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THE INTERACTION OF POTASSIUM WITH THE ACTIVATION OF ANOMALOUS RECTIFICATION IN FROG MUSCLE MEMBRANE

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SUMMARY

1. Inward rectification of frog muscle membrane was analysed with the Vaseline gap method.

2. Hyperpolarization, under voltage clamp, produced inward potassium currents, which had a component that activated with a time constant, τ_K.

3. The activation time constant τ_K of the inward potassium currents was voltage dependent. For a given external potassium concentration, the time constant was maximal for potentials near the potassium equilibrium potential, E_K.

4. The potassium chord conductance g_K, had a sigmoidal voltage dependency, increasing initially e-fold per 11·6 mV of hyperpolarization.

5. When the internal potassium concentration was fixed, raising external potassium induced a shift of the τ_K–V and the g_K–V relations in the positive direction along the voltage axis. That shift was comparable to the change in E_K.

6. No shift of the $\tau_K - V$ and the $g_K - V$ relations was observed when the internal potassium was reduced from 150 to 50 mM.

7. Changes of internal sodium concentration between 5 and 100 mM did not significantly effect the magnitude of inward rectification.

INTRODUCTION

Katz (1949) found that frog muscle had a potassium selective conductance which was not found in the squid axon. This conductance was termed anomalous rectification or inward going rectification (Adrian, 1969). Anomalous rectification is activated by hyperpolarizing potentials. However, the conductance is not only voltage dependent. When the external potassium concentration is raised, the potential at which the conductance is activated is shifted towards a more depolarized level (Hodgkin & Horowicz, 1959; Adrian & Freygang, 1962*a*, *b*). Therefore it appears as if the conductance is dependent upon both the membrane potential V, and the potassium equilibrium potential E_K. This property is referred to as $(V-E_K)$ dependence. A conductance strikingly similar to the inward rectifier of frog muscle, has been found in starfish egg cells (Hagiwara & Takahashi, 1974; Hagiwara, Miyazaki & Rosenthal, 1976). Internal dialysis of starfish eggs (Hagiwara & Yoshii, 1979) indicated that

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changes of E_K by decreasing the internal potassium did not shift the conductance voltage relation. Recently, a voltage clamp method (Hille & Campbell, 1976) introduced the possibility of controlling the internal ionic composition of frog muscle fibres. The aim of the present study was to use that method to examine the effects of internal as well as external potassium on anomalous rectification in frog muscle and to obtain a kinetic description of the conductance changes.

METHODS

Preparation. Segments of single muscle fibres were dissected from the dorsal head of the semitendinosus muscle of *Rana temporaria* or *R. catesbeiana*. Muscle fibres were isolated with one end intact (Vergara, Bezanilla & Salzberg, 1978). The experimental chamber and the recording methods were similar to those described by Hille & Campbell (1976) with small modifications. The membrane current was determined by recording the potential of the E pool (the cut end pool) after calibration with a differential recording across a resistor in series with the E pool (see Fig. 1 of Hille & Campbell, 1976). The diameter of the fibres was between 80 and 160 μm. The length of the test pool (A pool) was about 100 μm. The potential of the A pool was recorded with a separate electrode which was connected to a voltage follower. A few intracellular recordings from the segment of the fibre which was in the A pool were made with microelectrodes. It was found that this intracellular potential was within 3 mV of the ground potential (B pool) and that it was not altered when the external potassium concentration was raised. The temperature of the preparation was kept near 5 °C by a Peltier device (Cambion model 3959). A miniature thermistor (Fenwell Electronics GB38P11) was inserted into the A pool so that the probe was within 1 mm from the muscle fibre.

Detubulation. The effect of specifically reducing the tubular current was observed following hypertonic treatment (Howell & Jenden, 1967). Whole muscles were immersed in Ringer solution to which 1 M-ethyleneglycol was added. After soaking the muscle for 50 min at room temperature, an isotonic Ringer was introduced. Calcium and magnesium were elevated to 5 mM (Eisenberg, Howell & Vaughan, 1971). After 1 hr in isotonic solution, muscle movement, upon stimulation, was very slight. Single fibres of normal appearance could be obtained and mounted in the recording chamber.

Solutions. Internal solutions were made using EGTA (ethyleneglycol bis (β-aminoethylether)-*N*,*N*′-tetraacetic acid) as an anion. In the external solution methanesulphonate was used as a replacement for chloride.

TABLE 1. Composition of solutions

External solutions (mM)

	KCl	KOH	NaCl	NaOH	CH_2SO_3H†	$CaCl_2$	Ca gluconate	HEPES
Ringer	2·5	—	110	2·5	—	1·8	—	5
2·5 K*	—	2·5	—	120	112·5	—	1·8	5
10 K*	—	10	—	112·5	112·5	—	1·8	5
50 K*	—	50	—	72·5	112·5	—	1·8	5
100 k*	—	100	—	22·5	112·5	—	1·8	5

* Chloride-free

† pH was adjusted to 7·4 with methanesulphonate HEPES: *N*-2-hydroxyethylpiperazine *N*′-2-ethanesulphonic acid.

