PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 149, 40–45 (1975)

K^+ and Mg^{2+} Net Fluxes in Relation to Zero [Ca^{2+}] Perfusion and Subsequent Cardiac Contracture[1] (38739)

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Perfusion of myocardium with a Ca^{2+}-free medium causes uncoupling of excitation-contraction. Previous studies by Lee and Visscher (1, 2) have shown that the reintroduction of Ca^{2+} into the perfusion solution after 12 minutes of mechanical arrest with a Ca^{2+}-free, hypokalemic perfusate causes an irreversible sustained contracture in rabbit myocardium. A large net influx of Ca^{2+} and an efflux of creatine, phosphocreatine and adenine nucleotides are associated with the state of contracture (1, 2). Zimmerman and Hülsman (3) have demonstrated an efflux of myoglobin, lactate dehydrogenase and creatine phosphokinase from rat myocardium after restoration of Ca^{2+} to the perfusion medium following several minutes of Ca^{2+}-free perfusion. They have described this phenomenon as the calcium paradox.

In the present study, the mechanical response of rabbit myocardium to a restoration of Ca^{2+} in the perfusion medium was determined after variable periods of mechanical arrest with a Ca^{2+}-free medium. The duration of Ca^{2+}-free perfusion was found to be an important determinant of the myocardial response to the reintroduction of Ca^{2+} in the perfusion medium. The net fluxes of the intracellular cations, K^+ and Mg^{2+}, were measured during perfusion with the Ca^{2+}-free and Ca^{2+}-containing perfusion solutions. The time course of the observed efflux of K^+ and Mg^{2+} after Ca^{2+} restoration has been studied.

Methods. Hearts of Nembutal-anesthetized male, white rabbits (2–3 kg) were prepared for perfusion by the method of Langendorff as described previously (1). Each heart was subjected to the same sequence of perfusion solutions: first, with a standard salt solution (SSS) for 20 min so steady state conditions could be achieved; secondly, with a Ca^{2+}-free, hypokalemic arrest perfusate for 6–12 min; and, finally, with SSS for an additional 20 min. The composition of SSS was (m*M*): NaCl, 142.95; Na_2HPO_4, 2.05; KCl, 4.85; KH_2PO_4, 0.15; $CaCl_2$, 1.8; glucose, 5. The arrest perfusate contained 0 m*M* $CaCl_2$ and 2.85 m*M* KCl, but was otherwise identical in composition to SSS. In four experiments $MgCl_2$ (0.68 m*M*) was added to both the SSS and arrest perfusate.

Flow rates were constant at 20–24 ml/min except during zero [Ca^{2+}] perfusion when they were reduced to 17 ml/min. Perfusion solutions were bubbled with 100% oxygen and delivered to the hearts via a Harvard peristaltic pump (model No. 500-1200) from reservoirs maintained at 37°. Tension development and arterial pressure were monitored throughout each experiment.

Venous effluent samples were serially collected from the severed pulmonary artery. Arterial reservoir and venous effluent samples were analyzed for K^+ and Mg^{2+} by atomic absorption spectrophotometry (Jarrel-Ash, Model No. 82-800).

Results. Original records of tension development and arterial pressure are shown in Fig. 1. The upper panel of Fig. 1 illustrates recovery of rhythmic activity after restoration of calcium in the perfusion medium following 8 min of mechanical arrest induced by zero [Ca^{2+}] perfusion. Rhythmic activity returned to seven of eight hearts in this study when the period of arrest was 10 min or less as exemplified by this experiment. One heart developed contracture after 10 min of arrest. On the other hand, of 22 hearts studied after 12 min of arrest, 20 developed contracture as exemplified by the experiment shown in the lower panel of Fig. 1. Only two of the 22 hearts studied recovered rhythmic activity.

Efflux of potassium from 16 hearts during contracture after 12 min of arrest is shown in Fig. 2. A peak venous effluent concentration of K^+ of 6.8 m*M* is reached 45 sec after restoration of normal [Ca^{2+}] and [K^+]

[1] Supported by MSPHS Grant No. HE03212.

