The cardiac cycle: regulation and energy oscillations

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WIKMAN-COFFELT, JOAN, RICHARD SIEVERS, ROBERT J. COFFELT, AND WILLIAM W. PARMLEY. *The cardiac cycle: regulation and energy oscillations.* Am. J. Physiol. 245 (Heart Circ. Physiol. 14): H354–H362, 1983.—Cyclical changes in energy-related metabolites were observed in glucose-perfused but not pyruvate-perfused isolated working rat hearts. A chronological study of various phases of the cardiac cycle indicated maximum changes in metabolites occurred at half time to peak pressure (dF/dt_{max}). The high-energy phosphates ATP and phosphocreatine, as well as the glycolytic metabolites, glucose 6-phosphate and pyruvate, reached minimum values immediately prior to peak systole and maximum values during late diastole. The products of high-energy phosphate hydrolysis, ADP, inorganic phosphate, and creatine, as well as the regulator, adenosine 3′,5′-cyclic monophosphate, showed the phase alternate. It was necessary to study cyclical changes in a maximally stressed glucose-perfused heart because the cyclical changes were small and appeared to be the result of rate-limiting steps in glycolysis and the slow transport of NADH into the mitochondria. For stressing the heart, thereby increasing ATP utilization and augmenting cyclical changes, the afterload chamber was set at 110 mmHg, and the perfusate contained high concentrations of calcium (3.5 mM, free) and isoproterenol (5×10^{-9} M). When correction was made for binding and compartmentation of metabolites, data indicated that the free energy of ATP hydrolysis was preserved during the contraction process by a continuous binding and recycling of ADP.

high-energy phosphates; pyruvate; adenosine 5′-triphosphate

FEW STUDIES have been carried out analyzing cyclical changes occurring in the biochemistry of the myocardium in relation to the cardiac cycle, although it is well recognized that the chemical action and conformational changes occurring with troponin and myosin are phased with the cardiac cycle. Studies concerning cyclical changes were carried out with gated nuclear magnetic resonance (NMR) (7), analyzing oscillations in ATP and creatine phosphate in the rat heart; minimal values occurred at systole and peak values at diastole; inorganic phosphate cycled with the phase alternate (7). Enzymatic analyses showed similar cyclical changes in high-energy phosphates in the frog heart in vivo (16). Further studies concerning cyclical changes in the myocardium included oscillations in adenosine 3′,5′-cyclic monophosphate (cAMP) following stimulation of in vivo and isolated muscles from frog heart (3, 16, 34). The studies described here were carried out to determine if such analyses could be confirmed by in vitro analyses, following rapid freeze clamping of the heart at predetermined phases of the cardiac cycle. There was further interest in extending these studies to map more specifically the complete cardiac cycle. Such studies have recently been made possible by the development of a stimulator-triggered freeze clamp that decreases the temperature of the heart from 37 to −80°C in 5 ms at any predetermined phase of the cardiac cycle (31).

METHODS

Isolated rat heart perfusion. The isolated rat heart, a modification of that described by Neely et al. (22), was used for these studies. Media entered the left atrium and was ejected through the aorta. The media was a modified Krebs-Henseleit media (21) with added insulin (0.01%) and a low concentration of isoproterenol (5×10^{-9} M); media was flushed with 95% O_2-5% CO_2; free calcium in the media was 3.5 mM. The afterload chamber was set at 110 mmHg and the preload chamber at 15 mmHg. Stimulator leads were placed in the base of the right ventricle. Coronary flow was collected from the right ventricular outflow tract.

Oxygen consumption. Oxygen consumption was measured by two methods. In the first method, the pulmonary artery was cannulated and samples were collected for analyzing venous oxygen. Arterial oxygen was aspirated from the bubble chamber superior to the aorta. In addition, the heart was submerged (Fig. 1) and exchange of oxygen across the surface of the heart was measured by sampling the inflow and outflow for oxygen. The additive values gave the total oxygen exchange after carefully accounting for total flow in both conditions. In the second method the heart was submerged, and the coronary flow was allowed to drip into the bathing media in which the heart was submerged. The rate of inflow from both coronaries and bathing solution was carefully determined as well as oxygen content of each. The rate of outflow and oxygen content of outflow were analyzed as shown in the APPENDIX. Oxygen was analyzed using the Lex-O_2-Con as well as with the Corning gas analyzer. Calculations for each are described in the APPENDIX.

Stimulator-triggered freeze clamp. The stimulator-triggered rapid-freeze clamp bolts on the face of the perfusion apparatus. Specific details of both systems have been recently described (30, 31), including measurement of temperature in the heart during "smashing." The center of the heart drops from 37 to −80°C in 5 ms with

