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Sustained Postischemic Cardiodepression following Magnesium–Diltiazem Cardioplegia (42355)

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Abstract. Magnesium–diltiazem cardioplegia was evaluated in the intact, perfused rat heart to determine whether the joint administration of these agents would adversely affect myocardial contractile and high-energy phosphate recovery following intermittent, normothermic global ischemic arrest. Sequential metabolic and functional analyses were performed on isolated perfused rat hearts during each phase of the experimental protocol: control (10 min), normoxic cardioplegia (10 min), intermittent global ischemic arrest (two 15-min periods separated by 2 min infusion of the normoxic cardioplegic perfusate), and normoxic postischemic control reperfusion (60 min). Four different cardioplegic solutions were evaluated: 30 m*M* KCl, 30 m*M* KCl with 2 mg diltiazem/liter, 20 m*M* $MgCl_2$, and 20 m*M* $MgCl_2$ with 2 mg diltiazem/liter. Myocardial phosphatic metabolite levels and intracellular pH were analyzed nondestructively in the intact hearts by phosphorus-31 NMR spectroscopy. Corresponding measurements of peak left intraventricular pressure, rate of peak pressure development (*dP/dt*), and contraction frequency were performed at the midpoint during each 5-min interval of ^{31}P NMR signal averaging. Magnesium plus diltiazem-treated hearts were distinguished from all other groups by a marked delay in postischemic functional recovery consisting of a prolonged depression in contractility (34% of control, $P < 0.01$) that persisted throughout the first 50 min of postischemic reperfusion. Diltiazem in combination with magnesium cardioplegia was detrimental to postischemic functional recovery, despite a rapid restoration of high-energy phosphate stores. The apparent adverse interactive effects of excess magnesium and diltiazem suggest that elective ischemic arrest with magnesium cardioplegia in combination with diltiazem may be contraindicated clinically. The mechanistic basis and drug specificity of this response require further clarification. The present findings appear to exclude ATP and PCr production, and structural causes as the basis for the observed aberrant functional recovery from global ischemia of magnesium plus diltiazem-arrested hearts.

Myocardial recovery from prolonged elective ischemic arrest (e.g., bypass surgery) depends in part on the conservation of high-energy phosphate stores, the efficient utilization of these high-energy substrates in excitation–contraction coupling processes of contraction, and the avoidance of reperfusion injury at the cellular level (1, 2). During ischemia and reperfusion, the excess calcium accumulation that occurs has been attributed to disturbances in cell membrane organization, which disrupt intracellular regulation of ionized calcium (Ca^{2+}) (2–5). The resultant intracellular Ca^{2+} overload, most notable in the mitochondria, leads to impaired oxidative metabolism, inefficient ATP utilization for mechanical work, and cellular ATP depletion at a time when ATP is required for the repair of ischemia-induced structural damage and for the restoration of coordinated electromechanical activity (1, 6).

By virtue of their ability to retard calcium entry into myocardial cells, calcium-channel inhibitors appear to possess advantageous cardioprotective properties for the ischemic heart (7–11). Indeed, nifedipine, verapamil, and diltiazem have all been shown to ameliorate morphologic ischemic injury and improve postischemic functional recovery when used as cardioplegic agents (12–16). Recent work by Hearse and co-workers (17–20) suggests that prior administration of a slow calcium

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channel inhibitor together with their magnesium-based St. Thomas' solution under normothermic conditions may be a valid therapeutic alternative to hypothermia during elective ischemic arrest of the heart for bypass surgery. Although these results appear promising, the extent to which the specific cardioplegic agent (e.g., magnesium, potassium) employed may influence the ability of these drugs to protect the myocardium against ischemic injury has not been assessed. As a consequence, we undertook an initial series of studies to assess the relative effectiveness of diltiazem, as a cardioprotective adjunct during global myocardial ischemia, when used with either of two commonly used cardioplegic agents, potassium or magnesium. Diltiazem was selected for study because it (i) induces less cardiodepression at comparable doses than other calcium-channel inhibitors, (ii) has been shown to increase the recovery of contractile function and reduce the loss of ATP during ischemia, (iii) decreases tissue calcium and protects mitochondrial structure during anoxia, and (iv) prevents cell separation, alterations in gap junctions, and changes in mitochondrial ATPase activity during calcium paradox-related cell injury (21–26).

We tested the hypothesis that each membranoplegic cation would influence myocardial protection provided by diltiazem differently under normothermic, ischemic conditions. Isolated hearts were subjected to episodes of intermittent global ischemia followed by reperfusion. Postischemic metabolic and functional recovery (both rate and magnitude) were used as criteria to identify the relative effectiveness of diltiazem with either potassium or magnesium cardioplegia. Myocardial metabolite levels were determined continuously in the intact, isolated rat heart by phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P NMR). Since the recovery of ATP in the postischemic myocardium during reperfusion has been shown to parallel ventricular mechanical recovery (27), the rate and completeness of the recovery of heart high-energy phosphate stores and myocardial contractile function were analyzed and compared among the various treatment groups. Moreover, the magnitude of intracellular acidosis and the increase in intracellular inorganic phosphate (P_i) levels during ischemia, which have been reported to correlate inversely with myocardial functional recovery, were also analyzed in these hearts by ^{31}P NMR to determine their possible role in any cation-specific cardioprotection provided by diltiazem. In addition, morphologic analyses of the treated hearts were performed to elucidate possible structural correlates that may be involved in any differential recovery of hearts in the various pretreatment groups.

