BRIEF COMMUNICATION

HEART MUSCLE

INTRACELLULAR POTASSIUM AND INWARD-GOING RECTIFICATION

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ABSTRACT The cellular K content of frog ventricular strips is monitored using ^{42}K. Cellular K loss evoked by cardiac glycosides or a low extracellular K concentration is accompanied by a more than proportional decrease in the conductance of the resting membrane and the rate of rapid repolarization of the action potential. Voltage clamp experiments relate these changes to a decrease in the magnitude of an inward-rectifying K current. Current-voltage relations measured before and after changing the extracellular K concentration cross each other. This violation of the independence principle has previously suggested that extracellular K ions are required to open the rectifier channel (Cleemann and Morad. 1979. *J. Physiol.* 286: 113). Decreasing the cellular K content decreases the outward membrane current at all membrane potentials by an amount that is consistent with the independence principle. This suggests that the gating process is not sensitive to intracellular K ions. These findings are consistent with a previously published model for inward-going rectification.

INTRODUCTION

Anoxia, digitalis poisoning, rapid beating, and several other interventions result in loss of K from cardiac cells (1), yet little is known about the effect of the intracellular ionic concentrations on the various components of the membrane current. In this report the combination of voltage-clamp experiments and simultaneous isotope measurements makes it possible to relate variations in the intracellular K content to changes in an inward-rectifying K current. The results show that the inward rectifier current is strongly suppressed during the loss of cellular K. This suggests that the resting potential is less stable and more influenced by other current components. Previous experiments suggest that the inward rectifier channels have gates that open only when K ions are available from the outside (2). The present results indicate that intracellular K ions pass through the channels without being involved in the gating process.

METHODS

The single sucrose gap voltage-clamp technique is used to control the membrane potential and to measure membrane current and isometric force in frog ventricular strips (3). The effects of the extracellular series resistance are partially removed by using chopped current pulse clamping (4). The

^{42}K technique (5) is modified and made an integral part of the voltage-clamp setup thereby yielding a continuous record of the cellular K content throughout an experiment.

The β-radiation from the ^{42}K in the strip (0.1 mm^3) and in the surrounding perfusion chamber (1 mm^3) is monitored by a Geiger tube placed directly under the sucrose gap chamber. After equilibrating for 3 h the K content of the preparation is $\sim 10 \times 10^{-9}$ mol compared with $\sim 3 \times 10^{-9}$ mol in the surrounding bath. The 10×10^{-9} mol K in the preparation corresponds to a concentration of 167 mM in an intracellular volume constituting 60% of the 0.1-mm^3 test node. The 3×10^{-9} mol K in the 1-mm^3 bath corresponds to a concentration of 3mM. All solutions used during an experiment have the same specific activity (0.2–0.02 mCi/mg K). The counting efficiency is ~25%. The counts accumulated in an interval from 4 to 60 s are converted to an analog signal and registered on a pen recorder as seen in the original record in panel 2 *B*. Compensation for radioactive decay ($T_{1/2}$ = 12.4 h) is accomplished by automatically increasing the counting interval.

The cellular K content is lowered up to 50% by decreasing the extracellular K concentration, K_0, or by adding 10^{-4} M strophanthidin to the perfusate. Smaller reductions in the cellular content (2–10%) produced by rapid beating or passage of outward current are insufficient for the present purpose. In these experiments the rate of K loss is so low that the K concentration in the extracellular space can be expected to be close to that of the perfusate. The K concentration in the extracellular space of this preparation increases by ~1 mM when a voltage-clamp current of 1 μA is applied in the outward direction (6). The corresponding K efflux (10 pmol/s) is about 20 times larger than the rate of K loss observed in the present experiments (3 nmol/100min = 0.5 pmol/s). It is concluded that the extracellular K accumulation is small compared with the K concentration of the perfusate.

