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S Schaefer, ... , B Massie, M W Weiner

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Research Article

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Relationship between Myocardial Metabolites and Contractile Abnormalities during Graded Regional Ischemia

Phosphorus-31 Nuclear Magnetic Resonance Studies of Porcine Myocardium In Vivo

Saul Schaefer,^{**} Gregory G. Schwartz,^{**} Joel R. Gober,^{*} Andrew K. Wong,^{*} S. Albert Camacho,^{**} Barry Massie,[‡] and Michael W. Weiner^{*}

Magnetic Resonance Unit^{*} and Cardiology Section,[‡] Veterans Administration Medical Center, and Departments of Medicine and Radiology, University of California, San Francisco, California 94121

Abstract

The mechanisms responsible for changes in myocardial contractility during regional ischemia are unknown. Since changes in high-energy phosphates during ischemia are sensitive to reductions in myocardial blood flow, it was hypothesized that myocardial function under steady-state conditions of graded regional ischemia is closely related to changes in myocardial high-energy phosphates. Therefore, phosphorus-31 nuclear magnetic resonance spectroscopy was employed in an in vivo porcine model of graded coronary stenosis. Simultaneous measurements of regional subendocardial blood flow, high-energy phosphates, pH, and myocardial segment shortening were made during various degrees of regional ischemia in which subendocardial blood flow was reduced by 16–94%. During mild reductions in myocardial blood flow (subendocardial blood flow = 83% of nonischemic myocardium), only the ratio of phosphocreatine to inorganic phosphate (PCr/P_i), P_i, and [H⁺] were significantly changed from control. PCr, ATP, and PCr/ATP were not significantly reduced from control with mild reductions in blood flow. Changes in myocardial segment shortening were most closely associated with changes in PCr/P_i ($r = 0.94$). P_i and [H⁺] were negatively correlated with segment shortening ($r = -0.64$ and -0.58 , respectively) and increased over twofold when blood flow was reduced by 62%. Thus, these data demonstrate that PCr/P_i is sensitive to reductions in myocardial blood flow and closely correlates with changes in myocardial function. These data are also consistent with a role for P_i or H⁺ as inhibitors of myocardial contractility during ischemia. (*J. Clin. Invest.* 1990; 85:706–713.) high-energy phosphates • magnetic resonance spectroscopy • myocardial ischemia • phosphocreatine

Introduction

Myocardial contractile function declines rapidly after coronary artery occlusion (1, 2) and is closely coupled to blood flow during partial coronary stenosis (3, 4). However, the mechanisms responsible for these functional changes during ischemia are unknown. Since high-energy phosphates are required for contraction and are vulnerable to ischemia (5–7), they have

been postulated as important factors in ischemic contractile dysfunction. Their role in decreasing function may be related to the availability of energy substrate, such as adenosine triphosphate (ATP), phosphocreatine (PCr),¹ or the free-energy change of ATP hydrolysis ($-dG/d\epsilon$) (8–10), inhibition of crossbridge formation by products of ATP hydrolysis, such as inorganic phosphate (P_i) (11, 12), or intracellular acidosis (13–15). In addition, possible regulators of mitochondrial respiration, such as the concentration of adenosine diphosphate [ADP] (16) or the phosphorylation potential [ATP]/[ADP][P_i] (17), may be important in modulating contractility. These factors may operate either by directly influencing actinomycin crossbridge formation or by altering calcium transients necessary for excitation–contraction coupling.

Although the relationship between myocardial high-energy phosphates and function has been investigated in perfused hearts (18–21) and after total coronary occlusion in vivo (1, 2), there are only limited data concerning this relationship during partial reductions in regional myocardial blood flow (22, 23). We have previously demonstrated that changes in high-energy phosphates, especially the PCr/P_i ratio, correlate closely with changes in myocardial blood flow under conditions of steady-state regional ischemia (24). The purpose of the present study was to test the hypothesis that myocardial function is closely related to the changes in myocardial high-energy phosphates, particularly PCr/P_i. Therefore, phosphorus-31 nuclear magnetic resonance (NMR) spectroscopy was used to study an in vivo porcine model of graded coronary stenosis. During various degrees of regional ischemia, simultaneous measurements were made of regional subendocardial blood flow, high-energy phosphates, pH, and myocardial segment shortening.

Methods

Animal preparation. Fasted female Yorkshire-Landrace pigs ($n = 10$) weighing 35–40 kg were studied. After pretreatment with ketamine HCl (10 mg/kg i.m.) and halothane (5% by face mask) each animal was intubated via a tracheotomy and ventilated with a tidal volume of 10 ml/kg using a pressure-limited ventilator (Bird Corp., Palm Springs, CA). The ventilation rate and the FiO₂ were adjusted to maintain an arterial pH between 7.35 and 7.45 and a PO₂ > 100 mm Hg, respectively. Anesthesia was maintained with a combination of 0.5% halothane and intermittent intravenous sodium pentobarbital (1 mg/kg every 15–30 min). A no. 7 French sheath was placed in a carotid artery for blood pressure monitoring and arterial blood sampling. Lidocaine (1 mg/kg) was administered intravenously as a bolus and followed by

Address reprint requests to Dr. Schaefer, Magnetic Resonance Unit (IIM) Veterans Administration Medical Center, 4150 Clement Street, San Francisco, CA 94121.