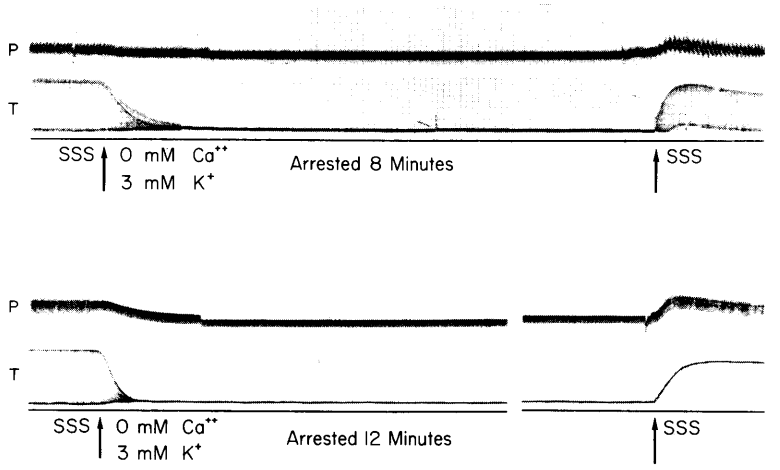

FIG. 1. Mechanical responses of rabbit myocardium after 8 and 12 min of excitation–contraction uncoupling. Both panels show original records of arterial pressure (P) and longitudinal tension development (T). After a period of equilibration with standard salt solution (SSS), hearts were mechanically arrested by perfusion with a Ca^{2+}-free, hypokalemic medium. The experiment in the upper panel illustrates the return of rhythmic contractions when SSS is reintroduced after 8 min of arrest. The experiment in the lower panel illustrates the development of contracture when SSS is reintroduced after 12 min of arrest.

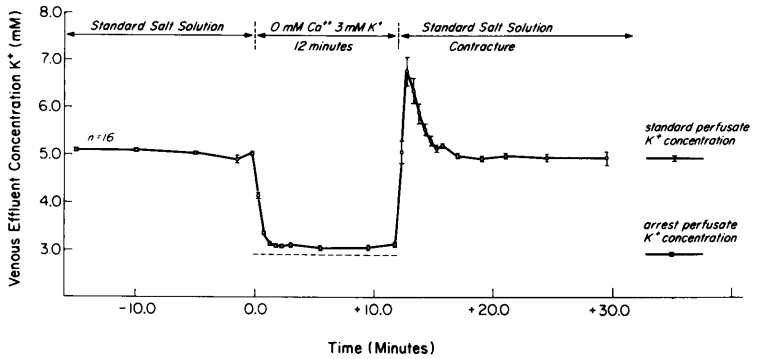

FIG. 2. Efflux of K^+ during irreversible contracture after 12 min of excitation–contraction uncoupling. $[K^+]$ in venous effluent during perfusion with SSS and Ca^{2+}-free, hypokalemic arrest solution are shown for 16 experiments. The time at which the arrest solution reached the heart is shown as zero in this and the following figures. Standard errors of the mean are shown as vertical bars. Arterial K^+ concentrations in SSS and arrest perfusate are shown to the right. The dashed line during the period of zero $[Ca^{2+}]$ perfusion also designates the arrest perfusate $[K^+]$. A large explosive efflux of K^+ is evident after restoration of SSS following 12 min of arrest.

to the perfusate. The large efflux is of short duration. The mean net efflux of K^+ during contracture from these 16 hearts calculated by the method of arteriovenous differences was 8.1 μmoles/g wet wt. Mean perfusate flow during contracture in these experiments was 21.4 ml/min and the mean heart weight was 7.9 g. Mean arterial perfusate $[K^+]$ in SSS was 4.94 $\pm$ 0.05 m*M* as shown to the right in Fig. 2.

A net efflux of K^+ was not observed in the case of hearts which recovered rhythmic activity after shorter periods of arrest nor from the two hearts which regained rhythmic contractions after 12 min of arrest. The flux of K^+ after 10 min of arrest is shown for two hearts in the upper panel of Fig. 3 and after 6 min of arrest for three hearts in the lower panel. When SSS is reintroduced, the $[K^+]$ in the venous effluent rises to its prearrest level. No net efflux of K^+ during recovery is detectable.

