Effects of diltiazem on phosphate metabolism in ischemic and reperfused myocardium using phosphorus^{31} nuclear magnetic resonance spectroscopy in vivo

Diltiazem may provide a protective effect to ischemic and reperfused myocardium through preservation of high-energy phosphate metabolism. To test this hypothesis, rabbits had a 1.3 cm solenoidal coil placed over the myocardium to be rendered ischemic. Data were acquired with a 22 cm bore nuclear magnetic resonance spectrometer at 2.0 T. Animals were treated with diltiazem (200 μg/kg intravenous bolus of drug followed by a 15 μg/kg/min continuous intravenous infusion, $n = 10$) or by an equal volume of saline ($n = 6$). The left circumflex artery was occluded and reperfused using a reversible snare while electrocardiogram-gated spectra were accumulated. Levels of phosphocreatine were decreased during occlusion in both groups; however, this decrease was attenuated in the diltiazem treated animals compared to control (in relative percent area: 7.8 ± 1.0 to 2.5 ± 0.5, $p < 0.01$). Levels of phosphocreatine promptly returned to baseline following reperfusion and there was no difference between the two groups. The inorganic phosphate metabolites of high-energy phosphate consumption increased with occlusion, though more so in the control group compared with the diltiazem-treated rabbits (in relative percent area: 72.5 ± 0.9 to 55.4 ± 1.3, $p < 0.01$). With reperfusion, levels of inorganic phosphates returned toward baseline in both groups; however, the diltiazem group had a more complete recovery relative to control (in relative percent area: 38.8 ± 2.1 to 47.6 ± 2.7, $p < 0.05$). Levels of adenosine triphosphate decreased in both groups relative to baseline; however, the amount of decrease was similar in the two groups. With reperfusion there was a definite though incomplete recovery of levels of adenosine triphosphate in the diltiazem-treated group (in relative percent area: 10.7 ± 1.0 at occlusion, 12.3 ± 0.4 during reperfusion, $p < 0.05$), but in the control group levels of adenosine triphosphate remained depressed (in relative percent area: 9.8 ± 0.6 at occlusion, 9.8 ± 0.8 during reperfusion, p = NS). During ischemia there was a trend toward attenuation of intracellular acidosis in the diltiazem group; however, this trend did not reach statistical significance. These data indicate that diltiazem provides a protective effect on myocardial high-energy phosphate metabolism during regional ischemia and reperfusion in the intact animal. (Am Heart J 1989;118:1210.)

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Phosphorus^{31} nuclear magnetic resonance (NMR) spectroscopy is a unique method for examining metabolic events in a noninvasive manner that is not destructive to tissue. With this technique, one can measure the relative intracellular concentrations of the high-energy phosphate molecules, phosphocreatine (PCr) and adenosine triphosphate (ATP), along with the "inorganic" phosphate (Pi) molecules released during their hydrolysis required for the energy-consuming processes of the cell. Previous studies in the intact animal[1-4] have examined the changes in high-energy phosphates during ischemia and reper-

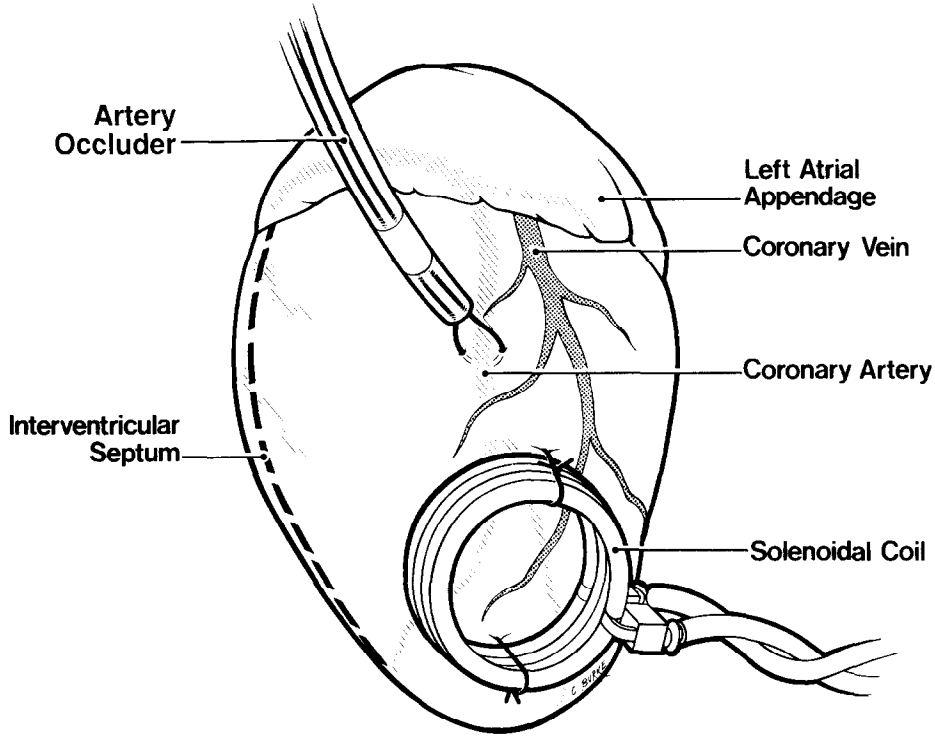

Fig. 1. Diagram illustrating a view of the left ventricle with the instrumentation used in the model. A suture is placed about the subepicardial marginal branch of the left circumflex coronary artery and is brought out through a plastic snare. The radiofrequency solenoidal coil is anchored distal to the suture over the region of myocardium to be rendered ischemic.