The cellular K content is proportional to the intracellular K concentration if the K is evenly distributed in a single intracellular space with a fixed volume. Rapid diffusion of ^{42}K in the intracellular space indicates adequate mixing (7). Monoexponential equilibration and washout curves for ^{42}K indicate that K is distributed in a single compartment. Some diffusion of ^{42}K into the sucrose gap is indicated by slow washout of a small fraction of the isotope (<10%) and by a rather high value for the estimated intracellular K concentration (150–200 mM compared with 125–135 mM in reference 5). The lower K content (8) and smaller counting efficiency in the gap probably help minimize this problem. Swelling is not observed in cardiac muscle that loses K because of inhibition of the Na-K exchange pump (9). These findings indicate that the measured K content is roughly proportional to the intracellular K concentration, K_i. Since the intracellular volume is not accurately known, the K content is indicated either as percent of the cellular content of the fully equilibrated preparation (Figs. 1 and 3) or as the absolute amount of K in moles (Fig. 2).

RESULTS

Fig. 1 shows results from an experiment where the strip of cardiac muscle loses 30% of the cellular K content when the K concentration of the perfusate, K_0, is lowered from 3 to 0.5 mM for 1.5 h.

Panel *A* shows how the total ^{42}K activity in a preparation equilibrated for 3 h may be separated into two fractions. The fraction corresponding to the K content of the bath is rapidly exchangeable and proportional to K_0. The slowly exchangeable cellular fraction is indicated by a smooth curve obtained from the measured curve by subtraction of the K content of the bath. The cellular fraction is assumed to be roughly proportional to the intracellular K concentration, K_i. Panels *B,C,D,* and *E* show action potentials measured before and during loss of cellular K. A comparison of panels *B* and *C* shows that a reduction in K_0 is accompanied by a decreased rate of rapid repolarization (white triangles) and a hyperpolarization of the resting potential. Panels *C*, *D*, and *E* show that the slow loss of cellular K is accompanied by a more than proportional reduction in the rate of rapid repolarization. From

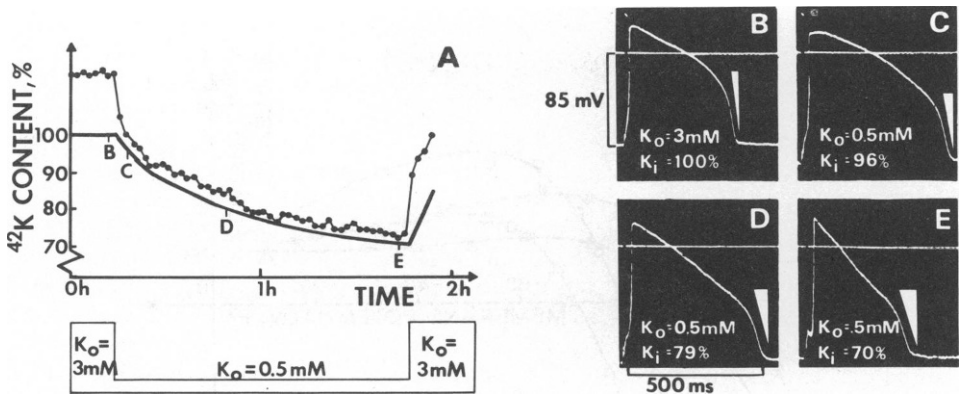

FIGURE 1 Cellular loss of K and decreased rate of rapid repolarization. *A* shows loss of K during perfusion with a Ringer solution of low K content (K_0 = 0.5 mM). The cellular K content (solid line) is obtained from the measured ^{42}K activity by subtraction of the ^{42}K content of the bath. The scale of the ordinate is the percentage of the cellular content of the fully equilibrated preparation. The action potentials shown in *B*, *C*, *D*, and *E* are measured at the times indicated in *A*. White triangles indicate the maximal rate of rapid repolarization. The rate of rapid repolarization is decreased when either the extracellular (*B* and *C*) or intracellular (*C*, *D*, and *E*) K concentration is decreased. The rates of rapid repolarization are 1.85 V · s^{-1} (*B*); 1.25 V · s^{-1} (*C*); 0.95 V · s^{-1} (*D*); 0.75 V · s^{-1} (*E*).

panel *C* to *E* the cellular K content is reduced by the factor 70%/96% = 0.73 while the rate of rapid repolarization is reduced by the fraction 0.75 V/s/1.25 V/s = 0.60. A small depolarization of the resting potential normally can be distinguished. Changes in the action potential plateau and the twitch tension are outside the scope of this report. It is noted that the rate of rapid repolarization is sensitive to changes in the cellular K content. A 30% reduction of the cellular K content gives a decrease in the rate of rapid repolarization comparable to that produced by a sixfold reduction in K_0 from 3 to 0.5 mM.