Internal solutions (mM)

	KOH	NaOH	TrisOH	EGTA*	HEPES
I	150	15	—	77	5
II	100	65	—	77	5
III	50	115	—	77	5
IV	50	10	105	77	5
V	140	10	—	73	5

* pH was adjusted to 7·4 with EGTA.

Ability to change ionic composition of internal solution. The solutions of pools C and E made contact with the internal solution of the fibre. Schwartz, Palade & Hille (1977) have calculated, from diffusion coefficients of the ions and the geometry of the preparation, that effective exchange of internal solution could be achieved in less than 30 min. It was, however, important to verify the success of that process. It is known that the reversal potential of the sodium channel in frog muscle can be described by the Goldman–Hodgkin–Katz equation (Campbell, 1976). In Ringer solution $[Na]_o \gg [K]_o$ and $P_K/P_{Na} = 0{\cdot}048$ (Campbell, 1976) for 5 °C the reversal potential should be obtained by

$$E_{Na} = 55{\cdot}2 \text{ mV} \log \frac{[Na]_o}{[Na]_i + 0{\cdot}048\,[K]_i}. \quad (1)$$

Fibres were cut in solutions with various concentrations of sodium and potassium. The reversal potential of the sodium current was measured in chloride-free Ringer solution. The range of different reversal potentials was −5 to +60 mV. For each experiment, the reversal potential was determined. If a deviation of more than a few mV from eqn. (1) was found, the preparation was discarded.

RESULTS

Resting properties of cut muscle fibres. It was important to compare the properties of the cut muscle fibres with known properties of intact fibres. For that purpose, fibres were cut in solution which had 140–150 mM-potassium, similar to the intracellular solution (Hodgkin & Horowicz, 1959; Armstrong & Lee, 1971; Venosa & Horowicz, 1973). It is known that for intact fibres at the resting potential, the chloride conductance is twice as large as the potassium conductance (Hodgkin & Horowicz, 1959). The input resistance of the obtained cut fibres was measured in Ringer solution and then in chloride-free solution. Only those fibres whose membrane resistance increased by more than a factor of 2 were used. Fibres which were selected by this method usually survived over an hour and had electrical properties which were similar to intact fibres.

Activation kinetics of inward rectification. Fig. 1*A* displays the time course of the inward currents which were turned on by stepping the membrane potential to different hyperpolarizing potentials. The currents were obtained from a fibre which had 140 mM-K internally and 100 mM-K externally. The holding potential was the calculated Nernst potential for potassium (−10 mV for 5 °C). I found that these conditions, namely, chloride free solutions, highly elevated external potassium, and temperature of 5 °C, provided relatively large currents and that the declining phase, which was observed at lower external potassium (see Fig. 4), was insignificant. During the initial few milliseconds, the ionic current was obscured by a capacitative surge. The duration of the capacity current was probably determined by the geometry of the tubular membrane, since the potential of the surface membrane settled orders of magnitude faster. Following the capacitative surge, a time dependent increase of inward current was observed. If we denote the ionic current just after the potential step and at steady state by $I(0)$ and I_s, respectively, then, assuming an exponential time dependency, the ionic current would be described by the following formula:

$$I(t) = I_s - [I_s - I(0)] \exp[-t/\tau(V)]. \quad (2)$$

As expected, plots of $I(t) - I_s$ were linear on a logarithmic scale (Fig. 1*A*). For potentials more negative than E_K, the time constant $\tau = \tau(V)$ was found to be a monotonically increasing function of the membrane potential (Fig. 1*B*). This method could not be used to reliably measure the time constant at potentials more positive

than E_K. The difficulty is illustrated in Fig. 2*A*. In this experiment, the conductance was first activated by stepping the membrane potential to −40 mV for a duration of 50 msec. Then, second pulses to different potentials were applied. A step to −50 mV produced a large current which developed instantly, indicating that the time dependent process was almost fully activated at −40 mV. Stepping to −28 mV produced a *time dependent decrease* of inward current, which was the result of the deactivating process. Almers' (1971) analysis of 'tail' currents suggested a decrease