fusion. Typical changes in high-energy phosphates assessed by phosphorus[31] NMR spectroscopy techniques during regional myocardial ischemia in the intact animal include a profound depletion of PCr with a concomitant increase in Pi, along with a decreased level of ATP. With reperfusion, levels of PCr and Pi return toward normal; levels of ATP, however, tend to remain depressed.

Calcium antagonists have a theoretical and experimental basis for exerting myocardial protection in the setting of ischemia and reperfusion related to the fact that myocardial injury in this setting is associated with calcium ion accumulation in the cytoplasm and mitochondrial matrix. Previous studies that used biochemical assays and phosphorus[31] NMR spectroscopy in the isolated heart preparation have shown various degrees of myocardial metabolic protection with calcium antagonists during ischemia and reperfusion.[5-13] Although considerable knowledge has been gained from in vitro studies of cardiac metabolic and physiologic functions, the ultimate description of these processes must be in terms of their regulation and integration in the intact, living animal. There are many advantages to examining the intact heart. Since the heart is maintained by the animal, the in vivo model does not have problems related to viability and instability compared with the buffer-perfused in vitro system. In addition, the in vivo model is more physiologic with adequate oxygen, blood pressure, osmolarity, temperature, electrolytes, and nutritional substrate provided by the animal.

To date, there have been no studies examining the effects of calcium antagonists in the intact animal using phosphorus[31] NMR spectroscopy. Since there may be significant differences between the isolated heart preparation and the intact animal model and since there have been variable results reported in the literature concerning the effect of calcium antagonists on high-energy phosphate metabolism during ischemia, we felt that our intact rabbit model of regional ischemia and reperfusion using phosphorus[31] NMR spectroscopy might offer additional insights into the mechanisms of cardioprotection. This is of particular interest, since the efficacy of calcium antagonists in the setting of acute myocardial infarction in humans remains controversial and largely unproven,[11, 14] further underscoring the importance of additional metabolic studies that may improve our

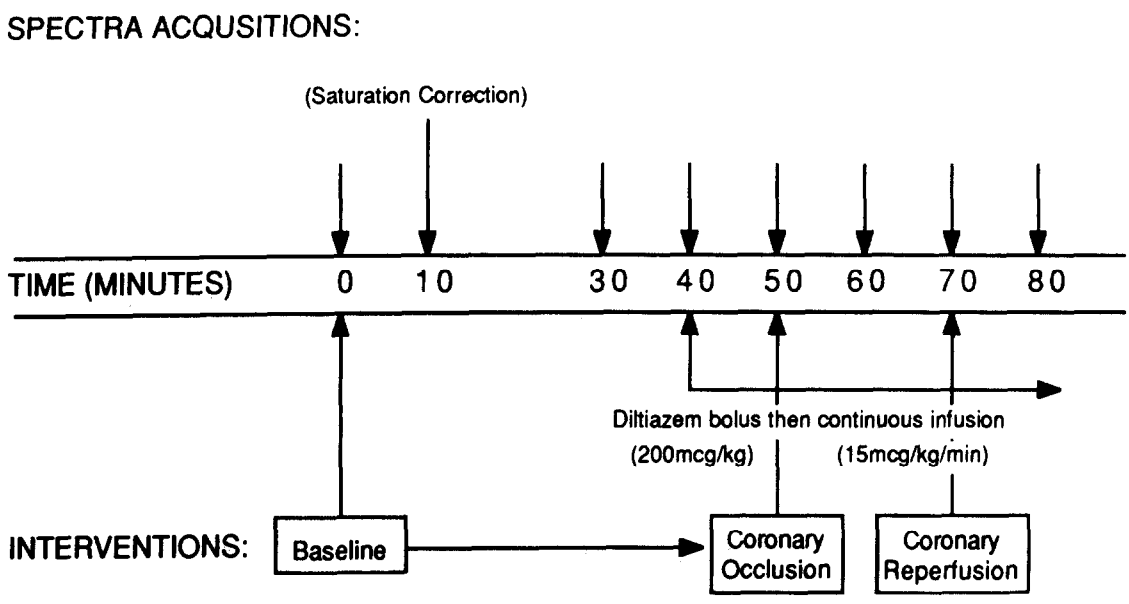

Fig. 2. Outline of the experimental protocol. *Arrows* at the top of the time line represent the beginning of each individual acquisition. See text for further details.

understanding of cardioprotective mechanisms. Accordingly, we undertook this study to examine the effect of diltiazem during regional myocardial ischemia and reperfusion on high-energy phosphate metabolism in the intact animal using phosphorus[31] NMR spectroscopy techniques.

