Potassium exchange and mechanical performance in anoxic mammalian myocardium

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Potassium exchange and mechanical performance in anoxic mammalian myocardium. Am. J. Physiol. 232(1): H85-H94, 1977 or Am. J. Physiol.: Heart Circ. Physiol. 1(1): H85 H94, 1977. — Mechanical performance and "K exchange were studied during anoxia in isolated, arterially perfused, interventricular rabbit septa at 42 beats/min and 28°C. The septa were perfused at 1.8 ml/min per g with a modified Tyrode solution having dextrose as the metabolic substrate. Developed tension declined to 16% of preanoxic control values during 60 min of anoxia, and returned to 65% of control during a 60-70 min recovery. Anoxia induced net potassium losses of 31 ± 2, 53 ± 2, and 90 ± 14 mmol K+/kg dry tissue (means ± SE) during 20, 40, and 60 min of anoxic stresses, determined by tissue probe analysis after asymptotic labeling. Potassium losses attributed to increased efflux of the ion from the cells during 20, 40, and 60 min of anoxia were determined from effluent analyses to be 32 ± 4, 60 ± 6, and 98 ± 11 mmol K+/kg dry wt. Potassium loss began within seconds of the onset of anoxia and reoxygenation immediately reversed the potassium loss. These data indicate that 1) function of the membrane Na-K pump is maintained through 60 min of anoxia with the entire net potassium loss attributable to increased efflux from the cells, 2) anoxia decreases the rate of exchange of potassium into the cells, an increased efflux of potassium out of the cells, or a combination of these two mechanisms. The objective of this study was to identify the mechanisms operating to control potassium exchange in perfused, anoxic, ventricular mammalian myocardium. A selective effect of anoxia on potassium efflux from the cells, with preservation of active transport of potassium during oxygen deficiency for periods up to 60 min, was demonstrated.

Experimental and Methods

Experimental preparation. The experimental preparation was the isolated, arterially perfused, interventricular septum of the male New Zealand white rabbit (1-2 kg) (16). The rabbit was heparinized (10,000 USP U) and anesthetized (180 mg pentobarbital) intravenously. The heart was rapidly excised through a sternotomy and placed in a 30-37°C oxygenated perfusate. The right ventricular wall and both atria were removed. The septal artery branch of the left coronary was cannulated with a PE-50 polyethylene cannula (Clay Adams), secured with the suture and perfused at a constant rate by a Harvard pump. The elapsed time from thoracotomy until the aorta was severed was 5-10 s, and the septal artery was routinely cannulated within 2-4 min. Approximately 80-100% of the septum was normally perfused, with delineation of the perfused area being easily determined by visual observation. A triangular piece of well-perfused tissue (0.7-1.1 g) was carefully dissected from the remaining heart and suspended by silk suture from the apex to a force transducer (Statham, model UC4) mounted on a geared slide that allowed alteration of the resting tension (Fig. 1). The base of the tissue, with the cannula to the lower left, was clamped between two opposing Harmon forceps, also mounted on geared slides. The transducer recorded only that vector of tension developed along its axis, but this proportion of the total tension remained constant throughout each experiment. A septum was accepted for study if a minimum of 15 g tension was developed at a resting tension of 7 g and remained stable. The temperature of the perfusate was maintained by passing a current through a 200 Ω resistor placed around the stainless steel needle immediately adjacent to the polyethylene cannula. Electrodes from a stimulator (Grass, model SD9) were attached to the Harmon forceps. A needle thermistor (Yellow Springs Instrument) was used to monitor core temperature of the septum. After cannulation and mounting, a dome was lowered over the septum into which nitrogen was passed (4 liter/min) by bubbling it through a water-containing well wrapped with heating coils. A thermistor placed in the water allowed feedback control to the heating coils' power supply (RFL Industries, /87/2 temperature controller).

This preparation allowed complete control of the envi-
Fig. 1. Diagram of apparatus for septal preparation. Front of hood has been removed to facilitate visualization. Septum is shown in position.

