Rubidium-82 Kinetics After Coronary Occlusion: Temporal Relation of Net Myocardial Accumulation and Viability in Open-Chested Dogs

Richard A. Goldstein

Division of Cardiology and Positron Diagnostic and Research Center, The University of Texas Health Science Center at Houston, Houston, Texas

Serial assessment of perfusion and viability during myocardial infarction has not been feasible, in part, because of the long half-lives of available tracers. Rubidium-82 (^{82}Rb) is a generator-produced, positron-emitting potassium analog with a short half-life (75 sec) that permits repeated studies. To determine the temporal relation of net myocardial ^{82}Rb accumulation to loss of viability during prolonged ischemia, a 2–3 mCi bolus of ^{82}Rb was given to 46 open-chested dogs while regional myocardial time-activity curves were obtained with beta probes at baseline, and serially after coronary occlusion lasting 1–6 hr. Hearts were then stained with triphenyl tetrazolium chloride (TTC) to assess the viability of the epicardium under the probe to a depth corresponding to the range of positrons. Irreversible injury occurred in two out of 16 experiments at 1 hr and ten out of 15 experiments at 3 hr and also at 6 hr ($p < 0.05$ vs. 1 hr). In viable myocardial samples, rubidium extraction increased with low flow as compared with nonischemic controls for all time periods but was unchanged (failed to increase) in nonviable tissue. Net ^{82}Rb accumulation decreased during 1 to 6 hr of occlusion in irreversibly injured samples (0.28 ± 0.19 to 0.16 ± 0.07, $p < 0.05$) but remained unchanged in myocardial tissue subsequently shown to be viable. For myocardial samples that were nonviable at 3 and 6 hr, changes in net accumulation of tracer became abnormal only after 6 hr of occlusion. The mechanisms primarily responsible for the decrease in net accumulation of ^{82}Rb at 6 hr appeared to be leakage of tracer after first pass. Therefore, failure to increase extraction at low flows may be an early indicator of cell death, whereas membrane leakage occurs several hours after loss of viability.

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Uptake of thallium-201 (^{201}Tl) has been used in the setting of acute myocardial infarction as an indicator of potentially viable myocardium based on the assumption that extraction is dependent upon the presence of an intact cellular membrane (*1–4*). Early studies in vitro supported this hypothesis, but recent in vivo studies suggest that initial uptake of tracer persists for a brief unknown duration after irreversible damage occurs (*5–7*). Unfortunately, serial assessment of transport kinetics in vivo is difficult with ^{201}Tl because of its 73-hr half-life. This physical property also limits its use to a single injection in acute infarction making it unsuitable for following the effects of thrombolytic therapy in patients.

Positron emission tomography (PET) can be used to quantify regional radiotracer concentrations (*8*). The use of PET, however, requires a cyclotron facility to produce tracers for most applications. The development of a rubidium-82 (^{82}Rb) generator has made it possible for positron imaging to be performed remote from a cyclotron. Rubidium-82, like ^{201}Tl, behaves in a manner similar to potassium. It has a much shorter half-life (75 sec) than ^{201}Tl and can, therefore, be given serially without an excessive radiation dose or background activity.

After experimental coronary occlusion followed by reperfusion, time-activity curves of ^{82}Rb have been used

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For reprints contact: Richard A. Goldstein, MD, University of Texas Medical School, P.O. Box 20708, Houston, TX 77225-0708.

to assess coronary artery patency and viability of the region at risk (*9*). Reperfused myocardium that is irreversibly injured demonstrates a net loss of activity after first-pass uptake whereas viable myocardium displays net accumulation of tracer. The current study was performed to determine the temporal relation of altered cellular ^{82}Rb kinetics to loss of viability in ischemic myocardium in the absence of perfusion.