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1. Abbreviations used in this paper: LAD, left anterior descending coronary artery; NMR, nuclear magnetic resonance; PCr, phosphocreatine; P_i, inorganic phosphate.

an infusion of 2 mg/min throughout the experiment. The heart was exposed via a median sternotomy and suspended in a pericardial cradle. A 1-cm length of the proximal left anterior descending coronary artery (LAD) was dissected free and encircled by a hydraulic occluder (In-Vivo Metric, Inc., Healdsburg, CA). A catheter was inserted in the left atrium for injection of radioactive microspheres and pacing wires were sutured to the left atrial appendage. A 24-gauge catheter was inserted into the terminal portion of the LAD to monitor distal coronary pressure. To identify the zone perfused by the LAD, the LAD was occluded transiently and the resulting regional epicardial discoloration noted. A two-turn, 2.3-cm diam phosphorus spectroscopy surface coil was lightly sutured with three 4-0 silk sutures into the epicardium over the center of this zone so as to not impair movement of the epicardium. Regional systolic function was measured using a pair of piezoelectric crystals connected to a sonomicrometer (Triton Technology, Inc., San Diego, CA). The crystals were inserted into the subendocardium below the coil, oriented at a 45° angle to the surface of epicardium, ~ 1 cm apart. The pig was placed in a plastic cradle lined with a circulating water blanket to maintain body temperature.

In order to determine the true ejection period, a no. 7 French pigtail catheter was inserted into the left ventricle through the carotid catheter sheath and simultaneous measurements of left ventricular pressure and left ventricular dP/dt were made using short lengths of high-pressure tubing while the intramyocardial electrogram was recorded from the crystals.

The animal was then placed into a 1-m bore, 2.0-T imaging and spectroscopy unit (Gyrosan, Philips Medical Systems, Inc., Shelton, CT). Ventilation and monitoring equipment (Gould Inc., Cupertino, CA) were placed outside the magnetic shield allowing continuous life support, monitoring of arterial and distal LAD pressures, and recording of myocardial contractility. Heart rate was maintained at 100 beats/min by atrial pacing using a pulse generator (model 5880A, Medtronic, Inc., Minneapolis, MN).

Spectrometer preparation. After tuning the surface coil, shimming was manually adjusted on protons using a Gordin-Timms arrangement (25), yielding line widths under 35 Hz. A hexamethyl phosphorous triamide external standard was placed in the center of the surface coil and the 90° flip angle pulse length for this standard was determined. The standard was then removed and spectroscopy was performed using single radio frequency pulses of 180°. This pulse length was chosen from pilot experiments and computer modeling of the surface coil radiofrequency field to achieve heavier weighting of signal from the subendocardium relative to the subepicardium (26). Spectroscopy was electrocardiographically gated to the onset of every fifth diastole, yielding a repetition time of 3 s. For each measurement, 40 free induction decays were summed, resulting in a total acquisition time of 2 min for each spectrum.

Experimental protocol. Baseline measurements of hemodynamics and contractility were obtained. Three spectra were obtained under control conditions to establish the baseline measurement of high-energy phosphates. The LAD occluder was then slowly inflated using a microsyringe (VWR Scientific, San Francisco, CA) to achieve a fall in distal LAD pressure. After a stabilization period of 5 min, hemodynamics and segment shortening measurements were recorded at 100 mm/s. Because of radiofrequency interference from the sonomicrometer cables, spectral acquisition occurred with the cables disconnected and placed within the radiofrequency shield of the magnet. Two successive spectra were acquired over a total of 4 min. These measurements were followed by left atrial injection of randomly chosen 2–3 million 15- μ m diam radioactive microspheres (^{51}Cr , ^{54}Mn , ^{57}Co , ^{85}Sr , ^{95}Nb , ^{113}Sn , or ^{153}Gd) with concurrent measurement of arterial and distal LAD pressures, the intramyocardial electrogram, and regional segmental function. The total ischemic time at each stenosis was 12 min. The occluder was released after the end of data acquisition and serial 2-min spectra were acquired until the levels of PCr and ATP returned to within 10% of control. This process was repeated in each animal from two to six times at different degrees of pressure drop across the LAD stenosis. Degrees of stenosis were chosen randomly to

cover a range of reductions in segment shortening, except that a severe stenosis was usually induced after several milder degrees of flow reduction. At the end of the experiment, the animal was killed with a lethal dose of sodium pentobarbital, the location of the coil was marked, and the heart was excised and placed in formalin.

