Resting Membrane Potential, Extracellular Potassium Activity, and Intracellular Sodium Activity during Acute Global Ischemia in Isolated Perfused Guinea Pig Hearts

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SUMMARY. Transmembrane potentials, extracellular potassium activity, and intracellular sodium activity were determined during acute global ischemia in Langendorff perfused guinea pig ventricles by microelectrode techniques. Resting membrane potential decreased with a sigmoidal time course from —82 mV to —49.5 ± 2.7 mV (so, n = 6) and extracellular potassium activity increased from 4 to 5 mM to 14.7 ± 1.3 mM (n = 8) during 15 minutes of ischemia. The estimated potassium equilibrium potential was 7 mV more negative than resting membrane potential prior to occlusion, but approached resting membrane potential during ischemia. An increase in extracellular potassium accumulation occurred when heart rate was increased abruptly from 60 to 170 beats/min. After rapid stimulation, a transient decrease of extracellular potassium activity occurred which was abolished in the presence of 10^{-6} M strophanthinidin. If the preparations were paced before and after aortic occlusion at a constant rate, potassium accumulation was independent of heart rate within a range of 50-170 beats/min. Intracellular sodium activity was 8.8 ± 2.8 mM (n = 8) prior to occlusion and decreased slightly to values between 4.7 and 7.6 mM after 10-15 minutes of ischemia. The results suggest that relative potassium permeability largely predominates over relative sodium permeability during the decrease of resting membrane potential after interruption of aortic flow. Furthermore, active sodium-potassium exchange compensates for the rate-dependent fraction of potassium efflux and maintains a low intracellular sodium activity. For reasons of electroneutrality, the potassium efflux underlying extracellular potassium accumulation must be balanced by an equivalent charge movement which is not carried by sodium. The most probable hypothesis regarding the charge carriers is that net potassium efflux occurs secondary to efflux of phosphate and lactate generated during ischemia. (Circ Res 52: 442-450, 1983)

CHANGES of cardiac electrical activity occur within a few minutes of coronary occlusion: both resting potential and action potential amplitude decrease, upstroke velocity slows, and the action potential shortens (for ref. see Janse and Kléber, 1981). These rapid changes in electrical activity are accompanied by the first phase of a triphasic accumulation of extracellular potassium. This initial phase is rapidly reversible if reperfusion occurs within 15 minutes of occlusion (Harris et al., 1954; Wiegand et al., 1979; Hill and Gettes, 1980; Hirche et al., 1980; Weiss and Shine, 1982). Following the rapid and reversible accumulation, extracellular K+ concentration ([K+]o) remains almost constant for 10-20 minutes before a final irreversible increase occurs. This last phase seems to be due to cell damage, since intracellular water and sodium increase (Schwartz et al., 1973).

The interdependence of metabolic changes, K+ accumulation, shifts of other ions, and the changes of electrical activity in the early phase of ischemia is not fully understood. In a closed system such as global ischemia, total potassium content would be expected to remain constant because no additional potassium can enter, or escape from, the system after circulatory arrest. Thus, the observation that [K+]o increases during global ischemia implies a net loss of K+ from the cells and/or a diminution of the extracellular volume due to a shift of water from the extra- to the intracellular space. The latter may play a role because osmotically active intracellular metabolites are known to be increased during ischemia (Tranum-Jensen et al., 1981). However, the major component of the increase in [K+]o probably is attributable to a net loss of cellular potassium. This, in turn, must result from decreased K+ influx, or increased K+ efflux, or both. If ischemia were to interfere with Na+/K+ pumping and thereby decrease K+ influx, both [K+]o and [Na+] would be expected to rise.

This study explores the relationship between resting membrane potential (RMP) and extracellular potassium activity during the initial phase of acute ischemia. We evaluated the mechanism of K+ accumulation during this phase by determining the effects of several interventions (rapid stimulation; application of strophanthinidin) on the changes in aK and by measuring intracellular Na+ activity (aNa). The results demonstrate that the resting membrane potential approximates the potassium equilibrium potential during
early acute ischemia. Furthermore, the results show that \(a_{K}^{\alpha}\) remains low and \(Na^+/K^+\) pump activity persists during early ischemia.