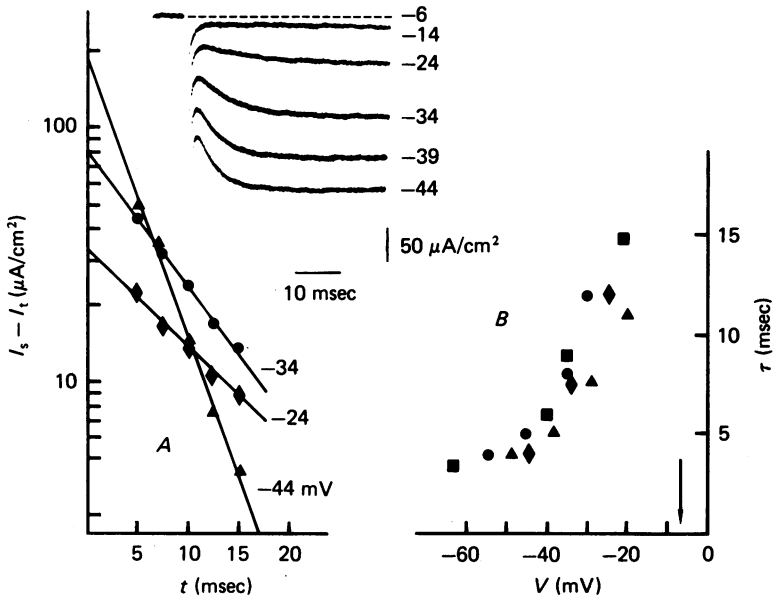

Fig. 1. The time course of inward rectification. *A*, semilog plot of the inward current. *B*, the activation time constant as a function of the membrane potential, four fibres. $[K]_o$ = 100 mM, $[K]_i$ = 140 mM, Temperature 5 °C.

of the time constants for potentials more positive than E_K. However, at potentials more positive than E_K, the ionic currents could not be clearly separated from the capacitative surge. This difficulty could occur if the turn-off time constants were *decreasing* for potentials more positive than E_K or if the activated channels *rectified inwardly*. The exponential relaxations between the non-conductive state and the conductive state might reflect a first order kinetic process. If the relaxation time constant is a function of the potential at any membrane potential, then the rate at which the conductance is turned off should be equal to the rate of the activation for that potential. The following three pulse experiments were carried out under these assumptions. The inward rectifier was activated by stepping the membrane potential to −40 mV for 50 msec. Then the potential was stepped to a more positive level at which the conductance was turned off. To examine the rate of the deactivation process, the membrane was stepped to −40 mV at various durations after the beginning of the second (deactivating) pulse. Owing to the capacitative surge, the 'tail' currents were measured about 5 msec after the beginning of the test pulse (○ in Fig. 2). The rate of the deactivation is apparent from the rate at which the tail

currents approached the steady state level. The time constant of that process was larger than 10 msec near E_K and decreased to less than 5 msec under depolarization to +10 mV or more. These experiments indicate that the voltage dependency of the time constant assumes a 'bell' shape with the maximal time constant at potentials close to E_K. The steady state level of the tail currents was dependent upon the ratio of the conductance at the deactivation potential to the conductance at the holding potential. As expected, the steady state tail currents were larger for a deactivation step of −18 mV and smaller for a deactivation step of −5 mV (Fig. 2*B*, *C*). These results indicate that g_K (−18 mV) > g_K (−10 mV) > g_K (−5 mV). A deactivation step to +10 mV produced an apparent increase in the tail current. This could have resulted from increased contamination by capacitative currents however, deviation from the first order process for large depolarizing potentials is also possible.