Spectral analysis. Spectra were analyzed using software (NMRi, Syracuse, NY), employing a convolution difference of 200 Hz, followed by an exponential multiplication of 15 Hz and phasing with a zero and first-order phase correction. Peak areas were fitted using Lorentzian line shapes for the phosphomonoesters, P_i , phosphodiesters, PCr, and the γ , α , and β resonances of ATP. Previous experiments under these conditions have not noted any significant changes in the line widths of PCr or ATP (24). For each spectrum, the following quantities were calculated as a fraction of control values: P_i , PCr, and ATP; the PCr/ P_i and PCr/ATP ratios; and the chemical shift of P_i in parts per million (ppm). Calculation of the ratios of high-energy phosphates (PCr/ P_i and PCr/ATP) was performed in order to minimize the variations in data acquisition between different animal preparations and because these ratios may have independent physiologic significance. The chemical shift of P_i was measured in order to calculate the change in pH with ischemia using the following equation: $\text{pH} = \text{pK} - \log \{(\delta_o - \delta_B)/(\delta_A - \delta_o)\}$, where δ_o = chemical shift in ppm, $\delta_A = 3.10$ ppm, $\delta_B = 5.75$ ppm, and $\text{pK} = 6.60$ (27). $[\text{H}^+]$ was calculated from the pH values as $10^{(-\text{pH})}$. Under control conditions, assignment of the P_i resonance was difficult owing to the large overlapping resonance from 2,3-diphosphoglycerate of the chamber blood. Thus, under control conditions, P_i was assumed to be the most upfield peak in that region, an assumption that would tend to underestimate the actual changes in P_i with ischemia. With ischemia, the P_i peak was easily resolved from the 2,3-diphosphoglycerate resonance.

Blood flow analysis. After formalin fixation, ~ 2-g sections of myocardium were cut from the region below the center of the surface coil and from two regions of myocardium distant from the ischemic region. Each section was then divided into subendocardial and subepicardial halves. After being blotted dry and weighed, segments were counted for 10 min in a gamma counter (Norland Corp., Ft. Atkinson, WI). These counts were corrected for the known radioactivities and decay rates of the isotopes, as well as for the weight of each sample (28). The counts in the subendocardial and subepicardial segments below the center of the coil were referenced to the mean counts in the segments in the two nonischemic regions. Thus, blood flow measurements were expressed as the ratio of blood flow in the myocardium below the center of the coil to the blood flow in distant nonischemic myocardium. These data were analyzed separately for subendocardial and subepicardial segments as well as for total transmural blood flow.

Function analysis. Using the timing calibrations performed at the beginning of the experiment, the end-diastolic dimensions were determined at the beginning of left ventricular contraction at the initial rise of the positive dP/dt signal. The end-systolic dimensions were determined at 20 ms before the peak negative dP/dt (4). Fractional systolic segment shortening was defined as $[(\text{end-diastolic length}) - (\text{end-systolic length})]/\text{end-diastolic length}$, and then normalized and expressed as a fraction of control segment shortening.

Postmortem examination of the position of the crystals confirmed their subendocardial location in all but two of the animals, which were excluded from analysis. In addition, if segment shortening did not return to within 10% of control values (usually following a severe ischemic episode), data obtained after this time were not included in the analysis. Therefore, prolonged myocardial dysfunction, i.e., “stunning,” was excluded as a factor in the analysis (29).

Data analysis. Results for the spectral and functional analysis were related to subendocardial blood flow for each animal. In order to compare the changes in function and metabolites for the eight animals, the normalized data were grouped for analysis over five levels of relative subendocardial blood flow (1.0 [control], 0.99–0.75 [level I], 0.74–0.50 [level II], 0.49–0.25 [level III], and 0–0.24 [level IV]). The mean and standard error for these variables at each of the mean levels of blood flow reduction for these levels were then calculated.