Methods. Animal care, which was provided by our AAALAC accredited animal facility, and the experimental procedures employed in the present studies were in compliance with established guidelines for humane animal care and treatment of vertebrate animals as set forth by the National Society for Medical Research and the National Academy of Sciences.

Heart perfusions. Albino male Sprague–Dawley rats (200–250 g) were heparinized and sacrificed by cervical dislocation, a procedure that eliminates potential anesthetic artifacts (28, 29). Each heart was excised from the chest cavity and immediately placed in prechilled (5°C) control perfusate causing hypothermic cardiac arrest. While in this buffer, the aorta was cannulated and retrograde aortic perfusion was started. Superfluous connective and adipose tissues were then excised, and a modified 3F Millar Instruments Mikro-Tip catheter was inserted through the left atrial appendage and mitral valve into the left ventricle. The catheter was sutured in position to achieve optimal recordings of myocardial contractile activity [left intraventricular pressure, rate of pressure development (dP/dt), and contraction rate]. The heart was then mounted in a NMR tube perfusion apparatus containing a volume of control perfusate at 37°C, and the entire perfusion apparatus was placed in the magnet probe assembly of the NMR system. Contractile and metabolic events of the heart were monitored to determine the stability of the preparation during a 30-min equilibration period. Past experience has shown that this 30-min time interval is sufficient to stabilize myocardial function and restore myocardial metabolite levels to preisolation levels. Hearts exhibiting unstable contractile activity or metabolic disturbances after this equilibration period were discarded.

Perfusion conditions. The crystalloid perfusion medium used in these studies was a modified Hartmann's solution containing 152 mM NaCl, 5.4 mM KCl, 1.05 mM $MgCl_2$, 1.8

m*M* $CaCl_2$ (1.2 m*M* for the cardioplegic solutions), 5.6 m*M* glucose, and 10 m*M* Tris buffer (Sigma) with an osmolality of 298 mOsm (30). The lower concentration of calcium used in the cardioplegic solutions has been shown previously in preliminary studies in our laboratory to reduce the rate of metabolic deterioration in potassium- and magnesium-arrested hearts under anoxic conditions without any discernable evidence of cell injury related to the calcium paradox phenomenon (unpublished results). These findings are consistent with recent results reported by Hearse and co-workers (18). The pH of the perfusate was 7.3 at 37°C throughout each perfusion experiment. This physiologic buffer was oxygenated continuously through sintered glass aerators to maintain stable perfusate O_2 saturation. An Electronics for Medicine (E for M) VR-12 fiber optics recording system was used to monitor and record the aortic perfusion pressure and the contractile events of the heart from appropriate pressure transducers. The aortic perfusion pressure was monitored from a Statham P23 pressure transducer attached to the perfusion column which was interfaced to the E for M recording system through a Model V2203A pressure amplifier module. Hearts were perfused under constant hydrostatic pressure (60–65 mm Hg) maintained by adjusting the flow of oxygen to the pressurized perfusate reservoirs. Calibrated tracings of left intraventricular pressure and dP/dt were obtained from the catheter-tip pressure transducer decribed previously through a Model TC-100 control unit (Millar Instruments) interfaced to a Model V2203A pressure amplifier module of the E for M recording system. Heart contraction rates were calculated from the peak to peak intervals between successive contractile events. Continuous coronary flow rate determinations were made by measuring the volume of coronary effluent aspirated at timed intervals throughout the course of the experiment. Actual flow rates and normalized values based on the contraction rate of the heart during each interval (μl/contraction cycle) were determined. The temperature of the perfusate was maintained constant (37°C) using a thermostatically regulated helical sleeve unit that surrounded the external perfusion lines leading to the common perfusion column of the NMR perfusion apparatus. In addition, the internal probe temperature of the NMR system was maintained at 37°C with a Nicolet variable temperature control unit. Normothermic as opposed to hypothermic conditions were used in the present study because the metabolic changes induced by ischemia occur more rapidly at 37°C and because, as stated, recent work suggests that calcium-channel inhibitors do not appreciably augment the cardioprotection provided by hypothermia (19).

