Rate-Dependent Changes in Extracellular Potassium in the Rabbit Atrium

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SUMMARY We measured levels of potassium ion in the extracellular space of isolated superfused rabbit atria continuously with double-barreled microelectrodes of which one barrel was a K+ liquid ion-exchanger microelectrode and the other a potential-sensing micropipette. Increases in heart rate resulted in transient increases in extracellular potassium (\(|K^+|\)). When the quiescent atrium was stimulated the maximal increase was 0.4 mM at rates of 60/min, 0.7 mM after 90/min, 0.9 mM at 120/min, 1.3 mM at 200/min, and 1.8 mM at 300/min. The increase was not sustained during continued stimulation but declined toward prestimulation levels. When the stimulus was terminated the extracellular potassium activity decreased below bathing solution values by 0.2 mM after 60/min, 0.5 mM after 90/min, 0.7 mM after 120/min, 0.9 mM after 200/min, and 1.0 mM after 300/min and subsequently returned to a value equal to that of the bathing solution. The magnitude of the decline in extracellular potassium activity during prolonged stimulation was markedly decreased when the bathing solution contained either zero potassium, ouabain, LiCl, or a decreased P\(_O_2\) such that an elevation in \([K^+]|\) persisted during stimulation. Moreover, the reduction in \([K^+]|\) that followed the cessation of stimulation also was inhibited. These results support a role of the Na-K pump in maintaining extracellular potassium activity during changes in cardiac rate.

AS CARDIAC rate is increased there is a transient loss of potassium from isolated preparations of cardiac tissue,\(^1\)\(^\text{,}^2\) from whole heart preparations in vitro,\(^3\)\(^\text{,}^4\) and from the heart in vivo.\(^5\) Although there is a difference of opinion among various investigators about the magnitude of unidirectional fluxes during rate changes, there is general agreement that a net efflux of potassium occurs over the first few minutes after a rate increase.\(^7\) This loss probably reflects the outflow of potassium in excess of inflow which contributes to the repolarization phase of the cardiac action potential. If there is a restriction to diffusion away from the cell membrane, a net potassium efflux would be expected to increase extracellular levels of potassium and establish a gradient between the extracellular space and the perfusion fluid. Recently Kline and Morad\(^6\) used potassium-sensitive electrodes to study frog ventricular tissue and showed that potassium activity increased by as much as 1 mM in the extracellular space of frog ventricular muscle in response to a single action potential. Potassium accumulated with successive action potentials, and \([K^+]|\) subsequently decreased after the period of stimulation. The magnitude of accumulation and the time course of decay following stimulation were dependent on the diameter of the strip and the depth of penetration of the electrode as would be expected in a superfused preparation with varying diffusion distances to the bathing solution (i.e., with varying thickness of unstirred layers).

Because the resting potential of the cardiac cell is influenced by the extracellular potassium activity,\(^8\) any changes in potassium distribution must be considered in evaluating the normal pattern of electrical activation and inactivation. This study was undertaken to examine the effects of prolonged stimulation on the extracellular potassium activity. The present report deals with the effect of changes in cardiac rate on extracellular potassium activities in isolated superfused rabbit tissue studied with a double-barreled microelectrode, one barrel of which was a K+ liquid ion-exchanger microelectrode and the other a potential-sensing micropipette. This electrode configuration is essential for these studies because the potential-sensing pipette indicates whether the electrode tip is in the extracellular space or in the intracellular fluid. Also, any extracellular voltage changes occurring during the cardiac action potential must be subtracted from the potassium electrode reading.