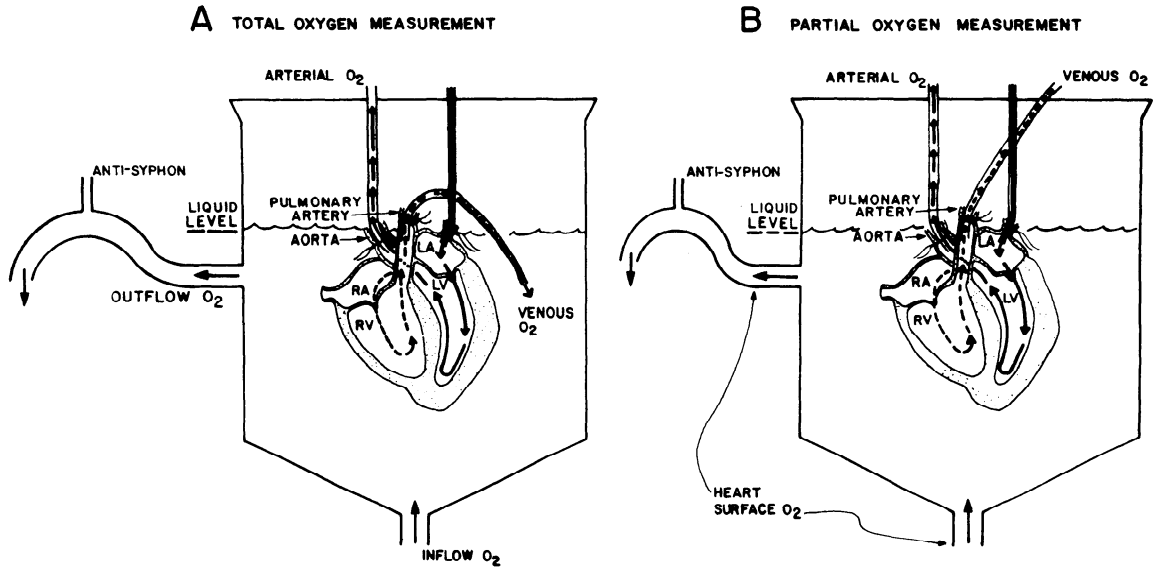

FIG. 1. Measurement of O_2 consumption. *A*: total O_2 measurement. *B*: partial O_2 measurement. For details and calculations, see APPENDIX.

TABLE 1. *Hemodynamic parameters for the perfused heart*

	End Diastolic Pressure, mmHg	Heart Rate, beats/min	LV Pressure, mmHg	Coronary Flow, ml/min	Oxygen Consumption, μmol $O_2 \cdot$g dry wt$^{-1} \cdot$beat^{-1}			
					Total		Coronary	
					Lex-O_2-Con		Lex-O_2-Con	
Working heart								
Glucose perfused	3.1 ± 0.2	330 ± 11	184 ± 7	24 ± 3	0.46 ± 0.04	0.47 ± 0.03	0.32 ± 0.03	0.35 ± 0.03
Pyruvate perfused	4.2 ± 0.3	325 ± 8	187 ± 4	23 ± 2	0.49 ± 0.03			
Langendorff heart								
Glucose perfused	3.7 ± 0.1	320 ± 9	185 ± 6	23 ± 2	0.33 ± 0.02			

Values are means ± SD; n = 6–13. Coronary flow at 37° and reservoir 30°C. LV, left ventricular. Modified Krebs-Henseleit media contained the following (in mM): NaCl 117, KCl 4.3, $CaCl_2$ 3.5, $MgSO_4$ 1.2, K_2HPO_4 0.8, $NaHCO_3$ 25, glucose 15 (or pyruvate 10), and Na-ethylenediaminetetraacetic acid 0.5.

the techniques used here. For the studies described here a single cardiac cycle was 180 ms. The heart was freeze clamped at 30, 40, 60, 110, and 180 ms following stimulation.

Tissue extraction. Preparation of acid extracts of the tissue was similar to the method described by Morgan et al. (21). The frozen wafer fell from the anvils into liquid nitrogen when the cylinders were retracted. Tissue was pulverized under liquid nitrogen. A 100-mg sample of the pulverized heart was removed, weighed, dried (110°C), and weighed again for assessing wet-to-dry weight. Acid (−200°C, 3 ml 10% perchloric acid) was pulverized separately, then added to the pulverized tissue. Pulverization under liquid nitrogen continued. The frozen mixture was transferred to a Sorvall tube and weighed, then transferred to a mortar. The weight of the cold tube and acid was used as a factor in calculating the final tissue weight (wet). The frozen mixture was brought to 0°C while pulverizing. After centrifugation of the mixture (20,000 *g* for 5 min), the extract was weighed, neutralized with 5 N KOH, and weighed again for determining volume. The mixture was centrifuged to remove $KClO_4$, and the supernatant was stored at −80°C.

Tissue assays. Standard enzymatic assay methods, using neutralized extracts, were carried out to determine concentrations of ATP, ADP, phosphocreatine, creatine, pyruvate, and glucose 6-phosphate (2, 4, 17, 19). Inorganic phosphate was determined as described earlier (26) and cAMP by a radioimmunoassay (27).

Animals. Two hundred rats were used for the studies described here. Rats were anesthetized with ether prior to perfusion (30).

Statistical evaluations. All statistical evaluations are standard deviations (SD), except as described in Table 4. Here the standard error (SE) of the means is given.

RESULTS

Cardiac performance as described in Table 1 was attained by perfusing the heart with a high perfusion pressure (110 mmHg), high calcium (3.5 mM), and a low concentration of isoproterenol (5×10^{-9} M). These conditions were used to increase work demands on the heart, augment oxygen consumption, and increase ATP utilization, thus emphasizing cyclical changes in high-energy phosphates.

Total oxygen consumption was measured by immersing the heart in a flowing media as shown in Fig. 1*A* and described in the APPENDIX. Combined analyses of oxygen exchange across the surface of the heart as well as arterial venous differences gave values of 0.46 μmol $O_2 \cdot$g dry wt$^{-1} \cdot$beat^{-1} as measured either with the Corning gas