Experimental design. A linear time course schema depicting the isolated perfused heart experiments performed to evaluate the experimental hypothesis is presented in Fig. 1. Experiments were performed in quadruplicate according to this schema. According to this protocol, hearts were perfused for 10 min with control buffer to obtain baseline control data for each heart. The hearts were then subjected to a 10-min period of normoxic arrest induced by continuous perfusion with one of the four cardioplegic test solutions: 30 m*M* KCl, 20 m*M* $MgCl_2$, 30 m*M* KCl + 2 mg/liter diltiazem, or 20 m*M* $MgCl_2$ + 2 mg/liter diltiazem. The arrested hearts were then subjected to two consecutive episodes of intermittent global ischemia consisting of 15 min of global ischemia and 2 min of reperfusion with the normoxic test solution followed by a second 15-min episode of global ischemia. The hearts were then reperfused for 60 min with the normoxic control buffer to assess the extent of metabolic and functional recovery. According to the second protocol, hearts (n = 6) were perfused with control buffer for 120 min to determine the temporal metabolic and functional stability of these preparations under the described perfusion conditions. Aortic flow rates averaged over the same 5-min period of ^{31}P NMR signal averaging were determined throughout the course of each experiment during the periods of actual heart perfusion. The dose of diltiazem selected for study (2 mg/liter or 4.4 μ*M*) was based on results from previous reports in rats demonstrating that this drug concentration afforded structural and metabolic protection of the myocardium during oxygen-deficient and calcium-paradox-related conditions (22, 26).

Phosphorus-31 magnetic resonance spectroscopy. P-31 NMR experiments were performed using a dedicated Nicolet NT-200 NMR system equipped with deuterium stabilization, variable temperature, and Fourier

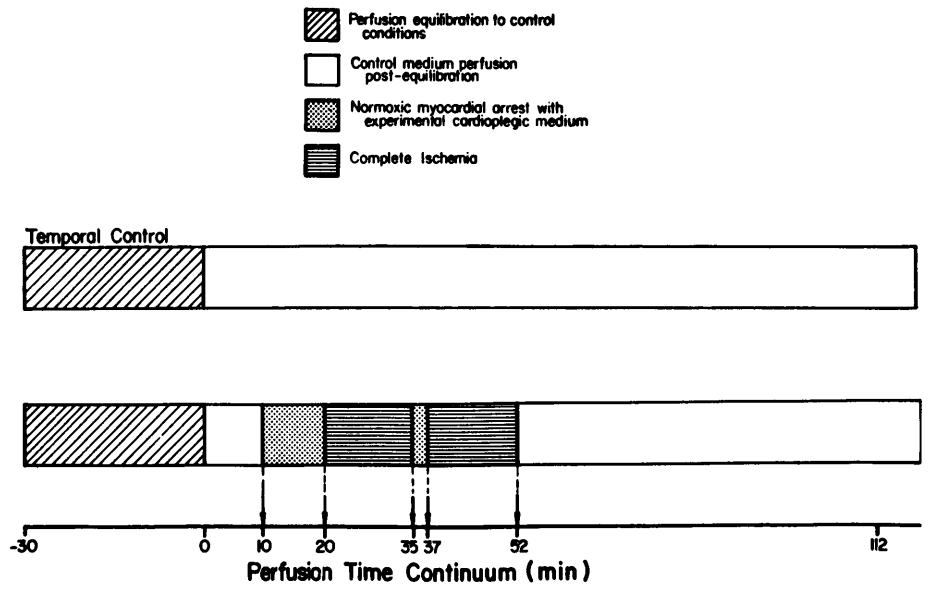

FIG. 1. Schematic representation of the temporal control and intermittent ischemia protocols.

transform capabilities operating at 80.99 MHz for P-31. The spectrometer configuration for the intact heart P-31 resonance signal acquisition and processing were as follows: pulse sequence, single pulse; pulse width, 8 μsec, 45° flip angle; acquisition delay, 200 μsec; number of scans, 368; number of data points per free induction decay, 8192; acquisition time, 0.819 sec; and sweep width, ±2500 Hz. In addition, a computer-generated exponential multiplication introducing 10-Hz artificial line broadening was applied to the free induction decay. The intact hearts were analyzed under nonspinning, proton-decoupled conditions (^{31}P proton decoupling requires only low-level decoupling; hence, no tissue heating occurs).

Data reductions, including peak area integrations and chemical shift measurements, were based on spectral analysis of the resonance signals detected from the heart and were effected by the spectrometer's computer. Spectra were generated for each 5-min period of signal averaging by Fourier transformation of the accumulated free-induction decay arising from the tissue ^{31}P resonance signals. Chemical identities of the various peaks were determined according to established procedures (31).

Adenosine triphosphate (ATP), phosphocreatine (PCr), and inorganic phosphate (P_i) were readily detected and quantitated in the intact heart by this spectroscopic technique. Changes in the levels of these various phosphatic metabolites provided the basis for the metabolic interpretations described herein. In addition, the intracellular pH was determined according to an established procedure that is based on the chemical shift position of intracellular P_i (31). The pH behavior of intracellular P_i is essentially identical to that of P_i in a solution resembling the ionic composition, ionic strength, and temperature found within the cell. As a consequence, these properties make possible the accurate estimation of the intracellular pH from the P_i chemical shift position in the intact tissue spectrum by extrapolating to a standardized P_i resonance shift–pH titration curve for P_i in solution.

