Time Course and Zonal Variations of Ischemia-induced Myocardial Cationic Electrolyte Derangements

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SUMMARY
Myocardial cationic electrolytes were determined at regular time intervals up to 24 hours after coronary artery ligation in the dog. Replicate electrolyte ratios were computed for different areas of the heart at each time interval. For purposes of statistical analysis, ratios from two border areas and four areas remote from the infarct were pooled as values for ZONE B and ZONE N, respectively, and compared with those from the infarct proper, ZONE I. Ischemia-induced tissue Mg++/Ca++ changes paralleled those of K+/Na+ with respect to time course and zonal variations. In ZONE I, both K+/Na+ and Mg++/Ca++ fell precipitously during the first hour, and the falls became more gradual thereafter, approaching those of extracellular fluid at 24 hours. Changes in ZONE B, which appeared normal histologically, followed a similar downward trend, but differed in magnitude from those in ZONE I (P < 0.01). Changes in ZONE N were small but did not always overlap values in sham-operated dogs. It was concluded that lowered tissue K+/Na+ and Mg++/Ca++ were sensitive, but not specific, indices of myocardial ischemia, and multiple samplings of ionic ratios were essential for proper interpretation of ischemia-induced myocardial electrolyte derangements.

Additional Indexing Words:
Coronary artery ligation Tissue ionic shifts Potassium Myocardial infarction Sodium

Tissue electrolyte derangements reflect cellular response to hypoxia, attributable to breakdown of the energy-dependent cell membrane “ionic pumps.”1-5 Previous studies in animals and in man have shown, indirectly by analysis of coronary sinus blood and directly by determining cardiac muscle electrolyte contents, that the onset of myocardial ischemia is followed almost immediately by efflux of potassium and influx of sodium in the injured cells.6-16 Altered myocardial electrolyte composition has been correlated with increased lactate production, lower coronary blood pH, left ventricular failure, dysrhythmia and electrocardiographic ST-segment depression.9, 10, 17-27 Analysis of the myocardial ionic ratio potassium/sodium (K+/Na+)28, 29 has been shown to be a sensitive chemical means for the detection of early, histologically apparent myocardial infarction (M1). Parallel changes of myocardial magnesium/calcium (Mg++/Ca++) ratio have also been anticipated but not previously documented.30 To explore these possibilities, the topography and chronology of myocardial K+/Na+ and Mg++/Ca++ changes in the first 24 hour period after coronary artery ligation in the dog were investigated. We sought answers to several questions. Is the pattern of ischemia-induced Mg++/Ca++ changes similar to those of K+/Na+? What is the time course of the changes in cation ratios? Are K+/Na+ and Mg++/Ca++ values in the border zone different from the normal myocardium and from the infarct? Which ionic ratio, K+/Na+ or Mg++/Ca++, is a more sensitive chemical indicator of myocardial ischemia?

Materials and Methods
Large transmural M1 of predictable size and location in the left ventricular anterior wall was produced in mongrel dogs of both sexes, weighing between 15 and 25 kg, by ligation and agar injection of the left anterior descending coronary artery as described previously.31 After sectioning the hearts transversely, representative slices were incubated in triphenyl tetrazolium chloride (TTC) solution. This macroscopic enzyme mapping technique31 sharply
delineated the site and size of the infarct long before it became grossly discernible to the unaided eye. It was thus visually possible to identify and divide a transverse slice of the heart into seven geographic areas: the infarct (ZONE I) flanked by two border areas (ZONE B), one on each side of the infarct, and the four normal areas (ZONE N) furthest away from the infarct. These areas were numbered 1 through 7 for identification (fig. 1). Only one slice from each heart was stained for mapping purpose and the adjacent unstained heart slice was used for tissue electrolyte analysis.

