle which is driven into the channel by depolarization.

Key-words. Inward rectification; skeletal muscle; voltage-clamp; potassium permeability.

The resting K^+ permeability of skeletal muscle shows inwardly rectifying properties[4,11], the permeability to K^+ being high when $V-V_K$ is negative and low when $V-V_K$ is positive[8]. We have used a three-electrode voltage-clamp method[3] to investigate this mechanism in frog (Rana temporaria) sartorius fibres. We present a model for inward rectification in which the conductance depends on the K^+ concentration within an aqueous pore and is reduced by a blocking particle driven into the pore by depolarization. Some previous models have invoked a carrier mechanism[2,10].

Muscles were immersed in solutions containing 10mM-K^+, 20mM-K^+, 40mM-K^+, 80mM-K^+ or 120mM-K^+, with sulphate as an impermeant anion. The composition of the 10mM-K^+ solution was: (mM) $5K_2SO_4$, $35Na_2SO_4$, $8CaSO_4$, 113 sucrose, 2 tris-maleate, pH7.2 – 7.3. In solutions with higher $[K]_o$, Na^+ was reduced accordingly. In 120mM-K^+, sucrose was 53mM. Each fibre was held at the calculated equilibrium potential for K^+, V_K, assuming $[K]_i$ to be 140mM[1,8], and the voltage-clamp was used to change the potential from this value in a step-like manner, so that current-voltage relations (for currents at the beginning of the voltage pulse) could be measured. (External Na^+ has a weak blocking action on inward potassium currents during extreme hyperpolarizations[14], but the block is time-dependent and so changes in $[Na]_o$ will not affect such instantaneous relations).

Fig. 1A shows mean current-voltage relations obtained in the five different $[K]_o$. In the 10, 20 and 40mM-K^+ solutions it was not possible to measure outward currents for depolarizations of more than 10 or 20mV since delayed rectification and contraction were activated.

It is clear from Fig. 1A that the conductance measured during hyperpolarization becomes lower as $[K]_o$ is reduced. We have attempted to model this effect by proposing that the maximum potassium conductance, $\bar{g}_K$, measured as $I_K/(V-V_K)$ when the membrane is hyperpolarized by 100mV from V_K, in any given $[K]_o$ is dependent on the binding of K^+ ions to a site within the permeability mechanism. Dubois & Bergman[6] have recently proposed a similar mechanism for the steady-state K^+ conductance at the frog node of Ranvier.

In muscle the effects of $[K]_o$ on the block of inward rectification caused by barium may be explained by supposing that two K^+ ions or one Ba^{2+} ion combine with a site in the permeability mechanism[12,13]. Therefore we propose that:

$$2K + R \rightleftharpoons K_2R$$

and:

$$\bar{g}_K = \frac{G_K [K]_R^2}{K_K + [K]_R^2} \qquad (1)$$

where G_K is the maximum conductance (i.e. when all sites are filled with K^+), $[K]_R$ is the concentration of K^+ at the site and K_K is the dissociation constant. The expression may be re-written:

$$\frac{1}{\bar{g}_K} = \frac{K_K}{G_K [K]_R^2} + \frac{1}{G_K} \qquad (2)$$

In Fig. 1B we have plotted $\frac{1}{\bar{g}_K}$ for each $[K]_o$ against $\frac{1}{[K]_R^2}$.

$[K]_R$ was calculated from:

$$[K]_R = [K]_o \exp(-\delta V_K F/RT) \qquad (3)$$

where $RT/F = 25mV$, V_K is the potassium equilibrium potential, and the binding site is placed a fraction δ of the electrical distance through the membrane, measured from the outside. We have taken $\delta = 0.2$ in Fig. 1B and it can be seen that the points fit a straight line. We have used this line to predict $\bar{g}_K$ in each $[K]_o$.

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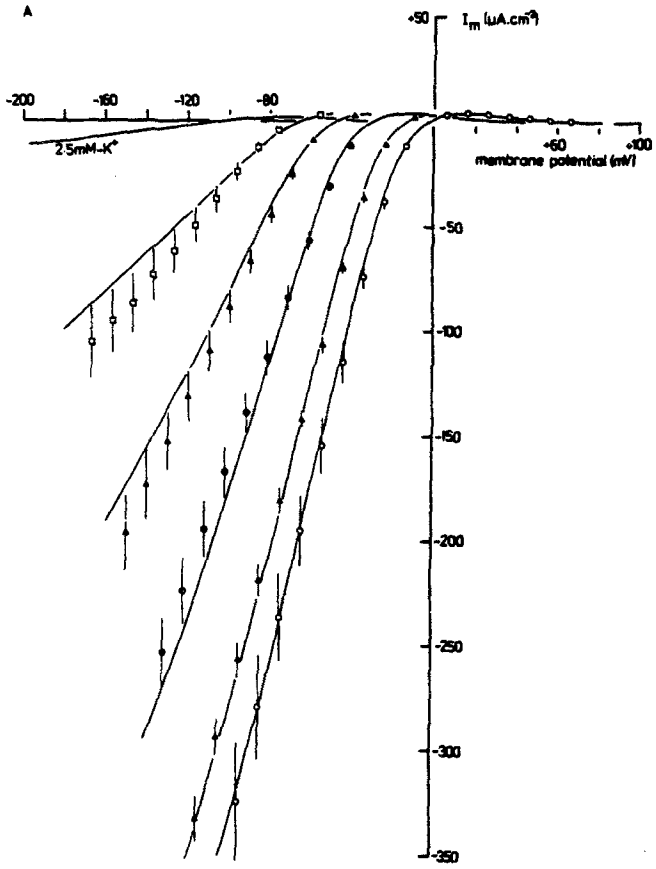