Heart morphology. At the conclusion of the recovery period, hearts were removed from the perfusion apparatus and fixed by continuous injection of cold 2.5% glutaraldehyde–0.15 *M* cacodylate buffer (pH 7.2) into the coronary ostia. To ensure uniform and complete fixation of the tissue of interest, minced tissue from the left ventricle was immersed in the glutaraldehyde–cacodylate buffer for 60 min. Tissue specimens were then dehydrated, prepared for embedding, and embedded in epoxy resin. The embedded tissue was then sectioned (1.0 μm) with glass knives on a Reichart ultramicrotome, stained with toluidine blue, and

examined with a Zeiss Photomicroscope III. Thin sections (40–60 nm) were cut using a diamond knife, mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with a Siemen's IA transmission electron microscope operated at 60 kV. Morphologic interpretaions were based on double-blind pathologist's report of the tissue specimens examined at the light and electron microscopic level.

Statistical methods. Six separate ventricular contraction cycles were analyzed to compute the average peak left intraventricular pressure (mm Hg), peak *dP/dt* (mm Hg/sec), and contraction frequency (beats/min) at each point in time (5-min intervals). Calibrated baseline pressure recordings obtained for the catheter transducer with each heart were used to detect any changes in left intraventricular end-diastolic pressure. The actual values obtained for the measured functional and metabolic parameters were expressed for each heart as percentages of the corresponding control values computed by averaging the values obtained during the initial 10-min control perfusion period. This internal referencing procedure facilitated meaningful quantitative and qualitative intergroup comparisons by indexing each heart to the same initial control reference point (100%). The values reported for each group represent the means ± SE at each point in time. The two-way analysis of variance procedure for repeated measures over time was used to determine statistically significant changes among the groups as a function of time. Individual variations among the different groups at the various points in time were examined for statistical significance using Duncan's multiple range test. A value of $P < 0.05$ was accepted as significant.

Results. *Temporal control perfusions.* Nonsignificant variations in myocardial contractility and energy metabolism were observed during prolonged (2-hr) heart perfusions under control conditions. At the conclusion of the 2-hr perfusions peak *dP/dt*, peak left intraventricular pressure, and contraction frequency were 91.3 ± 6.2, 94.1 ± 4.3, and 99.5 ± 12.6% of the respective zero-time control values. Similarly, myocardial ATP, PCr, and P_i levels were stable throughout the control perfusions, corresponding respectively to 91.9 ± 5.2, 97.5 ± 8.5, and 127.7 ± 24.6% of the zero-time control values after 2 hr of perfusion. A typical

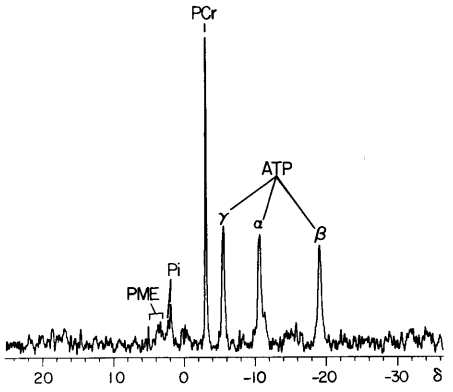

FIG. 2. Representative ^{31}P NMR spectrum obtained from an intact control-perfused rat heart under the described NMR acquisition and perfusion conditions. The principal intracellular metabolites are labeled as follows: PME-phosphomonoesters which include phosphorylated glycolytic intermediates, phosphoglyceride derivatives (primarily phosphocholine); adenosine monophosphate and inosine monophosphate; P_i, inorganic phosphate corresponding to mitochondrial and sarcoplasmic pools (26); PCr, phosphocreatine; ATP, adenosine triphosphate. The peak labeled α-ATP includes resonance contributions from esterified nucleotide phosphate metabolites such as nicotinamide adenine dinucleotide (NAD) and dinucleotide phosphate (NADP) in addition to ATP. The resonance peak areas are proportional to the relative concentration of each metabolite detected. The resonance peak signal-to-noise ratio was approximately 15:1 and 5:1 for PCr and β-ATP, respectively.

^{31}P NMR spectrum of a control-perfused rat heart is shown in Fig. 2. These results indicate that the duration of the perfusion period used in the experimental protocols was not a factor contributing to any functional or metabolic disturbances in the heart preparations that would compromise the results obtained by these methods. Thus, any deviations observed in myocardial contractility and metabolite levels were attributable solely to the experimental protocols.