Sixty-three dogs were used in this study, including four sham-operated dogs serving as "zero-time" or "normal" specimens. After coronary artery ligation, the animals were sacrificed at the following time intervals for the determination of myocardial electrolyte ratios: 15, 30, 45 and 60 minutes, and 2, 4, 6, 8, 10, 12 and 24 hours. There were at least four dogs in each of these intervals for which complete sets of ionic ratios were obtained for all seven areas of the heart. Complete sets of ionic data were obtained in 52 dogs; in the remaining 11 dogs, some of the ionic ratios were not available.

About 1 to 2 gm of fat-free myocardium, blotted free of surface blood with filter paper and chopped into small pieces, was dried to constant weight in a 100°C oven and ground into powder. Duplicate samples of 0.1 gm (±0.00001) powder were weighed into microkeldahl flasks and 3 ml concentrated HNO₃ and 1 ml 70% HClO₄ were added. The flasks were heated until all organic matter was oxidized, then evaporated to 1 ml to remove all HNO₃. The acid digest was diluted to 10 ml with distilled water, and from this solution suitable aliquots were diluted for the determination of electrolytes: K⁺, Na⁺, Mg²⁺, and Ca²⁺, by atomic absorption spectrometry. Results were calculated and expressed as μg/gm dry weight of tissue sample, and the ratios K⁺/Na⁺ and Mg²⁺/Ca²⁺ derived accordingly.

Histological evaluation of myocardial samples from the same seven areas was done on formalin-fixed, paraffin-embedded sections, cut at 6μ thickness, and processed for hematoxylin-eosin (H&E) and hematoxylin-basic fuchsin-picric acid (HBFP) stains.

Statistical evaluation of the data was done using multivariate analysis of variance techniques. The K⁺/Na⁺ and Mg²⁺/Ca²⁺ ratios, obtained from those animals for which complete sets of ionic ratios were available, were arranged in a 52 × 14 data matrix, the columns of which represent the two ratios from each of the seven areas of the myocardium. Since the raw data were presented in the form of ratios, an initial logarithmic transformation was carried out on the elements of the data matrix.

Results

The gross and microscopic findings of the myocardium at various time intervals after coronary artery ligation have been reported in detail previously, and will not be described again. Briefly, a homogeneous classic myocardial infarction developed only in ZONE I. The myocardium in ZONE B, while stained positively by the HBFP technique, was normal by conventional histological criteria.

Cellular electrolyte ratios in the seven different areas at various time intervals are presented in tables 1 and 2. The time course and zonal variations of myocardial K⁺/Na⁺ and Mg²⁺/Ca²⁺ are illustrated in figures 2 and 3, respectively, with time on the abscissa and mean ionic ratios on the ordinate. "Zero-time" or "normal" values of myocardial K⁺/Na⁺ and Mg²⁺/Ca²⁺ were calculated by pooling the respective ionic ratios at all locations in sham-operated animals: 5.05 ± 0.05 (range 4.07–5.80) for K⁺/Na⁺ and 7.08 ± 0.06 (range 6.09–7.98) for Mg²⁺/Ca²⁺.

There was no clear advantage of K⁺/Na⁺ or Mg²⁺/Ca²⁺ as a preferred chemical indicator of myocardial ischemia. Following coronary artery ligation, alterations of cellular Mg²⁺/Ca²⁺ paralleled those of K⁺/Na⁺ with respect to time course and zonal variations. Marked changes of myocardial K⁺/Na⁺ and Mg²⁺/Ca²⁺ from "normal" values (P < 0.01) already were evident at 15 minutes in ZONES I and B (tables 1 and 2). In ZONE I, both K⁺/Na⁺ and Mg²⁺/Ca²⁺ fell precipitously during the first hour, and the falls became more gradual thereafter. The K⁺/Na⁺ and Mg²⁺/Ca²⁺ ratios in ZONE I approached those of extracellular fluid at 24 hours (figs. 2 and 3). Changes of K⁺/Na⁺ and Mg²⁺/Ca²⁺ in ZONE B followed a similar downward trend but were significantly less in magnitude (P < 0.01) from those in ZONE I at all time intervals within the 24 hour period after coronary artery ligation.