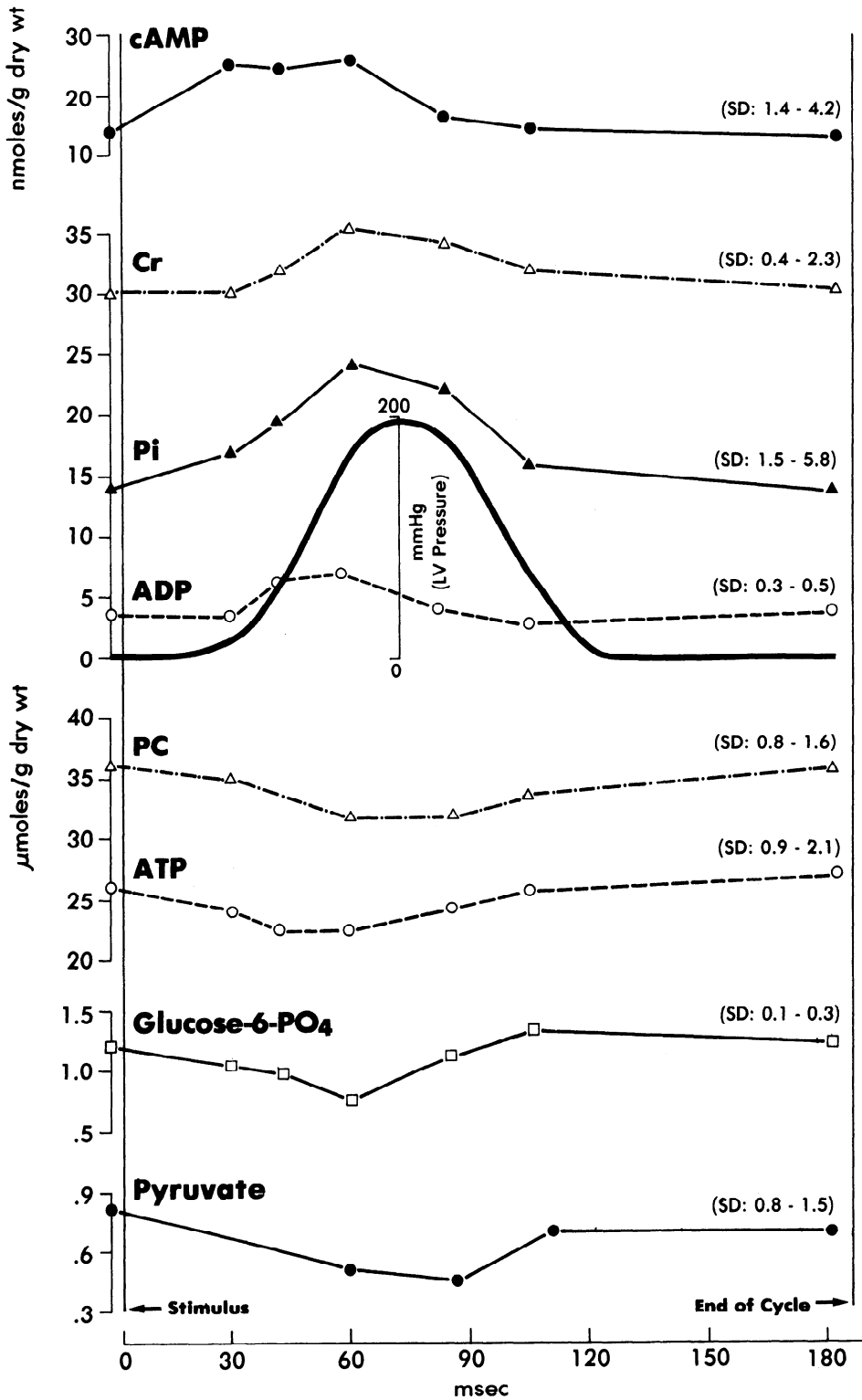

FIG. 2. Pressure tracing *curve* of a single cardiac cycle in rat heart (180 ms) is shown in center. Stimulus is given at point zero, immediately after the point in late diastole is taken. Heart is freeze clamped at 6 points in the cardiac cycle and analyzed for adenosine 3′,5′-cyclic monophosphate (cAMP) and various metabolites. Low-energy phosphates, inorganic phosphate (P_i), and ADP, as well as creatine and cAMP are shown in upper part of figure. High-energy phosphates, creatine phosphate (PC), and ATP, as well as glucose 6-phosphate and pyruvate are shown in lower part of figure. Range of SD are given for each. Each *point* is an averaged value for 10–16 rats.

analyzer (based on a derived value) or with the Lex-O_2-Con (based on a direct measured value; Table 1). Partial oxygen consumption was measured by *1*) differences in the arterial and venous oxygen and *2*) oxygen exchange across the surface of the heart as described in the APPENDIX. The arteriovenous differences gave an oxygen consumption value of 0.32 μmol $O_2 \cdot g$ dry $wt^{-1} \cdot beat^{-1}$. There was no significant difference in oxygen consumption between hearts perfused with the substrate pyruvate compared with glucose. Although the Langendorff-perfused heart was developing similarly high pressures (185 mmHg), it demonstrated a lower oxygen consumption (0.33 compared with 0.46 μmol $O_2 \cdot g$ dry $wt^{-1} \cdot beat^{-1}$). With the Langendorff preparation the aortic valve is neither being forced open nor is it ejecting perfusate, i.e., it is not doing physical work.

Cyclical changes in metabolites in relation to a left ventricular pressure tracing for a single cardiac cycle of 180 ms is shown in Fig. 2. The heart was freeze clamped at six predetermined points in the cardiac cycle for

analyses of cAMP, creatine inorganic phosphate, ADP, phosphocreatine, ATP, glucose 6-phosphate, and pyruvate. The cyclic nucleotide cAMP reached peak values between 40 and 60 ms poststimulation, and ATP reflected a similar time-dependent change but inversely related. All of the other metabolites reached maximum changes between 60 and 90 ms poststimulation.