Experimental perfusions: Metabolic effects. Serial ^{31}P NMR analysis of myocardial ATP, PCr, and P_i revealed significant, progressive metabolic changes during the period of ischemia; however, these changes were similar among the different experimental groups and no significant intergroup differences were detected. Predictably, the effect of short-term, intermittent ischemia on myocardial ATP levels was modest (Fig. 3). The ATP content of these hearts fell slightly in each group during

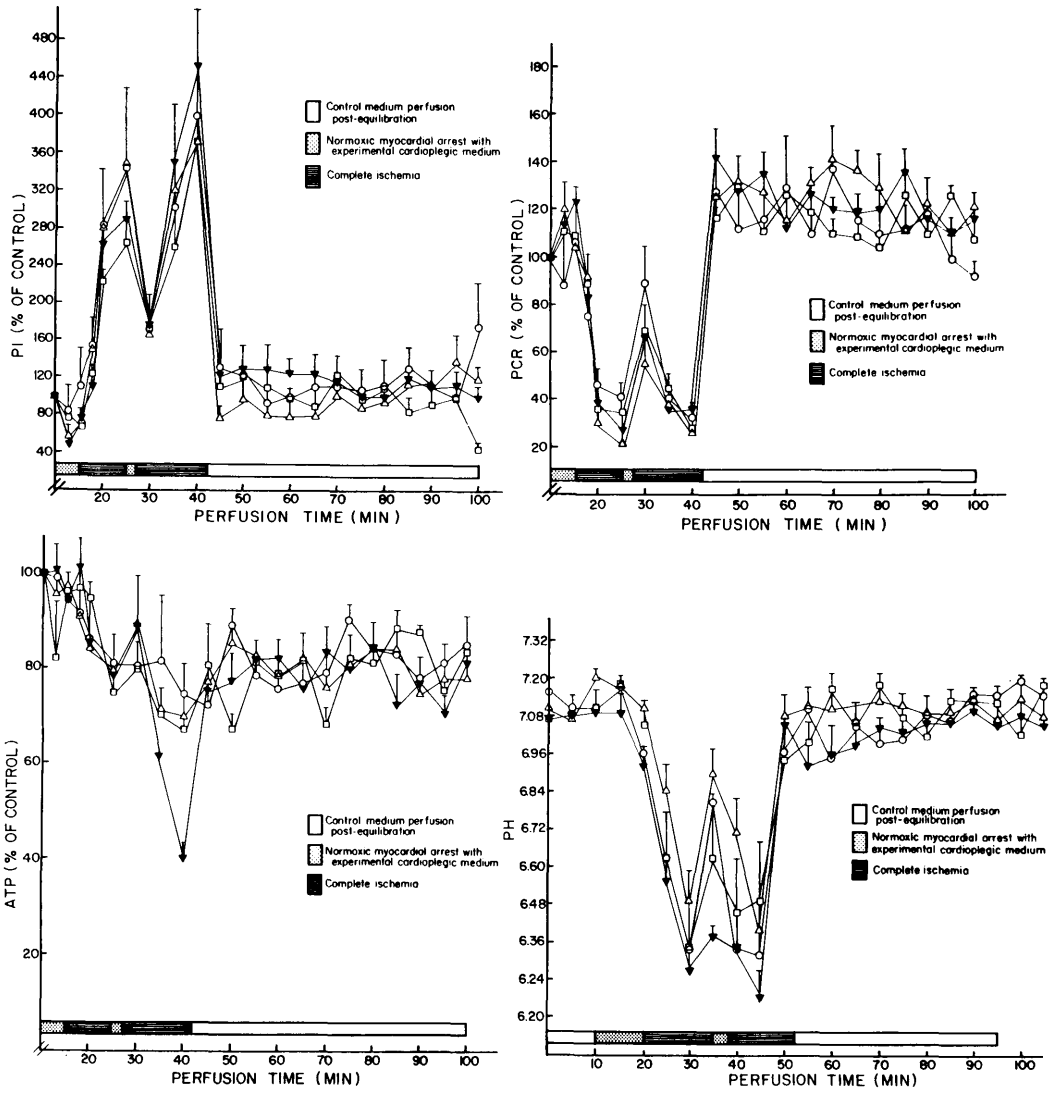

FIG. 3. Time sequence illustrating changes in myocardial ATP, PCr, P_i, and intracellular pH among the various perfusion groups as percentage of control. The values shown correspond to the various temporal phases of the intermittent ischemia protocol shown in Fig. 1. The vertical bars denote standard errors about the group means. ○, 30 m*M* KCl group; △, 20 m*M* $MgCl_2$ group; ▼, 30 m*M* KCl + 2 mg/liter diltiazem; □, 20 m*M* $MgCl_2$ + 2 mg/liter diltiazem.

the latter phases of the ischemic period, generally approaching 70% of the levels present during the normoxic control period. Control reperfusion of these hearts resulted in nearly complete restoration of myocardial ATP levels to between 80 and 95% of preischemic control levels.