MATERIALS AND METHODS

Animal Preparation

Forty-six dogs were anesthetized with sodium pentobarbital (30 mg/kg), intubated, and ventilated with room air by a volume respirator.* Arterial pressure was monitored through a transfemoral aortic catheter and lead II of the electrocardiogram was monitored using standard subcutaneous leads. A left thoracotomy was performed in the fifth intercostal space and the heart was suspended in a pericardial cradle, "open-chested." The left anterior descending coronary artery (LAD) was isolated just distal to the first major diagonal branch, where a snare occluder was placed. A heparinized catheter was then inserted into the aorta through the right carotid artery and connected to a withdrawal pump.* A catheter was also placed in the left atrium for injection of microspheres.

A pair of collimated beta radiation detectors (1 cm diameter) was positioned on the left ventricular epicardial surface between the LAD and second diagonal branches in order to measure positron radioactivity. One probe was covered by a 1-mm piece of lead to allow for on-line correction of gamma photons. The epicardial position of the unshielded probe was marked with methylene blue at the beginning of the experiment. The detected positron counts were processed by a pulse-height analyzer.† connected on-line to a Vax 11-780 computer that generated time-activity curves corrected for physical decay of ^{82}Rb.

Experimental Protocol

Two to three millicuries of ^{82}Rb in 10 ml of normal saline were eluted from a strontium-82/rubidium-82 (^{82}Sr/^{82}Rb) generator and injected as a bolus into a femoral venous catheter immediately followed by a 10-cc normal saline flush. An arterial sample was withdrawn from the aortic catheter at a constant rate and served as a reference for microsphere flow measurements and ^{82}Rb uptake. Rubidium-82 time-activity curves were recorded after each injection at control and serially following LAD occlusions of 1 (n = 16), 3 (n = 15), or 6 hr (n = 15). Animals were then killed and their hearts excised. The heart was then sectioned into 1-cm axial slices and incubated with triphenyl tetrazolium chloride (TTC) to determine whether the epicardial area under the unshielded beta probe had been reversibly (deep red staining) or irreversibly (pale staining) injured as previously described (*10–12*). Since the range of the rubidium positron is 3–4 mm, only the 4 mm of myocardium under the unshielded probe was used for comparison with ^{82}Rb measurements. Areas were reported as TTC negative if there was any evidence of irreversible injury within the sample.

Analysis of ^{82}Rb Time-Activity Curves

The beta radiation detectors are designed to have a high efficiency for positron particle radiation and a low efficiency for gamma photon radiation. To obtain positron radioactivity per se, the time-activity curve obtained with the lead shielded probe (gamma radioactivity only) was subtracted by computer from the probe without lead (positron and gamma radioactivity). Epicardial positron radioactivity was counted at one second intervals for 150 sec. The same pair of detectors were then placed over a 150-ml beaker containing the arterial reference sample for the same interval to determine the integrated delivered dose of ^{82}Rb. Since the surface area of the beaker is much greater than the probe diameter, the geometry and sample volume of blood and myocardium can be considered the same.

The net amount of ^{82}Rb accumulated by the heart was determined by dividing the positron counts at 150 sec by the delivered dose. This value represents the product of flow and the extraction fraction of ^{82}Rb less any tracer that has egressed from the myocardium during the observation period. The units for net accumulation are, therefore, ml/min/g × extraction fraction. First-pass extraction fraction was estimated using a previously described two-compartment model that expresses the beta probe activity as the sum of the instantaneous amounts of extracellular and intracellular rubidium (*13,14*). The counts recorded by the beta probe, P(t), can be expressed as follows:

$$P(t) = b_1 C_E(t) + b_2 C_E(x)\,dx, \qquad (1)$$

where b_1 and b_2 are constants and x is a dummy variable of integration. The concentration of the extracellular space is described by the following equation:

$$C_E(t) = bte^{-at}, \qquad (2)$$

where b and a are constants and C is the concentration of radioactivity in the extracellular space. Substitution of Eq. (2) into Eq. (1) gives the expression for beta probe activity, P(t). P(t) was then applied to a least-squares technique to best fit the equation by varying a, b_1, and b_2 iteratively over the first 30 sec from injection. Extraction fraction was then determined at peak activity by dividing the cellular activity by the total activity recorded at that time.