METHODS

Animal model (Fig. 1). Experimental procedures complied with the "Guiding Principles in the Use and Care of Animals" approved by the Council of the American Physiological Society as well as with state and federal laws. Sixteen healthy New Zealand white rabbits (3.75 ± 0.25 kg) were anesthetized initially with xylazine (10 mg/kg) intramuscularly followed by ketamine (40 mg/kg), also via intramuscular injection. A carotid artery was isolated and cannulated to allow monitoring of the mean arterial pressure. An internal jugular vein was isolated and cannulated with a polyethylene tube for venous access. Anesthesia was maintained with frequent small intravenous boluses of ketamine (1 to 2 mg) through this central venous line. The trachea was then isolated and animals were intubated via tracheostomy. Rabbits were then ventilated via a Harvard rodent ventilator (Harvard Apparatus Inc., S. Natick, Mass.) on a 95% oxygen and 5% carbon dioxide mixture. The strength of the magnetic field required that this respirator remain a remote distance from the animal. Accordingly, two approximately 5 m lengths of tube were attached to the inspiratory and expiratory ports of the ventilator. These tubes were then each connected to a one-way valve and joined at a T piece from which a short (6-inch) length was attached to the endotracheal tube. This setup, along with the alternating valve action of the Harvard respirator, minimized respiratory dead space. Arterial blood gasses were sampled in a series of pilot experiments and revealed pH and P_{CO_2} consistently in the normal range, with the oxygen saturation always above 95%, assuring adequate function of the respirator apparatus.

Animals then underwent a left lateral thoracotomy through the fifth intercostal space. The pericardium was excised allowing visualization of the lateral surface of the left ventricle. The large marginal branch of the left circumflex coronary artery, which runs subepicardially in the rabbit,[15] was identified. A 6-0 suture was then placed through the myocardium underneath this artery slightly distal to the atrioventricular groove. The suture was then brought through a polyethylene tube that served as a reversible snare occluder. The same solenoidal radiofrequency coil, described in detail below, was used in all experiments. It was placed on the surface of the myocardium distal to the suture snare. A brief (5- to 10-second) occlusion was performed to better identify the ischemic area of myocardium and aid in proper placement of the coil described above. Electrocardiograph leads were then placed on the animal's extremities and each rabbit was wrapped in an insulated covering prior to being placed in the spectrometer.

Reliability of the reversible snare occluder was validated in several ways: (1) A brief (5- to 10-second) occlusion as described above was done in each animal and resulted in the development of cyanotic, dyskinetic myocardium along with blanching of the coronary artery distal to the suture. (2) In a series of pilot experiments, radioactive microspheres were injected into the left atrium before, during, and after occlusion, using an identical suture snare. These studies revealed a near total ablation of subendocardial blood flow in the myocardium distal to the occlusion, which normalized with release of the suture snare. (3) Typical ST

segment elevation was seen relative to baseline tracings on the ECG monitor. (4) Typical changes in ^{31}P NMR spectroscopy, as previously reported in the literature,[3, 4, 16-18] were seen consistently during appropriate experimental conditions.

Nuclear magnetic resonance spectroscopy. Experiments were performed with a General Electric/Nicolet CSI-II NMR spectrometer, (General Electric NMR Instruments, Fremont, Calif.), with a 22 cm bore, operating at 2.0 T. The surface radiofrequency coil employed was constructed out of 0.8 mm copper wire and configured to four turns, with a diameter of 13 mm. The axial length of 6 mm assured near optimum circuit Q and the four-turn coil design resulted in concentration of the B1 field along the coil's axis, thus minimizing signal contamination from nonischemic tissue. A balanced tuning circuit[19] with grounding of the unbalanced side inside the magnet bore reduced capacitive losses and eliminated spurious resonances of the coaxial circuit formed by the bore and the radiofrequency cable. Insulation of the entire coil with a silicone rubber wrap prevented corrosion. The short T2 proton resonance from this insulating material was well separated from the water proton signal and posed no problem during shimming, when the receiver gain was set to the level of tissue proton signal. Sensitivity profiles of this coil from phantom studies revealed maximum sensitivity at the coil surface with 80% of the signal recovered from within 6 mm of axial distance. This coil, while within the rabbit, after being centered in the magnetic field, was tuned with the external tuning circuit to 34.62 MHz, the phosphorus resonant frequency at this field strength.