Methods

POTASSIUM-SENSITIVE ELECTRODES

The potassium electrode used in these experiments is a glass micropipette (one side of a double-barreled pipette)
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with a potassium-sensitive liquid ion-exchanger in the tip. At the tip a potential is developed which is proportional to the logarithm of the potassium activity of the solution surrounding the tip. The electrode measures this potential plus any intra- or extracellular membrane potentials. The latter potential can be subtracted from the ion electrode reading by using the value simultaneously recorded by an adjacent electrolyte-filled electrode. This gives the potential due to the potassium activity. The preparation of double-barreled electrodes has been reported by other groups. Double-barreled tubing was used to draw microelectrodes with a total tip diameter varying from 2 μm to less than 1 μm. The tips of the electrodes were dipped in a solution of 2-4% tri-n-butylchlorosilane in chloronaphthalene and dried at 250°C for 10-15 minutes. One barrel was then back-filled with liquid ion-exchanger (Corning 477317) to form a 1-cm column at the tip. The remainder of that barrel was filled with 0.5 m KCl. The other barrel, to be used to measure intracellular or extracellular potential, was filled with 1 m NaCl or 0.5 m Na2SO4. The potassium-sensitive barrel was calibrated in KCl solutions of 1 m, 0.1 m, 0.01 m, and 0.001 m. The slope of the calibration curve of electrodes used in these experiments was 55-57 mV at 37°C. The selectivity of the ion-exchanger for K over Na was checked by calibrating in mixed Na-K solutions at constant ionic strength and in Ringer’s solution with constant sodium and varying potassium concentrations. The selectivity coefficient was 47:1 to 50:1 for electrodes prepared in this manner. The experimental setup is illustrated in Figure 1A. The potassium electrode was connected via an Ag/AgCl wire to an Analog Devices 3113 amplifier (input impedance = 1014 Ω). The reference electrode was a broken tip electrode filled with 3 M KCl in the bath. The output was displayed on an oscilloscope and Brush recorder. The potential-measuring half of the double-barreled electrode was similarly connected and displayed. The time constant of the ion-sensitive electrode ranged from 75 msec to 200 msec. The response of the two barrels when the tip of the electrode is in the extracellular space and the tissue is stimulated at 120/min is illustrated in Figure 1B. The extracellular potential recorded by the NaCl-filled barrel never exceeded 0.20 mV and usually was less than 0.1 mV. Potassium activity was calculated as follows (see Walker):

\[ a_k^+ = (a_{bi}^+ + K_{ai} a_i^+) \exp \left( \frac{F Z}{n R T} (V_s - V_b - V_p) \right) - K_{ai} a_i^+ \]

\[ a_s^+ = \text{potassium activity in the space or intracellular fluid} \]

\[ a_{bi}^+ = \text{potassium activity of bathing solution} \]

\[ K_{ai} = \text{selectivity coefficient} \]

\[ a_i^+ = \text{potassium activity of bathing solution} \]

\[ Z = \text{valence of ion} \]

\[ n = \text{correction factor for non-ideal slope} \]

\[ V_s = \text{potential of potassium electrode in space or cell} \]

\[ V_b = \text{potential of potassium electrode in bath} \]

\[ V_p = \text{potential of electrolyte filled barrel} \]

\[ a_s^+ = \text{activity of ions in space or intracellular fluid} \]

All values of potassium measured by the electrodes are reported as activities. Activity may be converted to concentration by use of an activity coefficient of 0.74. A micropipette filled with 3 m KCl and connected to a microprobe system (W-P Instruments, model M701) was used periodically to record intracellular potential to monitor the condition of the tissue.

PREPARATION

New Zealand white rabbits were killed by a blow to the back of the neck. The heart was excised and the atria were dissected free. One atrium was opened and placed flat in the perfusion dish, with the endocardial surface facing upward. The tissue was superfused with a solution of the following composition: Na, 145 mM; Cl, 126 mM; HCO3, 25 mM; K, 4.8 mM; Mg, 1.2 mM; Ca, 1.4 mM; SO4, 1.2 mM; H2PO4, 1.0 mM; dextrose, 5.6 mM (pH 7.5 at 37°C aerated with 95% O2-5% CO2).