The septum was stimulated with 4 V, 10 ms duration pulses, at a rate of 42/min. The diastolic tension was 4-7 g. The tissues were perfused at 1.8 ml/min per g with solutions described below and under these conditions they remained stable for 7-9 h. Mechanical performance, ionic and water content reached a steady state in the preparation, after a gain of water and a loss of potassium, over the initial 60–120 min of perfusion (13). It was possible to record a number of parameters continuously and simultaneously: a) developed tension (DT), maximal rate of tension development (+dP/dt), maximal rate of relaxation (−dP/dt), rest tension (RT), and time-to-peak tension (TPT); b) isotopic activity of the whole muscle, monitored by a lead collimated Geiger-Muller probe (Ion Nuclear Medical, model 6222) opposing the tissue at a distance of 1–2 cm; c) radioisotopic activity of the venous effluent during washout of isotope from the previously labeled muscle.

Solutions and chemicals. The control solution for perfusion contained (in millimoles per liter): NaCl, 130; KCl, 4; NaH2PO4, 0.435; dextrose, 5.6; CaCl2, 1.5; MgCl2, 1; and NaHCO3, 12. After equilibration of the solution with 98% O2-2% CO2 (or 98% N2-2% CO2) the final pH and Pco2 were 7.3-7.4 and 13-17 mmHg at 28°C. Respiratory acidosis was induced by equilibration of the control perfusate with 70% O2-30% CO2 (or 70% N2-30% CO2), which obtained pH and Pco2 values of 6.4 and 225 mmHg, respectively, at 28°C. A high Pco2 solution (30-35 mmHg), relative to the control solution at the control pH of 7.3-7.4, was obtained by equilibrating a modified perfusate containing 28 mM NaHCO3 and 114 mM NaCl to maintain a constant sodium concentration with 95%-5% gases. 42K (New England Nuclear) was made up in an amount of potassium-free perfusate calculated to maintain a final concentration of 4 mM potassium.

Measurements and calculations. Oxygen content of the perfusate and the dome atmosphere were determined routinely on a Lex-O-Con oxygen analyzer (Lexington Instruments). Control perfusate equilibrated with 98%-2% gas contained 2.2-2.7 vol % oxygen, while the oxygen level in the anoxic perfusate sampled at the cannula tip was nondetectable (less than 0.1 vol %). Detection limits of the analyzer were verified by the Van Slyke technique. The dome atmosphere oxygen content during experiments was 2-3 vol %. The pH and Pco2 of
the perfusion solutions were determined on a Radiometer-Copenhagen blood gas analyzer at 37°C and converted to the tissue temperature of 28°C.

The tissue water content was determined as percent water at the end of each experiment after the excess fluid was carefully blotted and the tissue was dried at 100°C for 24 h. Quantitation of tissue potassium content and alteration in the content were determined by radioisotopic techniques from uptake and washout curves. The dried tissue was digested in warm concentrated nitric acid, distributed evenly over 20 planchets and dried. A sample of the labeled perfusate with the known potassium concentration of 4 mM was distributed evenly among 8 planchets and dried. Both groups of planchets were counted with a Geiger-Muller probe (Ultrascaler II, Nuclear-Chicago) and corrected for decay and background. The potassium concentration (mmol K+/kg dry tissue) of tissue, labeled until an asymptote was reached, was calculated from the specific activity of the perfusate and the activity of the digested muscle, and the net loss of potassium in response to anoxia was determined as a percentage of the content calculated from the tissue digest. When the effluent was analyzed for activity during isotopic washout of a previously labeled septum, it was collected, droplet by droplet, in individual planchets as it fell from the base of the septum. The elapsed time from the beginning of the washout until the drop was collected and the time required for formation of each droplet were recorded. The isotopic activity of each planchet was counted, corrected for decay and background, and plotted semilogarithmically as counts per minute per minute (counts/min²). Tissue probe activity was also corrected and plotted semilogarithmically as counts/min.

The quantity of potassium lost by efflux during a stress was calculated by integrating the altered area under the effluent curve (7). Extrapolation of the control as the base line during anoxia would underestimate the altered area under the curve, since at any time during the stress less 42K would remain in the tissue than would be predicted by the control. An advantage of the technique is that the base line for the loss by efflux can be determined from the decrease in the radioactivity of the whole tissue, as monitored by the tissue probe. Although a decrease in the counts per minute registered by the tissue probe at asymptote must represent a decrease in the net tissue potassium and cannot express an altered exchange rate, measurement of the isotopic activity of the effluent presents the rate and amount of 42K efflux from the tissue and may reflect the combined effects of an altered rate of exchange as well as a net tissue loss of the ion. Under steady-state conditions the rate of exchange of potassium is represented by the rate of efflux of 42K during washout and is calculated as ln 0.5/t1/2, where t1/2 represents the half time of the washout.