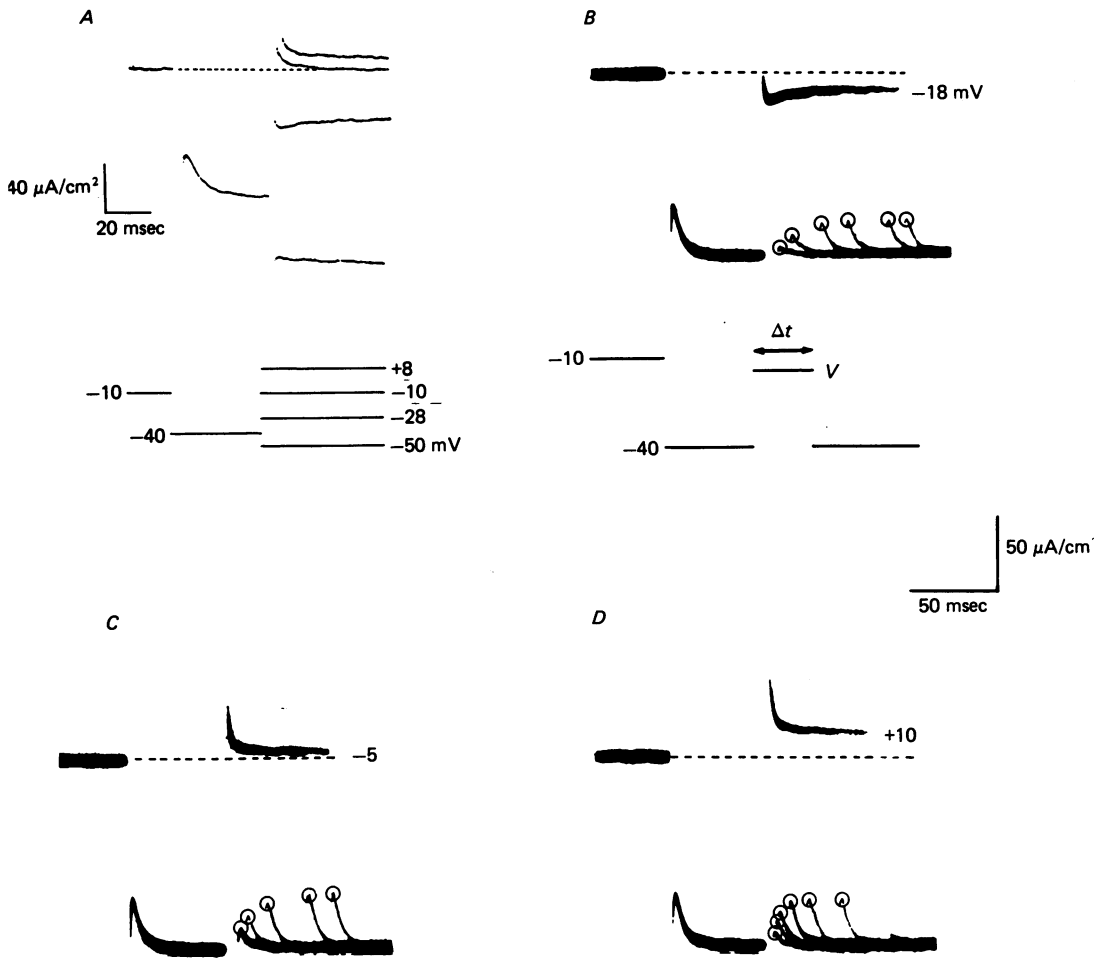

Fig. 2. *A*, double pulse experiment. Activation is produced with a 50 msec pulse to −40 mV, notice the conductance decrease after stepping to −28 mV $[K]_o$ = 100 mM, $[K]_i$ = 150 mM. *B*, *C*, *D*, three pulse experiment. The conductance is deactivated at three different potentials: −18, −5, +10 mV. Test pulses to −40 mV are superimposed. The circles indicate the 'tail' currents. $[K]_o$ = 100 mM, $[K]_i$ = 150 mM.

Detubulated fibres. The problems associated with the long lasting capacitative currents could be considerably reduced by a detubulation procedure (see Methods). Fig. 3*B* shows the inward Na current (obtained in Ringer solution) which lacked the secondary phase that is typical of fibres with an intact T system (Hille & Campbell, 1976). The dramatic decrease of capacitance (note the short capacitative current for the depolarizing step in Fig. 3*A*) allowed greater time resolution of ionic currents. The time course of the inward rectifier (in 100 mM-potassium externally) followed the same pattern of the usual cut fibre (Fig. 3*A*). The arrow in the figure indicates the response which is expected from ohmic behaviour. That value is smaller than the

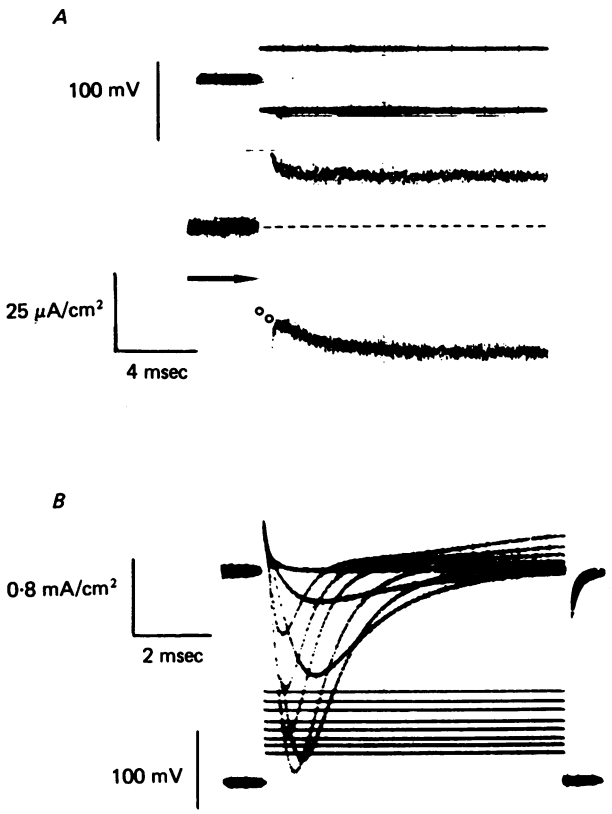

Fig. 3. Detubulated fibre. *A*, the response to a symmetrical voltage step. $[K]_o$ = 100 mM. Arrow indicates the 'expected' ohmic response. *B*, same fibre in normal Ringer. Note lack of 'hump' in the sodium currents.

instantaneous current ($I(0)$ in eqn. (2)) which is found by extrapolating (open circles) under the assumption of a single exponential time dependency. The non-ohmic behaviour of the membrane might reflect an instantaneous rectification. However, more experimentation is required to rule out a very fast gating process.