Potassium-free solution was prepared by replacing all potassium with sodium. Lithium chloride solution was prepared by substituting LiCl for 1/3 of the NaCl. The bathing solution was maintained at 36-37°C. The solution was perfused at a rate of 15-20 ml/min. The chamber volume was 3 ml. The Pco2 and Po2 of the solution in the bath were measured periodically with a gas analyzer (Instrumentation Laboratory, model 213). The atrium was stimulated electrically through bipolar electrodes connected to an Anapulse stimulator (W-P Instruments, model 302-T).

Potassium in the extracellular space was measured by advancing the microelectrode through the endocardium, identified by a sudden negative voltage step, and then into a myocardial cell. This was identified by a sudden negative voltage step and a cardiac action potential recorded by the electrolyte-filled barrel. This was followed by a positive

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**Figure 1**

A: schematic of the experimental set-up (not drawn to scale). The double-barreled electrode was placed in the space below the endocardium or below the first or second myocardial cell. B: the response of the potential-measuring electrode is shown in the top trace, that of the potassium electrode in the bottom trace. Between the arrows the tissue was stimulated at 120/min. The field potential recorded by the NaCl-filled barrel never exceeded 0.20 mV during stimulation, and usually was less than 0.1 mV.
voltage step back to zero potential as the electrode was advanced into the space below the cell. No attempt was made to record from spaces deeper than those encountered directly below the endocardium or after penetrating through one or two cardiac cells. When the electrode was inserted into a space in this manner, the reading of potassium activity matched that of the bath (control = 3.6 mM). The value remained stable over a period of several hours in control preparations. The extent to which the space was distorted or enlarged by the presence of the electrode is at present unknown.

**Results**

During the efflux of K⁺ which occurs during each action potential potassium ions will move across the cell membrane into the space surrounding the cell. There K⁺ may diffuse away from the cell to be picked up by capillary blood (or in the case of isolated, superfused tissue, by the bathing solution) or it may be returned to the cell by activity related to the Na⁺-K⁺ pump. The magnitude of the increase in extracellular potassium concentration will be dependent on the amount of potassium which leaves the cell with each action potential, the size of the extracellular space, the rate at which K⁺ diffuses away from the cell, and the rate at which it is being taken up by the cell.

**POTASSIUM ACCUMULATION IN RESPONSE TO INCREASES IN RATE**

When the tissue was at rest the extracellular potassium activity in a space between cardiac cells was the same as that of the bathing solution (3.6 mM). On stimulation of the tissue the potassium activity increased to a maximum value within 1–2 minutes and then began to decline over a period of 4–12 minutes until it reached “near control” values (Fig. 2). The maximum value of the initial rise was dependent on the stimulus rate. In six experiments, from baseline values of 3.6 mM, potassium activity increased to maximum mean values of 4.0 mM ± 0.058 (SEM) mM at 60/min, 4.3 ± 0.079 mM at 90/min, 4.5 ± 0.086 mM at 120/min, 4.9 ± 0.065 mM at 200/min, and 5.4 ± 0.113 mM at 300/min. The decline generally brought extracellular potassium to within 0.2 mM of value of the control bathing solution but occasionally the value stabilized further above or below control value (see Fig. 5, control values). When the stimulus was terminated, a decrease in extracellular potassium below the levels of bathing solution was recorded. The mean minimum value of potassium reached after the stimulus period was dependent on the rate and was 3.4 ± 0.029 mM at 60/min, 3.1 ± 0.032 mM at 90/min, 2.9 ± 0.100 mM at 120/min, 2.7 ± 0.065 mM at 200/min, 2.6 ± 0.062 mM at 300/min. The minimum value reached after stimulation depended on the length of stimulus period. If stimulus was terminated before the decline began, the extracellular potassium then fell to prestimulation values with no undershoot (Fig. 2B). At any given rate, the maximum undershoot reached after that rate occurred when the stimulus period was long enough to return potassium to the control value or to a new stable level. After the undershoot, potassium then returned gradually to control levels over several minutes (Fig. 2B). Absolute values of potassium loss or gain could not be calculated from the activity values because the size of the extracellular space was not known, nor was the amount of K⁺ lost to or gained from the superfusion fluid known.