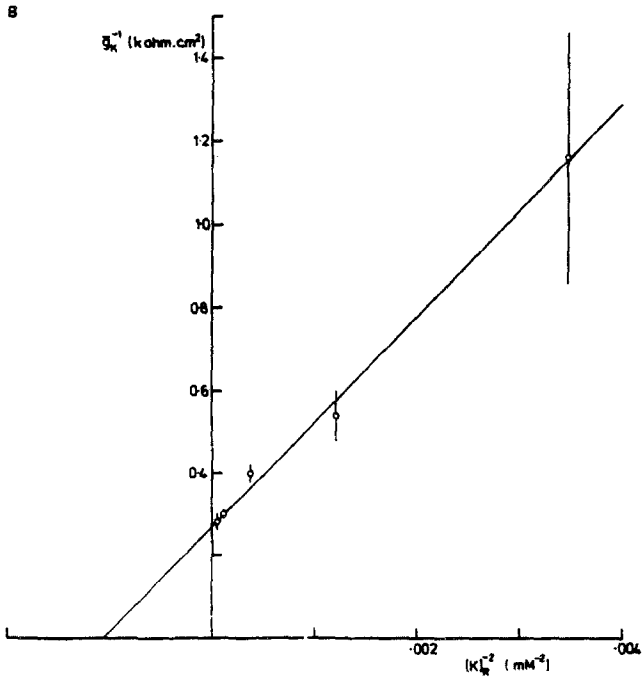

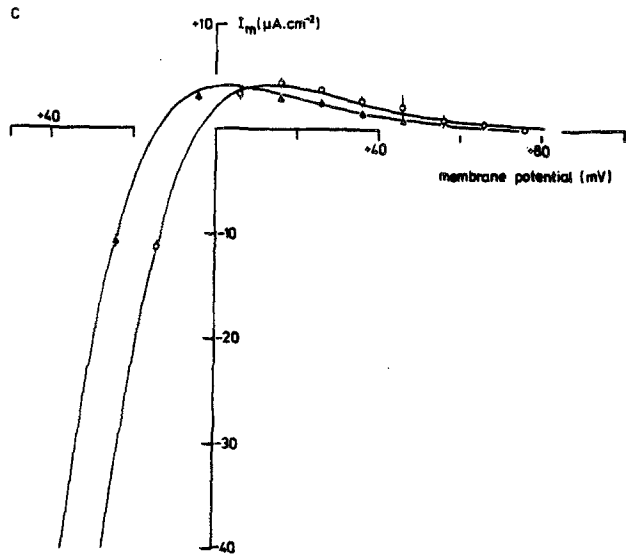

Fig. 1A Current-voltage relations obtained from fibres in: □, 10mM-K^+, holding potential (h.p.) -66mV; ▲, 20mM-K^+, h.p. -49mV; ●, 40mM-K^+, h.p. -31mV; △, 80mM-K^+, h.p. -14mV; ○, 120mM-K^+, h.p. -4mV. Ordinate: membrane current (μA.cm^{-2}); abscissa: membrane potential (mV). Points are means ±S.E. of mean from 5 or 6 fibres. A linear element, corresponding to a membrane resistance of 4175 Ω cm^2, obtained by extrapolating from currents measured during large depolarizations in the 80 and 120mM-K^+ solutions through the holding potential, has been subtracted from each current-voltage relation[4]. The lines were drawn from equations (8) and (9) with K_K = 950mM, K_S = 1mM, $[S]_i$ = 10mM, and δ = 0.2. $\bar{g}_K$ values (obtained from Fig. 1B) were (mmho.cm^{-2}): 120mM-K^+, 3.57; 80mM-K^+, 3.38; 40mM-K^+, 2.75; 20mM-K^+, 1.74; 10mM-K^+, 0.87; 2.5mM-K^+, 0.12.

B Reciprocal plot of membrane conductance against $[K]_R^2$ (calculated from equation (3) with δ = 0.2.) Ordinate: $\frac{1}{\bar{g}_K}$. Abscissa: $\frac{1}{[K]_R^2}$.
The straight line was fitted by eye. Its intercept with the abscissa gives K_K = 950mM.