**Methods**

**Perfused Heart Preparation**

Guinea pigs (weight 900 g) were anesthetized with ether and stunned by a blow to the head. The heart was removed rapidly and transferred to a tissue chamber. The aorta was cannulated and the heart perfused with cold Tyrode’s solution within 150 seconds after removal. Draining cannulas were inserted in both ventricles, and the heart was connected to the final perfusion apparatus and placed horizontally in a recording chamber (Fig. 1). The Langendorff perfusion apparatus incorporated a roller pump to collect the returning venous blood and propel it through an oxygenator and a thermostat back to the heart in a closed loop. The priming volume of the perfusion system was 400 ml. The hearts were perfused at constant pressure (45-55 mm Hg); myocardial flow rates varied between 100 and 150 ml/min per 100 g. The heart was superfused concurrently with the same solution, except for a small epicardial area (diameter approximately 7 mm) which was kept in open air. Temperature varied between 31°C and 33°C, but remained constant during individual experiments. The oxygenator was gassed with a mixture of oxygen and \(CO_2\); the flow rate of \(CO_2\) was adjusted to yield a \(pH\) between 7.34 and 7.37. A conventional glass electrode was used to measure \(pH\) of the perfusate close to the aortic inlet (see Fig. 1). Arterial oxygen content of the perfusate varied between 13 and 15 ml \(O_2\) per 100 ml. A filter (pore size 40 \(\mu\)m) was introduced into the perfusion tubing to prevent embolism by corpuscular fibrin aggregates. Normal perfusion fluid was composed of a mixture of washed bovine erythrocytes (hemoglobin concentration, 8 g/100 ml), dextran (mol wt, 70,000; 4 g/100 ml), insulin (1 U/liter), heparin (400 U/liter) and Tyrode’s solution of the following composition (mm): \(Na^+\), 149; \(K^+\), 4.5; \(Mg^{++}\), 0.49; \(Ca^{++}\), 1.8; \(Cl^-\), 145.8; \(HCO_3^-\), 11.9; \(H_2PO_4^-\), 0.4; and glucose, 20. Final \([K^+]\) in the perfusion fluid varied within a ±0.5 mM range among different experiments, due to potassium uptake by erythrocytes during storage and/or hemolysis. Therefore, in each experiment, \([K^+]_o\) was determined by flame photometry or by an ion-selective probe.

**Preparation and Calibration of Electrodes**

Floating electrodes for recording conventional and ion-sensitive signals were constructed by means of different techniques. Intracellular floating electrodes for the measurement of transmembrane potentials were pulled from borosilicate glass. DC tip resistances were 15-20 \(\Omega\) when filled with 3 mM \(KCl\). The tips of similar borosilicate glass microelectrodes were broken to an outer diameter of 30 \(\mu\)m to prepare floating electrodes for measurements of extracellular reference electrogams. The broken tips were fire-polished to produce a smooth surface with an opening of 3-4 \(\mu\m). This procedure prevented injury to the subepicardial cells of the contracting preparation. Extracellular microelectrodes were filled with a mixture of agar and 0.5 mM KCl, except those which served as a reference for extracellular \(K^+\)-sensitive electrodes which contained agar and 0.5 mM \(Na_2SO_4\).

Potassium-sensitive electrodes were prepared by two different methods. Floating extracellular \(K^+\)-sensitive electrodes for some of the experiments (shown in Figs. 2 and 3) were made from fire-polished micropipettes silanized in an oven (200°C for 30 minutes) with trimethylsilyldimethylamine (TMSDA; Fluka, Switzerland) and backfilled with potassium sensor (Corning 477317). Calibration curves were obtained from measurements of the potential difference between the \(K^+\)-sensitive electrode and a reference electrode in perfusion fluid and in Tyrode’s solution containing varying \([K^+]_o\) but constant total \([Na^+] + [K^+]_o\). In other measurements, \(K^+\)-sensitive electrodes were made from polyethylene tubing pulled to an inner tip diameter of 70-100 \(\mu\m). These tubes were filled with 0.5 mM KCl and sealed at the tip by a Valinomycin-PVC matrix membrane (valinomycin 4%). The fabrication and calibration of these electrodes were the same as described by Hill and Gettes (1978). The Valinomycin electrodes proved to be superior because they exhibited better DC stability and could be stored up to 2 weeks with minimal loss of sensitivity (<10%). An activity coefficient of 0.748 was assumed to evaluate voltages reflecting extracellular \(K^+\) activity (Bailen et al. 1970).

