Potassium Efflux from Amphibian Atrium during the Cardiac Cycle

A REEXAMINATION

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ABSTRACT

Fine fibers from amphibian atrium were mounted in a microperfusion chamber and loaded with $^{42}$K, followed by a rapid, nonisotopic wash during which the preparation was stimulated electrically. The stimulus was triggered and the "cold" effluent collected by a high-speed rotary fraction collector. Thus effluent corresponding to the same time interval in the cardiac cycle was always collected in the same tube. Large numbers of cycles were thus summed, decreasing the error in the tracer assay. Mechanical systole, previously shown to produce an artifact in the tracer efflux profile, was suppressed or prevented by various means. Under these conditions, a prolonged decrease in $^{42}$K efflux was observed, corresponding in time to the plateau of the action potential. Experiments were also carried out with $^{22}$Na, $^{45}$Ca, and $^{82}$Br, all of which showed primarily a single, positive efflux peak with its maximum within the first 50 msec of the action potential. Cell membrane permeability to K$^+$ appears to be markedly voltage dependent; and a diminished membrane permeability to K$^+$ apparently plays an important part in the generation of the plateau of the cardiac action potential.

KEY WORDS $^{22}$Na, $^{45}$Ca, and $^{82}$Br efflux from frog heart cardiac permeability to K$^+$ K$^+$ and cardiac membrane potential Ni$^{2+}$, zero Ca$^{2+}$ and plateau of heart action potential

A number of attempts have been made in recent years to study the transmembrane ionic flux patterns, particularly K$^+$ efflux, during the cardiac cycle by a combination of tracer and rapid sampling techniques (1-7). This work appears to have been flawed in part by the contractile event, which introduces a significant artifact in the tracer efflux profiles (4, 5, 7). In the present study, various means were employed to suppress or prevent contraction. In addition, muscle preparations of very small diameter were employed in an effort to minimize diffusion times within the fiber, and the rate of turnover of the liquid within the perfusion chamber was greatly increased. Under these circumstances, K$^+$ tracer efflux exhibits an initial brief augmentation followed by a marked suppression which lasts for approximately the duration of the plateau of the action potential, and then returns to the resting level along an exponential time course.