In contrast to these modest changes, marked changes in heart P_i and PCr levels were induced by the intermittent periods of ischemia. Myocardial P_i levels increased progressively during the ischemic episodes and oscillated with the intermittent phases of ischemia. Myocardial P_i approached 360 to 420% of the normoxic control levels at the conclusion of the ischemic period (Fig. 3). A corresponding decrease in myocardial PCr levels was observed during the ischemic episodes, which reflected an essentially stoichiometric change with respect to myocardial P_i. Myocardial PCr levels oscillated inversely with the changes in P_i during the intermittent phase of the isch-

emia, approaching 20% of normoxic control levels by the end of the ischemic period. Within 5 min after the initiation of reperfusion with the control buffer P_i levels had reverted to near baseline levels and remained essentially invariant thereafter. Similarly, reperfusion resulted in an abrupt restoration of myocardial PCr levels within the first 10 min. A slight overshoot (approximately +20%) in myocardial PCr relative to preischemic levels was observed, which persisted in most of the groups for the first 45 min of the reperfusion period.

Intracellular pH was stable (apparent pH between 7.1 and 7.2) throughout the control normoxic and normoxic arrest periods; however, immediately after initiation of global ischemia, intracellular pH rapidly decreased to between 6.5 and 6.3, indicative of intracellular acidosis (Fig. 3). This effect was manifest under normothermic conditions despite mechanical arrest of these hearts prior to the ischemic interval. Intracellular pH varied with the episodic nature of the ischemic insult. The most pronounced oscillations that occurred during the 2-min reperfusion phase with the normoxic cardioplegic solutions were observed in hearts arrested without diltiazem. Hearts arrested with potassium plus diltiazem exhibited the lowest rebound response during this transient 2-min reperfusion period. Following the initiation of control reperfusion, intracellular pH rapidly reverted to preischemic levels with the slowest response occurring in the hearts that had developed the most severe acidosis, the potassium plus diltiazem group; however, none of these differences were statistically significant.

Experimental perfusions: Contractile and coronary flow rate responses. The baseline levels of functional activity exhibited by hearts assigned to the different treatment groups during the 10-min control phase of the protocol were essentially the same for the various groups. In addition, the mean values obtained among these groups for the various contractile and flow determinations were comparable to the following average values obtained for the temporal control group (means ± SE): aortic flow rate, 9.5 ± 1.1 ml/min; contraction rate, 218 ± 25 beats/min; peak left intraventricular pressure, 74 ± 4 mm Hg; and peak dP/dt, 2866 ± 142 mm Hg/sec.

The diastolic cardiac arrest induced by each of the normoxic cardioplegic buffers occurred rapidly within 30 to 45 sec after the onset of perfusion with the cardioplegic crystalloid solutions. The periods of normoxic arrest and intermittent global ischemia, including the brief interval of reperfusion during the ischemic episode, were devoid of any evidence of contractile activity. Left intraventricular end-diastolic pressure following ischemic arrest was comparable to normoxic control values and subsequent electron microscopic analysis of these hearts (see below) provided no detectable evidence that functional recovery was complicated by myocardial contracture in any of the experimental groups. Postischemic control reperfusion was accompanied by a relatively rapid restoration of cardiac contractile activity in both magnesium- and potassium-arrested hearts with 80–85% of the recovery occurring within the initial 5 to 10 min of reperfusion (Fig. 4). Contractile recovery improved steadily in the magnesium group throughout the remainder of the reperfusion period, surpassing the potassium group after 25 min. The magnitude of the difference between these groups, however, was not statistically significant. The postischemic recovery of hearts from the potassium plus diltiazem group was delayed briefly, but within 10 min after initiating reperfusion myocardial contractile recovery was comparable to the non-diltiazem-treated hearts. The mechanical recovery exhibited by potassium plus diltiazem-treated hearts was maintained throughout the remainder of the reperfusion period at a level comparable to that exhibited by the potassium group. The restoration of aortic flow expressed as a function of heart rate occurred rapidly in all three of these groups without significant intergroup differences and coincided with the recovery of cardiac contractile function (Fig. 4).

Hearts comprising the magnesium plus diltiazem group were distinguished from all other groups by a significantly protracted delay in postischemic contractile recovery (Fig. 4). These hearts were characterized by a prolonged depression in mechanical function ($P < 0.01$), affecting both the magnitude and rate of peak left intraventricular pressure development which persisted throughout the initial 45–50 min of reperfusion. Despite this retarded mechanical recovery, coronary flow in these hearts recovered supramaximally during the first 5 min of reperfusion; a manifestation