Floating \(Na^+\)-sensitive microelectrodes were pulled from borosilicate glass and beveled to a tip diameter of 1 \(\mu\m). The electrodes were silanized in an oven (200°C for 30 minutes) using TMSDA and stored in a desiccator for several hours to several days. Subsequently, the electrodes

![Figure 1. Experimental arrangement. The perfused heart was placed horizontally in a perspex chamber and also superfused except at the measuring site (area of 7 X 7 mm) which was kept in open air.](image-url)
were backfilled with sodium sensor [10% neutral ligand ETH 227 + 0.5% sodium tetraphenylborate dissolved in o-nitrophenylcrylylether, kindly provided by Dr. Ammann (Steiner et al., 1979)] and calibrated with a set of solutions containing varying [Na+] but constant total [Na+] + [K+]. The ionic composition of the calibration solutions was made similar to that of the sarcoplasm (in mm): Na+ + K+, 142; MgCl2, 2; Tris, 10; pH 7.2. The DC shift of the Na+-sensitive electrodes was less than 3 mV/hour and the selectivity for Na+ vs. K+ was 50:1 (for details see Cohen et al., 1982). An activity coefficient for Na+ of 0.764 was used (Bates et al., 1970) to evaluate the calibration records in terms of Na+ activity. Both ion-selective and conventional microelectrodes were mounted on a fine AgCl-coated silver wire (diameter 20 ìm) to provide more stable records from both extra- and intracellular locations.

**Recording Procedure**

Measurements of extra- and intracellular potentials were made on the air-exposed part of the anterior left ventricular surface. Extracellular electrodes touched the epicardial surface, whereas intracellular electrodes penetrated the epicardium to an estimated depth of 125-200 ìm (Janse et al., 1978; Tranum-Jensen et al., 1982). Recording electrodes were connected to high-input impedance preamplifiers (Burr-Brown 3225 for conventional extra- and intracellular electrodes; Analog Devices 515 for ion-sensitive electrodes) and the signals amplified by a differential instrumentation amplifier. The recorded potentials were stored on an analog tape recorder (Ampex PR 2200) and displayed either on a UV fiberglass recording system (Electronics for Medicine VR 12) or on a pen recorder. K+-Sensitive recordings were filtered for display at low speed (low pass, corner frequency 1 Hz). A bipolar electrode was apposed to the surface of the ventricle for delivery of electrical stimuli that controlled the ventricular rate. In some experiments, the effect of stimulation frequency on extracellular K+ activity was investigated by superfusing the sinoatrial node with cold solution to lower the spontaneous heart rate.

Transmembrane potentials (Vm) were measured as the potential difference between an intracellular microelectrode and an extracellular reference microelectrode placed as close as possible to the impalement site. Extracellular K+ activity (ak) was measured from the potential difference (Vak) between the extracellular K+-sensitive electrode (VKE) and an extracellular reference electrode (Vref). Recordings of Vak showed a small bipolar extracellular electrogram due to the finite distance (<200 ìm) between the K+-sensitive electrode and the intracellular reference. The measurements of Vak were taken during the T-Q segment of the extracellular electrogram. Intracellular Na+ activity (akNa) was measured from the potential difference (VkNa − Vm) between a Na+-sensitive intracellular electrode (VKC) and a conventional 3 M KCl intracellular electrode (VNa). A single extracellular floating micropipette served as extracellular reference for both intracellular measurements (Vm and VNa). Both intracellular electrodes were impaled within a distance of 200 ìm. In preliminary experiments, an attempt was made to employ double-barreled floating electrodes in the hope of obtaining measurements from single cells. Unfortunately, the inertia of the large double-barreled tips did not allow stable intracellular measurements in the contracting preparation due to cell damage.
Several possible problems with the measurement techniques were addressed. The decline of $P_{O_2}$ from the air surface interface into the subepicardial cell layers was calculated because oxygen diffusion from the air to the site of recording theoretically could affect the results during ischemia. $P_{O_2}$ declines as a parabolic function of distance depending upon oxygen consumption and the permeation coefficient for oxygen (see Tsacopoulos et al., 1981). Partial pressure of oxygen is expected to fall from a level of 147 mm Hg at the surface to below 5 mm Hg at a depth of 90 $\mu$m. Since the estimated depth of electrode penetration is greater, it seems unlikely that oxygen diffusion into the myocardium could have affected the results during ischemia. In addition, several experiments were performed to determine whether the epicardial connective tissue sheath is a diffusion barrier for $K^+$ ions. The thin layer of tissue was removed and $a^K$ measured. No significant difference was noted between $a^K$ with the surface removed or intact.