Experimental procedure. A 120-min equilibration period was allowed to elapse before any experimental intervention. Anoxia was induced by switching to oxygen-free perfusate. In uptake experiments the tissue was labeled to 42K asymptote within 150–200 min. The specific activities of the 42K solutions used in an experiment were identical. For an isotope washout experiment, the tissue was labeled with 42K for 50–80 min prior to being switched to the nonlabeled solution to begin the washout. The control washout was continued for 30–40 min to permit accurate determination of the rate of potassium exchange before the tissue was made anoxic. Following the anoxic stress, the rate of exchange was measured after sufficient time elapsed for the tissue potassium levels to reach a new steady state, as determined from asymptotic uptake experiments.

Two 20-min anoxic stresses in the same muscle, separated by a 60- to 90-min recovery period, were induced during three uptake experiments to evaluate the sequential net potassium losses. Identical experiments, with the exception that the second stress took place during the washout, were performed in two septa to utilize a paired experimental design in comparing the net potassium losses (derived from asymptotic uptake) and potassium losses attributable to increased efflux (derived from washout curves). All other experiments were completed in separate septa. The role of anoxia-induced catecholamine release on potassium exchange was examined in two catecholamine-depleted septa.

Two hours prior to sacrifice 6-hydroxydopamine hydrobromide (Sigma) was administered intravenously (100 mg free amine/kg body wt). Catecholamine depletion was verified by showing no inotropic response during perfusion with a solution containing 1 × 10⁻⁴ M tyramine (Sigma), a concentration that had demonstrated a significant inotropic effect in control septa.

Experiments required 3.5-7 h for completion. At the end of each experiment the tissue was removed, blotted and weighed for later determination of water and isotopic content. The radioactive background for the probe was determined by removing the septum and counting the tissue remaining on the suture and between the forceps. A sample of the isotopic perfusate was retained for counting.

Results are expressed as means ± SE. Differences between groups of results were analyzed using the nonparametric randomization test for paired or independent samples. When the n was large, the Mann-Whitney U test was substituted for the randomization test. This work was completed between August and March and between the hours 0800–2000 of the day.

RESULTS

Mechanical. Responses of the mechanical performance parameters, developed tension, maximal rates of contraction and relaxation, time-to-peak tension, and rest tension of 16 septa to 60 min of anoxia are presented in Fig. 2. With the onset of anoxia DT, −dP/dt, and TPT fell rapidly, whereas +dP/dt was maintained and actually increased during the first 2 min of the stress. The decline of DT, +dP/dt, and −dP/dt were essentially symmetrical throughout the 60 min of anoxia after +dP/dt began to fall. During anoxia RT increased. The responses of the mechanical performance parameters to the 20 min of anoxia were unaffected by catecholamine
depletion with 6-hydroxydopamine. DT, \( +dP/dt \), and \( -dP/dt \) recovered symmetrically upon reoxygenation after the 20–60 min of anoxia. However, the magnitude and rate of recovery were depressed following the 60 min of stress compared to the recovery after the 20 min of anoxia. Reoxygenation induced a marked decrease in RT. Comparison of the recovery patterns following 20 and 60 min of anoxia are summarized in Table 1.

**Potassium exchange.** The in vivo potassium content of rabbit myocardium is approximately 360 mmol K\(^+\)/kg dry tissue (13). The mean tissue potassium content of 48 septa perfused for 3–7 h under control conditions was 294 ± 6 mmol K\(^+\)/kg dry tissue as determined from radioisotopic uptake and washout curves, representing a loss of approximately 18%.