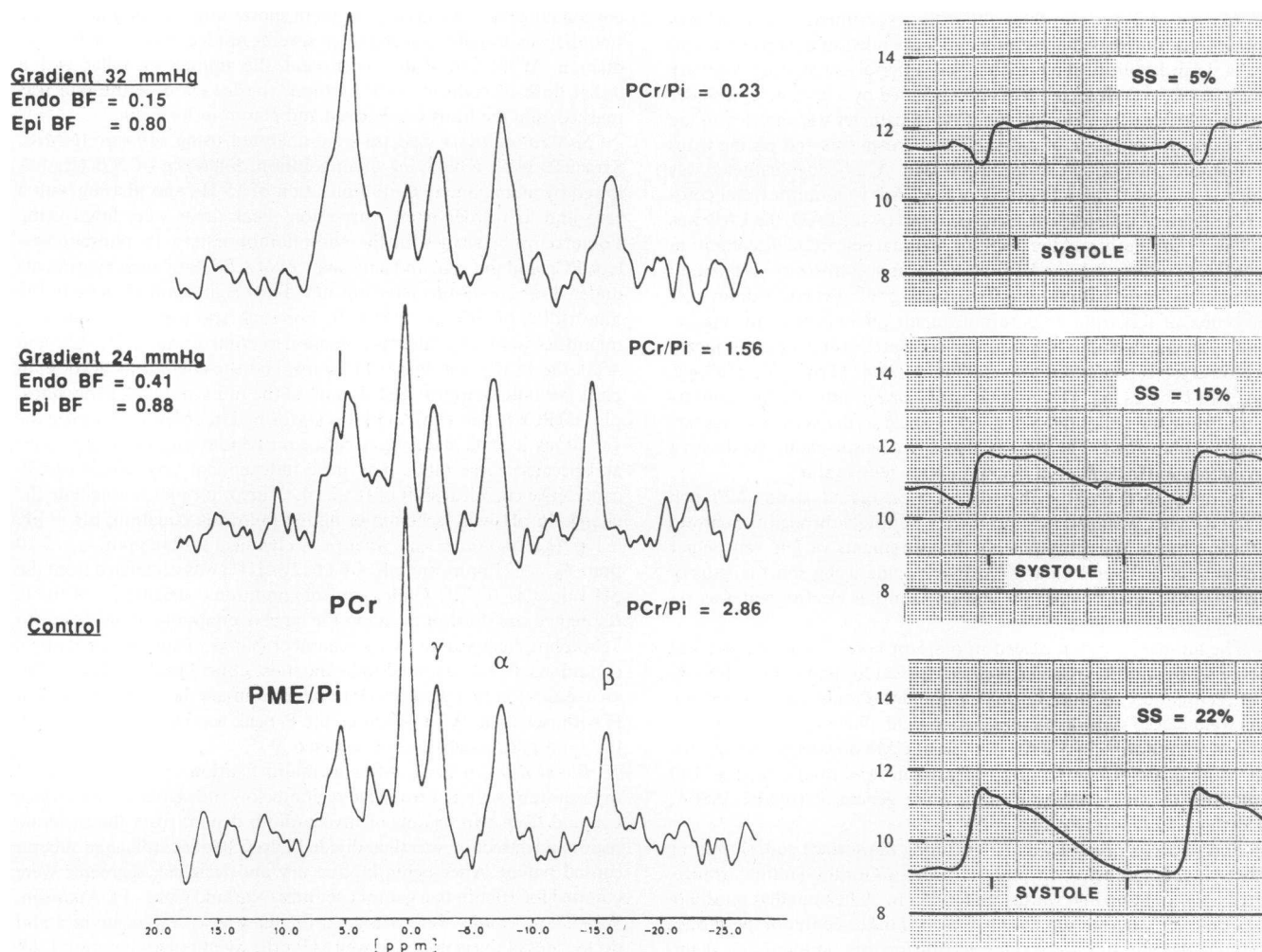


Figure 1. Representative spectra (left) and sonomicrometer tracings from subendocardial crystals (right) for three different degrees of coronary artery stenosis. Under control conditions (bottom), both the PCr/Pi ratio and percent myocardial segment shortening (SS) are normal. Under conditions of moderate regional ischemia resulting from a 24-mm Hg gradient between aortic blood pressure and LAD pressure (middle), subendocardial blood flow (Endo BF) and subepicardial blood flow (Epi BF) are reduced compared to normalized values of 1 under control conditions, with similar reductions in PCr/Pi and segment shortening. Under conditions of severe subendocardial ischemia resulting from a 32-mm Hg pressure gradient (top), PCr/Pi is markedly reduced and segment shortening is now only 5%.

Statistical analysis. Statistical analysis for the variables was performed using a commercial statistics program (Statview 512+, Brain Power, Inc., Calabasas, CA) operating on a MacIntosh SE microcomputer (Apple Computer, Inc., Cupertino, CA). Differences between control measurements and values obtained during periods of reduced blood flow were analyzed using a Student's two-tailed paired *t* test with the Bonferroni correction for multiple tests of significance (30). Differences between variables at any given level of blood flow reduction were analyzed using a Student's two-tailed paired *t* test. Correlation coefficients were calculated on the raw data using linear least square fits and are expressed as Pearson's *r*. A *P* value of < 0.05 was considered significant. All data are expressed as mean ± 1 SE.

Results

The mean systemic blood pressure did not change significantly between control conditions and the most severe period of stenosis (74.5 ± 5.2 vs. 70.3 ± 6.6 mm Hg, respectively [mean ± SEM]).

A representative set of spectra and sonomicrometry tracings for three levels of myocardial blood flow are shown in Fig.

1. The spectra demonstrate a fall in PCr, rise in Pi, and upfield shift of Pi (signifying intracellular acidosis) as the gradient between the aortic pressure and distal coronary artery pressure was increased to 32 mm Hg. Corresponding values for subendocardial and subepicardial blood flow show the increasing severity of ischemia, not only as a reduction in subendocardial blood flow, but also as a change in the ratio of subendocardial to subepicardial blood flow. The changes in myocardial segment shortening, corresponding to these flow and metabolic changes, are illustrated by tracings from the subendocardially placed crystals. Under control conditions, segment shortening was 22%, which then fell to 15% with moderate ischemia, and to 5% with severe ischemia.