Biochemical analyses were carried out during two phases of the cardiac cycle, as indicated by the arrows in the pressure tracings (Fig. 3), immediately prior to peak systole when the cross bridges were turning over rapidly and during diastole when there was minimal cross-bridge turnover. The heart was perfused with either glucose or pyruvate as the sole substrate (Fig. 4). In the glucose-perfused heart, ATP, phosphocreatine, glucose 6-phosphate, and pyruvate were significantly higher during diastole, the resting phase, whereas inorganic phosphate, creatine, and ADP were significantly higher during systole. There were, however, no significant differences in these values between systole and diastole when the heart was perfused with pyruvate. No significant differences were found in AMP values. The sum of changes in phosphocreatine and ATP ($\Delta PC + \Delta ATP$) were not significantly different from that of the end product, inorganic phosphate (ΔP_i), at half time to peak pressure (dF/dt_{max}), approximately 60 ms after stimulation; however, the standard error for inorganic phosphate was normally quite high. The latter therefore was not a good indicator of absolute values for cyclical changes. Phosphocreatine (PC) buffers ATP via creatine kinase ($\Sigma ADP + \Sigma PC \leftrightarrow \Sigma Cr + \Sigma ATP$). There is a rapid recycling of ADP back to ATP via creatine kinase; thus it is expected that ΔADP would equal ΔATP. The sum of changes in ADP (ΔADP) is not significantly different from ΔATP. ΔPC is not significantly different from ΔCr.

Total values shown in Fig. 4 were converted to cytosolic values as described by Kammermeier (12) for approximating the change in free energy of ATP hydrolysis ($\Delta G/d\epsilon_{ATP}$) during systole and diastole (Table 2). One modification was made relative to bound ADP, to make up for variances in bound ADP between systole and diastole. There are approximately 1.2 μmol myosin heads/g wet wt. Approximately 85% of the myosin contains bound ADP during diastole and 15% during systole. Assuming creatine kinase is in equilibrium at diastole and calculating ADP according to the creatine kinase equilibrium using the equation shown in Table 2, we found the free energy of ATP hydrolysis was 62.40 kJ/mol during diastole (Table 2). Calculations of the creatine kinase value according to the data obtained here show that creatine kinase is not in equilibrium at systole, thus systolic values cannot be calculated from this formula. If ADP is calculated according to the derived values in Table 2, then the free energy change between systole and diastole is greater for systole (49.54 kJ/mol) than for diastole (59.57). The latter would occur only if ADP remained free in the cytosol.

When inorganic phosphate was omitted from the perfusing media, there was a decrease in ATP, phosphocreatine, and creatine whether pyruvate or glucose was the sole perfusing substrate. However, in the Langendorff preparation in which the heart was not working and total oxygen consumption was low (Table 1), there was only a minimal decrease in ATP, phosphocreatine, and creatine when inorganic phosphate was not included in the perfusing media (Table 3).

A statistical analysis of cyclical changes of ADP, PC, and ATP in 13 cardiac cycles is given in Table 4, using the same hearts that were analyzed for oxygen consumption. Standard deviation of mean values are smaller for ΔADP than for ΔATP and ΔPC, perhaps due to the greater fractional variances in the former. The ratio of ATP produced to oxygen consumed ($\sim P/O$) was 3.1 for ΔADP and 4.6 for $\Delta ATP + \Delta PC$. SE was smaller for ΔADP; however, this value may be an underestimation as ADP is rapidly recycled back to ATP via PC.

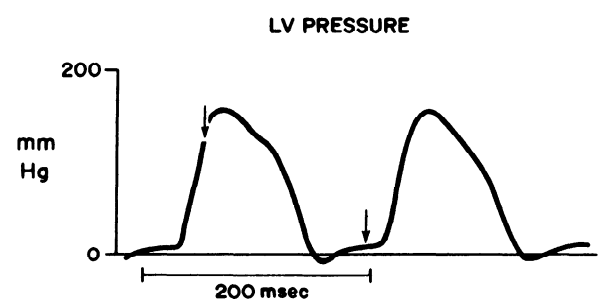

FIG. 3. Left ventricular pressure tracing for working rat heart preparation. *First arrow* indicates peak force where the heart was "smashed" and quick frozen for analysis of metabolites at systole. *Second arrow* indicates where the heart was frozen for analysis of metabolites at diastole.

DISCUSSION

Cyclical changes in high-energy phosphates occurred in the glucose-perfused heart developing high pressures but not in the hearts perfused with pyruvate. The observed cyclical changes in high-energy metabolites may be due to the slow rate of glycolysis (33) and the slow transport of NADH into the mitochondria (15). The slow rate of glycolysis has been attributed to lack of α-agonists in the perfused heart and the dependency of phosphofructokinase, a rate-limiting enzyme in glycolysis, on calcium for activation (5). Inorganic phosphate and ADP have also been shown to activate phosphofructokinase and the rate of glycolysis (29). Another rate-limiting enzyme in glycolysis, glyceraldehyde-3-phosphate dehydrogenase, has been shown to be inhibited by NADH (15), as well as phosphocreatine and ATP at physiological concentrations (25). Limiting amounts of available pyruvate in the glucose perfused heart may also inhibit pyruvic dehydrogenase, whereas larger concentrations in the pyruvate-perfused heart may activate it (8). Two metabolites of glycolysis, glucose 6-phosphate and pyruvate, were shown to oscillate with the cardiac cycle. These two metabolites were also shown to decrease with an increase in work imposed on the perfused heart (15). The low pyruvate-to-lactate ratio supports previous evidence for a slow rate of glycolysis in the glucose-perfused heart (15, 25, 33). Cyclical changes observed with freeze clamping of tissue may be the result of restricted oxidative phosphorylation in the glucose-perfused heart due to the slow delivery of pyruvate to the mitochondria and