Figure 3 illustrates the typical effect of 20 min of anoxia on the potassium content of a septum labeled to asymptote. Eight septa made anoxic for 20 min demonstrated a mean net loss of 31 ± 2 mmol K\(^+\)/kg dry tissue. The effects of 20 min of anoxia on the efflux of potassium were evaluated in four septa by inducing anoxia during a washout. The elevated appearance of \(^{42}\)K in the effluent was calculated to represent 32 ± 4 mmol K\(^+\)/kg dry tissue lost by efflux, which was not significantly different from the net loss of 31 ± 2 \( (P, 0.808) \) derived from asymptotic labeling. Three of the septa that were labeled to asymptote and that demonstrated a mean net loss of 30 ± 5 mmol K\(^+\)/kg dry tissue were allowed to recover for 60–90 min and then made anoxic for a second 20-min period. They demonstrated a mean net loss of 31 ± 7 mmol K\(^+\)/kg dry tissue. Paired experiments completed in two septa in which a 20-min anoxic stress at asymptote was followed by a 60- to 90-min recovery and a second 20-min stress during the washout showed a

**FIG. 2.** Responses of mechanical performance parameters to anoxia. Anoxia was induced at time 0. Reoxygenation was begun at 60 min. Values are means ± SE \((n, 16)\).

**TABLE 1. Recovery of mechanical performance parameters after anoxia**

<table>
<thead>
<tr>
<th>Anoxia</th>
<th>Max % recovery</th>
<th>Time to max recovery, min</th>
<th>Time to ½ max recovery, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Min</td>
<td>86</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>60 Min</td>
<td>65</td>
<td>43</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Maximum +dP/dt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Min</td>
<td>83</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>60 Min</td>
<td>70</td>
<td>65</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Maximum -dP/dt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 Min</td>
<td>83</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>60 Min</td>
<td>70</td>
<td>62</td>
<td>12</td>
</tr>
</tbody>
</table>

Values are means.
FIG. 3. Effect of 20 min of anoxia and recovery on tissue potassium levels. Total tissue $^{42}$K activity is shown with calculated potassium content (mmol K+/kg dry wt) at control, anoxia, and recovery times. Simultaneous mechanical performance envelopes are also presented. Note return of potassium levels during recovery to greater than control.

FIG. 4. Effect of 20 min of anoxia on tissue potassium levels during respiratory acidosis. Total tissue $^{42}$K activity is shown with calculated potassium content (mmol K+/kg dry wt) at control, during acidosis, and at end of anoxia. Simultaneous mechanical performance envelopes are also presented. Acidosis was induced at vertical arrow. Note increase in potassium content in response to acidosis.

mean net loss of 35 ± 4 mmol K+/kg dry tissue calculated from the asymptotic uptake, and a loss of 33 ± 8 calculated from the washout and attributed to elevated efflux of the ion.

Previous studies (13, 14, 16) have shown that elevated Pco2, respiratory acidosis, or elevated Mg++ levels eliminate the potassium loss associated with glycoside treatment and attributed to elevated efflux of the ion. Four septa, labeled to $^{42}$K asymptote with a solution containing 10–15 mM Mg++ and made anoxic for 20 min, showed a net loss of 26 ± 3 mmol K+/kg dry tissue, not significantly different from that of the untreated septa (0.214 < P < 0.281). Four septa made anoxic for 20 min with a solution containing an elevated Pco2 (30–40 mmHg, pH 7.3–7.4) demonstrated a net loss of 38 ± 1 mmol K+/kg dry tissue (P, 0.072). Two septa labeled to asymptote with control perfusate and stressed for 20 min with anoxic respiratory acidotic solutions (Fig. 4) demonstrated a net loss of 33 and 46 mmol K+/kg dry tissue (P, 0.266). Two catecholamine-depleted septa from rabbits pretreated with 6-hydroxydopamine demonstrated a net potassium loss of 39 and 40 mmol K+/kg dry tissue during 20 min of anoxia (0.044 < P < 0.088).

The effects of 40 and 60 min of anoxia on potassium
exchange were studied in 18 septa. The mean net loss of 53 ± 2 mmol K+/kg dry tissue during 40 min of anoxia (derived from asymptote uptake curves), and 60 ± 6 mmol K+/kg dry tissue during 60 min of anoxia, attributed to increased efflux (derived from washout curves), were not significantly different (0.228 < P < 0.400). The typical effect of 60 min of anoxia on potassium content and efflux are illustrated in Figs. 5 and 6. The mean net potassium loss of 90 ± 11 mmol K+/kg dry tissue during 60 min of anoxia was not significantly different from the mean loss of 98 ± 11 attributed to elevated efflux (0.528 < P < 0.648). The effects of anoxic stress on tissue potassium content are summarized in Fig. 7.