Electrocardiographic (ECG) gating was employed to reduce motion-related line broadening. ECG signals from the animals were fed into a standard monitor fitted with a custom-made R-wave trigger output assuring signal acquisition during the same period of the cardiac cycle. Local magnetic field homogeneity was optimized by shimming on the proton signal with a pulse width of 30 μsec, yielding water line widths between 0.3 and 0.5 ppm. Gated phosphorus[31] spectra were acquired over a period of 10 ± 1 minutes by averaging 256 transients with a 2.0-second interpulse delay. Also, one spectrum was acquired for 7 of the 10 animals with an 8.0-second interpulse delay prior to any intervention for calculation of partial saturation factors. Spectra were transformed with a line broadening of 10 Hz. The chemical shift for PCr was defined as 0 ppm by convention.[20]

Experimental protocol (Fig. 2). After acquisition of baseline spectra, including the spectra with an 8.0-second interpulse delay as described above, the diltiazem-treated group (n = 10) received a 200 μg/kg intravenous bolus of drug followed by a 15 μg/kg/min continuous intravenous infusion. The control group (n = 6) received an equal volume of normal saline. A post-drug (saline) spectra was then acquired in all animals. The suture snare was tightened to produce coronary artery occlusion without disturbing the rabbit's position within the NMR unit. Two more spectra were then acquired consecutively during the 20 ± 2-minute ischemic period. This duration of occlusion was chosen for two reasons. First, the lateral borders of ischemia are well established within 15 minutes of occlusion; second, though there will be some degree of damage to myocardium, some muscle will not be irreversibly damaged, with the greater proportion of this viable tissue being more subepicardial and thus closer to the surface coil.[21] Upon completion of the occlusion period, the suture snare was released, allowing myocardial reperfusion. Two more spectra were acquired serially during this reperfusion period, after which animals were killed. Heart rates were recorded at each time point described above and mean arterial pressures were recorded throughout the experiment.

Data analysis. The areas under the five phosphate spectral peaks were calculated with the aid of a Lorentzian curve-fitting program supplied with the CSI computer. Data were expressed as relative percent area for each respective peak compared with the total area. The ratio of PCr to Pi, an index of mitochondrial function,[22] was also calculated at each time point. The chemical shift of the Pi peak relative to PCr was measured at occlusion as an estimate of intracellular pH.[23] All data are presented as mean ± SEM. Comparisons within treatment groups were made by means of analysis of variance. When significant F statistics were obtained, paired t tests were used to distinguish which time periods differed significantly. Because multiple comparisons were performed, the Bonferroni inequality adjustment was used to modify the acceptable α level. Comparisons between treatment groups were made with unpaired t tests.[24]

RESULTS

Heart rate and mean arterial pressure decreased approximately 10% after infusion of diltiazem, though this decrease did not reach statistical significance. Heart rate and mean arterial pressure did not change significantly during occlusion or reperfusion in either group.

Given the heterogeneity of the Pi peak in the intact animal, accurate measurement of chemical shift for determination of intracellular pH was difficult during nonischemic periods. However, with an ischemic insult, levels of Pi rose to an extent that allowed us to reliably distinguish this compound from the 2,3-diphosphoglycerate of blood and other phospho mono- and diesters that also resonate at this frequency. Though a trend was noted, this experiment revealed no significant preservation of normal intracellular pH when the diltiazem group was compared with the control group during ischemia.

The results of the ^{31}P NMR measurements of high-energy phosphates are summarized in Table I and Figs. 3 to 6. The baseline data are those acquired after diltiazem (or saline) infusion, although there was no difference in the spectra for any group before or after diltiazem (or saline) infusion. Pi increased significantly during occlusion in both groups as a re-