C Predicted current-voltage relations drawn on a larger scale to show the fit for outward currents in 120mM-K^+ and 80mM-K^+ solutions. Symbols give experimental points: (○) 120mM-K^+, (△) 80mM-K^+.

In order to model the rectifying properties of the permeability mechanism we propose that the predicted linear conductance $\bar{g}_K$ is modified by a blocking cation, S^+, either present in the intracellular solution or held at a constant concentration at the inside of the membrane, which is driven into the membrane by depolarization where it competes with K^+ for binding to the site R:

$$2S + R \rightleftharpoons S_2R$$

and the dissociation constants for K^+ and S^+ are given by:

$$K_S = \frac{[S]_R^2 \left\{[R]_T - [S_2R] - [K_2R]\right\}}{[S_2R]} \quad (4)$$

$$K_K = \frac{[K]_R^2 \left\{[R]_T - [S_2R] - [K_2R]\right\}}{[K_2R]} \quad (5)$$

where $[R]_T$ is the total amount of site present. The concentration of sites filled by S^+ will then be given by:

$$[S_2R] = \frac{[R]_T}{1 + \frac{K_S}{[S]_R^2}\left\{1 + \frac{[K]_R^2}{K_K}\right\}} \quad (6)$$

We have also made the assumption that S^+ cannot pass through the pore to the outside solution and so it will accumulate at the level of the site according to a Boltzmann relation:

$$[S]_R = [S]_i \exp\left\{(1 - \delta)VF/RT\right\} \quad (7)$$

where $[S]_i$ is the internal concentration of S^+.

Since K^+ has access to the pore from both sides of the membrane it will not accumulate in the same way as S^+. It can be seen from Fig. 1A that for large hyperpolarizations the chord conductance tends towards a maximum value. If $\bar{g}_K$ does depend on $[K]_R$ as we have proposed (equation (1)) then this suggests that $[K]_R$ must be either independent of potential or only weakly potential-dependent. We have, therefore, made the simplifying assumption that $[K]_R$ is independent of potential and have calculated its value, in each $[K]_o$, from equation (3). The same assumption has been used to fit the block of inward rectification caused by Ba^{2+} [13].

If the fraction of channels blocked by S^+ is equal to $[S_2R]/[R]_T$ then from equations (6) and (7) the fraction of channels open (y) is given by:

$$1 - y = \left[1 + \frac{K_S}{[S]_i^2 \exp\left\{2(1 - \delta)VF/RT\right\}}\left\{1 + \frac{[K]_R^2}{K_K}\right\}\right]^{-1} \quad (8)$$

and the membrane current can be calculated from:

$$I_K = y.\bar{g}_K(V - V_K) \quad (9)$$

The lines in Figs.1A and 1C show current-voltage relations predicted from equations (8) and (9). We have measured K_K from the intercept of the line in Fig. 1B with the abscissa as 950mM. K_S was taken as 1mM and $[S]_i$ as 10mM. It can be seen that the model gives a good fit to our experimental results, the shape of the current-voltage relation in the depolarizing direction being accurately predicted (Fig. 1C). The predicted curve for the normal $[K]_o$ of 2.5mM is also shown in Fig. 1A. The predicted resting g_K is 0.1 mmho.cm^{-2} which agrees well with values measured experimentally by Hodgkin & Horowicz[8].

It is possible to obtain similar predictions varying K_S and K_K over quite a large range, but keeping the ratio between them constant. Also $[S]_i$ can be made larger or smaller by adjusting K_S appropriately. Identical predictions can be made by assuming that S is divalent and that only one S combines with one site R, provided that K_S is adjusted accordingly (to 0.1mM). We could not, however, predict curves of the correct shape if only one monovalent S combined with one site. An expression which is identical in numerical result to equation (8) can also be obtained if it is assumed that the dissociation constant K_S, rather than $[S]_R$, is potential-dependent. An alternative approach, which does not assume that $\bar{g}_K$ depends on K^+ binding to a site, is to calculate $[K]_R$ and I_K by assuming that $[K]_R$ behaves as predicted by constant-field theory[2,7,9] and by replacing eqn (9) with:

$$I_K = y.\bar{P}_K \frac{VF^2}{RT}\left\{\frac{[K]_i \exp(VF/RT) - [K]_o}{\exp(VF/RT) - 1}\right\}$$

We have found that we can obtain similar current-voltage relations in this way, taking $\bar{P}_K = 8 \times 10^{-6}$cm.$sec^{-1}$ and independent of $[K]_o$, but that the size of the inward currents declines rather more rapidly with reduced $[K]_o$ than is observed experimentally.

Armstrong[5] suggested a model for inward rectification in which an internal blocking particle, lying outside the electrical field of the membrane, is displaced by K^+ moving in through the membrane. His model predicts that I_K is an exponential function of membrane potential. Our model assumes that the potassium conductance depends on K^+ binding to a site in the membrane, and that an internal cation S^+ competes for binding to the site, blocking the channel when it binds. The model accurately fits our experimental measurements of potassium currents.

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