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Distribution of High-Energy Phosphates in the Heart (38018)

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(Introduced by M. M. Sayeed)

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Adenosine triphosphate is the immediate source of energy for cardiac contraction and along with creatine phosphate, it provides a readily available source of energy (1, 2). Energy production in the heart under normal conditions is largely due to oxidative phosphorylation (3). Whether or not there is a relationship between high-energy phosphates and development of tension in various chambers of the heart is not fully known. The aim of the present study was to determine the distribution of adenosine triphosphate (ATP), creatine phosphate (CP), and adenosine diphosphate (ADP) stores in the different cardiac chambers and to relate their concentrations to the pressures in the chambers.

Methods. Eight normal mongrel dogs were anesthetized with 30 mg/kg sodium pentobarbital. A cuffed endotracheal tube was inserted and connected to a Harvard respirator to maintain ventilation. A left thoracotomy was performed at the fourth intercostal space and the heart was exposed. Intracardiac pressures were measured using the Hewlett–Packard 1830C pressure transducers and a 7700 recorder. Myocardial samples weighing 250–500 mg and 1.5–2 mm thick were excised from the wall of each chamber of the beating heart. Major coronary arteries were avoided in the sampling. Epicardial samples were taken from the apex of the left ventricle (LV) and the outflow tract of the right ventricle (RV); transmural samples were taken from the body of the left atrium (LA) and of the right atrium (RA). The ventricular samples were taken simultaneously, as were atrial samples, and the order of sampling was randomized. All samples were immediately frozen between aluminum blocks continuously cooled in dry ice. The delay in obtaining all four samples and placing them in dry ice was timed using a second timer and was approximately 3 sec.

The frozen tissues were ground, weighed, homogenized in 10% trichloroacetic acid–HCl (0.1 *M*) mixture, and centrifuged at 6000 rpm for 15 min in a Sorvall RC2-B centrifuge at 2°. The supernatant was extracted four times with water-saturated ether and neutralized with 1 *M* Tris base. The ATP, ADP, CP, and pyruvate concentrations were assayed enzymatically (4).

Significance between the different samples was tested using the Student's *t* test.

Results and Discussion. The mural concentrations of metabolites in μmoles per gram wet tissue, the total of high-energy phosphates, and mean pressures in the four chambers are shown in Table I. The concentrations of ATP, ADP, and CP were progressively higher in chambers with higher pressures (Fig. 1), and the concentrations in the tissues from LV, RV, LA, and RA were significantly different ($P < 0.01$). The individual concentrations of ATP, ADP, and CP as well as the sum of concentrations of these high-energy phosphates in the intact beating heart were distributed in the following order: apex of the LV > outflow tract of the RV > body of the LA > body of

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TABLE I. Distribution of Metabolites in the Heart.[a]

	Right atrium	Left atrium	Right ventricle	Left ventricle
ATP	2.34 ± 0.17	2.91 ± 0.11	3.59 ± 0.14	4.60 ± 0.16
ADP	0.41 ± 0.030	0.46 ± 0.020	0.84 ± 0.053	1.28 ± 0.033
CP	2.00 ± 0.26	2.99 ± 0.091	3.16 ± 0.090	3.82 ± 0.079
Σ ATP, ADP, CP	2.75	6.36	7.59	9.70
ATP/CP	1.17	0.99	1.14	1.20
Pressure (mm Hg)	3 ± 1	8 ± 2	12 ± 2	49 ± 3

[a] Mean mural concentrations of metabolites in the four chambers of the heart expressed as μmoles/g wet tissue ± SEM of 8 dogs. ATP = adenosine triphosphate; ADP = adenosine diphosphate; CP = creatine phosphate. The mean pressure in each chamber is also shown.

the RA. The left ventricular concentrations of ATP and ADP in the present study were comparable to values reported for the LV by Pool and coworkers (5, 6); however, CP levels in the LV of 3.82 ± 0.079 μmoles/g were lower than the reported values of 12.2 ± 0.3 and 7.85 ± 0.68 μmoles/g in two separate studies by Pool and coworkers (5, 6). These differences in the present findings and those in the previous observations (5, 6) may be due to the fact that CP is the most labile of the high-energy phosphates (5); therefore, the lower CP concentrations in the present study may reflect the slight delay involved in sampling and freezing the four tissue samples in the present study. The other determinations were not affected since ATP and ADP are not as labile as CP (5). The ventricular and atrial samples were taken consecutively and the order was reversed in half of the studies; therefore the delays in sampling are expected to be comparable in all tissues. The rates of freezing in the different samples are also expected to be similar since the samples from the four sites were of approximately equal thickness. The samples were taken from uniform sites in each chamber to avoid the possible variations in distribution within a chamber.