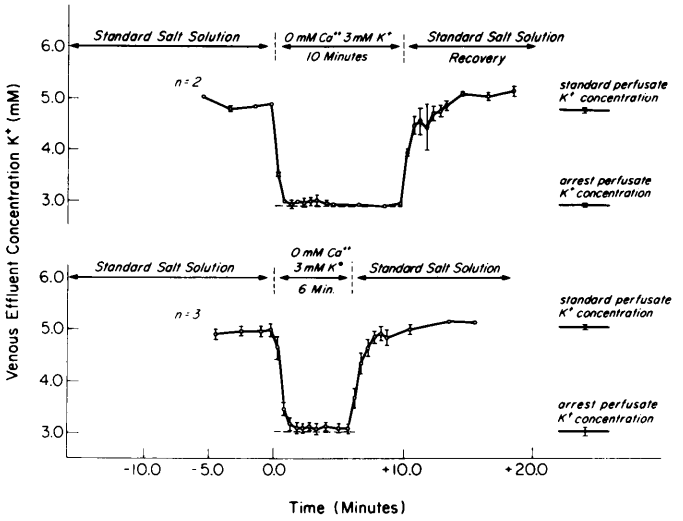

FIG. 3. Flux of K^+ during recovery of rhythmic activity after 6 and 10 min of excitation–contraction uncoupling. $[K^+]$ in the venous effluent from two hearts arrested for 10 min is shown in the upper panel and from three hearts arrested for 6 min in the lower panel. There is no detectable efflux of K^+ when rhythmic contractions return following restoration of SSS after 6–10 min of arrest. Compare with the efflux of K^+ during contracture shown in FIG. 2.

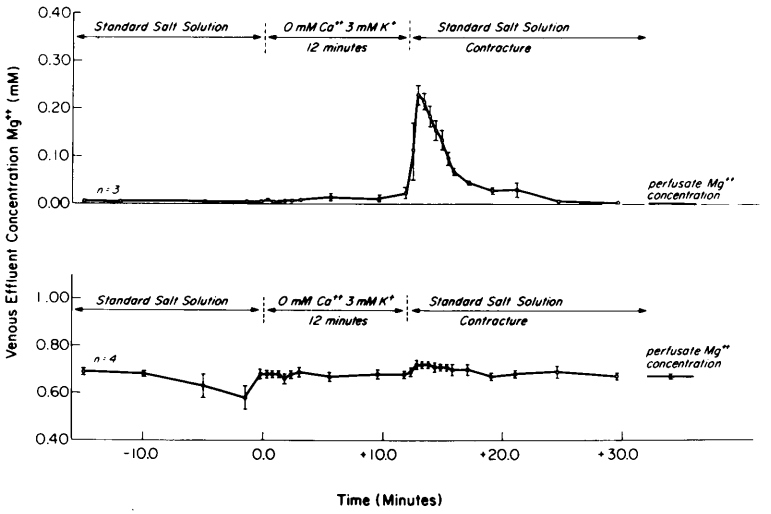

FIG. 4. Flux of Mg^{++} during irreversible contracture after 12 min of excitation–contraction uncoupling with and without Mg^{2+} in the perfusate. The upper panel shows $[Mg^{2+}]$ in the venous effluent from three hearts when Mg^{2+} was not incorporated into the perfusate. Return to SSS after 12 min of arrest causes a large efflux of Mg^{2+} which is associated with contracture. The lower panel shows $[Mg^{2+}]$ in the venous effluent from hearts of four experiments in which the arterial perfusate $[Mg^{2+}]$ was 0.68 m*M*.