Conductance voltage relation, effect of external potassium. Fibres which were externally perfused with lower potassium concentrations had a remarkable inward rectification. Fig. 4 (inset) displays the current time course from a fibre in 2·5 mM-external potassium. The rate at which the inward currents increased showed a similar

dependency on the voltage step for all the potassium concentrations. At concentrations of 2·5 and 10 mM-potassium, there was a prominent phase of current decline. That decline was attributed to the depletion of potassium inside the tubular system (Adrian & Freygang, 1962*a*; Almers, 1972). As expected for a depletion mechanism, the decline was slower and smaller in 100 mM-potassium. The rate of current decline was sufficiently slower than the rate of current increase to allow characterization of the

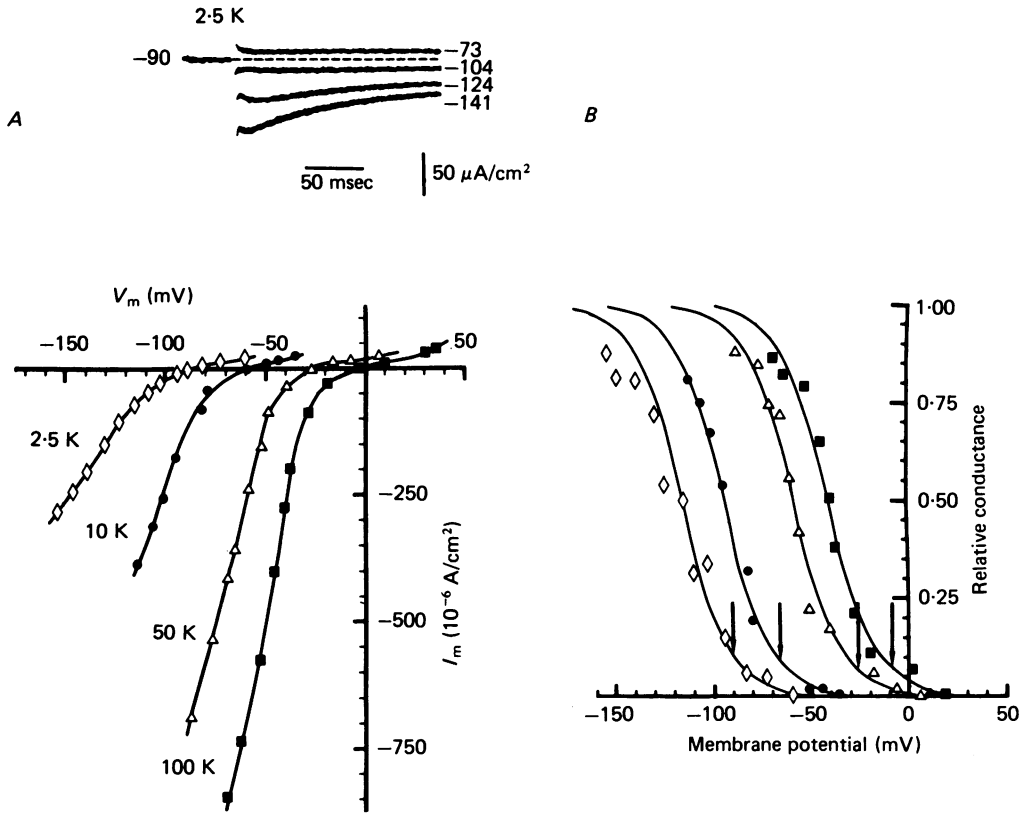

Fig. 4. Effect of external potassium. The current–voltage relation *A*, and the conductance–voltage relation *B*, from the same fibre in four external potassium concentrations. The lines in *B* are drawn according to eqn. (3). Arrows indicate E_K. $[K]_i = 150$ mM. Inset: currents from a fibre at 2·5 mM-$[K]_o$. Notice the remarkable phase of current decline.