Mean calculated blood flows (as a fraction of nonischemic myocardium) for the levels I–IV were as follows: level I, 0.80 ± 0.03 (*n* = 3); level II, 0.67 ± 0.03 (*n* = 5); level III, 0.38 ± 0.02 (*n* = 7); and level IV, 0.11 ± 0.02 (*n* = 7). Fig. 2 shows the reductions in segment shortening, PCr, and ATP by level analysis that resulted from reductions in subendocardial blood flow. While the relationship between segment shorten-

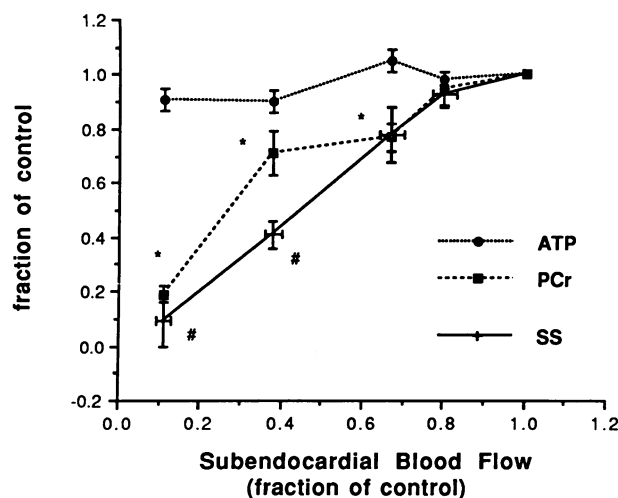


Figure 2. Normalized segment shortening (SS), PCr, and ATP (mean \pm SEM) as a function of subendocardial blood flow by levels of blood flow reduction. Horizontal error bars on segment shortening indicate SEM for each blood flow level. See text for details of analysis. * $P < 0.01$ vs. control (PCr); * $P < 0.01$ vs. control (SS).

ing (SS) and relative subendocardial blood flow (BF) was essentially linear ($SS = 1.07 \cdot BF - 0.04$, $r = 0.91$, $P = 0.0001$), segment shortening fell significantly only at level III using the above described analysis. ATP did not change significantly, whereas PCr was significantly reduced when subendocardial blood flow was reduced to 67% of control (level II). Whereas changes in PCr were similar to and closely correlated with those of segment shortening ($r = 0.90$, standard error of estimate [SEE] = 0.147, $P = 0.0001$), changes in segment shortening were greater than PCr when blood flow was reduced to 38% of control (level III).

Fig. 3 shows the changes in P_i with reductions in blood flow. For comparison with Fig. 2, segment shortening is shown in this and the following graphs without error bars. P_i rose significantly by level I and was increased by over twofold when blood flow was reduced to 38% of control. The increase in P_i was closely correlated to the reduction in segment shortening

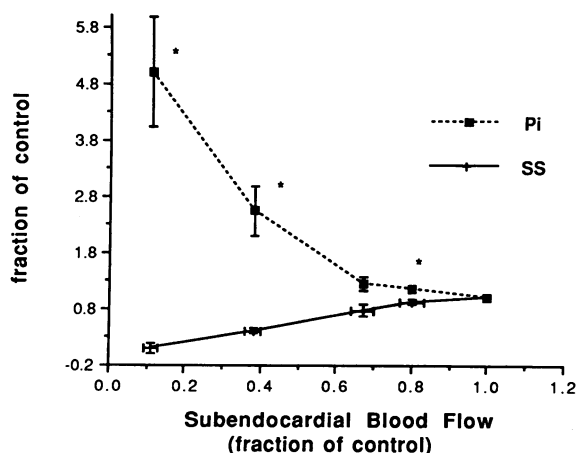


Figure 3. Normalized P_i (mean \pm SEM) as a function of subendocardial blood flow by levels of blood flow reduction with segment shortening (SS) shown as a solid line. See text for details of analysis. * $P < 0.01$ vs. control (P_i). Significance levels for SS as in Fig. 2.

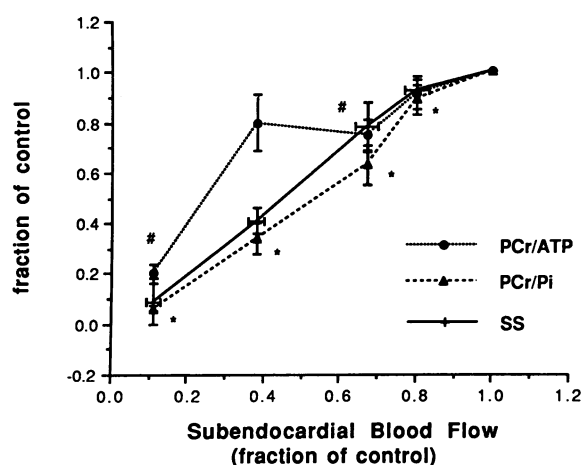


Figure 4. Normalized PCr/ATP and PCr/ P_i (mean \pm SEM) as a function of subendocardial blood flow by levels of blood flow reduction with segment shortening (SS) shown as a solid line. * $P < 0.01$ vs. control (PCr/ P_i); * $P < 0.01$ vs. control (PCr/ATP).

($r = -0.64$, SEE = 1.614, $P = 0.002$), as well as to the reduction in subendocardial blood flow ($r = -0.70$, SEE = 1.505, $P = 0.0001$).