The rate constant for the net transfer of ^{82}Rb, k_T, was obtained from a best fit monoexponential least-squares equation applied to the probe counts from 60–150 sec

after injection of tracer as previously described (*9*). The starting point at 60 sec was selected since first pass of the tracer was always complete within that time.

Microsphere-Determined Flow

Epicardial flow at baseline and following occlusion were measured with 15-μ diameter radioactive microspheres (strontium-85, tin-113, cobalt-57, or niobium-95) administered at the same time as the ^{82}Rb. Microspheres were given over 20–30 sec into the left atrium, and an arterial reference sample (the same used for ^{82}Rb measurements) was withdrawn at a rate of 16 ml/min from the ascending aorta for 2.5 min into a preweighed syringe. At the conclusion of the experiment, an epicardial biopsy in the same sample volume as the unshielded detector was trimmed of fat and weighed. Tissue samples were counted separately in a 1,000 channel pulse-height analyzer (*15,16*). A computer program was used to calculate flow using a least-squares analysis of the spectra.

Statistics

Differences in all variables were determined with a two-tailed, Student's t-test for unpaired samples, a Wilcoxon rank test or analysis of variance where appropriate. All values reported are the mean ± s.d.

RESULTS

Epicardial biopsy samples taken 1 hr after occlusion showed evidence of irreversible ischemic injury in two out of 16 experiments. By 3 and 6 hr of occlusion, ten out of 15 experiments for each group were nonviable by TTC staining, a significant difference from 1 hr occlusions ($p < 0.05$). In tissues that were subsequently viable at biopsy, net ^{82}Rb accumulation fell from 0.82 at control at 0.32 but did not change further with prolonged occlusion (Fig. 1). Myocardial blood flow fell from 1.18 to 0.50 ml/min/g with occlusion and remained constant up to 6 hr. Extraction fraction increased from 0.44 to 0.61 1 hr after occlusion and remained at this value throughout the observation period for tissue samples that were subsequently TTC positive.

In samples that were subsequently shown to be irreversibly injured, net accumulation of ^{82}Rb fell from 0.71 at baseline to 0.28 at 1 hr, 0.23 by 3 hr and 0.16 by 6 hr (Fig. 2). Microsphere-determined flow fell from 1.36 ml/min/g at baseline to 0.23 ml/min/g at 1 hr and was not significantly changed during the ensuing observation period up to 6 hr. Thus, the flow was lower in the nonviable tissues than viable samples. Extraction fraction averaged 0.47 at baseline. In contrast to viable tissue, however, there was no significant increase in extraction fraction at 1, 3, or 6 hr after occlusion.

Figure 3 illustrates an example of two time-activity curves at 3 and 6 hr after occlusion in a sample that was nonviable at necropsy. Flow was 0.42 ml/min/g at 3 hr and was unchanged at 6 hr. Net accumulation of ^{82}Rb was 0.24 at 3 hr and fell to 0.15 at 6 hr. The transfer rate constant measured 60 to 150 sec after tracer administration fell from $+0.64 \times 10^{-3}$ to -3.83×10^{-3} sec^{-1} by 6 hr indicating that the decrease in rubidium accumulation was due, in part, to leakage of tracer after first-pass uptake.

Mean values for the net transfer rate constant, k_T, are presented for each time period for viable and nonviable samples in Fig. 4. The rate constants were not significantly changed during the 6 hr of occlusion in viable samples. In irreversible injured samples, there was no significant change in the rate constants at 1 or 3 hr after occlusion. However, at 6 hr, the rate constants in these samples decreased to an average of -0.46×10^{-3}/sec which was significantly lower than the earlier values in nonviable samples and lower than those measured for viable tissue at all time periods.

