PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 158, 599–603 (1978)

Transmural Citrate Synthase and Lactate Dehydrogenase Levels in Hypertrophied Rat Left Ventricle[1] (40255)

RUSSELL T. DOWELL

Department of Physiology and Biophysics, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190

In recent years it has become apparent that inhomogenities exist or can develop between epicardial (EPI) and endocardial (ENDO) portions of the left ventricle (LV). Some investigators (1) consider blood flow to the ENDO portion of the LV to be marginally adequate under normal conditions. Biochemical studies (2) revealed a higher level of lactic acid in the normal rat ENDO while the levels of high-energy phosphate compounds (PCr and ATP) were concomitantly lower. Similar transmural gradients have been shown in the dog LV (3, 4). These gradients are accentuated when coronary perfusion pressure and blood flow are reduced (3, 4), suggesting that the ENDO is the primary site of metabolic alterations when ischemic conditions are encountered by the myocardium.

When a chronic pressure overload is imposed upon the heart, a substantial increase in myocardial tissue occurs. The additional heart tissue as well as hemodynamic factors associated with pressure overload (5) create additional energy and oxygen requirements. Under normal conditions, oxidative metabolism is preferentially utilized in the heart and elevated oxygen demands are met primarily by increasing coronary blood flow. However, recent studies have shown that total coronary blood flow (6) and flow per unit mass of tissue (7) are reduced below normal levels in hypertrophied hearts. The apparent failure to achieve adequate coronary blood flow in the hypertrophied heart presents the possibility that metabolic adaptations may be required. It seemed likely that the ENDO portion of the ventricle would be particularly vulnerable to chronic pressure overload stress. Therefore, the activity of selected enzymes associated with aerobic and anaerobic metabolism was measured in EPI and ENDO portions of LV in control animals. These enzyme activity levels were compared with those observed in the LV which had enlarged due to pressure overload.

Materials and methods. Animals and surgical procedures. Male, Sprague-Dawley rats initially weighing 250–275 g were used in these experiments. Pressure-induced LV hypertrophy was created by constricting the abdominal aorta above the renal vessels (5). Control animals received sham operations. Aortic constricted and control animals were studied 5 weeks following surgery at which time stable LV hypertrophy would be expected in aortic constricted animals.

Tissue preparation. Animals were weighed and then killed by cervical dislocation. The chest cavity was opened, the heart was rapidly excised and placed in crushed ice. Tibialis anterior (TA) muscles were dissected out and placed in ice. Atria, great vessels and the right ventricle were then carefully removed from the excised heart. The remaining LV plus interventricular septum was separated into EPI and ENDO portions by dissecting the ventricle at the mid-wall. These tissue samples were weighed separately and the combined values were taken as LV weight. TA muscles were trimmed of fat and connective tissue and weighted. EPI, ENDO and TA tissues were homogenized (5%, w/v) in 100 m*M* potassium phosphate buffer, pH 7.4, containing 5 m*M* glutathione using a ground-glass homogenizer. Homogenates were frozen and subsequently utilized for enzyme assay as described below. In a separate group of animals, isolated mitochondria were prepared from EPI and ENDO tissue. Tissue was homogenized in a solution containing 270 m*M* sucrose, 100 m*M* EDTA, 10 m*M* Tris (pH 7.4) and 10 m*M* KCl using a ground-glass homogenizer. Mitochondria were isolated by differential centrifugation, resuspended in

[1] This work was supported, in part, by NIH Grant Nos. HL16352, HL18839, and the American Heart Association, Texas Affiliate.

0037-9727/78/1584-0599$01.00/0

250 m*M* sucrose and frozen.

Assay methods. Spectrophotometric assays were conducted at 25°. Citrate synthase activity was measured in muscle homogenates and isolated mitochondria following detergent treatment with Triton X-100 to disrupt the mitochondria. Enzyme activity was assayed as described by Srere (8). Lactate dehydrogenase (LDH) activity was measured in muscle homogenates by the method of Pesce *et al.* (9). Enzyme activity was assayed at three different substrate concentrations, i.e. 0.33 m*M*, 1.0 m*M* and 10.0 m*M* pyruvate. The ratio of LDH activity at 0.33 m*M* pyruvate to the activity at 10.0 m*M* pyruvate was used as an indication of the relative amounts of the H and M isoenzyme present in tissue homogenates. All enzyme assays were conducted under conditions in which the kinetic reaction was linear and proportional to enzyme concentration. Reaction rates were corrected for any nonspecific activity. Enzyme activities in tissue homogenates are expressed as μmole/g wet tissue/min. Protein was measured in mitochondrial suspensions by the biuret method and enzyme activity expressed as μmole/mg mitochondrial protein/min.

