POTASSIUM EFFLUX AND ACCUMULATION IN HEART MUSCLE

EVIDENCE FROM K^+ ELECTRODE EXPERIMENTS

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It is well known that the ventricular muscle of vertebrate heart is a functional syncytium. Electron microscopic studies, however, have shown that the myocardium is composed of individual cells with well-defined cell borders attached to each other at specialized sites such as intercalated discs, desmosomes, and nexuses (1–3). Individual fibers (3–5 μm in diameter) are generally arranged within bundles of various sizes and shapes (average diameter, ~50 μm). Each bundle is surrounded by a thin layer of endothelial cells. The endothelial covering of the bundle may serve as a barrier to free ionic movement (2). The small intercellular spaces (0.01 – 5.0 μm) between the myocardial cells or beneath the endothelial cell sheath (1, 2) may serve as "ionic pools" under physiological or experimental conditions. Thus, small variations in the ionic content of such "pools" due to generation of an action potential or rapid beating may produce effective concentration changes around the ventricular cells to alter the electrophysiological characteristics of the cells.

K^+-sensitive microelectrodes (4) were used to examine the time course and the magnitude of K^+ efflux and accumulation in the frog ventricular strips. Frog ventricular muscle was chosen for its documented lack of *t*-tubular system (1, 2) and to avoid complications due to specific permeability changes at the *t*-tubular membrane (5) and accumulation of K^+ in the tubules (6).

K^+-sensitive microelectrodes were prepared by filling the tip of rapidly tapering glass microelectrodes, pretreated with a siliconizing agent, with K^+-selective resin (Corning liquid resin no. 477317 with K^+/Na^+ selectivity of about 60/1; Corning Glass Works, Science Products Div., Corning, N.Y.). K^+ electrodes with tips of 1-2 μm had a resistance of about 1000 MΩ and an electrical response time of 15–20 ms. The chemical response time of the electrode to variation of K^+ was found to be considerably faster (7). Double-barrel electrodes, with one barrel containing the K^+ sensitive resin and the other normal Ringer's solution, were used in experiments described below. The output

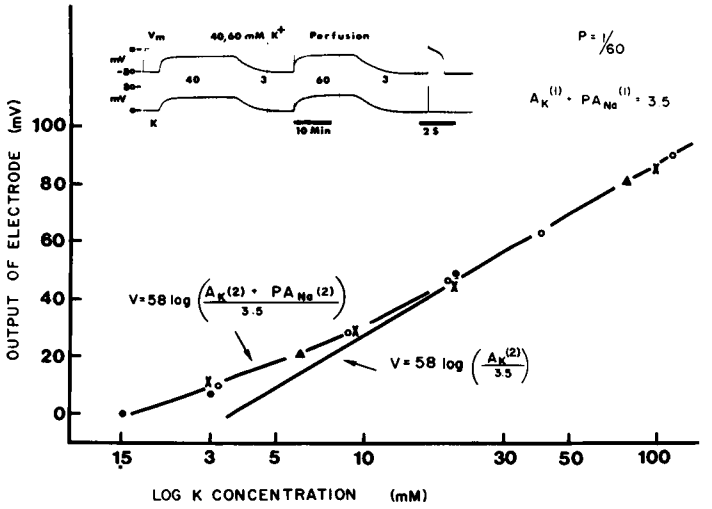

FIGURE 1 Response of potassium electrode versus log of potassium concentration. Test solutions are isotonic Ringer's with K variation accomplished by substituting KCl for NaCl. Different symbols represent four different electrodes of similar selectivity. The curved line is the Hodgkin-Katz equation with the appropriate Na:K selectivity of 1:60 (curve fitted by eye). The straight line has slope of 58 mV/decade and represents the Nernst plot. *Inset:* Response of membrane potential (intracellular microelectrode, top trace) and extracellular potassium electrode (single barrel; (bottom trace) to addition of potassium to bathing solution. K variation accomplished by perfusion with high K hypertonic Ringer's (40 and 60 mM K). A control action potential follows.

of the reference barrel (V_o) was monitored independently and subtracted electronically from the output of the K^+-sensitive electrode ($V_k + V_o$). Alternatively, the tip of the reference barrel was clamped to ground potential, which provided for a constant local reference potential for the K^+ electrode (8). Both precautions were routinely taken in order to isolate the potential caused by K concentration changes at the resin-preparation interface (V_k) from the potential fluctuations occurring in the extracellular space (V_o) during activity.

K^+ electrodes were calibrated by variation of K^+ in the bathing solution. The electrode calibration curves were fit by the following equation which is similar to that described by Hodgkin and Katz:

$$V(2, 1) = 58 \log [(A_K^{(2)} + PA_{Na}^{(2)})/(A_K^{(1)} + PA_{Na}^{(1)})],$$

where $V(2, 1)$ is the potential change in millivolts encountered in going from solution 1 to solution 2; A_K and A_{Na} are the K and Na activities of the solutions. The selectivity (P) of the electrode was determined for each electrode (9) (see Fig. 1 graph).