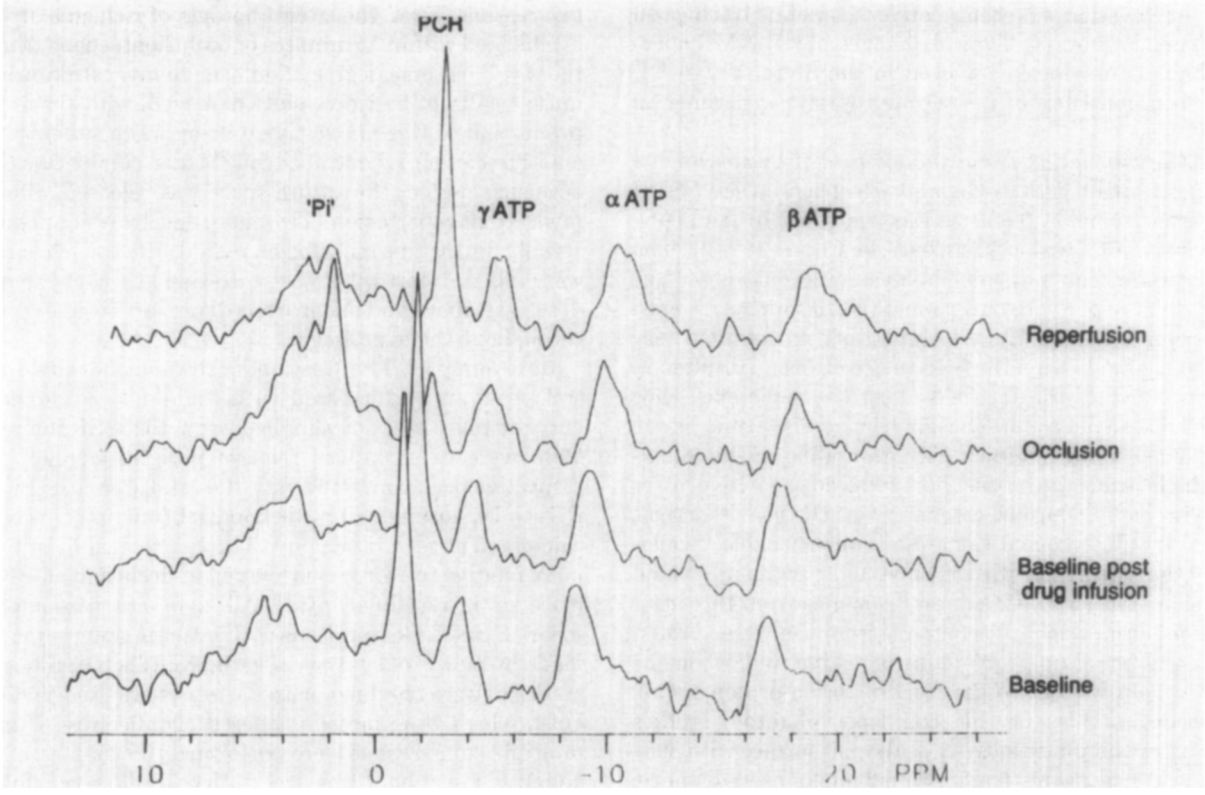

Fig. 3. Representative example of one experiment with spectra acquired at baseline, at baseline after infusion of diltiazem, at occlusion, and at reperfusion.

Table I. High-energy phosphates (relative percent area ± SEM)

	Baseline	*Occlusion*	*Reperfusion*
PCr			
Diltiazem	23.7 ± 1.5	7.8 ± 1.0*	21.7 ± 1.3†
Control	27.4 ± 1.3	2.5 ± 0.5*	23.2 ± 2.6†
Diltiazem vs Control	p = ns	$p < 0.01$	p = ns
Pi			
Diltiazem	28.8 ± 2.3	55.4 ± 1.3*	38.8 ± 2.1*†
Control	31.8 ± 1.1	72.5 ± 0.9*	47.6 ± 2.7*†
Diltiazem vs Control	p = ns	$p < 0.01$	$p < 0.05$
ATP			
Diltiazem	14.9 ± 0.7	10.7 ± 1.0*	12.3 ± 0.4*†
Control	14.5 ± 0.5	9.8 ± 0.6*	9.8 ± 0.8*
Diltiazem vs Control	p = ns	p = ns	$p < 0.01$
PCr/Pi			
Diltiazem	0.93 ± 0.16	0.15 ± 0.02*	0.58 ± 0.06*†
Control	0.87 ± 0.07	0.03 ± 0.01*	0.50 ± 0.09*†
Diltiazem vs Control	p = ns	$p < 0.01$	p = ns

ATP, Adenosine triphosphate; PCr, phosphocreatine; Pi, inorganic phosphate.
*$p < 0.05$ versus baseline.
†$p < 0.05$ versus occlusion.

sult of continued hydrolysis, with decreased replenishment of PCr and ATP during the ischemic insult (Fig. 3). However, the magnitude of this increase was less in the diltiazem-treated animals (55.4 ± 1.3% compared with 72.5 ± 0.9% for the control group, $p < 0.01$). The levels of Pi returned toward baseline with reperfusion, but this return was much more pronounced in the diltiazem-treated group

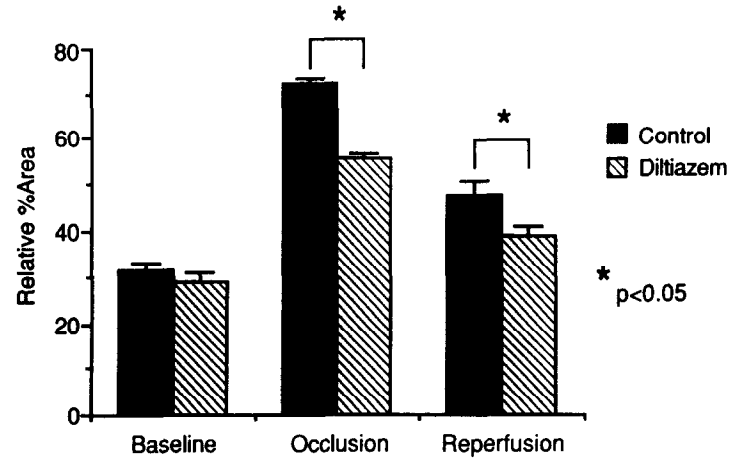

Fig. 4. Levels of inorganic phosphates during each experimental condition. See text for details.