**Calculation of Potassium Equilibrium Potential**

The potassium equilibrium potential ($E_K$) was calculated from the measured values of extracellular potassium activity as follows:

$$E_K = 60.3 \text{mV} \times \log \frac{a^K}{a^{K,\text{in}}} - f \times \Delta a^K$$

where 60.3 mV = Nernst slope for a temperature of 31°C; $a^K$ represents an assumed value of 100 mm for initial intracellular potassium activity (see discussion); $\Delta a^K$ is the difference between the actual $a^K$ and the extracellular $K^+$ activity prior to occlusion (i.e., the extra potassium accumulated); and $f$ is the extracellular to intracellular volume ratio assumed to be 0.33 (Page, 1962).

**Results**

**Relationship between Changes in Resting Membrane Potential and Extracellular Potassium Activity**

In nine experiments, $a^K$ was measured simultaneously with resting membrane potential (RMP) during a 15-minute period of ischemia. The ventricular rate was 150 to 180 per minute in all experiments. Figure 2A shows the result of an experiment in which transmembrane potential and $a^K$ were recorded continuously from an epicardial site between 2 and 6 minutes after coronary occlusion. The gradual increase of $a^K$ was accompanied by a decrease in RMP and action potential amplitude. Electrical alternans, which has been described by several investigators (see Janse and Kleber, 1981), occurred between 4.5 and 5.5 minutes.

The time course and magnitude of changes in RMP and $a^K$ during ischemia were similar in all nine experiments (see Fig. 3). Resting membrane potential was $-81.7 \pm 2.0$ mV (SD) prior to occlusion. After 8 minutes, RMP was $-55.3 \pm 3.0$ mV and by 15 minutes, $-49.5 \pm 2.7$ mV. Extracellular $K^+$ began to rise within the first minute of occlusion and reached average values of 11.0 ± 2.0 mm and 14.7 ± 1.3 mm after

**FIGURE 4. Effects of an abrupt change in heart rate on $a^K$ during three different time intervals: prior to occlusion, during occlusion, and during reperfusion. Upper trace: original recording of potential sensitive to $a^K$ ($V_{ap}$). Lower trace: $a^K$ calculated from $V_{ap}$. The spontaneous heart rate (60 beats/min) was increased abruptly to 170 beats/min for the times indicated by the bars. In the perfused preparation, the time course of reversion of $a^K$ to the steady state level during and after stimulation (left half of figure) suggested delayed activation and inactivation of the $Na^+/K^+$ pump. Rapid stimulation during ischemia (right half of figure) caused a more pronounced increase in $K^+$ accumulation. The decline of $a^K$ after stimulation implies that $Na^+/K^+$ pump activity resulted in a net uptake of $K^+$ from the extracellular space.
8 and 15 minutes, respectively. Prior to occlusion, the average potassium equilibrium potential was calculated to be −89.2 mV, 7.5 mV more negative than RMP. \( E_K \) approached RMP after 5 minutes of ischemia at an \( a_K \) of 9 mM. Fifteen minutes after the onset of ischemia, \( E_K \) averaged −49.4 mV, virtually identical to the average RMP. However, this apparently accurate relationship should be interpreted with caution since the calculated \( E_K \) depended on the assumed initial value of 100 mM for \( a_K \) (see Discussion).