Fig. 4 shows the relationship of PCr/ATP and PCr/ P_i to subendocardial blood flow, and compares them to the changes in segment shortening. The mean value of PCr/ATP under control conditions was 1.34 ± 0.06 . PCr/ATP did not change significantly until blood flow was reduced to 67% of control (level II), whereas PCr/ P_i was significantly reduced when blood flow was reduced to 80% of control (level I). The relationship between PCr/ P_i and subendocardial blood flow (BF) was linear ($PCr/P_i = 1.05 \cdot BF - 0.05$, $r = 0.94$, SEE = 0.125, $P = 0.0001$). Furthermore, the correlation between PCr/ P_i and segment shortening was the closest for any metabolic variable ($r = 0.94$, SEE = 0.197, $P = 0.0001$), and there were no significant differences between PCr/ P_i and segment shortening at any level of blood flow reduction. This is seen in Fig. 5, which shows the individual animal data for segment shortening as a function of PCr/ P_i . These data indicate that PCr/ P_i was sensitive to mild reductions in subendocardial blood flow and, furthermore, ac-

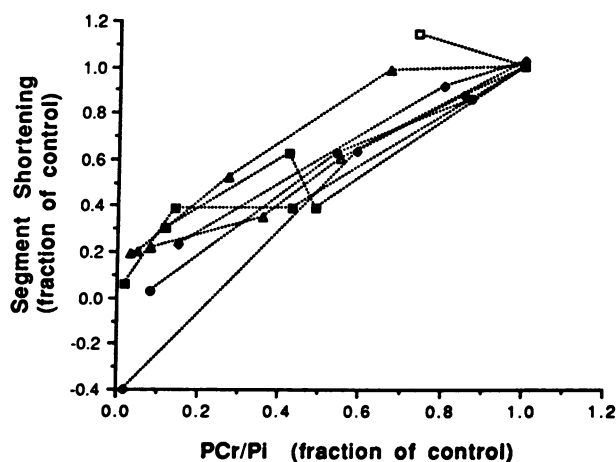


Figure 5. Normalized segment shortening (SS) as a function of PCr/ P_i for the eight animals. In seven of the eight animals, there was a monotonic decline in SS as PCr/ P_i declined during regional ischemia.

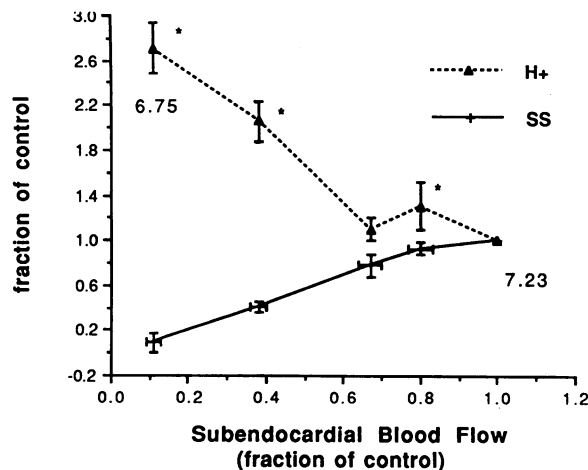


Figure 6. Normalized $[H^+]$ (mean \pm SEM) as a function of subendocardial blood flow by levels of blood flow reduction with segment shortening (SS) shown as a solid line. Calculated pH values at control and the most severe reduction in blood flow are shown. * $P < 0.01$ vs. control.

curately reflected the changes in myocardial segment shortening.

Intracellular pH was reduced from a mean of 7.23 ± 0.10 ($[H^+] = 58.9 \times 10^{-9}$ M) under control conditions to 6.75 ± 0.06 ($[H^+] = 177.8 \times 10^{-9}$ M) when blood flow was 11% of control (Fig. 6). Significant elevation of $[H^+]$ was observed when blood flow was reduced to 80% of control (level I) and, as with P_i , $[H^+]$ was increased by over twofold that when blood flow was reduced to 38% of control. Similarly, $[H^+]$ was negatively correlated to relative subendocardial blood flow ($r = -0.74$, SEE = 0.616, $P = 0.0001$). Although significant, the correlation between $[H^+]$ and segment shortening was less close than for other measured variables, except ATP ($r = -0.54$, SEE = 0.232, $P = 0.002$).

Discussion

This study examined the changes in myocardial function, high-energy phosphates, and pH of porcine myocardium during steady-state graded regional ischemia using ^{31}P NMR spectroscopy. Myocardial segment shortening in the subendocardium was found to decrease linearly as blood flow was reduced. The PCr/ P_i ratio was found to be a sensitive marker of regional myocardial ischemia and was closely correlated to changes in segment shortening in ischemic myocardium. Also, significant changes in P_i and H^+ were observed with only mild reductions in myocardial blood flow and segment shortening, indicating that these compounds are sensitive to myocardial ischemia and may have important roles as inhibitors of myocardial function during ischemia.

Myocardial blood flow and function

The relationship between myocardial blood flow and contractile function has previously been defined in perfused heart preparations (19–21) and in vivo models of coronary stenosis (3, 4). The perfused heart studies have noted an essentially linear relationship between flow and various measures of myocardial function (rate-pressure product, developed pressure, and dP/dt) over a wide range of flow rates. Similarly, the

in vivo studies have found virtually linear relationships between either coronary artery or myocardial tissue blood flow and indexes of myocardial function when flow is reduced below normal. The results of this study, namely a closely linear relationship between subendocardial blood flow and myocardial function (as measured by subendocardial segment shortening), are similar to these previous results, but extend these earlier in vivo observations by examining myocardial metabolism and function simultaneously.