Statistical methods. Within groups, EPI and ENDO values were compared using Student's paired *t* analysis. Comparisons between control and aortic constricted animals were made by unpaired *t* analysis. A *P* value of 0.05 or less was considered statistically significant.

Results. At the time of sacrifice, the combined control animals used in this study weighed 423 ± 7 g (Mean ± SE). Since the body weights of aortic constricted animals were similar to those of control animals (429 ± 6 g), the efficacy of 5 weeks of pressure overload in producing hypertrophy could be evaluated directly on the basis of LV weight. LV weight was 809 ± 19 mg in control animals and was significantly elevated to 1,099 ± 35 mg in aortic constricted animals, approximately a 35% increase in LV mass.

A citric acid cycle enzyme, citrate synthase, was utilized as a mitochondrial marker enzyme in these studies. An estimation of mitochondrial mass in the LV was calculated by summing the products of citrate synthase activity and tissue weight in the EPI and ENDO samples. As shown in Table I, the ENDO portion of the rat LV possesses a small (9%) but significantly lower citrate synthase activity when compared to the EPI portion in control animals. The same qualitative relationship was observed between the EPI and ENDO in aortic constricted animals; however, the magnitude of this difference (16%) was accentuated. When the individual portions of the LV were compared between control and aortic constricted animals, a significant reduction in citrate synthase activity was detected only in the ENDO portion of the LV. This alteration in enzyme activity cannot be accounted for by differences in separating LV tissue. In control animals, 62 ± 1% and 38 ± 1% of the total LV was designated EPI and ENDO, respectively. Identical values were obtained in aortic constricted animals. In addition, we have previously shown (5) no disproportionate transmural differences in heart tissue levels of protein, nucleic acids, and hydroxyproline in aortic constricted rats. Therefore, the present results would be the same whether expressed per gram tissue or normalized for protein, hydroxyproline or water content. When the total citrate synthase activity was calculated, total LV activity was significantly increased by approximately 25% in aortic constricted animals (Table I). If the citrate synthase activity per mitochondrion remains constant (an assumption dealt with below), then the total citrate synthase activity results indicate that mitochondrial mass was significantly elevated in LV of aortic constricted animals. However, it would appear that accumulated LV mass outstrips mitochondrial mass particularly in the ENDO portion of the enlarged LV.

The results from isolated mitochondria are shown in Table II. Neither mitochondrial

TABLE I. CITRATE SYNTHASE ACTIVITY IN LEFT VENTRICLES OF CONTROL AND AORTIC CONSTRICTED ANIMALS[a].

	Epicardium	Endocardium	Total LV[e]
Control (20)	122.0 ± 1.8	111.4 ± 2.6[c]	94 ± 3
AC[b] (20)	115.7 ± 3.3	97.4 ± 2.7[c,d]	118 ± 4[d]

[a] Enzyme activity is μmole/g/min. Values are mean ± SE with number of animals given in parentheses.
[b] AC = Aortic constricted.
[c] $P < 0.001$ *vs* epicardium by paired *t* analysis.
[d] $P < 0.001$ *vs* control.
[e] Total LV = summed products of citrate synthase activity and tissue weight in epicardium and endocardium.

protein yield (approximately 10 mg/g) nor mitochondrial citrate synthase activity were significantly different in the EPI and ENDO portions of LV from control animals. Although mitochondrial protein yield was reduced in the ENDO portion of LV from aortic constricted animals, this difference did not achieve statistically significant levels. Citrate synthase activity was not significantly different in mitochondria isolated from LV portions in aortic constricted animals. Therefore, the mitochondrial mass conclusions are substantiated by the isolated mitochondria results.

With the substrate (pyruvate) concentrations utilized for measuring LDH activity in the present experiments, maximum enzyme activity in LV homogenates was seen at a pyruvate concentration of 0.33 m*M* (Table III). In control animals, enzyme activity at different pyruvate concentrations as well as the 0.33/10.0 m*M* pyruvate ratio were not significantly different in the EPI and ENDO portions of the LV. Abdominal aortic constriction had no significant effect on either the LDH activity measured at three different pyruvate concentrations or the 0.33/10.0 m*M* pyruvate ratio. Because a change in LDH isoenzyme pattern would produce an altered 0.33/10.0 m*M* pyruvate ratio, the LDH results indicate that no significant alteration in isoenzyme pattern occurred in the LV which had enlarged in response to a pressure overload stimulus.