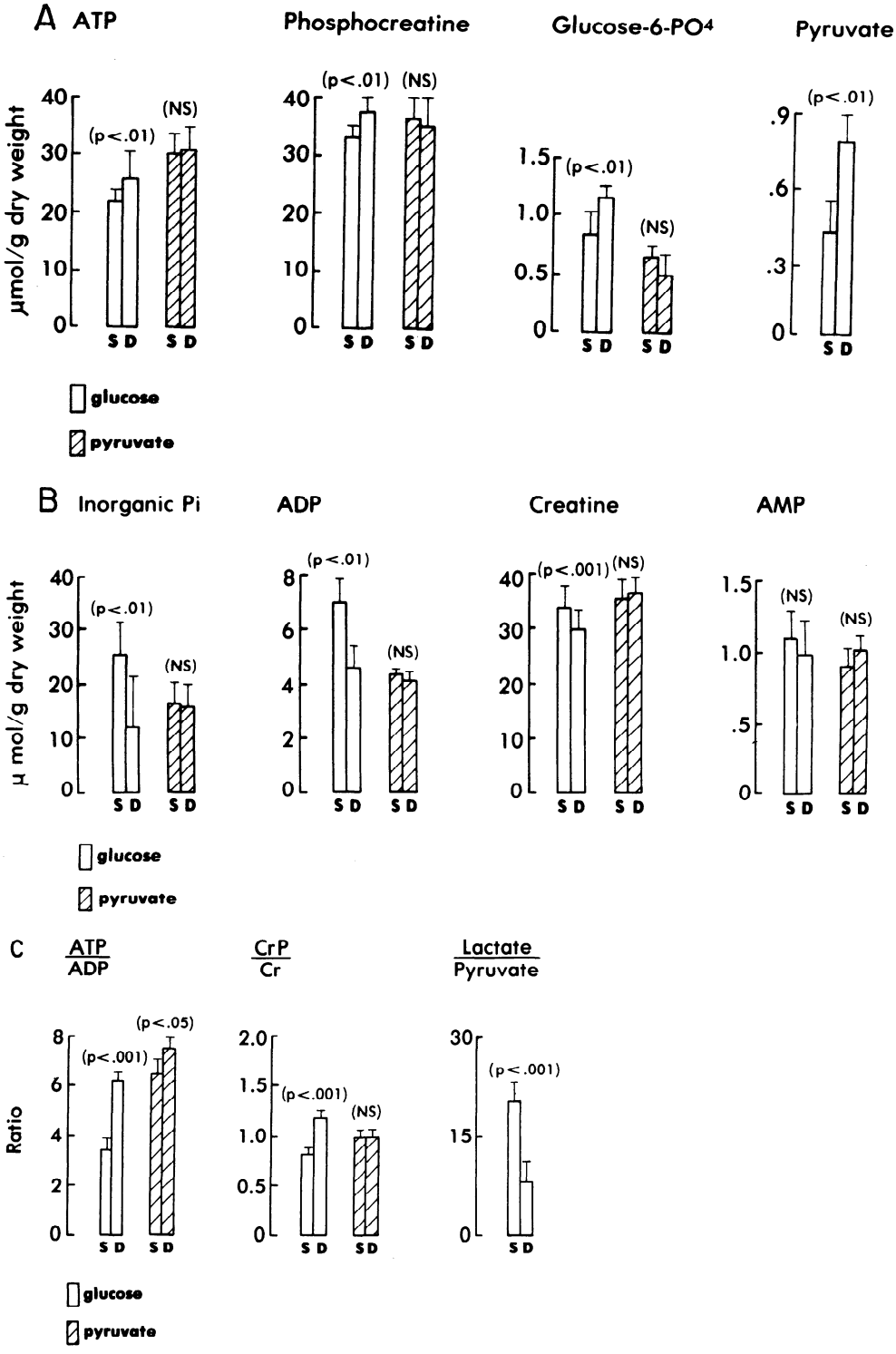

FIG. 4. Analyses of high-energy phosphates, phosphocreatine and ATP, as well as glycolytic metabolites, glucose 6-phosphate and pyruvate (*A*) and low-energy phosphates, ADP, AMP, and inorganic phosphate (P_i) as well as creatine (*B*), and ratios of ATP/ADP, PC/Cr, and lactate/pyruvate (*C*) at systole (S) and diastole (D) in the cardiac cycle. For these studies heart was either perfused with glucose (*open bars*) or pyruvate (*stippled bars*). SD is shown. *P* values are for unpaired *t* test. Each *bar* represents an average of 10–16 rat hearts. NS, no significant difference, i.e., $P < 0.05$. Hearts were freeze clamped after 20 min of perfusion. Pyruvate could not be determined in pyruvate-perfused heart because of the contamination of media containing pyruvate. The media contained 10 mM pyruvate.

thus may not pertain to general cardiac energetics. The high afterload (110 mmHg), the high calcium concentrations (3.5 mM, free) and the addition of isoproterenol (5×10^{-9} M) to the perfusion media increased the workload on the heart, thereby augmenting oxygen consumption and ATP utilization. An increase in energy demands on the glucose-perfused heart demonstrates rate limitations in glycolysis based on the slow transport of NADH to the mitochondria (13) and thereby emphasizes cyclical changes in high-energy phosphates. Preliminary data indicated that low concentrations of isoproterenol increased both basal concentrations of cAMP as well as cyclical changes in energy-related metabolites. Although cAMP activates phosphokinases and phosphorylases and has numerous and complex ramifications on myocardial metabolism, it nevertheless does not activate important rate-limiting steps in glycolysis (5), including the ones causing cyclical changes in metabolites observed here.