The upper panel of Fig. 4 demonstrates a net efflux of Mg^{2+} from three hearts during contracture when Mg^{2+} was absent from the perfusion solutions. The peak venous effluent concentration of Mg^{2+} of 0.23 m*M* occurs, like that for $[K^+]$, 45 sec after restoration of normal $[Ca^{2+}]$ and $[K^+]$ in the perfusate. The mean net efflux of Mg^{2+} during contracture from these three hearts was 2.4 μmoles/g wet wt. Mean perfusate flow during contracture in these three experiments was 23.0 ml/min and the mean heart weight was

8.3 g. The lower panel of Fig. 4 demonstrates a marked suppression of the Mg^{2+} efflux from four hearts during contracture when Mg^{2+} was incorporated into the SSS and arrest perfusate. The peak venous effluent [Mg^{2+}] during contracture is 0.72 m*M* in these experiments, only 0.04 m*M* greater than arterial perfusate [Mg^{2+}]. There was, however, an efflux of K^+ during contracture from each of these hearts similar in magnitude to the efflux shown in Fig. 2.

Discussion. The duration of mechanical arrest with the Ca^{2+}-free, hypokalemic perfusate is an important determinant of the myocardial response to the restoration of normal [Ca^{2+}] and [K^+] in the perfusion medium. Return to SSS after 12 min of arrest ordinarily causes irreversible myocardial contracture. A large explosive efflux of K^+ and Mg^{2+} is associated with the state of contracture. The K^+ content of rabbit myocardium after 10 min of perfusion with Ringer–Locke solution is 52.5 μmoles/g wet wt (4). Thus, the mean loss of K^+ of 8.1 μmoles/g during contracture after 12 min arrest represents approximately 15% of the total myocardial K^+. As to Mg^{2+}, the fractional loss in experiments in which there is no Mg^{2+} in the perfusate, assuming 9.0 μmoles/g wet wt, is 27%. The efflux of Mg^{2+} is diminished during contracture when Mg^{2+} is present in the perfusate at 0.68 mM. When the duration of arrest is 10 min or less, restoration of Ca^{2+} usually results in a resumption of rhythmic activity. No efflux of K^+ occurs during the period of recovery. The [K^+] was reduced from 5 m*M* in SSS to 3 m*M* in the Ca^{2+}-free medium because Lee and Visscher (1) have previously found that contracture does not occur as regularly after 12 min of arrest when the [K^+] in the arrest perfusate is 5 m*M* as it does when the concentration is 3 m*M*.

Electron microscopic examination of rat myocardium by Zimmerman and coworkers (5) after restoration of Ca^{2+} in the perfusion solution has revealed the existence of two types of cells. The first type are intact, severly contracted cells and the second type are damaged, noncontracted cells in which myofibrils are partially disintegrated or even apparently absent altogether. The large efflux of Mg^{2+} and K^+ found in this study as well as the efflux of larger molecules found in previous studies (1, 3) during contracture probably occurs from the severely damaged myocardial cells. Severe cell membrane damage may be attributed (a) to the reexposure of the cell membrane to calcium ion or (b) to the mechanical disruption subsequent to the state of contracture. If cell membranes only were severely damaged as a result of the reexposure to calcium ions, some efflux of K^+ might be expected to occur from hearts that recovered normal rhythmic activity. Since no such efflux is observed in this situation, it seems probable that cell damage great enough to allow efflux of large molecules does not precede the contracture, but is secondary to it.

In previous studies in which the experimental conditions were similar to those of the present study (1, 2), the total calcium content of rabbit ventricular muscle was found to be approximately 30% greater than normal when irreversible contracture occurred. A sixfold increase in the rate of influx of isotopic calcium during the early phase of contracture has been demonstrated (2). In a recent study (6), the flux of calcium into "cell-associated" compartments was 1.7 μmoles/g during the first 2 min of contracture, but only 0.5 μmoles/g during the first 2 min of recovery of rhythmic contractile activity. The contracture thus appears to be related to a cellular influx of Ca^{2+} after its restoration in the perfusion medium.