Mediators of myocardial function during ischemia

Two major mechanisms have been postulated as the cause of reduced myocardial function during ischemia. These are inhibition of myocyte contraction by by-products of ATP hydrolysis and glycolysis or inadequate energy to maintain contractile function.

BY-PRODUCTS OF ATP HYDROLYSIS AND ANAEROBIC GLYCOLYSIS

As oxidative phosphorylation is inhibited by ischemia, the products of ATP hydrolysis, namely P_i and ADP, increase in the cytosol while anaerobic glycolysis produces lactic acid, thus lowering tissue pH. Previous studies have suggested that increasing amounts of P_i , ADP, and/or H^+ may be responsible for contractile dysfunction.

P_i . Increased inorganic phosphate may directly inhibit contractile function since the release of P_i from the actin-myosin crossbridge is associated with force development (11). Thus, an increase in P_i (either total P_i , $H_2PO_4^-$, or HPO_4^{2-}) may inhibit crossbridge formation in vivo. Evidence for an inhibitory role for P_i comes from studies of skinned cardiac muscle showing a large negative effect of P_i on maximal force production and myofibrillar Ca^{++} sensitivity (11), although in hypoxic isolated ferret hearts there is a strong inverse correlation between maximum Ca^{++} -activated pressure development and P_i (12). Studies of skeletal muscle have had similar findings both in vitro (31) and in vivo (32).

The present study also demonstrates a strong inverse relationship between P_i and myocardial function, as well as sensitivity of P_i to small reductions in myocardial blood flow. For example, when blood flow was reduced by 20%, P_i had increased 17% while contractility was reduced by 7%. Although this observation is not proof of a causal role for P_i , it shows that, in vivo, P_i is sensitive to myocardial ischemia. These data, together with previous observations of its effect in isolated muscle and perfused hearts, further emphasize the potential of P_i as an important inhibitor of contractile dysfunction.

In contrast to these findings, P_i was not closely correlated to myocardial function at high perfusate flow rates in a perfused heart study of graded ischemia (19). These investigators noted that function (measured as rate-pressure product) varied linearly over a wide range of perfusate flows, whereas changes in PCr and P_i only occurred at relatively low flow rates. They also noted that P_i was maintained at flow rates sufficiently low to reduce PCr. However, it is difficult to determine the normal coronary flow rate of a heart perfused with red cell-free perfusate, and the governing mechanisms operating at high flow rates may be different than those operating when flow is below normal. For example, at high flow rates, nonmetabolic mechanisms (such as mechanical distension from increased coronary flow) may be dominant in determining myocardial function, whereas at low flow rates, metabolic factors may predominate.

Furthermore, in this perfused heart study, the heart was perfused only with glucose, resulting in a lower PCr/ P_i ratio than observed in vivo under more physiologic substrate conditions (33).

H^+ . The accumulation of H^+ ions, producing intracellular acidosis, has also been postulated to produce myocardial dysfunction during ischemia. Several prior studies have shown diminished contractility with intracellular acidosis (13–15) and a reduced sensitivity of the myofibrillar ATPase to Ca^{++} as pH is reduced from 7.0 to 6.2 (34). Recently, Watanabe et al. (35) using a similar porcine model of graded regional ischemia and plunge electrodes in the midmyocardium, demonstrated that local potassium concentrations and pH were more sensitive to reductions in coronary blood flow than segmental function. In their study, midmyocardial $[K^+]_e$ and pH_e were changed at approximate myocardial blood flows of 0.7–0.8 ml/min per g, whereas segment shortening was only altered when blood flow was in the range of 0.5–0.6 ml/min per g. However, in a perfused ferret heart preparation under hypoxic conditions (12), maximal Ca^{++} -activated pressure was not correlated with intracellular pH, whereas in a rat model of acute ischemia (18), $[H^+]$ was the slowest-changing variable, lagging far behind functional changes.

The magnitude of the changes in $[H^+]$ observed in this study agree with results of previous perfused heart and in vivo studies of graded ischemia (19–21, 24, 35). Statistically significant changes in $[H^+]$ occurred at mild reductions in blood flow (80% of control) that corresponded to an approximate absolute flow rate of 0.8 ml/min per g. Although the correlation between segment shortening and $[H^+]$ was less strong than for P_i or PCr/ P_i , this may have resulted from the inaccuracies inherent in assigning the P_i resonance under control conditions.

INADEQUATE ENERGY SUBSTRATE

Myocardial dysfunction may be related to inadequate energy substrate, such as insufficient ATP concentrations to maintain contractile function, or inadequate energy supply, expressed as the free energy of ATP hydrolysis, $-dG/d\epsilon$, where $-dG/d\epsilon$ is a function of $[ATP]$, $[ADP]$, $[P_i]$, free $[Mg^{++}]$, and pH. Previous studies have shown the NMR observed $[ATP]$ to be stable during partial or early total ischemia (1, 2, 19, 24). In the present study, the myocardium was able to maintain cytosolic ATP concentration, presumably owing to the reduced energy requirements for ATP related to reduced contractility during ischemia. This decrease in ATP utilization, presumably combined with recruitment of alternate pathways for generation of ATP (via the creatine kinase reaction and glycolysis), served to maintain ATP at normal levels. These findings indicate that ATP concentration is not an important mediator of myocardial dysfunction under conditions of graded regional ischemia.