Because cardiac tissue is continuously active, it was of interest to investigate the effect of changes in rate beginning from values other than zero. Therefore, rate was increased from 60/min and the maximum accumulation was measured. In each case the potassium activity returned to control values within 4–12 minutes during continued stimulation. Figure 3 shows the extracellular accumulation as the stimulus rate was increased from a control value of 60/min. It can be seen that increases of about 0.15–0.50 mM occurred as the rate was raised from 60/min to frequencies within the physiological range. When the rate was decreased from a higher value to a lower value an undershoot occurred with potassium returning to control over several minutes.

To test the possibility that both the decline in potassium activity during continued stimulation and the undershoot following stimulation might reflect an increase in activity of the sodium pump the following procedures were used.

**EFFECT OF ZERO POTASSIUM**

Na⁺ pump activity has been shown to be dependent on the presence of extracellular potassium for a variety of
preparations. This is thought to reflect the coupling of K⁺ uptake to Na⁺ extrusion. Therefore, we studied the effect of a potassium-free bathing solution on the response to increased rates of stimulation. Control potassium activity responses were obtained at 120 stimuli/min. With the tissue quiescent, the superfusing solution then was changed to one in which the potassium was substituted by sodium. Figure 4 shows the result of such an experiment. The extracellular potassium activity, as measured by the electrode, never reached zero but fell to between 0.3 and 0.8 mM (mean value = 0.7 mM in four preparations) during the 40-minute period in zero potassium. With such initial activities, K⁺ increased on stimulation to as much as 2.0 mM (mean value = 1.6 mM) and remained elevated during the stimulus period. No undershoot was seen on termination of the stimulus.

**EFFECT OF DECREASED PO₂**

The Po₂ of the bathing solution, equilibrated with 95 % O₂, 5 % CO₂, was 505–520 mm Hg when measured with the chamber open to the atmosphere. When the preparation was superfused with solution which had been equilibrated with 95 % N₂, 5 % CO₂, the Po₂ measured in the bath dropped to 40 mm Hg. After 1 hour of superfusion with the latter solution, resting extracellular potassium in four preparations increased by 0.15–0.25 mM above that of the bathing solution and established a gradient between the space and the bath. This finding indicated that the preparation was losing potassium. The response of one of the preparations to stimulation under these conditions is illustrated in Figure 5. Both the decline in extracellular potassium activity during continued stimulation and the undershoot after the stimulus period were decreased. When stimulation was maintained for 5–10 minutes the extracellular potassium stabilized at 0.3 mM to 0.7 mM (mean = 0.55 mM) above the prestimulation values as compared to −0.2 mM to +0.2 mM (mean = 0 mM) for the same preparations when it was oxygenated. The undershoot phase after stimulation in the preparation exposed to a low oxygen tension reached a maximum value of 0.2–0.5 mM (mean = 0.3 mM) below the prestimulation values. This compared to a value of 0.5–0.9 mM (mean = 0.75 mM) during control stimulation of the same preparations.

**EFFECTS OF LITHIUM**

Lithium substitutes for sodium in generating the cardiac action potential but is not transported by the sodium-pumping mechanism. Full substitution of Li for Na rendered the tissue inexcitable after several minutes. Therefore, solutions in which 1/3 of the NaCl had been substituted by LiCl were used in three experiments. After 30 minutes in the lithium solution two of three preparations showed a slight increase in resting extracellular potassium (3.7 mM and 3.8 mM). During the stimulus period (180/min) the decline in extracellular potassium following the initial rise was reduced (Fig. 6). In the three preparations the average decline was to 0.7 mM above prestimulation levels during lithium perfusion as compared to 0.1 mM in the control solution. The average undershoot following stimulation was only 0.2 mM with lithium substitution as compared to 0.8 mM in control solution containing a normal sodium concentration.