Frog ventricular strips 0.5–1.0 mm in diameter were equilibrated at room temperature (22–24°C) for an hour in Ringer's solution containing 120 mM NaCl, 3 mM KCl, 2 mM $NaHCO_3$, and 0.2–1.0 mM $CaCl_2$, pH = 7.6–7.8, before the start of the experiment. The preparation was electrically stimulated at 12 shocks/min.

Placement of the K^+ electrode in the extracellular space of the ventricular strip was

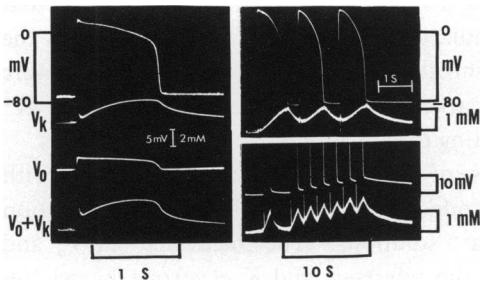

FIGURE 2

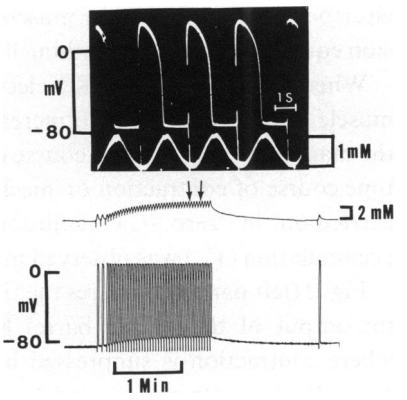

FIGURE 3

FIGURE 2 *Left:* Responses of intracellular microelectrode and extracellular double barrel potassium electrode during a single action potential. The strip was perfused in "zero" Ca^{2+} Ringers to suppress contraction. The four traces are (top to bottom): (1) membrane potential; (2) potassium response (V_k, subtraction of reference barrel from K-selective barrel); (3) reference barrel indicating extracellular potential artifacts (V_o); and (4) K-selective barrel ($V_k + V_o$). Since reference barrel has been used to compensate for the electrical artifacts in the extracellular space, V_k represents the response of the K-selective barrel only to changes in K concentration. For small accumulations seen during single action potentials, the K electrode is nearly linear in its response to K concentration. *Right, top:* Membrane depolarization (top trace) and potassium response (V_k, tip of reference barrel clamped to ground) for three successive action potentials. Muscle was equilibrated in 0.2 mM Ca^{2+} Ringer's. Due to shortened stimulus interval, K accumulation does not return to base level before next action potential. *Right, bottom:* Depolarization of the resting potential (foot of action potential at increased gain) and potassium response (V_k, tip of reference barrel clamped to ground) during rapid stimulation. Note progressive summation of K accumulations and progressive depolarization per beat (0.2 mM Ca^{2+} Ringer's). Right and left panel traces are from different strips.

FIGURE 3 The effect of increased frequency of stimulation on the time course of membrane potential (bottom trace) and K^+ accumulation (middle trace). Upside down arrows marking a segment of middle trace denote the time at which expanded tracing of action potential and K^+ accumulations were obtained (upper panel). Note that summation of K^+ accumulation within an action potential causes the steady accumulation of K^+ and membrane depolarization. Temperature 15°C,and $[Ca]_o$ = 50 μmol; diameter 1.0 mm. Double-barrel electrode.

generally accompanied by a transient increase (lasting 1–2 min) in K^+ concentration, resulting from local injury or the rupture of the myocardial cells at the locus of impalement. Potassium concentration in the bathing fluid was then varied and the response of the K^+ electrode (placed in muscle) was monitored. The upper inset of Fig. 1 shows the response of the extracellular K^+ electrode (lower trace) and the intracellular electrode (standard glass microelectrode, upper trace) as the K concentration was changed from 3 mM to elevated values of 40 and 60 mM K. Comparison of such recordings at various K^+ concentrations produces a direct relation between the response of the two electrodes. Withdrawal of the K electrode from the muscle into the surrounding bathing fluid did not alter response of the electrode. Thus, the K^+

electrode when placed in the muscle appears to be in a space which is in a rapid diffusion equilibrium with the bathing fluid.

When the double-barrel K^+ electrode is placed in certain extracellular areas in the muscle, K^+ activity seems to increase during a single action potential. To insure that the magnitude and the time course of accumulation is not substantially modified by the time course of contraction or mechanical shortening, a number of experiments were carried out in "zero"-Ca^{2+} solution. No marked alteration in the time course of K^+ accumulation (V_K) was observed in contracting or noncontracting ventricular strips.