The recovery patterns of tissue potassium content upon reoxygenation following 20, 40, and 60 min of anoxia, determined by asymptotic uptake experiments, are summarized in Fig. 8 as the percentage of recovery of the potassium lost during the stress. The maximal recovery of potassium was depressed following increased periods of anoxia. New steady-state values were reached within 65 min after 20 and 40 min of anoxia. Whereas 121 ± 9% of the lost potassium was recovered following 20 min of anoxia, only 94 ± 11% and 59 ± 8% were recovered following 40 and 60 min of anoxic stresses.

In addition to the characteristic potassium loss, three invariable anoxia-induced alterations in potassium exchange were observed. 1) An immediate onset of 42K loss from the tissue with anoxia. Elevated levels of 42K consistently appeared in the effluent within 30-45 s of perfusion with oxygen-free solution, as illustrated in Fig. 6, while the tissue probe confirmed a net loss of tissue potassium within 1 or 2 min of the stress (Fig. 3). 2) A rapid reversal of anoxia-induced potassium loss upon reoxygenation. Within 30-45 s of reoxygenation, elevated 42K appearance in the effluent stopped (Fig. 6) and the tissue probe during uptake experiments confirmed the onset of recovery of tissue potassium (Figs. 3 and 5). 3) A decreased rate of exchange of potassium following recovery of potassium to a new steady-state level. Analyses of the exchange rate constants (λ) from the washout experiments are summarized in Table 2 and demonstrate a significant (P < 0.001) decrease in the exchange rate of potassium following anoxia.

Water content. The mean percentage of water content during recovery from 20, 40, and 60 min of anoxic stress are summarized in Table 3. The mean water content of five control septa perfused for 3 h was 85.3 ± 0.3%. Five experimental septa were allowed the usual 2-h equilibration period but were made anoxic for the additional 60-min period and a significant increase to 86.6 ± 0.3% water was obtained (0.016 < P < 0.032). The percentage of water of septa allowed to recover for 30-70 min showed a maintained elevation over the control level.

DISCUSSION

The responses of mechanical performance parameters to anoxic stress are in qualitative agreement with previous reports on the cat papillary muscle (18) and may reflect differential alterations in the intensity and duration...
tion of the active state during the onset of anoxia and recovery.

After the initial 120-min equilibration period the mechanical performance parameters and potassium levels remained constant for 5-6 h. Under control conditions, the mean tissue potassium content of the 48 septa used in these experiments was 294 mmol K+/kg dry tissue, as determined by radioisotopic techniques. Gerlings et al. (4) have reported that the Tyrode-perfused heart may be oxygen limited and that the preparation is unsuitable for studies carried out under levels of inotropic stimulation. Comparison of the potassium content and exchange rate of the septa used in these experiments (perfused with a modified Ringer solution of 2.2-2.7 vol % oxygen, at 1.8 ml/min per g wet tissue wt) to the values of septa perfused under identical conditions with
RABBIT
28°C 42 BEATS/MIN

FIG. 8. Recovery of potassium lost during 20, 40, and 60 min of
anoxic stresses. Note that ordinate presents percentage of recovery
of potassium lost during each stress. Values are means ± SE.

TABLE 2. 42K rate constants (λ, min⁻¹) before
and after anoxia

<table>
<thead>
<tr>
<th>Anoxic Stress</th>
<th>Control Recovery</th>
<th>Probe</th>
<th>Effluent</th>
<th>Probe</th>
<th>Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 Min (3)</td>
<td></td>
<td>0.026</td>
<td>0.034</td>
<td>0.019</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>±0.003</td>
<td>±0.004</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.005</td>
</tr>
<tr>
<td>40 Min (3)</td>
<td></td>
<td>0.025</td>
<td>0.026</td>
<td>0.017</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
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<td>±0.001</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.001</td>
</tr>
<tr>
<td>60 Min (4)</td>
<td></td>
<td>0.018</td>
<td>0.020</td>
<td>0.013</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>±0.000</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.001</td>
<td>±0.001</td>
</tr>
</tbody>
</table>

P = 0.001

Values are means ± SE. Number in parentheses is number of
experiments.