Materials and Methods

The method is similar in principle to that used previously by us and by others (1-5). Fine fibers from the atrium of the bullfrog (Rana catesbeiana) were used throughout. This preparation has been shown to function for several days in a steady state with respect to mechanical activity and tissue K$^+$ (8). Loading with tracer was followed by a rapid nonisotopic wash during which the preparation was stimulated electrically. The stimulus was triggered and the effluent collected by a high-speed precision rotary fraction collector.
Diagram of the perfusion system (not to scale). The atrial trabecula (A) (2.45-4.95-mm length, 0.05-0.20-mm diameter, and 4-20 μg dry weight) is shown mounted in the platinum clip (B) which positions it in the center of the 0.72-mm channel (C) of the lucite perfusion chamber (D). During isotopic loading, the appropriate solution is driven through the chamber by a Holter model RL155 peristaltic pump or by gravity at about 0.1-0.2 ml/min. The nonisotopic wash which is collected for radioassay is delivered by syringes (E) driven by a Bronwill infusion pump model 1836 at a rate of 34.3 ml/minute. This produces a turnover of the ambient fluid volume once every 1.5—3 msec, and an average linear velocity of the perfusion stream of about 1.5 m/sec along the length of the trabecula. The stimulus to the preparation is carried by the platinum electrodes (F), which connect with a Tektronix type 161 pulse generator via a Bioelectric Instruments type ISA 100 stimulus isolator. The exit orifice of the perfusion chamber is positioned a few mm above the upper lucite disc (G) of the rotary fraction collector, on the edge of which is mounted the contact which triggers the stimulus. From the dimensions of the system and the fluid flow rate, the time for liquid to move from the muscle fiber to the upper surface of disc (G) can be estimated. The perfusion chamber is positioned so that liquid in contact with the preparation when the stimulus is triggered reaches the upper surface of disc (G) just as the knife edge barrier which separates adjacent sample apertures passes beneath the effluent stream. That the chamber occupies the correct position has been checked stroboscopically with the collector in motion. The apparatus offers fractionation options of 15, 30, or 60 samples per cardiac cycle, and corresponding collector. Consequently, effluent corresponding to the same time interval in the cardiac cycle was always collected in the same sample tube. Usually 60 or 120 cycles were summed in this way, giving a favorable signal-to-noise ratio in the determination of the tracer. (On the basis of previous experiments [8] it is estimated that approximately 75% of the tracer remains in the fiber after the nonisotopic wash.) The essentials of the system are presented in Figure 1. The contractile event, which was shown to produce an artifact in the tracer efflux profile (5), was suppressed in most experiments by omitting Ca\(^{2+}\) or adding Ni\(^{2+}\) (9) to a normal or low Ca\(^{2+}\) Ringer solution. In other experiments, a high resting tension and the frictional drag of the perfusion stream were the only factors operating to reduce the distortion due to the mechanical event. In preliminary experiments employing a sensitive tension-measuring system (8) it was shown that the solutions employed to suppress contraction produced complete quiescence within a few cardiac cycles. Fibers immersed in the inhibitory solutions for periods corresponding to the exposure time during an experiment showed prompt recovery when returned to the normal medium. The normal Ringer solution employed throughout had the following composition (mm): NaCl 110, KCl 2.5, CaCl\(_2\) 1.1, MgSO\(_4\) 1.87, Na\(_2\)HPO\(_4\) 1.65, NaH\(_2\)PO\(_4\) 0.37, glucose 5.5; pH 7.4. All experiments were carried out at 21° ± 1°C.

Radioassay was carried out in a Packard liquid scintillation spectrometer, Model 3375, each aqueous sample of about 3.4 ml being mixed with approximately 4 volumes of scintillation liquid (Aquasol, New England Nuclear Corp.).

Detailed studies of the transmembrane potential were carried out under conditions approximating those of the various efflux experiments to permit correlation of tracer profiles with the electrical events of the cardiac cycle. Sources and preparation of the isotopic solutions, composition of the normal Ringer fluid, recording of transmembrane potentials and other experimental details have been given previously (3, 5, 8), or will be mentioned in the text, where appropriate.

Results

Efflux Profiles of Potassium-42

In 12 experiments in which contraction was suppressed by omitting Ca\(^{2+}\) from normal Ringer's solution or adding Ni\(^{2+}\) to normal or low-Ca\(^{2+}\) Ringer's solution, the \(^{42}\)K efflux

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uniformly exhibited a small augmentation with its peak in the first fraction (50 or 100 msec) of the cycle. This was followed by an abrupt decrease of approximately 500-1500 msec after which ensued a rapid return to resting values. The rates of decrease and increase in activity during these latter two phases could be fitted by a single exponential.

Figure 2 presents the results of an experiment in which $^{42}K$ efflux was recorded under conditions of inhibition by zero Ca$^{2+}$ and Ni$^{2+}$ successively in the same muscle preparation. Depression of tracer efflux under the influence of Ni$^{2+}$ outlasts that produced by zero Ca$^{2+}$, a uniform finding.

Figure 3 presents the results of one experiment of four carried out in a contracting preparation in which tension and frictional drag of the perfusion stream suppressed the mechanical event sufficiently to prevent the unevenness in flow rate produced by a contraction of normal amplitude, as shown in Figure 4. The flow record exhibits a minor disturbance following the stimulus scarcely beyond the usual limit of variability, and the tracer efflux curve shows the abrupt depression as seen with inhibition by zero Ca$^{2+}$ and Ni$^{2+}$ but appreciably shorter than that usually seen with zero Ca$^{2+}$ and Ni$^{2+}$.