Fig. 2 (left panel) compares the time course of the action potential (upper trace) with the output of the double-barrel K^+ electrode (trace marked V_k) in a preparation where contraction is suppressed in low Ca^{2+} solutions. The traces marked V_o and $V_o + V_k$ illustrate the electrical output of the reference and K electrode barrel, respectively. The potential recorded at the reference (Ringer filled) barrel continuously decreases (an attenuated action potential type recording), while the potential recorded with K^+ selective barrel increases during the plateau. The electrical artifact due to the generation of the action potential (V_o) is electronically compensated to yield only the time course of the increase in K^+ activity (V_k) in the extracellular space during the action potential.

In normal Ringer solution K^+ accumulates with a similar time course (Fig. 2, top right panel). Note that the transient increments of K^+ sum at the end of each depolarization-repolarization cycle to produce a base-line increase in K^+ activity. In the bottom right panel of Fig. 2, the preparation is stimulated more rapidly. The membrane potential trace is magnified so that alterations in the foot of the action potential can be easily monitored. Note that the small and transient K^+ increments accompanying each action potential sum to produce the depolarization of the resting membrane upon increasing the frequency of stimulation. In ventricular strips bathed in normal Ringer's, generally K^+ accumulation of about 1.0 mM was measured during a single action potential. Assuming a volume to surface ratio of 0.88 μm (10) and extracellular subendothelial volume of 10%, the K^+ accumulation was consistent with 3 $\mu A/cm^2$ compensated K^+ current measured during the plateau with a new voltage clamp technique.[1]

Fig. 3 shows the effect of increasing the frequency of stimulation (from 12 to 60 shocks/minute) on the resting membrane potential (bottom trace) and K^+ accumulation (middle trace). Increasing the frequency of stimulation results in membrane depolarization and an increase in activity of K^+ in the extracellular space. The summation of K^+ accumulated within each action potential seems to be responsible for the base-line increase in K^+ activity (see also Fig. 2). Termination of electrical stimulation results in decay of K^+ activity and repolarization of the membrane.

Remarkable similarity in the time course of membrane depolarization and K^+ accumulation as well as membrane repolarization and decay of K^+ activity were con-

[1]Goldman, Y., and M. Morad. Ionic membrane conductance during the time course of the cardiac action potential. Submitted for publication to *J. Physiol.*

sistently seen in every experiment. The time constant of decay of the smaller K^+ accumulation during each action potential is about 10–20 times faster than the slow K^+ buildup seen with a sequence of rapid stimuli ($\sim$20 s). One possible interpretation of such a finding is that K^+ efflux during a single action potential is confined to spaces beneath the subendothelial sheath, so that the accumulated K^+ would diffuse across the endothelial sheath into the larger extracellular space or be reabsorbed by the cells. The 1–2 s decay times for K^+ accumulation are consistent with previously estimated diffusion half time of 0.15–2.0 s for endothelial sheath (2). For persistent beating at higher frequencies, K^+ accumulates not only in the more confined subendothelial spaces, but also in the less confined and larger extracellular spaces between the bundles.

The magnitude of accumulated potassium depends on the strip diameter and the depth of electrode penetration in the muscle. In a particular strip, more accumulation is seen for deeper punctures and for higher rates of stimulation. The time course of accumulation depends primarily on the strip diameter; larger strips have slower time constants of accumulation and decay ($\approx$20 seconds for 600 μm strip). A simplified cylindrical free diffusion model with multiple compartments closely approximates the time course of build up or decay of accumulated potassium (unpublished observations).

As the ventricular strips are cooled to 10°C, the magnitude of K^+ accumulation increases and the time required for removal of accumulated potassium lengthens. The Q_{10} of this process is about 2.0. The addition of a digitalis analogue, acetyl strophanthidin (10^{-6} to 10^{-5} M) to a strip beating at low frequencies causes a steady increase in the accumulation of K^+ in the extracellular space (generally 1–2 mM above bath level). This finding and the high Q_{10} of the process indicates that the removal of accumulated K^+ is in part dependent on a metabolically mediated mechanism.

These studies suggest that K^+ efflux occurs continuously during the plateau of the action potential and terminates on repolarization. The duration of K^+ accumulation is strictly related to the duration of the action potential. Accumulation of K^+ seems to vary in magnitude in different places in muscle. Histological evidence supports such a variability in K^+ accumulation. The magnitude of K^+ accumulation seems to provide a good approximation to the magnitude of net outward current during the plateau. Although the magnification of K^+ accumulation seems to be responsible for the resting membrane depolarization during a sequence of rapid stimulation, the action potential shortening seems to be less directly related. On step increases of the frequency of stimulation, the action potential often reaches its shortened equilibrium state within one or two beats while K^+ accumulation requires more beats. The present studies do not support the hypothesis that shortening of action potential at higher frequencies is related to K^+ accumulation in extracellular space. However, it is still likely that in small clefts (0.01–0.1 μm) adjacent to the membrane (where 1–2 μm K^+ electrode cannot be placed), large K^+ accumulation may occur as to determine the duration of subsequent action potential.

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