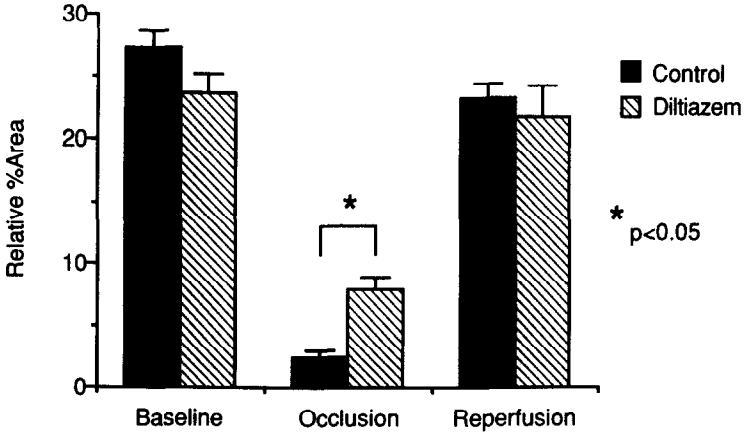

Fig. 5. Levels of phosphocreatine during each experimental condition. See text for details.

(38.8 ± 2.1% compared with 47.6 ± 2.7% for the control group, $p < 0.05$). Levels of PCr decreased significantly with occlusion in both groups (Fig. 4), though they were much more pronounced in the control group (2.5 ± 0.5% compared with 7.8 ± 1.0% for the diltiazem group, $p < 0.01$). With reperfusion, this decrease promptly returned to baseline values in both groups and there was no difference between them. Levels of the β resonance of ATP decreased with occlusion to the same extent in both groups (Fig. 5). With reperfusion, the amount of β-ATP in the diltiazem-treated group increased toward baseline levels and was significantly more than at occlusion. However, β-ATP levels for the control group remained depressed at levels similar to those found during occlusion and were significantly less when compared with levels in the diltiazem-treated group (12.3 ± 0.4% compared with 9.8 ± 0.8% for the control group, $p < 0.01$) (Fig. 6).

The ratio of levels of PCr to Pi was also calculated at each time point. This ratio decreased significantly with occlusion and returned toward normal with reperfusion. However, the decrease in this ratio at occlusion was less in the diltiazem group when compared with control values (0.15 ± 0.02 to 0.03 ± 0.01, respectively, $p < 0.01$).

DISCUSSION

Previous experimental studies have shown that calcium antagonists reduce tissue injury during ischemia and reperfusion and a variety of mechanisms have been proposed.[5, 14] These include: (1) reduction of myocardial oxygen demands by negative inotropic and chronotropic effects[6, 7]; (2) coronary artery vasodilitation and enhancement of blood flow to the ischemic region[10, 25, 26]; and (3) attenuation of loss of diffusible purine precursors, thus making more raw material available for ATP resynthesis.[8, 9] In addition, direct actions of calcium antagonists relative to myocardial preservation in the setting of ischemia

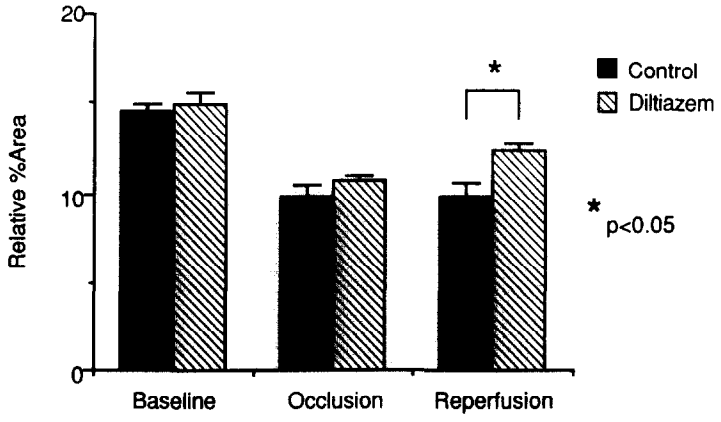

Fig. 6. Levels of adenosine triphosphate during each experimental condition. See text for details.

and reperfusion have also been hypothesized. These agents impede the influx of calcium into the cell via one of the entry mechanisms (the slow channels) and thus may attenuate mitochondrial calcium overload induced by ischemia and reperfusion, decreasing the availability of calcium to stimulate intracellular proteases, lipases, and ATPases (adenosinetriphosphatases).[6, 7, 27] Finally, there may also be a direct protective effect on mitochondrial ultrastructure, thus preserving cellular respiratory function and myocardial high-energy phosphates.[11]

Our results indicate that diltiazem provides a direct protective effect on myocardial high-energy phosphate metabolism during regional ischemia and reperfusion in the intact animal. In our study, during occlusion, levels of PCr and ATP decreased significantly with a concomitant reciprocal increase in inorganic phosphate metabolites. With reperfusion, the inorganic phosphate metabolite accumulation was reversed and PCr promptly returned to normal. Levels of ATP either remained depressed or only partially returned toward baseline levels. With diltiazem, we noted an attenuated decrease of PCr during ischemia, an attenuation of the increase in Pi during ischemia with an enhanced recovery during reperfusion, and an enhanced recovery of ATP during reperfusion. Furthermore, the PCr/Pi ratio, an index of mitochondrial function,[22] was significantly greater in the diltiazem group during ischemia compared with the control group.