$^{42}K$ efflux from an amphibian atrial trabecula (3.8-mm length and 24.6 μg dry weight). Counts/min are corrected for system background and isotope decay. The arrow indicates the position of the stimulus and the beginning of the cardiac cycle, which is divided into 30 segments of 100-msec duration. The upper curve (circles) of the activity record presents the tracer efflux pattern obtained with a washing solution devoid of Ca$^{2+}$. A 30-second preliminary flush, to remove ambient and extracellular tracer and suppress contraction, was followed by a 3-minute collection period, superimposing a total of 60 cycles. The decrease of tracer efflux below diastolic levels lasts approximately 800-900 msec. The lower curve (squares) was recorded from the same preparation following reloading with tracer. An approximately 4-minute exposure to 2.5 mM Ni$^{2+}$ in $^{42}K$-Ringer's solution followed the standard 90-minute incubation in $^{42}K$-Ringer's solution to permit the Ni$^{2+}$ effect on the action potential to stabilize before the sampling period. This was followed by a 30-second preliminary flush with nonisotopic Ni$^{2+}$ (2.5 mM)-Ringer's solution and a 3-minute collection period as above. Duration of the decreased efflux is 1500-1600 msec. Sample weight variations of approximately ± 1% are shown, a finding observed routinely when the mechanical response was suppressed. Variations from the mean (± 2 SD) are shown.
$^{42}$K efflux during the cardiac cycle

**Figure 3**
$^{42}$K efflux from an amphibian atrial trabecula (4.5-mm length and 15.7-μg dry weight); presentation as in Figure 2. After isotope loading, the preliminary flush and sampling wash were carried out with normal Ringer's solution so that the contractile event was not suppressed by chemical means. 100 msec per sample.

**Figure 4**
$^{42}$K efflux from an amphibian atrial trabecula (4.3-mm length and 17-μg dry weight); presentation as in Figure 2. Circles represent data from the preparation contracting in normal Ringer's solution. Squares give the data from the same preparation rendered quiescent by exposure to 7.5 mM Ni$^{2+}$-Ringer's solution containing 0.11 mM Ca$^{2+}$ (1/10 the normal level). 100 msec per sample.

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The more usual tracer efflux pattern from actively contracting preparations, reminiscent of earlier results (5), is shown in Figure 4. The flow record displays significant fluctuations during the period corresponding to the contractile event, while the tracer efflux shows a shallow, irregular augmentation during the same period. Also shown in Figure 4 is the $^{42}$K efflux pattern from the same preparation following suppression of the contractile event by a combination of Ni$^{2+}$ and low Ca$^{2+}$. The typical prolonged depression is seen.

Two experiments in which both the mechanical and electrical response were abolished by perfusion with isotonic sucrose showed no fluctuations in $^{42}$K efflux.

**ACTION POTENTIALS IN SOLUTIONS WITH NICKEL AND WITHOUT CALCIUM**

Since it is technically impossible at present to measure the transmembrane potential in the same preparation in which the tracer study is being carried out, the effects of the various contraction-inhibiting solutions on the characteristics of the action potential, primarily the duration, were studied in similar preparations suspended in an open chamber accessible to the impaling electrode and bathed in a flowing Ringer solution of the desired composition. Figure 5 presents typical tracings recorded from trabeculae incubated in the various solutions. Action potential durations are approximately 750, 1100, and 1750 msec in normal, zero-Ca$^{2+}$, and Ni$^{2+}$ Ringer’s solutions, respectively. Although considerable variation was sometimes noted between different preparations, the order illustrated in the figure was usually observed in any given preparation. Plateaus measured 200–400 msec less than the total action potential. In favorable experiments in which the rate of development of the change in duration could be followed, it was found that the prolongation induced by the absence of Ca$^{2+}$ approached full development in approximately 60 seconds and that due to Ni$^{2+}$ in a similar period. These effects were promptly reversed by return to the normal medium.