**Effects of Heart Rate on Extracellular Potassium Accumulation**

Abrupt changes in heart rate lead to fluctuations of extracellular potassium activity in different isolated cardiac tissues, caused by the delay between the rate-dependent change of K\(^+\) efflux and active, pumped K\(^+\) influx. Therefore, changes in heart rate provide a means to study activation and inactivation of the Na\(^+\)/K\(^+\) pump (Kunze, 1977; Kline and Morad, 1978). In three experiments, spontaneous heart rate was lowered to 60/min by local superfusion of the right atrium with cold perfusate. As shown in Figure 4, a sudden increase in heart rate to 170/min during normal perfusion resulted in a transient increase of \( a_K \) followed by a decrease toward the former steady state level. A transient undershoot of the steady level of \( a_K \) was observed after the train of rapid stimuli, consistent with delayed inactivation of active K\(^+\) influx. Both the time course and magnitude of changes in \( a_K \) were similar to those reported from isolated cardiac tissue (Kunze, 1977). After aortic occlusion, rapid stimulation led to a pronounced K\(^+\) accumulation. The rate of accumulation was fastest immediately after the onset of stimulation followed by a gradual slowing. A transient decline of \( a_K \) was again present after rapid stimulation. Since extracellular washout was prevented during the period of coronary occlusion, this transient depletion (1.2 mM) is assumed to reflect a net potassium movement from the extracellular space.

In two experiments, strophanthidin (10\(^{-6}\) M) was added to the perfusion fluid in order to assess the contribution of changes in active K\(^+\) influx to the variation of \( a_K \) before and after coronary occlusion (Fig. 5). Measurements were performed 30 minutes after the administration of the cardiac steroid. The experimental procedure was the same as described for Figure 4. The left part of Figure 5 shows that the initial increase of \( a_K \) after the onset of stimulation (170/min) was not followed by a delayed decrease. Instead, \( a_K \) remained at a level 1 mM higher than \( a_K \) of the perfusion fluid. After rapid stimulation, \( a_K \) declined to the former baseline level without transient undershoot. During ischemia (Fig. 5, right), rapid stimulation (170/min) caused an immediate increase in the rate of K\(^+\) accumulation and there was no distinct decline in the rate of K\(^+\) accumulation during rapid stimulation, in contrast to experiments without strophanthidin (e.g., Fig. 4). Furthermore, there was no decline in \( a_K \) following cessation of rapid stimu-
rate was increased abruptly to 170/min, 5 minutes prior to occlusion. In the latter case, extracellular K⁺ activity initially rose but then declined to approach the former steady state level before occlusion (cf. Fig. 4). During ischemia, the time course of ak was almost identical at both rates. This result, confirmed in two other experiments, suggests that potassium accumulation during early ischemia is largely independent of heart rate (within a 50 to 170/min range), providing active K⁺ influx is allowed to reach a steady state prior to occlusion, and heart rate during occlusion is constant.

**Intracellular Sodium Activity during Acute Ischemia**

Intracellular sodium activity (aka) was measured before and after aortic occlusion in eight experiments. These experiments tested the hypothesis that the net movement of potassium ions from the intracellular to the extracellular space is compensated by an inward movement of sodium ions. Intracellular Na⁺ activity was determined by means of two separate microelectrodes impaled within a distance of 200 μm. With this technique, measurements were obtained before and at different times after interruption of aortic flow. Figure 7 shows original recordings of transmembrane potential (Vm) and potential measured by the Na⁺-sensitive electrode (VNa). Two simultaneous records before aortic occlusion are given in panel A. Resting membrane potential (RMP) was −81 mV; 35 sec after the impalement VNa was −136 mV. The potential difference (Vdiff) between VNa and RMP was −55 mV, corresponding to aNa of 9.9 mM. In panel B, recordings from the standard intracellular microelectrode (upper trace) and the Na⁺ electrode (lower trace) are displayed at a rapid paper speed. The action potential recorded by the Na⁺ electrode was distorted by its low frequency response. The potential reached a steady resting level only 400 msec after the upstroke. This poor electrical response time limited the range of heart rates over which aka could be measured. In panel C, simultaneous recordings of Vm and VNa are depicted, from the same experiment, after 15 minutes of ischemia. Vm was −52 mV and VNa −112 mV; Vdiff of −60 mV corresponded to an aka of 7.6 mM, i.e., 2.3 mM less than that prior to aortic occlusion. The combined results of eight experiments (in seven preparations) are summarized in Figure 8. Heart rate varied between 70 and 120/min in the different experiments. Resting membrane potentials and aka are given for each experiment; up to four successful simultaneous impalements were obtained in a single experiment. The time course of RMP was comparable to the data presented in Figure 3. RMP decreased from a value of −79 ± 3.6 mV to values ranging from −47 to −54 mV between 10 and 15 minutes after aortic occlusion. During normal perfusion, the mean aka was 8.8 ± 2.8 mM (so, n = 8). During ischemia, no increase or a slight decrease in aka was recorded. Between 10 and 15 minutes after aortic occlusion, when resting membrane potential was approximately −50 mV, eight measurements (five experiments) were obtained, ranging from 4.7 to 7.6 mM. Mean aka before occlusion from these five experiments was 7.9 mM.