Since there were only small, insignificant changes in heart rate and mean arterial pressure, the improved high-energy phosphate levels during ischemia and reperfusion cannot be fully explained by decreases in myocardial oxygen demands. Furthermore, high-energy phosphate changes were measured in the central ischemic zone where myocardial contractile function would be expected to be abolished. Thus changes in heart rate or loading conditions are likely to have only minimal contributions to decreasing myocardial oxygen demands in the specific region of ischemic myocardium sampled and are unlikely to explain the metabolic changes that we observed. Although we did not measure myocardial blood flow in these experiments, others[8, 28] have reported no improvement in blood flow following treatment with calcium antagonists in occlusion-reperfusion studies. Bush et al.[28] found no increase in coronary collateral flow by diltiazem despite a decrease in infarct size. Similarly, Lange et al.[8] demonstrated improvements in high-energy phosphates with calcium antagonists during ischemia-reperfusion that could not be explained by differences in regional blood flow. Thus it is unlikely that enhancement of blood flow to the ischemic region fully explains our results.

Kloner et al.[11] previously demonstrated that calcium antagonists attenuate ultrastructural damage during ischemia-reperfusion, with disproportionate preservation of mitochondrial structures in relation to other organelles. Some cells that exhibited edema, nuclear change, and glycogen loss had relatively well-preserved mitochondria. These ultrastructural observations, in concert with our metabolic results, further support the hypothesis that calcium antagonists produce a protective effect on mitochondrial structure and function. Thus diltiazem may decrease mitochondrial calcium accumulation, protect the electron transport system, and thereby enhance high-energy phosphate production.

There are several other potential explanations for the high-energy phosphate sparing effect of diltiazem. First, since intracellular calcium concentration is lowered by the calcium antagonist, particularly during reperfusion, less calcium efflux is required. As a result, decreased ATP hydrolysis occurs since calcium efflux is an energy-dependent phenomenon.[29]

Second, a lower intracellular calcium concentration may reduce the amount of ATP consumed by the contractile apparatus.[30] Third, increased intracellular calcium may cause mitochondrial calcium overload that can compete with ATP synthesis for respiratory energy.[31] Finally, diltiazem has been demonstrated to prevent the loss of mitochondrial oxidative phosphorylation that is caused by the increased intracellular phosphate concentration that occurs during ischemia.[32]

In our studies, diltiazem was administered prior to occlusion and reperfusion. The rationale for this was twofold. First, we wished to evaluate the metabolic effects of diltiazem using ^{31}P NMR, both during occlusion and reperfusion. Second, previous studies have demonstrated that calcium antagonists only reduce infarct size when administered prior to coronary occlusion and reperfusion,[28, 33-35] and failed to limit infarction when given only prior to reperfusion.[33, 34]

Only two previous studies of calcium antagonists in the setting of myocardial ischemia and reperfusion have been reported using phosphorus[31] NMR techniques, and both of these have been limited to the isolated heart model. Lavanchy et al.[12] studied diltiazem using a retrograde perfused rat heart model with 24 minutes of global low-flow ischemia followed by 30 minutes of reperfusion. In agreement with our results, they observed no difference between the diltiazem and control groups in the extent of ATP depletion or intracellular acidosis during ischemia, but they did observe a 30% reduction in Pi accumulation in the diltiazem-treated animals. However, their results differed from ours in that they noted no difference in the extent of PCr depletion with ischemia and no improvement in ATP levels of their diltiazem group during reperfusion. Of interest, they did notice a better recovery of ATP in the diltiazem group with standard biochemical assays. The apparent discrepancies between their results and ours most likely can be explained by differences in the experimental models. In the isolated heart model, changes in loading conditions and other physiologic factors are less profound when compared with the intact model. Thus the effect of diltiazem on these physiologic factors may contribute at least in part to its beneficial effects with regard to ATP restitution noted in our model.

Kirkels et al.[13] recently reported a study using a globally ischemic rat Langendorff preparation pretreated with the calcium antagonist anipamil, a highly lipophilic verapamil-type calcium antagonist. While they noted no significant cardioprotective effects of this calcium antagonist on high-energy phosphates during ischemia, intracellular acidosis was attenuated. On reperfusion, hearts from the anipamil-pretreated group recovered significantly better than untreated hearts with respect to replenishment of ATP and PCr, with restitution of low levels of Pi and return of intracellular pH toward normal when compared with the control group. These results are analogous to our findings, with the exception of the attenuation of deleterious effects on high-energy phosphates that we noted during ischemia. This difference in findings during ischemia may be explained on the basis of the model employed and the mechanism of action of anipamil. In a globally ischemic isolated heart preparation used by Kirkels et al. with a prolonged duration of ischemia (30 minutes), the degree of ischemia produced may be so great as to prevent any protective effects of a calcium antagonist. It is also possible that there may be small differences in the mechanisms of action of anipamil when compared with diltiazem in the setting of ischemia.