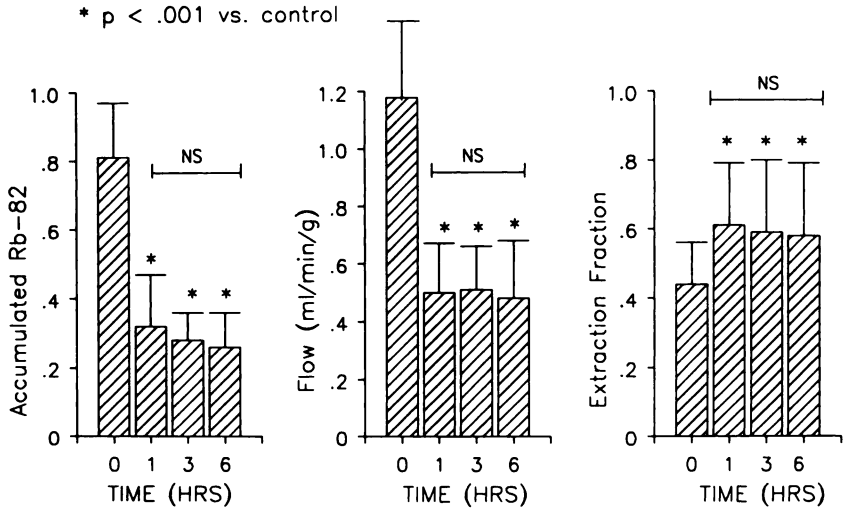


FIGURE 1
Epicardial accumulation of ^{82}Rb (normalized for delivered dose), flow determined by microsphere in same sample volume and estimated ^{82}Rb extraction fraction in potentially viable samples. Values are mean ± s.d. at baseline and 1, 3, and 6 hr after coronary occlusion

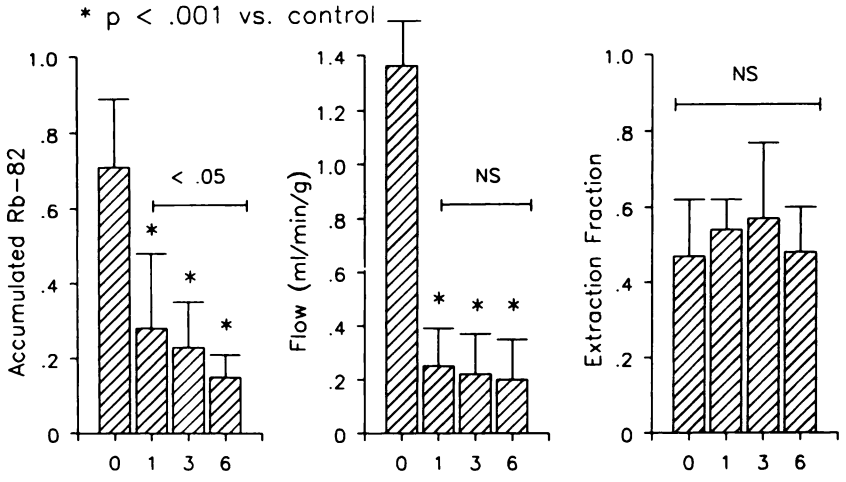


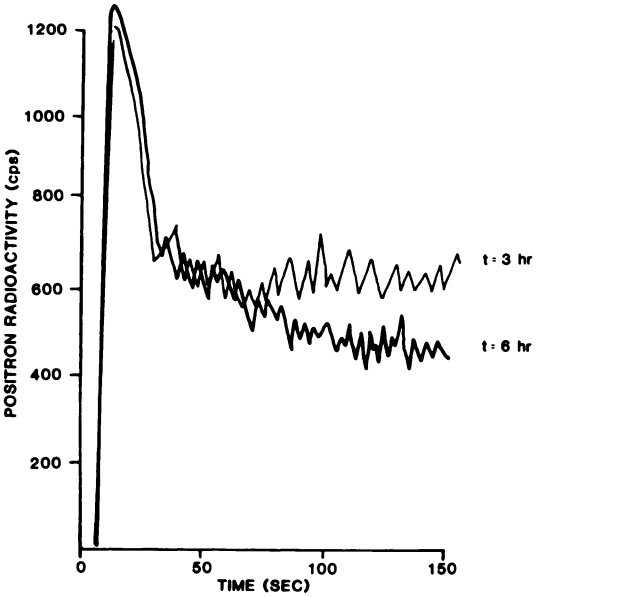
FIGURE 2
Epicardial accumulation of ^{82}Rb (normalized for delivered dose), flow determined by microsphere in same sample volume and estimated ^{82}Rb extraction fraction in irreversibly injured samples. Values are mean ± s.d. at baseline and 1, 3, and 6 hr after coronary occlusion