The major current component at rest and during rapid repolarization is an inward-rectifying K current (2). The experiment illustrated in Fig. 2 tests if this component of the membrane current exhibits variations similar to the rate of rapid repolarization. Panel *B* shows how a reduction in the cellular K content is accomplished by reducing K_0 and adding strophanthidin to the perfusate. As previously shown (2), reducing K_0 (○ → ●) shifts the current voltage relation toward more negative potentials and reduces both the maximal outward membrane current and the conductance at the reversal potential. The current-voltage relations cross at −70 mV. Reducing the cellular K content from 10 to 7 nmol (● → ▲) results in a further reduction of the membrane conductance at rest. A new crossover point is not produced since the outward membrane current is reduced at all membrane potentials. The schematics in the panels *C* and *D* illustrate the different effects of the extra and intracellular K concentrations.

In Fig. 1 readmission of normal K_0 is followed by rapid uptake of K. In the experiment illustrated in Fig. 2, the uptake of K is blocked by strophanthidin and a current-voltage relation (△) is measured after readmission of normal K_0 at a time when the cellular K content has a stable low value. Comparing this current-voltage relation to the previously measured current voltage relations again demonstrates a qualitative difference between the effects of

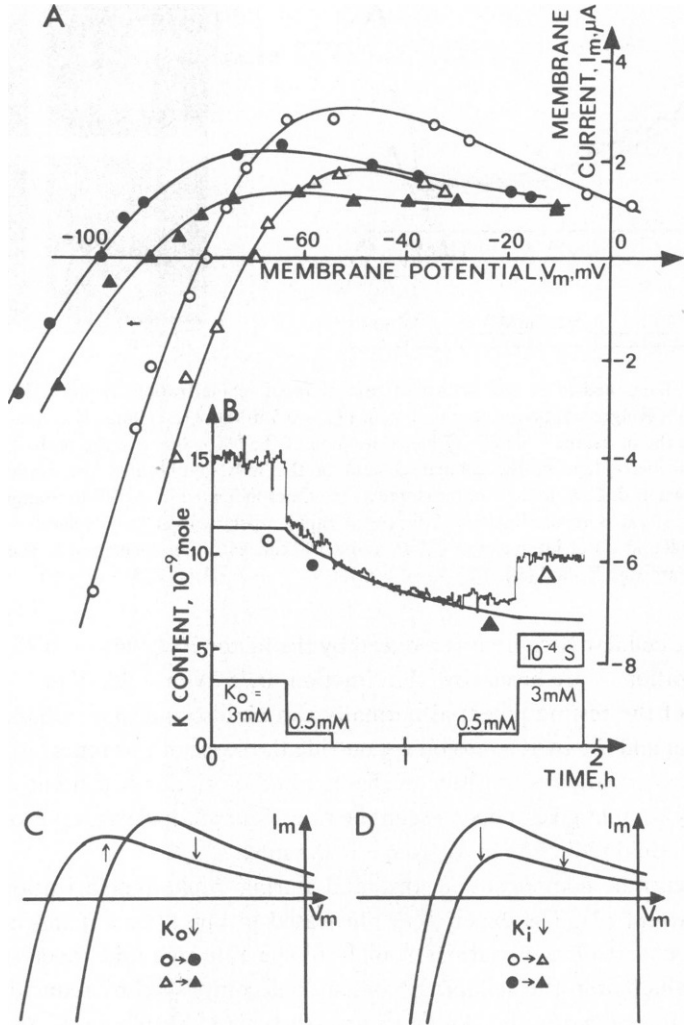

FIGURE 2 The effect of the extra- and intracellular K concentrations on the inward-rectifying K current. The current-voltage (*I-V*) relations in *A* show the initial membrane current, I_m, plotted vs. the clamped membrane potential, V_m. *B* shows how the cellular K content is lowered by decreasing K_0 and adding 10^{-4} M strophanthidin (10^{-4} S). The smooth curve is the cellular K content in nmol. The symbols close to the smooth curves indicate when the different *I-V* relations were measured. At membrane potentials < -60 mV the current is measured at the beginning of the clamp pulse. Inward current activated > -60 mV is disregarded by extrapolation from the slowly changing part of the current trace. Measurements of the rate of extracellular K accumulation have shown that the current component measured in this way is carried mainly by K ions (1). The *I-V* relations show that decreasing K_0 results in hyperpolarization, a decrease in the maximal outward current, and a point where the *I-V* relations cross. Decreasing the cellular K content causes depolarization and decreased outward membrane current at all membrane potentials. *C* and *D* demonstrate the different effects of extracellular and intracellular K using current-voltage relations derived from a simple model (See the text). The conductances at rest are: 240 μmho (K_0 = 3 mM, K_i = 100%), 150 μmho (K_0 = 0.5 mM, K_i = 90%), 100 μmho (K_0 = 0.5 mM, K_i = 60%), and 180 μmho (K_0 = 3 mM, K_i = 60%).