Using techniques other than NMR, other investigators have reported beneficial metabolic effects of calcium antagonists in intact animal models. Lange et al.[8] studied the effects of verapamil on adenine nucleotides in anesthetized dogs who underwent 15 minutes of coronary artery occlusion followed by 4 hours of reperfusion. High-energy phosphates were assayed using standard biochemical techniques. They noted a protective effect of this calcium antagonist during reperfusion, similar to our results with respect to high-energy phosphates. During the preceding ischemic period, they also noted a protective effect of verapamil on ATP that was even more pronounced than the effect we noted with diltiazem. In their verapamil-treated group there was no significant fall in ATP levels during ischemia when compared with baseline values, while in the untreated controls there was a marked fall in ATP. While we noted a trend toward attenuation in the fall of ATP with occlusion in the diltiazem group when compared with the control group, levels in both groups were significantly decreased from baseline. However, it should be noted that there are significant sampling differences in the two models. Lange et al.[8] used subendocardial sampling, where the ischemia is more profound and the beneficial effects of calcium antagonism may be more pronounced. Our model used a surface coil with transmural sampling and a greater contribution of signal from the subepicardial region, where the ischemia is less pronounced and thus the beneficial effects of diltiazem may be minimized.

Several comments should be made regarding our method for expressing the peak areas. There is presently no generally accepted approach to this aspect of the data analysis. We have chosen to express each

peak area as a percent of the total area of the five peaks (Pi, PCr, α-, β- and γ-ATP), in a large part because of the simplicity of this approach. This method corrects for the fact that positioning of the animal might unavoidably vary during the experiment, slightly changing the total spectral area. However, it does not correct for changes that might occur in the total amount of NMR-visible phosphorus that might occur between ischemic and nonischemic tissue. We have also not applied saturation correction factors, though we have calculated them (1.4 for PCr, 1.0 for all other peaks). Since we are performing identical analyses on two different animal populations, we feel that our approach is appropriate.

In conclusion, we report a protective effect by pretreatment with the calcium antagonist diltiazem on myocardial high-energy phosphates in the setting of ischemia and reperfusion. Our study corroborates previous studies in the isolated heart preparation that use phosphorus^{31} NMR spectroscopy techniques. Furthermore, our study extends the scope of the current literature by reporting the cardioprotective effect of calcium antagonists during regional ischemia and reperfusion in the intact animal using noninvasive, tissue-sparing phosphorus^{31} NMR spectroscopy techniques.

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Influence of positive inotropic agents on intracellular calcium transients. Part I. Normal rat heart

This study, which was designed to evaluate the effects of positive inotropic agents on intracellular calcium transients ($[Ca^{2+}]i$), is the first to analyze calcium transients in the whole heart. The positive inotropic agents that augment intracellular cyclic adenosine monophosphate (cAMP) (dibutyryl cAMP, amrinone, and isoproterenol) caused an increase in developed pressure and $[Ca^{2+}]i$ transients and a decrease in diastolic $[Ca^{2+}]i$. On the other hand, the glycoside digoxin and the α-adrenoceptor agents, phenylephrine and dobutamine, also caused an increase in $[Ca^{2+}]i$ transients and developed pressure. However, unlike the agents that increase [cAMP]i, they induced an elevation in diastolic $[Ca^{2+}]i$. With all the positive inotropic agents, developed pressure increased commensurately with the percentage changes in amplitude of the $[Ca^{2+}]i$ transients. (AM HEART J 1989;118:1219.)

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Intracellular calcium plays a key second-messenger role in cardiac contraction by maintaining the resting level, or diastolic, intracellular calcium transients ($[Ca^{2+}]i$) at a very low concentration, so that a large signal/background ratio can be obtained with only a small absolute change in $[Ca^{2+}]i$. Calcium enters the cell through voltage-sensitive calcium channels. In the heart there are the low-voltage–sensitive calcium channel (L channel) and the high-voltage–sensitive (N channel). The voltage-sensitive calcium channels can be modulated by receptor-mediated events. For example, adenosine 3′:5′-cyclic phosphate (cAMP) activates protein kinase C. Phosphorylation of calcium channels by protein kinase C can bring about different patterns of Ca^{2+} channel modulation at the single-channel level. Examples of stimulatory mechanisms include the unmasking of covert Ca^{2+} channels[1] and the altered probability of the Ca^{2+} channel opening because of changes in millisecond gating kinetics[2,3] or changes in channel availability.[4] In addition to regulating calcium channels indirectly through activation of cytoplasmic kinases, guanine nucleotide binding protein (G protein) can regulate calcium channels directly.[2,5] The L calcium channel is activated by cAMP[5,6] and the G protein.[2,5] β-Adrenoceptor agonists increase cAMP, while some of the α-adrenoceptor agonists increase G-protein.[7]

Calcium flows out of the heart by means of calcium adenosinetriphosphatase (Ca^{2+} ATPase).[8,9,10] The Na^+/Ca^{2+} exchanger is regulated by Ca^{2+} concentrations within the dynamic physiologic range, promoting $[Ca^{2+}]i$ entry as well as efflux and thus playing a critical role in modulating the resting level of

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