DISCUSSION

Diffusible radiotracers such as thallium and rubidium have been used to assess regional myocardial perfusion (*17,18*). Some studies suggest that initial uptake of these tracers requires active transport and thus cellular viability, while other studies suggest that it is a passive process (*1–7*). In cell culture preparations where perfusion is not the limiting factor for delivery of tracer, the ability of cells to extract thallium decreases with loss of viability (*5*). However, in open-chested dogs, Melin and colleagues found that loss of viability was not associated with decreased uptake of thallium obtained from a biopsy 5 hr after the onset of ischemia (*6*). This observation was taken as evidence that uptake was a passive (perfusion limited) rather than an active (Na-K ATPase) process.

In the current study, radioactivity was detected with beta radiation probes that allow measurements of tracer kinetics in a discrete region of the myocardium at very frequent time intervals (*9,13,14*). Counts do not include activity from the ventricular blood pool or opposite myocardial wall because of the limited range of positrons. Using a short-lived tracer such as ^{82}Rb, serial assessment of extraction and release characteristics of tracers can be readily determined.

This study indicates that there is a decrease in net myocardial accumulation of ^{82}Rb (normalized for dose) from 1 to 6 hr after experimental coronary occlusion



Time (hrs)	Rb-82 Accumulation	Flow (ml/min/g)	k_T ($\times 10^{-3}$ sec^{-1})
3	.24	.42	0.64
6	.15	.44	-3.83

FIGURE 3
Epicardial time-activity curves of ^{82}Rb at 3 and 6 hr after occlusion in sample that was irreversibly injured at 6 hr. Decrease in ^{82}Rb accumulation at 150 sec is in part due to increased egress of tracer (i.e., negative k_T)

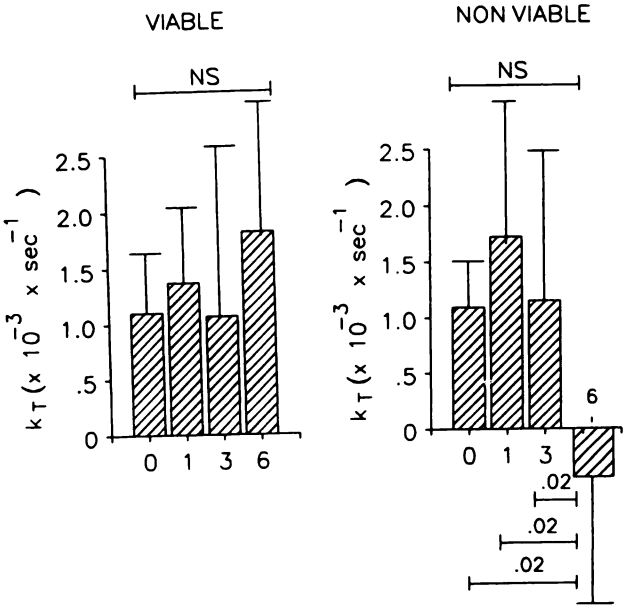


FIGURE 4
Rubidium-82 transfer rate constants at baseline and 1, 3, and 6 hr after occulusion in viable and nonviable samples. Values are mean ± s.d.