TABLE 3. Percent of water content

<table>
<thead>
<tr>
<th>Anoxic Stress</th>
<th>Recovery Period, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0  30  60-70  90</td>
</tr>
<tr>
<td>20 Min</td>
<td>86.4±0.2(5)</td>
</tr>
<tr>
<td>40 Min</td>
<td>86.1±0.5(4)</td>
</tr>
<tr>
<td>60 Min</td>
<td>86.6±0.3(5)</td>
</tr>
</tbody>
</table>

0.016 < P < 0.032

Values are means ± SE. Number in parentheses is number of
experiments. * Compares control to 60 min of anoxia.

In addition, a previous study by Poole-Wilson and Langer (13) on the isolated, Tyrode-perfused septum,
showed no alteration in the net 42K exchange during administration of epinephrine (1 x 10⁻⁶ M), a concentration
which induced 120% increases in tension and +dP/dt. Thus, the ionic and mechanical stability of the septum,
complete control of the environment presented to the septum, and accessibility of the mechanical and
potassium-exchange parameters for measurement made the isolated, arterially perfused septum an excellent
model for study of the parameter responses to anoxia.

The specific activity (counts/min per mol K⁺) of the whole tissue and the perfusate are equal when 42K
uptake becomes asymptotic. Therefore, inducing anoxia at asymptote permitted quantitation of the net potas-
sium loss during anoxia. Inducing anoxia during the linear washout phase of a previously isotopically-
labeled septum permitted quantitation of the potassium loss that was attributable to efflux from the cells, since
the only source of 42K available for presentation in the effluent during this phase of washout was the 42K previ-
ously taken into the cells during the radioisotopic labeling. In addition, under steady-state conditions, the λ
values represent the rate of exchange of potassium. The parallel rates of washout of the whole tissue and the
effluent indicate that the isotope in the effluent is being washed out of a kinetically homogeneous compartment.

It is clear from these data that maintained perfusion, with total lack of oxygen, induced net loss of potassium
from the tissues (as determined from asymptotic uptake curves). Inhibition of active transport of potassium into
the cells, increased efflux of potassium out of the cells, or a combination of these two processes could have
resulted in the net loss of potassium. If the membrane potassium pump had been significantly impaired, re-
sulting in decreased transport of potassium into the cells, we would have expected to have observed a signifi-
cantly greater net loss, as measured by the tissue probe, than that attributable to efflux from the cells (7). The
nearly identical values of potassium loss calculated from uptake and washout experiments for anoxic pe-
riods of 20-60 min indicated that the entire net loss of potassium occurred through increased efflux from the
cells. No evidence for inhibition of active transport of potassium into the cells was demonstrated. The de-
creased rates of recovery of the potassium lost during anoxia, following increased lengths of anoxic stress
(Fig. 8), might indicate a depressed ability of the Na-K pump to transport potassium during the recovery pe-
riod. However, the decreased rates of recovery were associated with correspondingly depressed rates of po-
tassium exchange (Table 3). Additional corroborative evidence that Na-K pump function remained intact dur-
ing recovery was presented by the rapid reversal of the anoxia-induced potassium loss at the time of reoxygena-
tion, in both uptake and washout experiments. The conclusion drawn is that these results indicate that the
integrity of the membrane Na-K pump is maintained, under the experimental conditions, through anoxic pe-
riods up to 60 min and during the ensuing recovery period.
Our results that indicate that active transport of potassium is maintained during anoxia are in agreement with those of Calkins et al. (1) in isolated diaphragm, and Goerke and Page (5) in cat papillary muscle, while our observation that anoxia induces a net loss of potassium in myocardium confirms the classical observation of Lowry et al. (8) and the more recent reports by Scheuer and Stezoski (15) and Sybers et al. (17). However, Polimeni (11), Goerke and Page (5), Page et al. (9), and Page and Klegerman (10) did not demonstrate anoxia-induced potassium losses. The inability to demonstrate a net potassium loss in the heart of the acutely hypoxic rat (11) may have presented from the differential effects of hypoxia and anoxia and hypoxic-induced reflex hyperglycemia and hyperkalemia, as suggested by Polimeni, Goerke and Page (5), Page et al. (9), and Page and Klegerman (10) attributed the absence of anoxic-induced potassium losses in the isolated rat heart and cat papillary muscle to failure to exhaust reserves of high-energy compounds in the quiescent or nonpaced preparations. A net loss of potassium was observed in each of our experiments with septa paced at 42 beats/min.