From the resting membrane potentials shown in Figure 8 and the relationship between RMP and aka (Fig. 3), a theoretical estimate was made for the in-
increase of $\Delta V_{\text{K}}$, which should occur if extracellular K$^+$ accumulation was fully compensated by intracellular Na$^+$ accumulation. Thus, depolarization to $-50$ mV (see Fig. 3) was accompanied by an increase in $aK$ of 11 mm. Therefore, assuming an extra- to intracellular space ratio of 0.33, a 3.6 mm increase of $aK$ should have occurred after 10 to 15 minutes of ischemia. For the type of Na$^+$ electrodes employed, an increase of $aK$ of this magnitude (i.e., from 8 to 11 mm) would be expected to change $V_{\text{K}}$ by approximately 8 mV. A potential difference of that size certainly should have been detectable with these methods but was not observed.

**Discussion**

Intracellular potentials and extracellular K$^+$ activity were measured simultaneously in guinea pig hearts to evaluate the relationship between resting potential and the potassium equilibrium potential after cessation of myocardial perfusion. A model of global ischemia was used to reduce spatial differences that occur with regional ischemia. The results demonstrate a magnitude and time course for changes in $aK$ during ischemia similar to those reported for intramural $aK$ during ischemia in vivo (Hill and Gettes, 1980). This close agreement for $aK$ suggests that local variations in ischemic metabolism, evaporation from the epicardial surface, and $O_2$ diffusion into the air-exposed ventricle were unlikely to have affected the results significantly. In addition, the decrease in resting potential described qualitatively in the perfused pig heart (Downar et al., 1977) and in isolated measurements during regional ischemia (Kléber et al., 1978) has been verified and more precisely defined by the present results. Moreover, the results reveal that changes in resting potential and $aK$ both follow a similar sigmoidal time course, suggesting (not surprisingly) a close relationship between the transsarcolemmal K$^+$ distribution and the resting potential. This relationship can be evaluated by comparing RMP with estimates of EK. The results demonstrate a closer approximation of RMP to EK as ischemia progressed (the two values concurring around $-50$ mV). The close correspondence between EK and RMP arises partly because the initial $aK$ was assumed to be 100 mm. This was not an unreasonable assumption, since measured values for guinea pig papillary muscle (99 mm, Wier, 1978; 106 mm, Baumgarten et al., 1981) and the mean of many of the reported values for $aK$ (86 mm, Lee and Fozzard, 1975; 116 mm, Cohen et al., 1982) are close to this value. Deviations of initial $aK$ ($\pm 10$ mm) would have shifted EK by $\pm 2$ to 3 mV but would not have affected the conclusion that EK is closer to RMP during ischemia than during normal perfusion. In addition, since total tissue K$^+$ is constant in the absence of extracellular washout, changes in $aK$ during ischemia were estimated (3–4 mm after 15 minutes of occlusion) and allowed for in the calculation of EK. A similar relation between RMP and EK at elevated [K$^+$], has been described in normoxic myocardium (Lee and Fozzard, 1975).

The implication of a close relation between EK and RMP during ischemia is that relative K$^+$ permeability ($P_K$) predominates over relative Na$^+$ permeability ($P_{Na}$). The $P_K/P_{Na}$ ratio may be even higher in ischemic than normal myocardium at elevated $aK$. Comparison of upstroke velocities and action potential amplitudes during ischemia and during perfusion with increased $[K^+]_o$ suggests that potassium conductance is increased and/or fast inward sodium current is decreased in myocardial ischemia (see Janse and Kléber, 1981). Additionally, Vleugels et al. (1980) have measured an increase in slope conductance at EK and time-independent K$^+$ outward current during hypoxic perfusion.