Compared with the "normal" values, there were

Figure 1

Top) Triphenyl tetrazolium chloride (TTC) treated heart slice four hours after coronary artery ligation, showing a sharply demarcated transmural infarct in the anterior wall of the left ventricle (pale unstained area). Bottom) The seven numbered areas, denoting the infarct, ZONE I (1); border areas, ZONE B (2 and 3); and normal myocardium, ZONE N (4, 5, 6 and 7).

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Table 1

<table>
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<th>Zone I</th>
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<th>Zone III</th>
<th>Area</th>
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*No data available.

Operated dogs (SO) was 5.14 ± 0.22 (range 4.67-5.58), at 15 min after coronary artery ligation K+/Na+ fell to 4.28 ± 0.10 (range 3.96-4.53). The Student’s t-test of unpaired data showed that the difference is highly significant (P < 0.01). The corresponding values for Mg2+/Ca2+ were 6.62 ± 0.15 (range 6.17-6.88) and 5.90 ± 0.13 (range 5.55-6.16); P < 0.02. Although the biological importance of these differences may be debatable, they tend to support the precision of the chemical analyses. Only 9 of 54 area 4 K+/Na+ values in dogs with coronary artery ligation exceeded the lowest K+/Na+ for the SO samples, and none exceeded the highest SO value (table 1). With respect to myocardial Mg2+/Ca2+

small but significant decreases of both K+/Na+ and Mg2+/Ca2+ in ZONE N. Taking area 4 as an example (see tables 1 and 2), whereas K+/Na+ from sham-

Figure 2

Time course and zonal variations of mean myocardial K+/Na+ ratios after coronary artery ligation. The small open circles in the left upper corner indicate ‘zero’ time normal values, and areas 1 through 7 are shown in the diagram on the right with the infarct zone (area 1) shaded.
Table 2
Myocardial Mg++/Ca++ after Coronary Artery Ligation in the Dog

<table>
<thead>
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<th>Time</th>
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<td></td>
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<td>Sham-operated (zero time)</td>
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*No data available.

Discussion

The relationship of ionic shifts of the cations potassium-sodium-calcium and cardiac function has been known to cardiologists and myocardial biologists for some time,1-8 while the importance of magnesium in heart muscle has been appreciated only recently.35 The causes of potassium efflux from the myocardium include acute ischemia, congestive heart failure, acidemia, arrhythmias, increased preload and afterload, electric shock, glycosides, various symp-
pathomimetic amines, and a host of so-called electrolyte-steroid cardiopathies. Ischemia, however, constitutes the most common and important cause of rapid myocardial potassium depletion. The underlying mechanism appears to be ischemia-induced reduction of cellular oxidative energy formation which is indispensable for the active return of systolically extruded potassium during diastole. Efflux of potassium is coupled with influx of sodium and, as a corollary, myocardial ischemia-triggered cellular loss of magnesium is coupled with a gain in calcium.

Net loss of potassium from ischemic myocardium was first demonstrated by Harris et al. in 1954 by sampling the coronary sinus blood following coronary artery ligation, and they suggested that the cation released was responsible for the supervening ventricular tachycardia and fibrillation so frequently observed. The numerous studies that followed have confirmed ischemia-induced myocardial electrolyte shifts, but left the cause-and-effect role of potassium efflux and ventricular dysrhythmia unresolved.

Our investigation concerned the practical value of myocardial electrolyte composition as a chemical means for the detection of early, histologically apparent, myocardial infarction. For this purpose, determination of electrolyte ratios K+/Na+ and Mg2+/Ca2+ were considered more informative than quantitative values of individual ions. Since the numerator and denominator in the ionic ratio change in opposite directions, even slight shifts in individual ions would magnify the altered electrolyte ratios. Fifteen minutes was chosen as the earliest time interval after coronary artery ligation at which the myocardial K+/Na+ and Mg2+/Ca2+ ratios were determined, because this would allow a period of stabilization for the injured cells, time for recording intraoperative findings of the compromised heart, and unhurried procurement of tissue samples for morphologic and chemical studies. Moreover, even if the analysis of myocardial electrolytes was to become a routine procedure to study biopsy or autopsy material, it was envisioned that there would be few occasions, if ever, when such material could be obtained sooner than 15 min after the onset of ischemic injury.