Abdominal aortic constriction would not be expected to exert a major influence on TA muscle; therefore, the citrate synthase and LDH enzyme activities in this tissue served both as an "internal control" for the LV responses and to demonstrate that metabolic discrimination can be achieved by the methods utilized in this study. As shown in Table IV, the citrate synthase activity in TA muscle homogenates is approximately sevenfold lower than that measured in the LV. In con-

TABLE II. CITRATE SYNTHASE ACTIVITY IN MITOCHONDRIA ISOLATED FROM LEFT VENTRICLES OF CONTROL AND AORTIC CONSTRICTED ANIMALS[a].

	Mitochondrial enzyme activity μmole/mg protein/min	
	Epicardium	*Endocardium*
Control (9)	1.37 ± .07	1.50 ± .12
AC[b] (6)	1.42 ± .13	1.71 ± .11

[a] Values are mean ± SE with number of animals given in parentheses.
[b] AC = Aortic constricted.

TABLE III. LACTATE DEHYDROGENASE ACTIVITY IN LEFT VENTRICLES OF CONTROL AND AORTIC CONSTRICTED ANIMALS[a] LACTATE DEHYDROGENASE.

	Pyruvate 10 m*M*	Pyruvate 1.0 m*M*	Pyruvate 0.33 m*M*	0.33 m*M* Pyruvate/10.0 m*M* Pyruvate
Control (16)				
Epi[c]	165 ± 5	482 ± 11	533 ± 13	3.24 ± .05
Endo[d]	150 ± 5	444 ± 12	498 ± 12	3.33 ± .05
AC[b] (16)	168 ± 6	480 ± 13	528 ± 14	3.17 ± .05
Epi	168 ± 6	480 ± 13	528 ± 14	3.17 ± .05
Endo	151 ± 6	439 ± 14	482± 13	3.22 ± .04

[a] Enzyme activity is μmol/g/min. Values are mean ± SE with number of animals given in parentheses.
[b] AC = Aortic constricted.
[c] Epi = Epicardium.
[d] Endo = Endocardium.

TABLE IV. CITRATE SYNTHASE AND LACTATE DEHYDROGENASE ACTIVITY IN TIBIALIS ANTERIOR MUSCLES OF CONTROL AND AORTIC CONSTRICTED ANIMALS.[a]

		Lactate Dehydrogenase			
	Citrate synthase	Pyruvate 10 m*M*	Pyruvate 1.0 m*M*	Pyruvate 0.33 m*M*	0.33 m*M* Pyruvate/10 m*M* Pyruvate
Control (10)	16.1 ± 0.8	333 ± 17	582 ± 27	485 ± 18	1.46 ± .03
AC[b] (11)	17.1 ± 0.9	338 ± 21	574 ± 28	473 ± 19	1.42 ± .04

[a] Enzyme activity is μmole/g/min. Values are mean ± SE with number of animals given in parentheses.
[b] Aortic Constriction.

trast to LV tissue, maximal LDH activity was observed at 1.0 m*M* pyruvate in TA muscle homogenates and the 0.33/10.0 m*M* pyruvate ratio was substantially lower. These results are in keeping with the known metabolic properties of rodent TA muscle which, when compared to heart tissue, possesses (a) a low aerobic metabolic capacity, (b) a much greater capacity to utilize anaerobic metabolism, and (c) a predominance of the muscle (M) isoenzyme of LDH. Citrate synthase and LDH values were nearly identical in TA muscles of control and aortic constricted animals. Therefore, the enzymatic alterations noted in the LV of aortic constricted animals is related to pressure-induced LV enlargement rather than any nonspecific response elicited by the experimental procedures utilized in these experiments.

Discussion. Considerable quantities of myocardial tissue are synthesized and accumulated during pressure-induced cardiac enlargement. If cardiac enlargement is to represent a completely effective adaptive mechanism, then all myocardial components should accumulate in proportion to the overall increase in heart mass. Myofibrillar proteins accumulate approximately in proportion to the increase in heart mass during cardiac enlargement (10). Additional quantities of ATP are required to support synthetic processes and contractile processes in the accumulated myofibrils; therefore, the relative mitochondrial mass is of particular interest due to the heart's dependence upon aerobic metabolism. During the very early stages of pressure overload (hours, days), mitochondria may be preferentially accumulated (10, 11). At later time periods (days, weeks), however, mitochondrial cytochrome content per gram tissue (11) and the relative cardiac muscle cell volume occupied by mitochondria (10) are substantially reduced. Results were obtained in the present study which confirm and extend these conclusions. LV mass was elevated by approximately 35% following 5 weeks of abdominal aortic constriction. Estimated mitochondrial mass was elevated by approximately 25% in the enlarged LV; however, accumulated myocardial tissue outstripped mitochondrial mass particularly in the ENDO portion of the ventricle. Significantly lower citrate synthase activity in the ENDO portion of the LV may be partially responsible for the lower high-energy phosphate levels in the ENDO of normal rats (2). Citrate synthase activity is further reduced in the ENDO as the result of pressure-induced cardiac enlargement. Although it is recognized that the maximum oxidative capacity of mitochondria cannot be determined by measuring citrate synthase activity, the above response indicates that a potential aerobic metabolic deficit could exist when the total tissue is considered.