activation by measurement of the peak currents. The peak current voltage relation (Fig. 4*A*) illustrates the properties of anomalous rectification (Almers, 1971; Standen & Stanfield, 1978). The currents which were produced by hyperpolarizing steps were large compared with the small outward currents. The potential at which the conductance turned on was dependent upon the external potassium concentration. That property was termed $(V-E_K)$ dependency (Hodgkin & Horowicz, 1959). Hagiwara & Takahashi (1974) found that the chord conductance $g_K = I/V-E_K$ of the starfish egg could be described with the following expression:

$$g_K/g_{K\,max} = \left[1+\exp\left(\frac{\Delta V - V_h}{\nu}\right)\right]^{-1}, \qquad (3)$$

where $\Delta V = V - E_K$, V_h indicates the half activation level and ν is the slope factor. Recently, Ciani, Krasne, Miyazaki & Hagiwara (1979) and Hille & Schwartz (1978) formulated models which relate to that expression. I found that the results from frog muscle could be fit with eqn. (3). It was assumed that for large, positive steps, the major component of the current is leakage current (Adrian & Freygang, 1962*b*). The normalized conductance voltage relations for different external potassium are illustrated in Fig. 4*B*. The lines are derived from eqn. (3) with $V_h = 25{\cdot}0$ mM and $\nu = 11{\cdot}6$ mV. The arrows in the Figure indicate the equilibrium potential for potassium E_K. When the external potassium was changed from K_1 to K_2, the potential of half activation was shifted by a value close to $(E_{K_2} - E_{K_1})$. However, the steepness of the conductance voltage dependency was unaltered by changes of

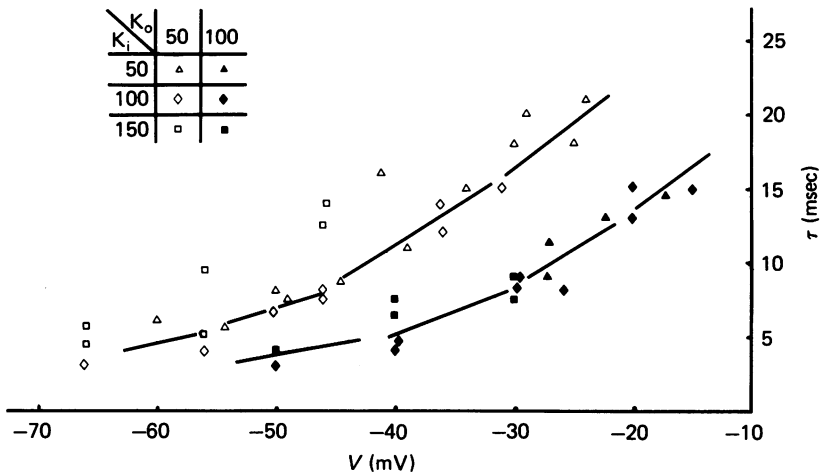

Fig. 5. Effect of internal potassium. The voltage dependence of the activation time constant at 50 mM-$[K]_o$ (open symbols) and at 100 mM-$[K]_o$ (filled symbols). The internal potassium is 150, 100 and 50 mM. Data from four different fibres.

external potassium since the same slope factor gave a reasonable fit in all the cases. It should be emphasized that the conductance was obtained by measurement of peak currents rather than instantaneous currents. Therefore the conductance voltage relation represents both the activation of the inward rectifier and the rectification of the single channels.

Effect of internal potassium. Fibres were cut in solutions which had 100 or 50 mM-potassium (solns. II, III). The holding potential in all cases was the calculated equilibrium potential of potassium. Under these conditions, the membrane had a strong, inward rectification. Furthermore, the inward currents had a time course similar to fibres with 150 mM-internal potassium. Fig. 5 displays the voltage dependency of the activation time constant for fibres with 50, 100 or 150 mM-internal potassium and 50 or 100 mM-external potassium. For comparable potentials, the time constant was independent of the internal potassium. For example, at −30 mV the time constant was about 10 msec when the external potassium was 100 mM and internal potassium was 50, 100 or 150 mM. However, changes of external potassium from 100 to 50 mM shifted the voltage dependency about 15 mV in the hyperpolarized

direction. For example, with 50 mM external potassium, a time constant of 10 msec was obtained at about −45 mV membrane potential for 50, 100 or 150 mM-internal potassium.

Conductance–voltage relation. The conductance voltage relation for fibres with 50 or 100 mM-potassium could be described by eqn. (3). Fig. 6 compared results from fibres with 50 mM-external potassium and 50 or 150 mM-internal potassium. At a membrane potential of about −50 mV, 0·5 of the conductance is turned on for both conditions.