The cause of contracture may be an increase in cell membrane permeability to Ca^{2+} during zero [Ca^{2+}] perfusion. Saari and Johnson (7) have concluded from uptake studies that capillary membrane permeability to calcium ion is increased after periods of Ca^{2+}-free perfusion in the rabbit heart. The observed partial separation of the basement membrane from the plasma membrane of rat myocardial cells after several minutes of Ca^{2+}-free perfusion (8, 9) indicates that the cell membrane itself is greatly altered and that its permeability may change during zero [Ca^{2+}] perfusion. If the magnitude of the permeability increase is sufficient after 12 min of arrest to allow a large influx of Ca^{2+} upon its reintro-

duction into the perfusate, Ca^{2+} saturation of myofilament sites coupled with inadequate sequestration by intracellular sites would cause contracture. When the $[Ca^{2+}]$ in the arrest perfusate is 0.09 m*M*, restoration of normal $[Ca^{2+}]$ and $[K^+]$ in the perfusate is followed by recovery of rhythmic contractions (10). Thus, small quantities of Ca^{2+} in the arrest perfusate apparently inhibit changes in membrane permeability during arrest and thereby prevent contracture when normal $[Ca^{2+}]$ is reintroduced into the perfusate.

Tomlinson and coworkers (9) have, however, postulated that damage to Ca^{2+} storage structures rather than changes in cell membrane permeability during Ca^{2+}-free perfusion is responsible for the contracture. Swelling of mitochondria and sarcoplasmic reticulum were observed in rat myocardium after 10 min of Ca^{2+}-free perfusion. These observations are, however, not incompatible with a permeability change.

Wingrad (11) treated strips of frog ventricle with EDTA containing media and found that contracture developed in these treated fibers when exposed to 140 m*M* KCl in the presence of 10^{-5}–10^{-8} *M* $[Ca^{2+}]$. Since the relationship between tension development in these EDTA treated fibers and $[Ca^{2+}]$ in bathing solutions was similar to that which had been found in other studies of isolated myofibrils and mechanically skinned muscle fibers which had not been treated with EDTA, the contracture found in the EDTA treated frog ventricle was attributed to rapid entry of Ca^{2+} into the sarcoplasm as a consequence of the increased Ca^{2+} permeability. This study appears to demonstrate that when Ca^{2+} rapidly equilibrates with the sarcoplasm, contracture can develop. It should be noted, however, that Winegrad (11) employed a high K^+ concentration in his contracture medium while we did not. Nevertheless his studies add to the evidence that rapid influx of Ca^{2+} can occur after periods of zero $[Ca^{2+}]$ in interstitial water.

The diminished efflux of Mg^{2+} during contracture when Mg^{2+} is incorporated into the arterial perfusate suggests that the electrochemical gradient between intracellular free and interstitial Mg^{2+} is considerably reduced in this situation. Since the cell membrane permeability is sufficiently large during contracture to allow efflux of molecules such as myoglobin (3), the potential difference across the membranes of these altered cells is probably minimal or nonexistent. Thus, the flux of Mg^{2+} is probably determined by its concentration gradient. A reversal of the Mg^{2+} flux might be expected to occur during contracture when the arterial perfusate $[Mg^{2+}]$ is sufficiently elevated. Preliminary studies have confirmed this prediction. This preparation could be useful for estimating the intracellular concentration of non-sequestered Mg^{2+} in myocardial sarcoplasm.

Summary. Following within 45 sec after the development of contracture induced by restoring normal ionic composition perfusion conditions after a 12 min period of mechanical arrest in the rabbit heart caused by zero $[Ca^{2+}]$ perfusion, there is an explosive efflux of K^+ and Mg^{2+}. After shorter periods of Ca^{2+}-lack arrest, the restoration of $[Ca^{2+}]$ to normal causes recovery of rhythmic contraction and no K^+ efflux. The K^+ and Mg^{2+} effluxes are ascribed to the effects of the contracture itself and not simply to the loss of Ca^{2+} during zero $[Ca^{2+}]$ arrest nor to the restoration of normal perfusate $[Ca^{2+}]$, except insofar as the latter operates to induce the contracture. It is suggested that cell membrane permeability progressively increases during zero $[Ca^{2+}]$ arrest and that an abnormally large influx of Ca^{2+} after restoration of normal perfusate $[Ca^{2+}]$ induces the contracture.

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Received September 6, 1974. P.S.E.B.M. 1975, Vol. 149.