in irreversibly injured tissue subject to constant low levels to flow. Possible mechanisms for this fall in ^{82}Rb accumulation in the face of constant flow include a decreased extraction of avilable tracer or accelerated washout. Accumulation is initially dependent on delivery of tracer and extraction by the cells. Extraction fraction in viable samples was increased above baseline due most likely to prolonged residence time for the tracer at low flow. In samples that were subsequently irreversibly injured, extraction fraction did not significantly change after occlusion at any time period. It is, therefore, unlikely that an abnormality in extraction could account for the decrease in net accumulation of ^{82}Rb. However, the failure to increase extraction fraction despite a prolonged residence time may be an early harbinger of cell death that preceeds evidence of irreversible injury by TTC staining.

Extraction fraction was determined using a model previously validated in our laboratory over a wide range of flow and under varying metabolic conditions including acidosis, alkalosis, and digoxin administered to inhibit the Na-K ATPase pump (*13,14*). Despite a good agreement between flow measured using this estimate of ^{82}Rb extraction fraction and microsphere-determined flow, there are several possible limitations to this approach during ischemia. The curve fitting for extraction fraction is limited to the first 30 sec after injection and assumes that there is no leakage of tracer during this interval. The data presented indicates that some leakage of tracer is present after 6 hr of occlusion although the effect of leakage on the measurement of extraction derived over the first 30 sec is probably small. A more significant problem is the low signal to noise ratio and the rounding off of the peak activity which could produce large errors in estimation of first-pass extraction. This problem would be even more marked using positron tomography.

The net transfer rate of ^{82}Rb, k_T, was unchanged in potentially viable myocardium up to 6 hr after occlusion but was significantly decreased after 6 hr of ischemia in nonviable samples. The negative values of k_T indicate that leakage of tracer occurred in excess of continued uptake. Following permanent occlusion, as in this study, the change in k_T was not present until 6 hr despite TTC evidence of irreversible damage to the myocardium at 3 and 6 hr. In contrast, k_T consistently predicted viability following reperfusion (*9*). After reperfusion, all potentially viable samples exhibited net uptake after first-pass delivery (positive k_T) and all irreversibly injured samples displayed net loss of activity (negative k_T). The explanation for the difference in ^{82}Rb kinetics observed between permanent occlusion and reperfusion is that reperfusion into nonviable myocardium accelerates the loss of vascular and cellular membrane integrity. Other contributing factors may be the lower signal to noise ratio after permanent occlusion which increased scatter in k_T values and the probability of an admixture of varying degrees of ischemic injury in the sample volume.

In summary, this study suggests that following permanent coronary occlusion, there is a relative decrease in ^{82}Rb membrane extraction in ischemic myocardium that precedes TTC evidence of irreversible injury. However, because extraction normally increases at low flow, the impairment in membrane function appears as a failure to increase extraction at low perfusion levels. Following loss of viability, cell membrane function is further impaired resulting in a leakage of tracer out of the cell, thereby causing a fall in net accumulation of tracer. Although leakage of ^{82}Rb from the cell occurs later in ischemia, it is more readily identified and followed as a marker of viability because net negative balance after first pass delivery of the tracer (i.e., negative k_T) is always associated with nonviable tissue. Low flow states, as with permanent occlusion, may delay the time at which k_T becomes negative compared to reperfusion where k_T becomes negative immediately for nonviable tissue. The net loss or washout of ^{82}Rb from the myocardial sample volume under the beta probe may then depend on sufficient flow to washout the ^{82}Rb which has leaked out from the cells but remains in the counting field. Rubidium-82 kinetics may therefore be useful for monitoring myocardial viability during acute infarction, especially in patients with reperfusion of a previously occluded coronary artery. The short half-life and availability of the tracer from a generator allows frequent serial sampling for assessment of therapeutic interventions in the absence of a cyclotron.

FOOTNOTES

* Harvard Apparatus Co., Inc., S. Natick, MA.
† EG and G Ortec, Oak Ridge, TN.

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