**TRACER AND ELECTRICAL RECOVERY RATES**

Whereas the initial change in $^{42}$K efflux following the stimulus is a biphasic event consisting of a brief positive and prolonged negative wave, recovery to resting values follows an uncomplicated course to which a single exponential can be fitted. The initial phase of electrical recovery from the plateau to the level of the resting potential can also be described by a single exponential. Table 1 compares the tracer and electrical recovery rates measured in the two types of contraction-suppressing solution. The half-times for the tracer and electrical measurements are not statistically different when values determined in the same test solution are compared, and the differences between the values obtained in the two types of contraction-inhibiting solution are statistically significant. Rapid repolarization in normal Ringer’s solution cannot be distinguished from that in...
TABLE 1
Comparison of Rates of the Initial Phase of Rapid Repolarization and K Efflux Recovery to Diastolic Levels

<table>
<thead>
<tr>
<th>Condition</th>
<th>Recovery Slope τ^1/2 (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ca]₀ = 0 (6)</td>
<td>187 ± 24 P ≤ 0.001</td>
</tr>
<tr>
<td>[Ni]₀ = 1.25-2.5 mm</td>
<td>330 ± 27</td>
</tr>
<tr>
<td>Normal Ringer's solution</td>
<td>194 ± 26</td>
</tr>
<tr>
<td>[Ca]₀ = 0</td>
<td>210 ± 20</td>
</tr>
<tr>
<td>[Ni]₀ = 2.5 mm</td>
<td>337 ± 44</td>
</tr>
</tbody>
</table>

Values are msec (mean ± 1 SE).

solutions with no Ca²⁺. Tracer data with normal Ringer’s solution are inadequate for statistical comparison but lie in the range noted in solutions with no Ca²⁺.

EFFLUX CURVES WITH OTHER TRACERS
Bromide-82.—In five experiments in which contraction was suppressed by either a combination of Ni²⁺ and low Ca²⁺ or solely by absence of Ca²⁺, tracer efflux displayed a single early positive peak. In three experiments carried out at 50-msec resolution, the efflux peak occurred in the first fraction of the cycle. These experiments provided a total of six cycles in which the recovery slope from the early peak could be fitted with a single exponential, yielding a half-time of 28 ± 2 msec (± 1 se). Data from one of these cycles is presented in Figure 6.

Sodium-22 and Calcium-45.—Preliminary experiments with these tracers have yielded sharp positive peaks with a maximum in the first 50 msec of the cycle. The initial rapid decline appears to be followed by a period of more gradual return to resting values.

Discussion
The principal questions which require an answer concern the relation of the observed tracer efflux profiles to those that actually occur at the membrane and the fidelity with which the tracer record represents the movement of carrier ion.

The 8²Br peak (Fig. 6) embraces a membrane event, diffusion through the extracellular space, and hydraulic washout of the tracer. The half-time of the peak is 28 ± 2 msec, corresponding to a recovery slope of 210 ± 20 msec. The tracer data with normal Ringer’s solution are inadequate for statistical comparison but lie in the range noted in solutions with no Ca²⁺.

**Figure 6**

8²Br efflux from an amphibian atrial trabecula (3.85-mm length and 12.4-mg dry weight); presentation as in Figure 2. Ringer’s solution with zero Ca²⁺ was used to suppress contraction. The plot represents the combined total of 60 cycles, divided into 30 segments of 50-msec each. Sample weights lay within ± 1%.

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ambient medium. If we assume the membrane event coincides with the depolarizing spike of the action potential (approximately 1 msec), the duration of the Br tracer peak is accounted for in practically its entirety by the last two factors. Since the tracer recovery slope can be readily fitted with a single exponential, the extracellular space and surrounding medium may be treated formally as a single compartment with a turnover half-time of 25–30 msec in series with the cell which is generating 42K flux transients with apparent half-times of about 150–350 msec. The extent to which the 42K transients are distorted decreases as the difference between the rates of the two processes increases. Using a simple two-compartment model (8, 10), graphic estimates indicate that beyond the first 50 msec or so following a change, the recorded 42K transient should closely approximate that at the membrane.