**EFFECT OF OUABAIN**

The cardiac glycosides inhibit a Na-K ATPase which controls Na-K transport across the cell membrane. When the tissue was bathed with solution containing ouabain, 3 × 10⁻⁶ M, there was an increase in extracellular potassium activity of the resting tissue. In two preparations this value stabilized at 3.9 and 4.0 mM after 25 minutes. Stimulation (180/min) caused the customary rise in potassium followed by a slight decline with stabilization at 0.7 and 0.5 mM above prestimulation values. No undershoot occurred after the stimulus period. The effect was reversible if the preparation was exposed to ouabain for no longer than 40 minutes (Fig. 7).

**Discussion**

Extracellular potassium activity has been shown to increase in response to an increase in cardiac rate and then
increased rate. The data presented here support an initial net K$^+$ efflux on
begins rising during the early part of the plateau phase. If
rate varies with rate, the contribution of these two phases
plateau phase might offset the increase in efflux during
have studied the problem by tracer techniques. Langer$^7$ hypothe-
may be variable. Kline and Morad$^8$ used potassium-sensi-
tive electrodes and showed that extracellular potassium
concentration temporarily fell below control values. Simi-
lar changes in potassium activity have been seen in the
fluid collected from both isolated and intact whole hearts. These
data correlate well with results of studies by Parker
et al.$^5$ that showed that in normal human subjects whose
hearts were paced at rates above resting level, potassium
concentration in the coronary sinus effluent initially rose
and subsequently declined toward control values in spite
of maintained stimulation. Their measurements also re-
lected the undershoot following stimulation with return to
control over a time course of 10 minutes. Gilmore and
Gerlings$^4$ and Gilmore et al.$^{16}$ have shown an initial potas-
sium loss from the isolated, blood perfused dog heart
when heart rate was increased. Potassium concentration in the
coronary venous effluent returned to control within 2
minutes in spite of maintained stimulation at a constant
rate. When rate was decreased, coronary venous potas-
sium concentration temporarily fell below control values.
Recently Kriz et al.$^{17}$ have reported that extracellular
potassium activity of the cat lumbar spinal cord increases
with peripheral nerve stimulation but that this increase is
not maintained. There is also a temporary decrease below
prestimulation levels after cessation of stimulation.

It might be anticipated that the initial increase in extra-
cellular potassium activity as rate in increased would be
accounted for by an increased potassium efflux during the
repolarization phase of the action potential and that, as
the frequency of action potentials increased, so would potassium efflux. However, there is disagreement as to the
effect of rate changes on cardiac potassium efflux among
those who have studied the problem by tracer techniques.
Increases, decreases, and no change in K$^+$ fluxes have
been reported (for review see Langer$^{7}$). Langer$^7$ hypothe-
sized that a decrease in potassium permeability during the
plateau phase might offset the increase in efflux during
repolarization. Because the duration of the action poten-
tial varies with rate, the contribution of these two phases
can be variable. Kline and Morad$^6$ used potassium-sensi-
tive electrodes and showed that extracellular potassium
begins rising during the early part of the plateau phase. If
one assumes no changes in extra- or intracellular volume,
the data presented here support an initial net K$^+$ efflux on
increased rate.$^{14}$