The ~P/O reported here for ΔADP was 3.1 where ADP was determined from an acid-extracted homogenate. (Acid extraction of ADP releases the nucleotide from bound sites.) A correlation of ~P/O during work

TABLE 2. *Metabolite values during peak dF/dt and diastole in rat hearts*

	Metabolites Extracted, μmol/g wet wt	Cytosolic Fraction, mmol/l
ATP		
Systole	3.32	7.55
Diastole	3.95	9.12
ADP		
Systole	1.02	1.48
Diastole	0.68	0.22
P_i		
Systole	3.26	3.25
Diastole	2.07	0.51
PC		
Systole	4.80	12.00
Diastole	5.46	13.65
Creatine		
Systole	5.25	13.13
Diastole	4.50	11.25
$\Delta G/d\epsilon_{ATP} = \Delta G^0_{obs(ATP)} + RT \ln \frac{[ADP][P_i]}{[ATP]}$		
Systole		49.54
Diastole		59.57
$\Delta G/d\epsilon_{ATP} = \Delta G^0_{obs(ATP)} + RT \ln \frac{[Cr][P_i]}{[PC][H^+][K_{CK}]}$		
Assumption: $(ADP)_{free} = \frac{1}{K_{CK}} \frac{[ATP][Cr]}{[PC][H^+]}$		
Diastole		62.40

Dry weight values are corrected for 15% dry weight fraction. Values are for 9 animals each. Calculations for cytosolic fractions are taken from Kammermeier (10, 12) except for corrections made in variance in dry to wet weight and ADP-myosin binding during systole. Calculations for cytosolic ATP: μmol ATP/g wet wt − 0.3 × 5/2 = mmol/l cytosolic fraction where 0.3 represents the amount compartmentalized and bound (10, 12), and 5/2 results from assuming a cytosolic water content of 40% wet wt (12). Calculations for ADP: [(μmol ADP/g wet wt) − (compartmentalized + bound)] × 5/2 where compartmentalized is 0.40 (12); bound is 0.15 for diastole and 0.03 for systole, based on the percent myosin-ADP binding. 30% of the dry wt is myosin, giving 0.18 mmol myosin heads/1; assume a 15% myosin-ADP binding in systole (23) and 85% during diastole. Calculations for cytosolic inorganic phosphate (P_i): (μmol/g wet wt) − (0.08 × mol P_i/g wt wt) − 1.7 × 5/2 where 0.08 represents 8% bound (12); 1.7 is the value for mitochondrial P_i (1.3 μmol/g), and extracellular P_i is (0.4 μmol/g). Calculations for cytosolic phosphocreatine (PC): (μmol PC/g wet wt) × 5/2. Calculations for cytosolic creatine (Cr): (μmol Cr/g wet wt) × 5/2.

TABLE 3. *Metabolite values in perfused heart with varying conditions (diastole)*

	ATP	PC	ADP	Cr
No phosphate in perfusing media				
Glucose (sole substrate)				
Working heart	13.3 ± 2.6	20.6 ± 1.8	3.9 ± 0.4	23.1 ± 2.3
Langendorff	28.6 ± 2.1	34.1 ± 3.1	4.0 ± 0.2	34.2 ± 3.8
Pyruvate (sole substrate)				
Working heart	14.5 ± 1.6	26.2 ± 3.4	4.2 ± 0.8	21.2 ± 3.2
Phosphate (1.2 mM) in perfusing media				
Glucose (sole substrate)				
Working heart	25.1 ± 2.1	34.1 ± 3.1	4.0 ± 0.2	29.8 ± 2.3
Langendorff	27.2 ± 3.2	42.6 ± 3.2	3.7 ± 0.3	35.1 ± 4.0
Pyruvate (sole substrate)				
Working heart	32.8 ± 4.8	35.0 ± 4.8	4.4 ± 1.0	34.9 ± 3.4

Values are means ± SD in μmol/g dry wt for 6–9 hearts. PC, phosphocreatine; Cr, creatine. Developed pressure for working heart was 160 ± 8 mmHg; developed pressure for Langendorff preparation was 125 ± 6 mmHg.

TABLE 4. *Statistical evaluation of cyclical changes with 13 cardiac cycles*

	ADP	ATP	P_i	PC
Systole				
Range	6.0–9.4	18–25	15–28	28–36
Mean	7.1	21.7	20.9	33.6
±SE	0.11	0.75	1.24	0.89
Diastole				
Range	3.0–6.5	20–28	7–23	30–38
Mean	4.4	24.1	16.4	35.7
±SE	0.28	0.75	2.2	0.86
Δ Value				
	+2.6	−1.5	+2.6	−0.9
	+2.9	−0.4	+3.0	−1.9
	+2.8	−3.5	+4.5	−2.5
	+2.8	−3.8	+4.5	−2.5
	+3.0	−2.0		−2.8
	+3.3	−2.8	+9.0	−2.8
	+3.5	−4.1	+11.4	−3.5
	+3.5	−3.3	+9.0	−2.0
	+2.7	−3.1	+4.2	−1.6
	+2.9	−1.8	+4.8	−2.9
	+2.8	−2.1	+3.0	−1.4
	+2.5	−2.0	+6.4	−1.2
	+2.9	−0.7	+6.8	−2.0
Mean	2.89	2.39	5.8	2.15
±SE	0.09	0.32	0.81	0.21

Values are in μmol/g dry wt. Delta values are for going from late diastole to dF/dt, i.e., immediately prior to peak systole. Analyses carried out on hearts used for measuring oxygen consumption. P_i, inorganic phosphate; PC, phosphocreatine.

performance in relation to ATP utilization based on ΔATP + ΔPC values is difficult to assess in unpaired analyses as shown here as the standard deviation of the mean value is quite high for the small changes observed. Nevertheless, with this in mind, ~P/O for ΔATP + ΔPC was 4.6. Concurrent ATP synthesis most likely occurs throughout the cardiac cycle, resulting in a possible underestimation of ATP utilization using techniques described here. Such studies can be analyzed more accurately with saturation transfer NMR in which utilization of ATP is analyzed independently of concurrent synthesis (14). Measuring oxygen consumption while the heart is submerged in a perfusing bath appears to give an accurate value for total oxygen consumed per beat. Oxygen consumption, as measured by arteriovenous differences, was similar to that described for blood-perfused rat hearts (6, 11) and for the working rat heart perfused with a similar Krebs-Henseleit solution with minor modifications (22, 30). Each of the values obtained with the isolated perfused working heart were lower than those obtained for the stressed rat heart in vivo (13).