extracellular K ($\Delta \rightarrow \blacktriangle$) and of intracellular K ($\circ \rightarrow \Delta$). The changes in the maximal outward current correspond to the changes in the rate of rapid repolarization. Again a cellular loss of about 30% is as effective as a sixfold reduction of K_0.

During voltage-clamp experiments lasting for hours it is often observed that an increasing fraction of the applied current appears to leak through the sucrose gap without reaching the test node (4). The current-voltage relations in Fig. 2 show decreasing currents in spite of this possible artifact. However, procedures that are less sensitive to leakage through the gap must be preferred when the effects of intracellular K are quantified. The experiments illustrated in Fig. 3 were done to obtain frequent estimates of the membrane conductance in a way which is insensitive to the slow deterioration of the sucrose gap. The rate of electrical relaxation, which is the inverse of the time constant of the membrane, is determined from the membrane response to a pair of current pulses. In the absence of time-dependent current components, the rate of electrical relaxation is proportional to the membrane conductance (10). The results in Fig. 3 show that the relative change in the rate of relaxation of membrane voltage is larger than the relative change in the cellular K content. This is observed both with low K_0 during K loss (filled symbols) and with normal K_0 during uptake or while fully equilibrated (open

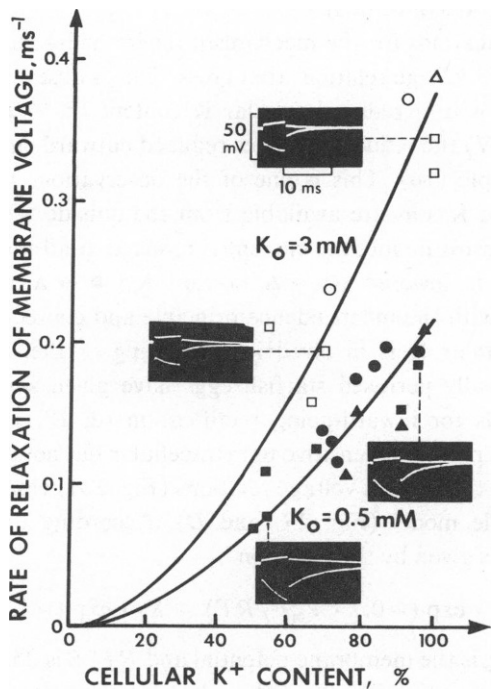

FIGURE 3 The rate of relaxation of the membrane voltage plotted versus the cellular K content. The rate of relaxation of membrane voltage is first measured under control conditions (K_0 = 3 mM, K_i = 100%, open symbols) then during K loss (K_0 = 0.5 mM, filled symbols) and finally during recovery (K_0 = 3 mM, open symbols). Triangles, circles and squares correspond to different experiments. The insets show the response of the resting membrane to consecutive pairs of current pulses, 1.5 μA and -4.5 μA in amplitude. The rate of electrical relaxation is found by analyzing the decaying membrane potentials that follow the current pulses. To avoid complications from lack of linearity, only the potential traces within +5 to −15 mV are subject to analysis. The curves approximating the measured rates of electrical relaxation are proportional to $K_0^{0.3} K_i^{1.7}$

symbols). These results confirm that the membrane conductance of the resting membrane is very sensitive to variations in the cellular K content.