Our findings showed that there was little to choose between K+/Na+ and Mg2+/Ca2+ as a chemical indicator of myocardial ischemia. Both ratios dropped precipitously in the infarct (ZONE I) after coronary artery ligation; at 15 min K+/Na+ changed from the mean "normal" value of 5.05 to 2.70, and Mg2+/Ca2+ from 7.08 to 2.47. The falls became more gradual thereafter; and at 24 hours the values of both K+/Na+ and Mg2+/Ca2+ approached those in extracellular fluid. It was noteworthy that both K+/Na+ and Mg2+/Ca2+ also fell sharply in the myocardium bordering infarcts (ZONE B). The changes patterned those of the infarct in time course, but were of less magnitude and did not drop to the values of ionic ratios in extracellular fluid at the end of the 24 hour experimental period (figs. 2 and 3). It would appear that the extent of myocardial ischemia following coronary artery ligation was more widespread than the size of the eventual infarct would indicate. However, since infarction did not develop in the border areas, it became apparent that even what seemed to be drastic changes of tissue K+/Na+ and Mg2+/Ca2+ did not always signal irreversible ischemic injury to the myocardium. While decreased myocardial ionic ratios have been enthusiastically endorsed as sine qua non of early, histologically apparent MI, the time course and zonal variations of ischemia-induced myocardial K+/Na+ and Mg2+/Ca2+ ratios have not been sufficiently emphasized previously.

With regard to K+/Na+ and Mg2+/Ca2+ in Zone N, although the changes were small and there was some overlap between the sham-operated dogs and dogs with coronary artery ligation, the differences were nevertheless significant by the criterion of Student's t-test. A number of investigators have previously reported a small decline in the absolute values of myocardial K+ and Mg++, concomitant with a small rise of Na+ and Ca++, in apparently normal tissue distal to infarcts in both dogs and man. Recently, this phenomenon has also been observed in the rat heart and its cause attributed to an expansion of the extracellular space. It should be emphasized that the K+/Na+ and Mg++/Ca++ ratios will both fall, not only if the cations run down their electrochemical gradients, but also if the extracellular space expands. The changes found in ZONE B appear to be too great to be entirely explicable by a change in the extracellular space, but even here a sizable swelling of this compartment could account for a large part of the results.

Ischemia-induced myocardial electrolyte derangements occur probably coincidentally with the shift to anaerobic metabolism. With regard to the rapidity of the tissue electrolyte changes, our study shows measurements dropping to 40% of control values in 30 min whereas the data of earlier work by other investigators do not reach 40% of control for almost 120 min. A number of factors might have contributed to the differences noted from 30 to 120 min, including methods of producing experimental myocardial infarction, sampling and electrolyte measurement. It is possible that our method of coronary artery ligation, augmented by the injection of agar in achieving almost total myocardial ischemia, could also have
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affected the diffusion of electrolytes in and out of the ischemic areas.

To reiterate, there seems little doubt that lowered tissue K+/Na+ and Mg++/Ca++ ratios are sensitive chemical indices of blighted, jeopardized myocardium which may or may not be irreparably damaged by ischemia. The result of a single determination of myocardial K+/Na+ or Mg++/Ca++ ratio has only limited diagnostic value. Multiple samplings are needed to establish topographic differences of ionic shifts and to locate the focus of maximum injury in the heart. The time interval following the onset of ischemia appears to be another important variable which needs to be understood in order to properly assess the significance of altered myocardial ionic ratios. In clinical situations, this may be extremely difficult if not impossible to determine with any degree of surety. Valid interpretation of tissue K+/Na+ and Mg++/Ca++ changes also demands prior knowledge of the appropriate clinical setting, taking into consideration possible adverse effects of various drugs and physical treatments (such as cardiac glycosides, insulin, artificial pacing and electric countershock) on myocardial ionic shifts.

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