On the assumption that one rate-limiting barrier controlled potassium efflux during anoxia, this barrier must have restrained at least 90 mmol K+/kg dry tissue, the mean potassium loss during 60 min of anoxia. Intracellular organelle groups, such as mitochondria, could not have contributed this magnitude of potassium loss (3). Therefore, the conclusion is drawn that the cell membrane is the rate-limiting site of potassium efflux from the cytosol during anoxia.

The mechanism of the increased potassium efflux across the cell membrane during anoxia is unknown. Gerlings et al. (4) have suggested that catecholamines may potentiate the net loss of potassium during limited oxygen availability. However, the net potassium loss during 20 min of anoxia was not significantly decreased in the catecholamine-depleted septa. The inability to block the anoxia-induced potassium efflux with Mg2+, PCO2, or respiratory acidosis, reported to block potassium efflux associated with administration of glycoside (14, 16), indicates that anoxia either opens new efflux pathways or increases the permeability of existing pathways and renders them insensitive to the blocking agents. Qualitatively similar responses of potassium efflux, reported by Scheuer and Stezoski (15) in the isolated rat heart, closely followed high-energy phosphate changes. Lai and Scheuer (6) have more recently reported altered NAD/NADH ratios 8 s after the onset of hypoxia. Thus, the increased efflux in our experiments may have been associated with altered cellular energy levels. Yet the rapidity of the onset and reversal of efflux in these studies during 20 and up to 60 min of anoxia indicates dissociation of potassium fluxes from the characteristic depletion and repletion patterns of high-energy phosphate stores as reported by Scheuer and Stezoski (15) and Poole et al. (12). Furthermore, without invoking highly compartmentalized energy storage at membrane sites association of the increased efflux with depletion of cellular high-energy levels is unlikely in view of the energy stores evidently available at the cell membrane for maintenance of the Na-K-ATPase pump activity. The “on-off” characteristic of the anoxia-induced efflux suggests the possibility of a direct effect of oxygen on rate-limiting efflux sites at the cell membrane. Coburn et al. (2) have recently proposed the existence of an O2 sensing system in smooth muscle, distinct from oxidative phosphorylation. We are unaware, however, of any previously proposed direct effect of molecular oxygen on myocardium. We are emphasizing that additional work must be completed to determine the mechanism of the increased potassium efflux during anoxia, and that a direct effect of oxygen is suggested as one possible explanation for the observed phenomenon.

Anoxic periods as short as 20 min can depress the potassium exchange rate observed following recovery from the stress. The parallel probe and effluent λ values after recovery indicate that the altered rate is not a result of the formation of an additional, more slowly exchanging kinetic compartment. The recovery of potassium levels to significantly above control (Fig. 8) following 20 min of anoxia, with no evidence of compartmentalization, indicates that the extra potassium load is distributed homogeneously within the cell. Death of a significant portion of the cell population after 40 and 60 min of anoxia may have masked similar elevations in potassium levels of the remaining viable cells (Fig. 8). The relationship between the depressed exchange rate and the elevated cellular potassium levels is not known.

In conclusion, these findings indicate that it is necessary to reevaluate the currently accepted concept that total oxygen deficiency results in depletion of high-energy levels, which, in turn, results in loss of the ability of the cells to maintain the intracellular milieu by active membrane transport processes. The findings clearly show that the classical observation of loss of cellular potassium by myocardial cells during anoxia can be accounted for by increased potassium efflux, and, under the controlled experimental conditions of the study, that the membrane Na-K pump continues to function through periods of up to 60 min of total oxygen deprivation. No evidence of inhibition of the pump during the ensuing recovery was obtained. The mechanisms of the increased efflux during anoxia and the depressed exchange rates following recovery are unclear at the present time. One possible explanation is that the removal of oxygen, perhaps by a direct effect on rate-limiting efflux sites at the cell membrane, may make existing potassium efflux paths more permeable or induce formation of new efflux paths, while reoxygenation may decrease the membrane efflux permeability, setting a new lower rate of potassium efflux that results in elevated, steady-state cellular potassium levels if the anoxic period is brief (20 min).

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