Calculating the ratio of ATP to CP, it was found that the values do not vary greatly at the four sites (Table I). This would suggest that the observed concentrations do reflect an equilibrium in the creatine kinase reaction at different sites of the myocardium. The enzyme creatine kinase converts ATP and creatine to ADP and CP. It is, however, not possible to calculate the equilibrium constant for the creatine kinase reaction at different sites since creatine determinations were not carried out in the present study. The sum of high-energy phosphate is progressively increased from RA to LV, suggesting that the concentrations of these compounds were indeed different at various sites of the myocardium.

The results of Pool *et al.* (6) indicate that ATP concentrations were higher in the left than in the right ventricle in 7 out of

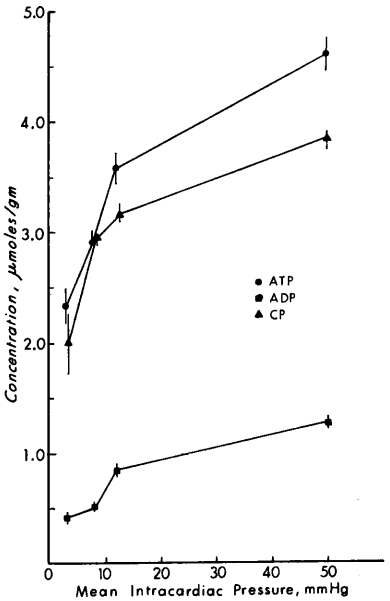

FIG. 1. Relationship between the mean intracardiac pressures generated by the four chambers and the concentrations of ATP, ADP, and CP in μmoles/g wet tissue. The bars represent ±SEM of 8 dogs.

10 instances. However, the differences in mean concentrations of ATP and CP in the two ventricles in their results were not statistically significant (6) as in the present study. In addition, our findings show that the concentrations of ATP, ADP, and CP vary with the mean intracardiac pressure in the chambers of the heart and with external work in a nonlinear fashion. Similar differences have also been demonstrated in the regional distribution of coronary blood flow. Flow per gram is greater for the left than for the right ventricular free wall (7).

The possibility that regional differences in the distribution of high-energy phosphates could be due to regional differences in energy production or energy utilization or both appears more likely. Previous observations that utilization of energy stores is directly related to the mechanical activity of the myocardium (8, 9) and that the lowest levels of CP are present in the endocardium where the tension is highest (10) suggest that energy stores should be lowest in the left ventricle since it performs the most work and has greater tension development per unit weight than the other chambers. Therefore, the finding that energy stores are highest in the LV suggests that there may be regional differences in energy production responsible for the observed distribution. This conclusion is supported by findings in isolated skeletal muscle myofibrils that the development of tension is correlated with the increase in the rate of ATP regeneration by the mitochondria (11). In addition, there is a greater concentration of mitochondria in the left than in the right ventricle (12), indicating that the left ventricle may be more active in the synthesis of high-energy phosphates through oxidative phosphorylation than the right ventricle.

The present findings are of interest in regard to the measurement of high-energy phosphate compounds in failing and hypertrophied hearts. They may help to explain the lack of significant change in the levels of high-energy phosphates observed in hearts with stable left or right ventricular hypertrophy (6, 13). The observed relationship between external work or pressure and the distribution of high-energy phosphates suggests that decreases in the left ventricular levels in stable ventricular hypertrophy are not likely since left ventricular external work was shown not to be significantly different from normal values (16). However, a decrease in ventricular external work associated with cardiac failure has been shown to be accompanied by a decrease in the levels of high-energy phosphates (6, 13). These findings are consistent with the observations that mitochondria isolated from ventricles in stable hypertrophy have normal oxidative phosphorylation (12), while several studies have shown that hypertrophied ventricles in prolonged and severe congestive failure have depressed mitochondrial function (15). Whether the decrease in the reserves of high-energy phosphates and alterations in pathways of energy production can themselves account for the reduced contractility in the failing heart has yet to be established. Nevertheless, the present studies indicate that cardiac muscle should not be regarded as a homogeneous unit and the study of regional energy producing and consuming reactions may lead to a better understanding of the processes involved in the hypertrophying and failing heart.

Summary. Dogs were anesthetized with sodium pentobarbital and ventilated with a respirator. Pressures were monitored in each chamber. Tissue samples from the beating heart were rapidly removed from uniform sites in the left ventricle (LV), right ventricle (RV), left atrium (LA), and right atrium (RA) and analyzed for ATP, ADP, and creatine phosphate (CP). The concentrations of ATP, ADP, and CP were distributed in the following order: LV > RV > LA > RA. This is the same order as the mean pressures developed in the various chambers, but the relationship between mean pressure and high-energy phosphate stores was not linear. The finding that ATP, ADP, and CP concentrations were highest in the LV, the chamber which performs the most work, suggests that differences in the distribution may be due to differences in the rates of production.

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