A major emphasis of the present study was to investigate the mechanism underlying the reversible increase in $aK$ that occurs during the first 15 minutes of ischemia. The current findings, together with those of Tranum-Jensen et al. (1981), would suggest that the increase in $aK$ can be attributed largely to a net efflux of K$^+$ from the myocardial cells. Certainly, a shift of water from the extra- to the intracellular space must be discussed as a possible factor contributing to the increase in $aK$. Such a shift is expected because new osmotically active particles (e.g., lactate, phosphate) are formed in the intracellular compartment during ischemic metabolism. On the other hand, quantitative considerations argue that transsarcolemmal water movements influence $aK$ to a rather small extent. Thus, Tranum-Jensen et al. (1981) measured a 20 mOsm increase in extracellular osmolality after 15 minutes of ischemia in Langendorff perfused pig hearts. In the worst case (i.e., no new osmotically active particles added to the extracellular space), the water shift required to account for that increase in extracellular osmolality would amount to a 6% loss of extracellular fiber water and a concomitant 2% gain of intracellular fiber water. This calculation certainly argues against a significant effect of extra- to intracellular water movement, since an 80% loss of extracellular volume would be required to explain a rise in $aK$ from 3 to 14.7 mm. Thus, the rise in $aK$ during ischemia must have resulted from a net loss of K$^+$ from the cells.

The relative contributions of changes in passive K$^+$ influx and in active K$^+$ influx to the potassium accumulation were studied by measuring the effect of heart rate on $aK$ under two conditions: (1) during and after rapid stimulation and (2) during constant stimulation at different rates. The first setting allowed discrimination between instantaneous changes of K$^+$ influx and delayed variations in active K$^+$ influx (Kunze, 1977; Kline and Morad, 1978). Rapid stimulation during ischemia resulted in an immediate increase in the rate of change of $aK$, reflecting an increase of influx at the high rate of stimulation. The slowing in the rate of rise of $aK$ that developed during stimulation and the subsequent K$^+$ depletion after stimulation strongly suggest that the mechanism for active K$^+$ inward transport, i.e., the Na$^+$/K$^+$ pump, remains at least partially functional during ischemia. This interpretation is supported by the blocking effect
of $10^{-6}$ M strophanthidin on these changes. Similar effects of rapid stimulation on $\Delta_{ak}$ have been reported recently by Weiss and Shine (1982).

In a second series of experiments, K$^+$ accumulation was measured at constant stimulation rates of 50 and 170 beats/min. If K$^+$ influx (at 170 beats/min) was allowed to reach a steady state prior to occlusion, $\Delta_{ak}$ was found to be independent of heart rate during ischemia. This result suggests that, even during ischemia, active K$^+$ inward transport is still capable of fully compensating for the frequency-dependent increase in K$^+$ efflux between rates of 50-170 beats/min, and it is in accordance with measurements reported from in situ pig and dog hearts (Wiegand et al., 1979; Hill and Gettes, 1980). However, the findings differ from the results of Weiss and Shine (1982), who found an increase in extracellular K$^+$ accumulation if heart rate was changed from 60-120 beats/min prior to circulatory arrest and kept constant during the ischemic period. Any explanations for this discrepancy are necessarily speculative, but it seems possible that the relatively low O$_2$ content of their perfusate (Tyrode’s solution, no oxygen carrier) could have limited the maintenance of oxygen delivery during the increased oxygen consumption expected at higher rates. Such a situation might lead to changes in intracellular metabolism prior to interruption of the circulation (e.g., decrease in levels of energy-rich phosphates, intracellular acidosis) which could then influence extracellular K$^+$ accumulation during the subsequent ischemic period.