Total oxygen consumption was only slightly higher in the pyruvate-perfused heart; however, there were no observable cyclical changes in high-energy phosphate. This may be due to the lack of the dependency of ATP synthesis on glycolysis, thus eliminating a slow step in metabolism; large concentrations of pyruvate are provided the mitochondria. (It was not possible to measure

intracellular pyruvate in the pyruvate-perfused heart because the extract contained large quantities of pyruvate from the media.)

The rapid decrease in ATP following stimulation is most likely due to the enzymatic action of actomyosin. With the influx of calcium following membrane depolarization, calcium is rapidly bound to troponin C, resulting in subsequent conformational changes in the thin filaments, thus exposing actin to cross-bridge formation (6). The rapid decrease in ATP following membrane depolarization occurs at a point in the pressure curve where aequorin studies indicate calcium is bound to troponin (1). The rapid binding of calcium to troponin derepresses troponin, thus allowing actin and myosin to interact to form the enzyme actomyosin. With the repetitive binding and hydrolysis of ATP as well as transfer and release of products, myosin goes through several conformational changes resulting in subsequent filament shortening and production of ventricular pressure, as observed in the pumping action of the heart. These analyses account for the early drop in ATP and the alternate cycling of ADP. According to the $\Delta ATP + \Delta PC$ values, myosin heads go through approximately 4.5 cycles during the shortening process. Since cross-bridge turnover occurs within microseconds, 4.5 cycles is well within the range of shortening time. (According to ΔADP values the number of cycles would be 3.1.) On the basis of the pressure tracing in Fig. 3, maximum shortening takes approximately 40 ms. Data obtained by saturation transfer ^{31}P NMR of skeletal muscle (8) gave about a 3% drop in ATP levels at systole and a larger decline in phosphocreatine. In contrast, the early precipitous drop in ATP obtained here may be due to the following differences in conditions. *1)* Concurrent ATP synthesis is occurring here; also limiting rates in glycolysis takes place, thereby affecting energy phosphate levels. *2)* Acid hydrolysis used in preparation of tissue as described here releases nucleotides from bound sites thus allowing for analyses of bound ATP and ADP not observed with ^{31}P NMR. The latter, at least in the case of myosin, may cause alterations between systole and diastole.

The percent change in ATP, PC, and P_i in relation to systole vs. diastole agrees with the NMR values for the perfused working heart under similar loading conditions (7). The sum of changes in ATP ($\Delta PC + \Delta ATP$) are not significantly different from those of inorganic phosphate (ΔP_i). These values, however, need to be analyzed with caution since the standard errors for P_i are high. The sum of ADP is not the result of total ATP hydrolysis. Due to the buffering action of phosphocreatine ($\Sigma ADP + \Sigma PC) \Leftrightarrow (\Sigma Cr + \Sigma ATP$), ADP is rapidly recycled back to ATP. If one assumes that the ADP formed via ATP hydrolysis remains free during systole when work is being performed, then the accumulation of end-product, i.e., the ADP, would rise sufficiently high to lower the free energy of ATP hydrolysis ($\Delta G/d\epsilon_{ATP}$) to a state in which it would be very near to hindering the sarcoplasmic reticulum pump (12). When the change in free energy for ATP hydrolysis falls to 48 kJ/mol, the calcium pump begins to fail (2). Furthermore, it is at peak systole that the sarcoplasmic reticulum begins to bind calcium. The binding and recycling of ADP is most likely rapid enough to prevent this from occurring. The rapid buffering action of phosphocreatine and myokinase as well as the rapid uptake by mitochondria may keep free ADP low at all times.

Calculation of creatine kinase values from data obtained for various phases of the cardiac cycle indicate, however, that creatine kinase is not always in a state of equilibrium. Also intracellular changes brought about by substrate and workload appear to cause creatine kinase equilibrium shifts; the creatine kinase equilibrium (diastolic values) favors higher phosphocreatine concentrations relative to ATP in the heart perfused by the nonworking Langendorff method compared with that obtained with the working heart. The equilibrium favors higher ATP concentrations relative to phosphocreatine when the working heart is given pyruvate as the substrate compared with when it is given glucose as the substrate.

Cyclical changes in high-energy phosphates observed here with the glucose-perfused working rat heart and reported earlier using gated ^{31}P NMR for analyses (7) may result from dependency of the glucose-perfused heart on glycolysis and the slow transport of NADH to the mitochondria. The cyclical changes in glucose 6-phosphate as well as the pyruvate-to-lactate ratios support this hypothesis. The decrease in the pyruvate-to-lactate ratios during systole may be due to the accumulation of NADH at this time, causing activation of lactic dehydrogenase and favoring formation of lactate. When a factor essential to mitochondrial synthesis of ATP such as P_i is limiting, then there is a general decrease in high-energy phosphates and nucleotides throughout the cycle in both the glucose-perfused and pyruvate-perfused hearts. The high rate of creatine kinase enzymatic activity, as predicted from equilibrium studies (18, 20), appears to rapidly buffer ATP thus keeping ADP concentrations low. Free ADP is detrimental to the cell, lowering the free energy of ATP hydrolysis. Thus, with the differential binding of ADP by myosin between systole and diastole and the high creatine kinase activity, free ADP remains low in the cell.

APPENDIX

Measurement of O_2 Consumption

For total analyses of O_2 consumption (Fig. 1*A*), coronary flow from the venous outflow tract was allowed to drip into the bathing chamber and three analyses were made. Samples were taken from *1)* the inflow, *2)* the outflow, and *3)* the aorta. O_2 content was analyzed in all three. Rate of flow was taken for the inflow and outflow; the difference was attributed to coronary flow. Calculations were made as described below for total O_2 consumption. Extraction of O_2 by the surface of the heart was analyzed independently of coronary extraction (Fig. 1*B*). Two measurements were made for arteriovenous differences. Coronary flow was collected and determined by suturing the right ventricular outflow tract and collecting coronary flow in a volumetric container. Arterial samples were determined as described earlier (30) and coronary extraction calculated as described below. Surface extraction was determined from the inflow and outflow after coronary flow was diverted.