Several explanations may be presented to account for
the fact that the net efflux is not maintained during stimu-
lation. One explanation would involve changes in mem-
brane permeabilities to potassium during the stimulus pe-
riod which would allow efflux to equal influx. Another
explanation, used by Kriz et al.$^{17}$ to interpret their results and
also favored by this investigator, is that potassium efflux initially is increased over influx as a result of in-
creased frequency of activation. Potassium accumulates in
the extracellular space. The sodium-potassium pump in-
crease its activity, probably because intracellular sodium
concentration is elevated by the increased frequency of
action potentials. Potassium is returned to the cell coupled
to the extrusion of sodium. Potassium influx increases
thereby to match potassium efflux. On cessation of stimu-
lation the increased activity of the sodium pump brings
extracellular potassium activity to levels below that of the
bathing solution because influx is greater than efflux. The
pump activity subsequently declines with time and potas-
sium activity returns to normal with influx equal to efflux.
The results of experiments in which we interfered with
sodium pump activity support this proposal. Unfortu-
nately in cardiac tissue any one of these manipulations
intended to block the pump probably has direct or indirect
membrane effects not mediated through the pump. This
is suggested by various changes in the shape and duration of
the action potential reported by other investigators.$^{9, 14, 18}$
Any contribution of these factors to the shape and time
course of the extracellular potassium curves during and
after continued stimulation awaits analysis of the mem-
brane ionic currents and concentrations under each condi-
tion. In spite of the uncertainty of these influences, all four
methods used either inhibited or decreased the decline and
undershoot phases of the response of extracellular potas-
sium activity to rate increases. Gerlings$^2$ and Gilmore et al.$^{19}$ have shown that the blood-perfused dog heart when made hypoxic showed a sustained potassium loss as rate was increased but that the nonhypoxic heart showed only an initial loss of potassium. This is supported by the effect of low Po$_2$ in the
present experiments. Kriz et al.$^{17}$ also showed that potas-
sium accumulation in cat spinal cord was sensitive to
hypoxia. The sodium pump lag, proposed by Langer$^7$ could, therefore, account for the initial rise and then
delcine in extracellular potassium activity. Gilmore et al.$^{15}$ contend that if the pump lag is involved in the frequency-
dependent loss or gain of potassium it must be sensitive to the rate at which frequency is changed. This they show not to be the case for isolated perfused dog heart. Langer$^{20}$ responded that the pump-lag model need not be sensitive to rate of change. Support of either contention is depend-
ent on knowledge of the kinetics and ion sensitivity of the
pump; this knowledge is not yet available. However, the
experiments presented here indicate that the sodium pump is
at least in part responsible for the decline in extracellular
potassium activity during continued stimulation as well as the
undershoot that follows stimulation because both are
eliminated or decreased by procedures which inhibit the
pump.

The increased pump activity may be initiated by an
increase in intracellular sodium concentration. Although
in squid axon the active sodium efflux is related in a
sigmoidal fashion to [K$^+$]$_o$ up to 100 mm,$^{21}$ this may not be

**Figure 7** The response of potassium in the extracellular space of
to decline toward prestimulation levels while the stimula-
tion is maintained. When stimulus rate is decreased or stimula-
tion terminated, extracellular potassium activity temporarily falls below that of the bathing solution. Similar
changes in potassium activity have been seen in the$
expenses 7x28 to 589x815'$.
the case for cardiac tissue. Haas\textsuperscript{18} showed that dinitrophe-nol (DNP)-sensitive sodium efflux is relatively independent of potassium concentration in the range of 1.35-27 mm. If the undershoot following stimulation is an indication of the pump activity just prior to terminating the stimulation, then, at this time, with the extracellular potassium close to (and occasionally below) the normal (prestimulation) value, the activity of the pump is nevertheless enhanced. Thomas\textsuperscript{22} measured intracellular Na\textsuperscript{+} and showed that the pump rate is primarily sensitive to internal sodium concentration if the extracellular fluid contains at least 1 mm K\textsuperscript{+}.

In the intact heart the actual magnitude of rate-related changes in potassium activity, as stated earlier, will be dependent on the efflux during the action potential, the pump-related influx, the volume of the extracellular space and the diffusion distance to and the barrier presented by the pathway from the cells to the blood flowing within the capillaries. If the capillary wall is not a significant barrier to K\textsuperscript{+} movement, then the shorter diffusion distances between the cells and capillaries in the intact heart, as compared to the superfused preparation described here, tend to minimize the extracellular changes because capillary flow carries away the excess potassium during increases in rate and returns potassium during decreases in rate. On the other hand, an area of the heart receiving a decreased coronary flow would approach and possibly surpass what is seen in the present experiments.

References

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