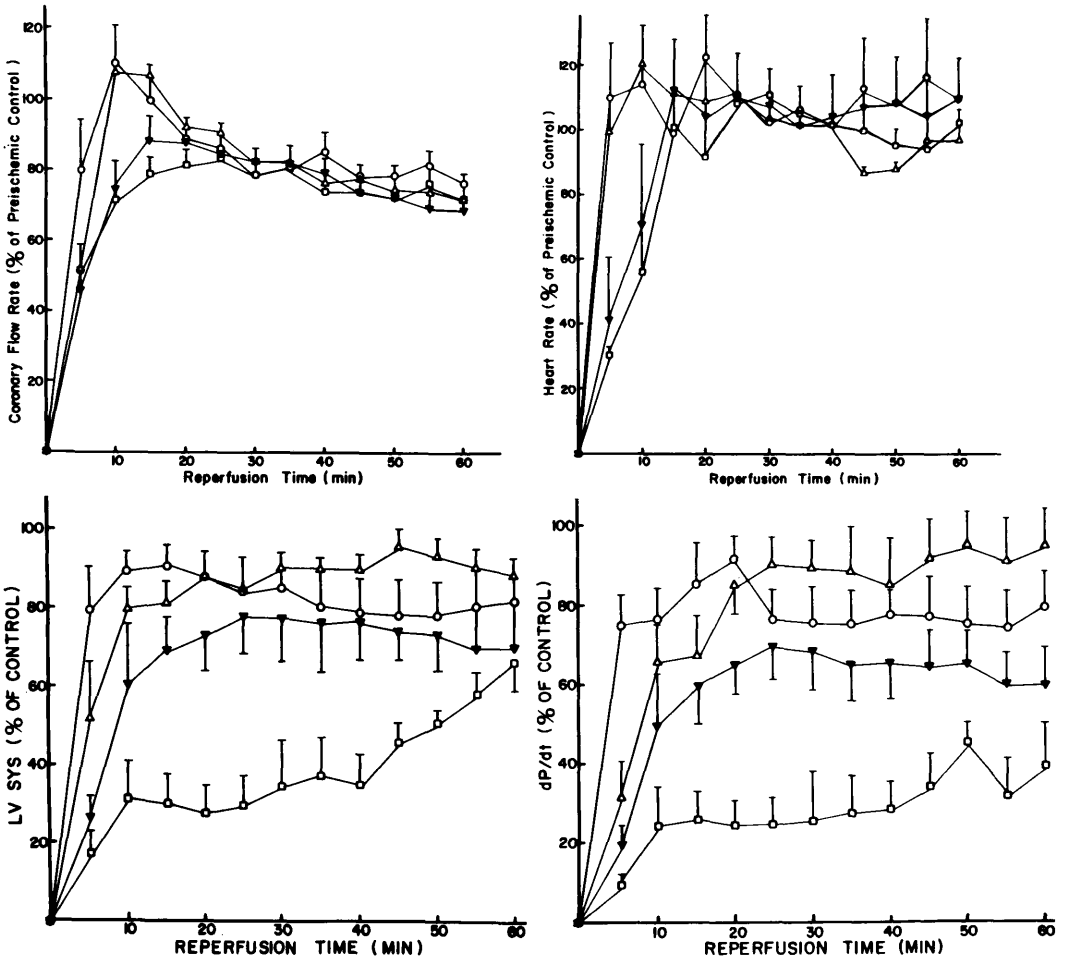

FIG. 4. Time-dependent recovery of left intraventricular pressure, *dP/dt*, heart rate, and coronary flow rate as percentage of control during postischemic reperfusion with control buffer. Symbols denoting the different groups are the same as in the legend of Fig. 3. The mechanical recovery of hearts treated with 20 m*M* $MgCl_2$ + 2 mg/liter diltiazem was significantly different from the recoveries of the other groups.

of a relatively slow contraction rate in combination with a coronary flow rate (ml/min) which was similar to that exhibited by hearts in the other groups (Fig. 4). This response differed from the other groups, but was transient, lasting only during the initial 5 min of reperfusion.

Morphology. Light and electron microscopic evaluation of tissue sections obtained from the left ventricle revealed distinctive changes in both groups of hearts treated with diltiazem. The mitochondria were clustered and appeared more prominent and electron dense in all tissue sections examined from these hearts. These structural changes appeared to be a generalized effect of diltiazem on heart mitochondria. Since these effects were observed in both potassium and magnesium hearts treated with diltiazem, the observed aberrant functional recovery of the magnesium plus diltiazem-treated hearts appeared unrelated to these structural perturbations.

Discussion. Diverse cardioplegic intervention procedures have been developed and extensively tested during the last two decades (4, 6, 32, 33–38). The focus of this research has been to devise a therapeutic pretreatment regimen that will protect the ischemic myocardium during cardiopulmonary bypass and open heart surgery, and optimize postischemic functional recovery of the heart. As part of these efforts, various chemical cardioplegic

formulations and hypothermic conditions have been studied alone and in combination with one another (4, 6, 12, 18, 19, 25, 34–36). Magnesium and potassium cardioplegia under normothermic conditions have been shown previously to reduce the deleterious effects of intermittent myocardial ischemia on myocardial ATP stores and promote a rapid restoration of postischemic functional recovery (3, 4). Recent attempts to minimize ischemic and reperfusion injury associated with calcium overload have focused on agents that act to reduce calcium movement into cardiac cells during periods of accentuated vulnerability. By virtue of their mechanism of action, the membrane calcium-channel inhibitors, such as diltiazem, have been considered to be promising cardioprotective adjuncts to conventional cardioplegic regimens (12, 13, 15–26). Diltiazem appeared to be a particularly promising drug in this regard because of its reported protective effect on cell membranes, mitochondrial function, and cellular metabolism under ischemic, anoxic, and calcium-paradox-related conditions (22–26). Since calcium-related cell injury generally occurs during reperfusion following even short ischemic intervals, it has been postulated that diltiazem in combination with a membranoplegic cation may provide superior protection as compared to the cation alone under normothermic conditions (17, 19, 20). The cation specificity of this effect, however, has not been investigated previously. The results of the present study suggest that the postischemic recovery of diltiazem-treated hearts depends on the cation used to induce myocardial arrest. Diltiazem at the dose administered was actually detrimental to postischemic functional recovery in magnesium-arrested hearts.