There is some evidence from isolated rat heart studies (8, 9) that $-dG/d\epsilon$ declines rapidly during global ischemia or anoxia and may fall below the energy requirements (~ 45 kJ/mol) for sodium or calcium transport, functions necessary for excitation-contraction coupling. However, the role of $-dG/d\epsilon$ in contractile failure has been challenged by observations in skinned cardiac muscle fibers (36) that changing $[ADP]$ and $[P_i]$ altered contractility despite constant $-dG/d\epsilon$. Also, in studies of acute ischemia in an isolated guinea pig heart (18), the time course of the change in $-dG/d\epsilon$ lagged that of myocardial function and the phosphorylation potential ($[ATP]/[ADP][P_i]$).

It must be noted that the relationships between metabolites and changes of systolic function found in this study do not necessarily imply causality. It is possible that very small changes in a metabolite (such as P_i , or H^+) can have significant effects on myocardial contractility. However, thresholds of blood flow reductions at which the measured variables were significantly reduced can be compared to determine relative roles in relationship to contractile function. The data are also consistent with relationships between high-energy phosphates, pH and myocardial function suggested by other experiments. Although these results have been compared to prior studies of perfused hearts (employing both graded ischemia and acute total ischemia), there are significant differences in the findings that may be due to the differences between the in situ animal preparation used here and the perfused heart. It is also likely that the mechanisms governing myocardial contractility in the steady-state condition of graded ischemia differ from those in acute, severe ischemia. In addition, other variables not measured in this study may effect myocardial function. These variables include the loss of myocardial distension when flow is reduced (37), alterations in the duration or amplitude of the action potential (38), and changes in calcium fluxes (39).

Limitations

The primary limitations of this study relate to the difficulties in accurately measuring metabolites and pH using ^{31}P NMR. While the peak resonances of PCr and ATP can be measured with great confidence in these spectra, the P_i resonance overlies a large resonance from the 2,3-diphosphoglycerate of chamber blood. Because of this overlap, accurate measurement of the intensity and chemical shift of the P_i resonance was difficult under control conditions. With ischemia, the P_i resonance was easily observed and its area and chemical shift accurately measured. Thus, while the calculation of pH and $[H^+]$ was subject to greater error under control conditions, the assumption of the P_i resonance as the most upfield peak allowed relatively consistent assignment of the P_i chemical shift within an experiment. These difficulties would tend to obscure small changes in P_i and H^+ concentration that might otherwise be observed. In these studies, the changes in P_i and $[H^+]$ were observed at small reductions in blood flow, indicating that the sensitivity of the measurements to ischemia were not severely affected.

Another issue relates to the acquisition of data from the ischemic region of the left ventricle. Since lateral spatial localization in this experiment was provided by the surface coil, all tissue within the sensitive volume of the coil could have contributed to the overall signal. More normally perfused tissue peripheral to the region in which blood flow was determined may have contributed to the spectra and diluted the changes in metabolites seen during ischemia. However, contamination of the spectra by normal myocardium was avoided by using a small-diameter surface coil within a large ischemic region. In this way, the lateral extent of the sensitive volume of the coil was well within the ischemic myocardium.

Localization across the depth of the myocardium was also considered in this experiment. The pulse-acquire sequence did not provide precise localization to the subendocardium of the ischemic myocardium. As in previous experiments (24), the radio frequency pulse was selected to obtain spectra weighted to the subendocardium. However, this weighting was only relative, and the spectra were contaminated by signals from the

subepicardium. Since blood flow in the subepicardium was higher than the subendocardium at any given degree of coronary artery stenosis, this effect would serve to minimize the metabolic changes when compared to subendocardial blood flow. Techniques that more precisely localize to one or more layers of the myocardium have been demonstrated (40, 41), but require longer times for spectral acquisition.

Finally, the hemodynamic status of our preparation may have affected the results. Our mean blood pressures of ~70 mm Hg may have placed the animals at a metabolic point where small reductions in perfusion pressure made them regionally ischemic. It is possible that animals operating at higher perfusion pressures would have exhibited different functional and metabolic responses to small reductions in coronary blood flow. However, the similarity of the functional data in this study to previously published conscious animal preparations argue against this being a significant factor (4, 42).

Summary

Phosphorus-31 NMR spectroscopy was employed in an vivo porcine model of graded ischemia to determine the relationship of myocardial function and high-energy phosphates to graded reductions in regional myocardial blood flow. The PCr/P_i ratio was found to relate directly to the change in segment shortening as subendocardial blood flow was reduced. Changes in P_i and [H⁺] occurred with only mild reductions in subendocardial blood flow and were relatively greater than changes in contractility, supporting their possible role as inhibitors of myocardial contractility. ATP was maintained throughout the range of blood flow reduction. These data indicate that changes in myocardial high-energy phosphates are closely related to the functional changes seen with steady-state regional ischemia in vivo.

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