Arterial inflow

$$\text{fraction of } O_2 \text{ (mmHg) entering prior to coronary extraction} = \frac{\text{coronary flow (ml/min)}}{\text{outflow (ml/min)}} \times \text{arterial } O_2 \text{ (mmHg)}$$

Pump inflow

fraction of O_2 (mmHg) entering prior to extraction by the surface of the heart

$$= \frac{\text{inflow (ml/min)}^*}{\text{outflow (ml/min)}} \times \text{inflow } O_2 \text{ (mmHg)}$$

Total O_2 Measurements

1) Analyses with Corning gas analyzer

$$\frac{(\text{arterial inflow } O_2 + \text{pump inflow } O_2) - \text{outflow } O_2 \text{ (mmHg)}}{760 \text{ (mmHg)}} \times \frac{\text{solubility of } O_2 \text{ at T°C (ml/ml } H_2O \text{ at 1 atm)}}{22.4 \text{ ml/mol}} \times \frac{\text{outflow (ml/min)}}{\text{dry wt of heart (g)}\dagger} \times \frac{10^3 \text{ (conversion to } \mu\text{mol)}}{\text{beats/min}}$$

$$= \mu\text{mol } O_2 \cdot \text{g dry wt}^{-1} \cdot \text{beat}^{-1}$$

Temperature of reservoir and coronary inflow can be regulated independently. When this is done compensation is made for oxygen solubility at the various temperatures. O_2 solubility at 27°C is 0.02736 and at 37°C is 0.0238. Linearity between these two differences is assumed. Arterial and inflow O_2 are between 650 and 700 mmHg. This high partial pressure is achieved by flushing the media vigorously with 95% O_2-5% CO_2 at a cooler temperature and then subsequently raising the temperature. The latter is achieved by insertion of countercurrent heat exchangers (30) and thermocouples to regulate temperature.

2) Analyses with Lex-O_2-Con

$$\frac{(\text{arterial inflow } O_2 - \text{pump inflow } O_2) - \text{outflow } O_2 \text{ (ml/ml } H_2O)}{22.4 \text{ ml/mmol}} \times \frac{\text{outflow (ml/min)}}{\text{dry wt of heart (g)}} \times \frac{10^3 \text{ (conversion to } \mu\text{mol)}}{\text{beats/min}}$$

$$= \mu\text{mol } O_2 \cdot \text{g dry wt of heart}^{-1} \cdot \text{beat}^{-1} \text{ (total } O_2\text{)}$$

Partial O_2 Measurements

Coronary extraction. 1) Analyses with Corning gas analyzer

$$\frac{\text{arterial } O_2 - \text{venous } O_2 \text{ (mmHg)}}{760 \text{ mmHg}} \times \frac{\text{solubility of } O_2 \text{ at T°C (ml/ml } H_2O \text{ at 1 atm)}}{22.4 \text{ ml/mmol}} \times \frac{\text{coronary flow (ml/min)}}{\text{dry wt of heart (g)}} \times \frac{10^3}{\text{beats/min}}$$

$$= \mu\text{mol } O_2 \cdot \text{g dry wt}^{-1} \cdot \text{beat}^{-1} \text{ (partial } O_2\text{)}$$

2) Analyses with Lex-O_2-Con

$$\frac{\text{arterial } O_2 - \text{venous } O_2 \text{ (ml/ml } H_2O)}{22.4 \text{ ml/mmol}} \times \frac{\text{coronary flow (ml/min)}}{\text{dry wt of heart (g)}} \times \frac{10^3}{\text{beats/min}}$$

$$= \mu\text{mol } O_2 \cdot \text{g dry wt}^{-1} \cdot \text{beat}^{-1} \text{ (partial } O_2\text{)}$$

Heart surface extraction. 1) Analyses with Corning gas analyzer

$$\frac{\text{inflow } O_2 - \text{outflow } O_2 \text{ (mmHg)}}{760 \text{ mmHg}} \times \frac{\text{solubility of } O_2 \text{ at T°C (ml/ml } H_2O \text{ at 1 atm)}}{22.4 \text{ ml/mmol}} \times \frac{\text{outflow (ml/min)}}{\text{dry wt of heart (g)}} \times \frac{10^3 \text{ (conversion to } \mu\text{mol)}}{\text{beats/min}}$$

$$= \mu\text{mol } O_2 \cdot \text{g dry wt}^{-1} \cdot \text{beat}^{-1}$$

2) Analyses with Lex-O_2-Con

$$\frac{\text{inflow } O_2 - \text{outflow } O_2 \text{ (ml/ml } H_2O)}{22.4 \text{ ml/mmol}} \times \frac{\text{outflow (ml/min)}}{\text{dry wt of heart}} \times \frac{10^3 \text{ (conversion to } \mu\text{mol)}}{\text{beats/min}}$$

$$= \mu\text{mol } O_2 \cdot \text{g dry wt}^{-1} \cdot \text{beat}^{-1}$$

* Inflow into the reservoir is approximately 50 ml/min. The reservoir contains 110 ml of media.

† Dry/wet weight of pulverized tissue from smashed hearts was approximately 0.14 when prepared and dried as described here.

The authors are grateful to H. E. Morgan and P. Kochel for the stimulating and helpful discussions and assistance in developing the procedures for pulverizing the tissue and assaying enzymatic activity.

This work was supported in part by Susan and Don Schleicher. J. Wikman-Coffelt is the recipient of Career Development Award HL-00466 from the National Heart, Lung, and Blood Institute.

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Received 13 December 1982; accepted in final form 30 March 1983.

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