The major findings of the present study were twofold: the combination of diltiazem with magnesium cardioplegia at a perfusate calcium concentration of 1.2 m*M* resulted in a protracted cardiodepression during the postischemic recovery phase, despite a rapid and complete restoration of intracellular pH and myocardial high-energy phosphate stores, and diltiazem offered no significant advantage to either potassium or magnesium in terms of additional preservation of high-energy phosphate stores or amelioration of intracellular acidosis. Under the conditions similar to those used in the present experiments, 30 min of sustained normothermic (37°C) global ischemia has been shown in previous studies to cause a marked loss of myocardial ATP and the formation of ischemic contracture bands (1–3, 33, 37, 38). The intermittent administration of oxygenated cardioplegic perfusate after 15 min of global ischemia appears to have prevented major ischemic injury. Since hearts arrested with potassium and magnesium exhibited a greater than 85% recovery of both ATP and contractile function following ischemia, it may be difficult to demonstrate a significant additive effect of diltiazem at this level of recovery. Thus, longer ischemic episodes may be required to show any significant advantage with diltiazem in terms of the preservation of myocardial high-energy phosphate stores and enhancement of contractile recovery under the conditions of the present experiments.

Until now, potential confounding factors that may complicate the use of calcium channel inhibitors as cardioprotective agents have not been identified. The present findings provide the first evidence that calcium-channel inhibition in combination with magnesium cardioplegia may actually interfere with the functional recovery of the myocardium following episodes of intermittent, normothermic ischemia. This aberrant recovery was observed neither in hearts treated with potassium and diltiazem, nor in hearts arrested with magnesium alone, which suggests that this anomaly may be related to an undefined synergistic effect of diltiazem and magnesium. This interpretation is consistent with the findings reported, but not explained by Yamamoto *et al.* (17). In their study hearts pretreated with increasing doses of diltiazem in combination with their magnesium-based cardioplegic solution exhibited a dose-dependent decline in postischemic myocardial contractile recovery at 35 min of reperfusion. The time dependence of this observation and the possibility of a synergistic interaction between diltiazem and magnesium were not considered. Evaluation of the metabolic and morphologic condition of the hearts in the present study failed to reveal any significant derangements in cellular structure or tissue high-energy phosphate levels that might suggest a mechanistic basis for this effect. The results, however, do not exclude other possible sites of action. The possibility exists that the combined actions of

diltiazem and magnesium may produce a persistent interference with excitation–contraction coupling processes that link ATP utilization to work performance by cardiac cells. Reportedly, the sensitivity of calcium-channel binding sites for calcium-channel inhibitors is modified by various factors, including transmembrane voltage fluctuations, temperature, protein phosphorylation, and conformational changes in sarcolemmal phospholipids (7, 9–11, 19, 39). Based on recent evidence, these factors may alter the transition dynamics of calcium-channel binding sites, converting them from low- to high-affinity binding and visa versa, thereby enhancing and diminishing, respectively, the affinity of diltiazem and other calcium-channel inhibitors for these sites (7, 9–11). It is conceivable that the sites involved in the action of diltiazem combined with the sites involved in the chemical cardioplegia induced by magnesium may be responsible for the observed response.

Hypothetically, the displacement of calcium (Ca^{2+}) from sarcolemmal binding sites by magnesium (Mg^{2+}) may involve the cation-binding site of the calcium channel (40). The putative binding of Mg^{2+} to this site has several possible ramifications. The affinity of diltiazem for its channel binding site may be enhanced when magnesium occupies the cation-binding site of the channel, giving rise to a more stable diltiazem complex at the site of the calcium channel. This interpretation is consistent with recent work reported by Triggle, Janis, and co-workers (40). The resultant effect may be to reduce the rate of dissociation of both magnesium and diltiazem from these sarcolemmal binding sites, which would produce a sustained period of cardiodepression such as that observed in the present study.

In conclusion, evidence has been presented which demonstrates that magnesium in combination with diltiazem produces a protracted cardiodepression of the isolated rat heart following intermittent, normoxic global ischemia. This effect, which was not observed with potassium cardioplegia, cannot be explained on the basis of defective myocardial high-energy phosphate production, changes in intracellular pH, or selective structural damage. The exact mechanism responsible for this effect awaits elucidation. This anomaly requires further characterization to identify not only the mechanism involved in the rat heart, but also the applicability of these findings to the human heart. The growing use of these agents in cardiac surgery further emphasizes the need for clarification of this issue.

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