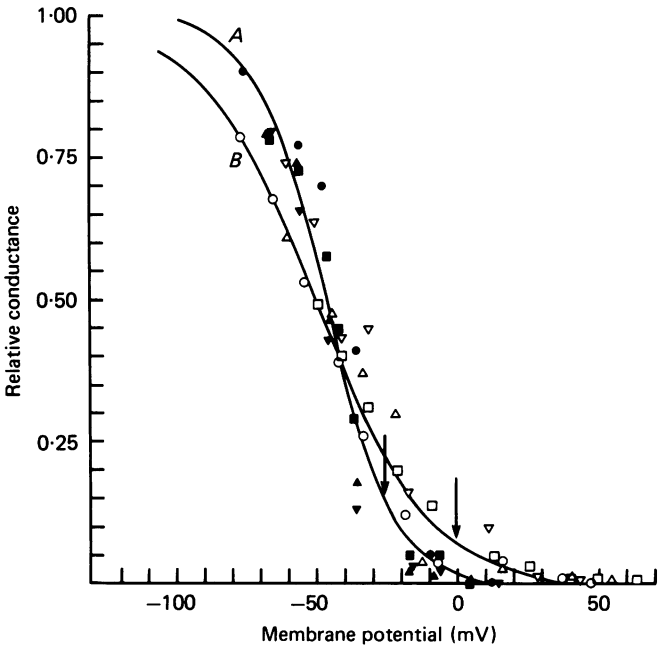

Fig. 6. Effect of internal potassium. The conductance–voltage relation at 50 mM-$[K]_o$ and 150 mM-$[K]_i$ (*A*), or 50 mM-$[K]_i$ (*B*). Four fibres for each condition.

The arrows in the Figure indicate the equilibrium potential of potassium which is shifted by 26 mV. The steepness of the voltage dependency was found to be different. 'Best fit' was obtained for $\nu = 11{\cdot}6$ mV with 150 mM-internal potassium and $\nu = 20$ mV for 50 mM-internal potassium (four fibres for each condition). These results indicate that the position of the conductance voltage relation on the voltage axis depends upon the membrane potential and upon $[K]_o$ but not upon $[K]_i$. At the present the significance of the decrease in the slope factor with low internal potassium is unknown.

Effect of internal sodium. Hagiwara & Yoshii (1979) have shown that in starfish egg an increase of internal sodium increases the magnitude of inward rectification. The sodium effect in egg cells was most prominent at internal concentrations of 35 mM. Experiments were done using fibres which were cut in solutions that had between 115 to 10 mM-sodium. The results from these experiments did not suggest significant sodium interaction with the inward rectifier. A few experiments were done using solutions with 0 mM-sodium (the internal sodium in these experiments calculated

from the reversal potential of the sodium channel (eqn. (1)), was 3 mM). Even under these conditions, the inward rectification was not significantly smaller from that observed in normal internal sodium. These results are comparable to the findings in intact muscle fibres (P. R. Stanfield, personal communication).

DISCUSSION

This paper describes an analysis of inward rectification in frog muscle, with the voltage-clamp methods of Hille & Campbell (1976). The preparation has several advantages over methods that utilize micro-electrodes. Namely, complications due to contraction or its suppression are avoided, control of the membrane potential is improved and the ionic composition of both internal and external solutions can be changed. Hille & Campbell have found that, although the sodium currents were normal in their preparation, the outward potassium currents were markedly reduced. However, the properties of the inward rectifier in this preparation are found to be similar to its properties in the intact cells.

One of the major questions concerned with anomalous rectification in frog muscle is whether the rectification is a product of a time dependent gating or a reflection of rectifying channels. With the present method, I have obtained a kinetic description of anomalous rectification which is similar to the starfish egg (Hagiwara *et al.* 1976) and confirms Almers' observation (1971). The time course of the conductance increase, upon hyperpolarization, could be described as an instantaneous jump followed by an exponential increase. At 5 °C the time constant was about 15 msec for hyperpolarizing steps of 10 mV and 5 msec for steps of 30 mV. The rate at which the conductance returned to the resting level, was also voltage dependent. Measurements of turn-off rates suggest that the $\tau - V$ relation is bell shaped, with a maximum near E_K. It is concluded from these findings that inward rectification is produced, to a large extent, by a time dependent gating mechanism. However, additional rectification due to single channel rectification or faster kinetics appear to be present.

I have found that the conductance can be described with the empirical expression which describes anomalous rectification in starfish egg (Hagiwara & Takahashi, 1974). The conductance was about 10% of its maximum value at rest and for small hyperpolarizing steps it increased e fold for 11·6 mV. However, the steepness of the conductance voltage relation decreased for lower internal potassium. The position of the τ–V relation and the g–V relation, on the voltage axis, depends on external potassium. When the external potassium was increased, the shift along the voltage axis of these relations was comparable to the change in E_K. Similar results, with elevated potassium, have been found in intact fibres (Standen & Stanfield, 1980). Armstrong (1975), Hille & Schwartz (1978) and Standen & Stanfield (1978) suggested that inward rectification is produced by an internal blocking particle which competes with potassium for a binding site inside the channel. Another model has been proposed by Ciani *et al.* (1978). In their model, inward rectification is produced by a voltage sensitive gating particle which can be stabilized by binding to potassium. In both models, the steady state conductance is $(V - E_K)$ dependent, at a fixed internal potassium concentration. At present, the kinetic consequences of the models

are not available. However, preliminary analysis indicates that the external potassium dependency of the activation time constant requires that the complexation of the gating particle with potassium is the rate limiting step in the model of Ciani *et al.* (S. Ciani, personal communication). Recently, Hagiwara & Yoshii (1979) have shown that in the starfish egg, changes of internal potassium do not alter the shape of the conductance voltage relation. In frog muscle fibres, I have found that both the $g-V$ and $\tau-V$ relations are not shifted on the voltage axis when the internal potassium is between 50 and 150 mM. These last results indicate that internal potassium does not have access to the binding site which is proposed by the two models.