Concerning the early, positive tracer peak noted with all ionic species tested, the limitations of sampling time (50 msec minimum) permit one to conclude only that the event occurs within the first 50 msec of the cardiac cycle. Since the mechanical latency of the preparation employed is of the order of 100 msec, the early spike cannot be related to incompletely suppressed mechanical activity. Rather, it seems reasonable to suppose that it coincides with the depolarizing spike of the action potential. Although the 82Br peak cannot be interpreted in terms of quantitative anionic efflux, the prominent tracer transient suggests that Cl efflux may make a measurable contribution to membrane depolarization in the amphibian atrium.

The relation of 42K to K+ flux in the present work may be inferred from prior studies of 42K exchange kinetics in the amphibian atrial trabecula (8). The bulk of cellular K+ (90–95%) in this preparation behaves as an apparently kinetically homogeneous compartment which exchanges directly with the extracellular space. If we assume that the tracer in the present experiments arises predominantly from this compartment, which would appear to be a reasonable assumption, then the observed tracer patterns (with the possible exception of the early spike) should correspond closely with changes in carrier flux. In this connection it is interesting to note that Fozzard and Sleator (11) employing membrane resistance measurements in guinea pig atrial muscle, have constructed a K+ efflux curve that closely resembles the present results.

Since measurements of tracer movements and the action potential cannot be made simultaneously, the relationship of the K+ flux pattern to the electrical event must be inferred from potential measurements made separately but under similar conditions. As shown in

\[
M_0 = P_k \times \frac{FV}{RT} \times \frac{[K_i] \times e^{-\frac{VF}{RT}}}{1 - e^{-\frac{VF}{RT}}},
\]

where \(M_0\) is the outward flux, \(P_k\) is the relative permeability to potassium, \(V\) is the membrane potential, \([K_i]\) is the activity of the cell potassium and the other terms have their customary meaning. The points are based on the observed 42K efflux into Ni2+, zero Ca2+, and normal Ringer's solution in the present work, and on prior studies of exchange kinetics (8). Permeability during diastole is assigned a value of one.
Figures 2, 3, and 5, the durations of the two events exhibit a correspondence over a range of approximately 500–1500 msec and, of the several ions studied, K+ is the only one that shows a prominent positive inflection corresponding to the period of electrical repolarization. Similarity of the time course of repolarization and the tracer recovery slope (Table 1) strengthens the conclusion that the augmentation in K+ efflux occurs simultaneously with repolarization. Based on the previously determined absolute flux rates for K+ (8) and an assumed membrane capacity of 3 μf/cm², the increase in outward K+ current involved in regaining the resting K+ efflux levels should be ample to account for membrane repolarization. However, in the absence of independent quantitative data on K+ influx, the net outward K+ current (if any) during repolarization cannot be determined with certainty.

During the period in which membrane depolarization increases the electrical gradient for outward movement of K+, this ion actually exhibits a sharp reduction in efflux, a response indicative of a diminished membrane permeability to K+. When the relative permeability of the membrane to K+ is calculated using the “constant field” equation (12-14), it is seen to be strongly dependent on membrane potential (Fig. 7). It may be suggested that the initial depolarization, through an effect on fixed charges in the membrane, produces a potential-dependent change in the conformation of membrane structures involved in the “channels” for K+ transport, and that the resulting sharp drop in membrane permeability to K+ plays an important part in the generation of the plateau of the action potential. The results of exchange kinetics studies (8) are consistent with a diminished influx as well as efflux during this period. Since the exchange kinetics studies were carried out in preparations contracting normally in a balanced Ringer’s solution and since the characteristic 42K efflux profile has appeared in the present studies both in the presence and absence of the contraction-suppressing solutions, we conclude that the observed changes in tracer movement reflect physiological fluctuations in K+ transport.

References
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