DISCUSSION

The results in the three figures are tied together by the membrane capacitance. The rate of rapid repolarization is related to the maximal outward current measured 20–40 mV above the resting potential and the rate of relaxation of membrane voltage is related to the membrane conductance at rest. In both cases a comparison gives estimates for the membrane capacitance in the range from 1 to 2 μF. Direct measurements (11) or estimates based on the surface-to-volume ratio (12) give similar values. Thus all the results indicate that the inward-rectifying K current is suppressed by loss of cellular K. This suggests that conditions which cause K loss may give a less stable resting potential. For instance, arrhythmia caused by hypokaleamia (13) may reflect a decrease in the membrane conductance which is not only produced by the decreased K_0 (14) but also by a decreased intracellular K concentration. The membrane depolarization and the subsequent development of tonic tension seen in digitalis toxicity (15) may also in part be brought about by a decrease in the membrane conductance, which is secondary to the loss of cellular K.

The results have implications for the mechanism for inward-going rectification. In Fig. 2, reducing K_0 gives current-voltage relations that cross. This is observed with a normal cellular K content (○ → ●) and with a reduced cellular K content (△ → ▲). Above the cross-over points (−70 and −62 mV) the reduced K_0 gives reduced outward current in clear violation of the independence principle (16). This is one of the observations that suggests that the K channels open only when K ions are available from the outside. Fig. 2 clarifies the role of intracellular K. The outward membrane current is reduced at all membrane potentials when the cellular K content is lowered (○ → △ normal K_0; ● → ▲ reduced K_0). This is in quantitative agreement with the independence principle and consequently it is not necessary to assume that intracellular K is involved in the gating of the inward rectifier channel. Experiments with internally perfused starfish eggs have given similar results (17). These results show that models for inward-going rectification (2, 18, 19) have been correct in assuming that the gating process is sensitive to extracellular but not to intracellular K. In fact, the measured changes in the current voltage relations (Fig. 2 *A*) closely resemble the changes calculated from a simple model (Fig. 2 *C* and *D*). According to this model the inward rectifying K current I_K, is given by the equation

$$I_K = AK_0 \cdot [K_i \cdot \exp(-0.7 \cdot V_m F/RT) - K_0 \cdot \exp(-1.7 \cdot V_m F/RT)]$$

where A is a constant, V_m is the membrane potential and RT/F is 25.4 mV (Eq. 2.4, $y = 0.15$, reference 2). From this equation it follows that the membrane conductance at the reversal potential is proportional to $K_i^{1.7} \cdot K_0^{0.3}$ (Eq. 2.16, reference 2). The measurements in Fig. 3 verify this relationship. These theoretical considerations indicate that a reduction of the inward rectifier current is linked directly to a decreased intracellular K activity.

In this light it seems unlikely that other ionic changes that accompany cellular loss of K have a substantial effect on the inward rectifying K current. Cellular uptakes of Na and Ca, for instance, may well explain suppression of the action potential plateau and increased contractility but are probably not responsible for decreasing the rate of rapid repolarization.

In this context it may be mentioned that the opposite effect has been observed in starfish eggs where some intracellular Na is required to activate inward-going rectification (17).

The reduction in the cellular K content in Fig. 2 is accompanied by a reduction in outward current which does not depend strongly on the clamped membrane potential. Similar current shifts have been interpreted as resulting from an ouabain-sensitive electrogenic pump (20). This is an unlikely explanation in Fig. 2 since other experiments have shown that the ouabain-sensitive K flux in these preparations is typically <1 pmol/s or 0.1 μA under the experimental conditions used in Fig. 2.

The interpretation of electrophysiological experiments with cardiac muscle has often suffered from lack of detailed knowledge about ionic composition of the intracellular fluid. By measuring the cellular K content it has been possible to shed some light on the stability of the resting potential and the mechanism for inward-going rectification.

I thank M. Morad and R. Orkand for reading the manuscript. This work is supported by National Institutes of Health grant HL 24667.

Received for publication 5 February 1981 and in revised form 6 May 1981.

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