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REFERENCES

ADRIAN, R. H. (1969). Rectification in muscle membrane. *Prog. Biophys. molec. Biol.* **19**, 339–369.

ADRIAN, R. H. & FREYGANG, W. H. (1962*a*). The potassium and chloride conductance of frog muscle membrane. *J. Physiol.* **163**, 61–103.

ADRIAN, R. H. & FREYGANG, W. H. (1962*b*). Potassium conductance of frog muscle membrane under controlled voltage. *J. Physiol.* **163**, 104–114.

ALMERS, W. (1971). The potassium permeability of frog muscle membrane. Ph.D. Thesis, University of Rochester, Rochester, N.Y.

ALMERS, W. (1972). Potassium conductance changes in skeletal muscle and the potassium concentration in the transverse tubules. *J. Physiol.* **225**, 33–56.

ARMSTRONG, C. M. (1975). K pores of nerve and muscle membranes. *Membrane, A Series of Advances*, vol. 3, ed. EISENMAN, G., pp. 325–358. New York: Marcel Dekker.

ARMSTRONG, W. McD. & LEE, C. O. (1971). Sodium and potassium activities in normal and 'sodium-rich' frog skeletal muscle. *Science, N.Y.* **171**, 413.

CAMPBELL, D. T. (1976). Ionic selectivity of sodium channel of frog skeletal muscle. *J. gen. Physiol.* **67**, 295–307.

CIANI, S., KRASNE, S., MIYAZAKI, S. & HAGIWARA, S. (1978). A model for anomalous rectification: electrochemical-potential-dependent gating of membrane channels. *J. membrane Biol.* **44**, 103–134.

EISENBERG, R. S., HOWELL, J. N. & VAUGHAN, P. C. (1971). The maintenance of resting potentials in glycerol-treated muscle fibres. *J. Physiol.* **215**, 95–102.

GAGE, P. W. & EISENBERG, R. S. (1967). Action potential without contraction in frog skeletal muscle fibres with disrupted transverse tubules. *Science, N.Y.* **158**, 1072.

HAGIWARA, W., MIYAZAKI, S. & ROSENTHAL, P. (1976). Potassium current and the effect of cesium on this current during anomalous rectification of the egg cell membrane of a starfish. *J. gen. Physiol.* **67**, 621–638.

HAGIWARA, S. & TAKAHASHI, K. (1974). The anomalous rectification and cation selectivity of the membrane of a starfish egg cell. *J. membrane Biol.* **18**, 61–80.

HAGIWARA, S. & YOSHII, M. (1979). Effects of internal potassium and sodium on the anomalous rectification of the starfish egg as examined by internal perfusion. *J. Physiol.* **292**, 251–265.

HILLE, B. & CAMPBELL, D. T. (1976). An improved Vaseline gap voltage clamp for skeletal muscle fibres. *J. gen. Physiol.* **67**, 265–293.

HILLE, B. & SCHWARTZ, W. (1978). Potassium channels as multi-ion single file pores. *J. gen. Physiol.* **72**, 409–442.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HOWELL, H. N. & JENDEN, D. M. (1967). T-tubules of skeletal muscle; morphological alterations which interrupt excitation–contraction coupling. *Fedn Proc.* **26**, 553.

KATZ, B. (1949). Les constantes électriques de la membrane du muscle. *Arch. Sci. Physiol.* **3**, 285–300.

SCHWARTZ, W., PALADE, P. T. & HILLE, B. (1977). Local anesthetics: effect of pH on use dependent block of sodium channels in frog muscle. *Biophys. J.* **20**, 343–368.

STANDEN, N. B. & STANFIELD, P. R. (1978). Inward rectification in skeletal muscle: A blocking particle model. *Pflügers Arch.* **378**, 173–176.

STANDEN, N. B. & STANFIELD, P. R. (1980). Rubidium block and rubidium permeability of the inward rectifier of frog skeletal muscle fibres. *J. Physiol.* **304**, 415–435.

VENOSA, R. A. & HOROWICZ, P. (1973). Effects on sodium efflux of treating frog sartorius muscles with hypertonic glycerol solutions. *J. membrane Biol.* **14**, 33–56.

VERGARA, J., BEZANILLA, F. & SALZBERG, B. M. (1978). Nile Blue fluorescence signals from cut single muscle fibres under voltage or current clamp conditions. *J. gen. Physiol.* **72**, 775–800.