In the final series of experiments, intracellular Na$^+$ activity determined prior to occlusion (8.8 ± 2.8 mm) agreed with values obtained in sheep and guinea pig ventricular strands measured with the same type of Na$^+$-sensitive ligand (7.9 mm, pacing at 0.5 Hz; Cohen et al., 1982). The observation that $\Delta_{aN}$ remained low (4.7-7.6 mm, after 10 to 15 minutes of ischemia) suggests that the normal regulation of active Na$^+/K^+$ exchange by extracellular K$^+$ and intracellular Na$^+$ is little affected in early ischemia. In normally perfused tissue, changes in the activity of the Na$^+$/K$^+$ pump are due mainly to variations in $\Delta_{aK}$ because pump activation by [K$^+$]o becomes saturated at [K$^+$]o above 4-5 mm (Glitsch et al., 1976; Gadsby, 1980; Glitsch et al., 1981). Therefore, when $\Delta_{ak}$ is elevated, only those changes in K$^+$ influx linked to concomitant changes in Na$^+$ influx involve a change in the activity of the Na$^+$/K$^+$ pump (e.g., rate-dependent fraction of K$^+$ efflux, see Fig. 6). Depolarization of the cells possibly may contribute to a low $\Delta_{aK}$ during ischemia. A change of RMP by +30 mV after 15 minutes of ischemia reduces the electrochemical gradient for Na$^+$ ($E_{Na} - E_m$, assumed $E_{Na} = +60$ mV). This, in turn, diminishes the energy requirement by 22% for maintenance of $\Delta_{aN}$ in the static situation, i.e., when changes in Na$^+$ influx are neglected. Measurements of energy-rich phosphate compounds indicate that ATP levels decrease relatively slowly during the initial phase of ischemia. Furthermore, ADP is largely converted to ATP and AMP, the thermodynamic buffering systems and glycolysis tending to maintain a high ATP potential (Jennings et al., 1981). In addition, the effect of a potential-dependent change of Na$^+$ influx on $\Delta_{aN}$ should be considered. This has been studied in sheep Purkinje fibers where depolarization induced a decrease in Na$^+$ influx and, hence, in $\Delta_{aN}$ (Eisner et al., 1981).

For reasons of electroneutrality, the net efflux of potassium ions must be balanced either by an equivalent influx of cations or by an equivalent efflux of anions. The present results exclude the possibility of a net influx of sodium, but they do not provide direct information concerning the identity of the charges which do maintain electroneutrality. For instance, an increase in intracellular Ca$^{2+}$ cannot be excluded. However, even inward movement of all Ca$^{2+}$ ions available in the extracellular space would not be sufficient to compensate for the millimoles of extracellular K$^+$ accumulated. Moreover, a significant shift of Ca$^{2+}$ ions into the intracellular compartment would be expected to increase resting tension, an effect not observed by Weiss and Shine (1982). Therefore, the most probable hypothesis is that net K$^+$ efflux occurs concomitantly with efflux of anions. In fact, such a mechanism is suggested from experiments in which anaerobic metabolism was induced by severe restriction of coronary flow. Analysis of venous effluent in this situation demonstrated that the time course of the increase in potassium, phosphate, and lactate was identical. The quantity of lactate and phosphate even exceeded that of potassium, probably by the amount of protons released (Mathur and Case, 1973). Loss of potassium associated with efflux of anions also could have its counterpart in skeletal muscle brought into a state of fatigue at low extracellular pH. Under such conditions, the resulting loss of intracellular lactate ions is greater than the efflux of H$^+$ (Mainwood and Worsley-Brown, 1975), and an increase in net K$^+$ efflux also occurs (Mainwood and Lucier, 1972). Mainwood and Lucier (1972) provided a hypothesis which might explain the relationship between intracellular metabolic acidosis and net potassium efflux during ischemia. Generation of weak acids inside the cells by breakdown of energy-rich phosphates and glycolysis will result in binding of the protons to negative charges of intracellular proteins which may have a buffer capacity as high as 60 mm per unit pH and per kg sarcoplasmic fluid (Heisler and Piiper, 1971). A loss of permeant anions so formed, associated with an efflux of cations, is then to be expected as a result of the decrease in fixed negative charges and the rise in freely movable anions (Boyle and Conway, 1941). Decrease of myocardial intracellular pH during ischemia, determined indirectly by nuclear magnetic resonance (Garlick et al., 1979), showed the same time course as the rise in $\Delta_{ak}$, further supporting the above hypothesis of anion loss.

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