The combination of reduced ENDO citrate synthase activity per gram tissue as shown in the present studies and reduced coronary blood flow described by other investigators (6, 7) presents the possibility that myocardial energy requirements may not be adequately supported by aerobic metabolism in the enlarged LV. When the energy demands placed upon the heart exceed aerobic capability, an alternative (anaerobic) source of energy production may be utilized. Tissues which are dependent upon aerobic metabolism have a greater proportion of the heart form of LDH (H-LDH) which is inhibited by low concentrations of pyruvate (9). Tissues which function well under anaerobic conditions contain a greater proportion of the muscle form of LDH (M-LDH) which maintains its enzymatic activity in the presence of high pyruvate concentrations (9). Thus, H-LDH favors the aerobic metabolism of pyruvate via the citric acid cycle while M-LDH favors anaerobic formation of lactate. Under conditions of chronic hypoxia, heart tissue has the capacity to augment its potential for utilizing anaerobic metabolism as evidenced by LDH isoenzyme alterations (12, 13). However, there was no evidence of enhanced M-LDH isoenzyme composition in ENDO tissue of control rat LV despite the possibility that this portion of the ventricle may be subjected to relative ischemic conditions. Several investigations have indicated an increased M-LDH isoenzyme composition in pressure overload hypertrophied heart tissue (14, 15). In the present studies there was no evidence of elevated LDH enzyme activity in enlarged LV when measured at three different substrate concentrations. Furthermore, the isoenzyme composition of LDH was unaltered as indicated by the 0.33/10.0 m*M* pyruvate concen-

tration ratio. The divergent LDH isoenzyme responses reported by others in enlarged hearts may be related to (a) the overload stimulus employed, (b) the stage of cardiac enlargement at which the measurements were taken, (c) the relative magnitude of the enlargement achieved, and (d) the methods used for isoenzyme evaluation.

The effectiveness of cardiac enlargement as a compensatory response must ultimately be reflected in the enlarged heart's functional characteristics. A depressed contractile state has been reported in papillary muscles from pressure overloaded cat (16) and rat (17) hearts. Clinical observations (18) in patients having enlarged but non-failing LV have associated depressed ventricular function with the hypertrophic condition. Inadequate coronary blood flow and/or cellular and subcellular lesions may contribute to depressed function in the enlarged heart; however, the present studies provide evidence that seemingly appropriate citrate synthase and LDH isoenzyme responses do not accompany pressure-induced cardiac enlargement. In view of these results, it seems likely that inadequate energy production may play a role in the development of depressed function in the heart which has enlarged in response to sustained pressure overload.

Summary. Transmural levels of citrate synthase and lactate dehydrogenase (LDH) were measured in left ventricles which had enlarged by approximately 35% due to pressure overload. Citrate synthase activity in whole homogenates and isolated mitochondria served to estimate potential oxidative capacity and mitochondrial mass. In control animals, the endocardium had a small (9%) but significantly lower citrate synthase activity when compared to the epicardium. This difference was accentuated (16%) in the enlarged ventricle due to reduced enzyme activity in the endocardium. Total citrate synthase activity was elevated approximately 25% in enlarged ventricles while enzyme activity in isolated mitochondria was not significantly altered. It appears that accumulated left ventricular mass outstrips mitochondrial mass particularly in the endocardial portion of the enlarged ventricle. LDH activity was utilized as a marker for potential anaerobic metabolic capacity. Pressure overload had no significant effect on either LDH enzyme activity measured at three different substrate concentrations or LDH isoenzyme composition. The present studies suggest that seemingly appropriate enzymatic adaptations do not accompany pressure-induced left ventricular enlargement.

1. Kirk, E. S., and Honig, C. R., Amer. J. Physiol. **207,** 661 (1964).
2. Takenaka, F., and Higuchi, M., J. Mol. Cell. Cardiol. **6,** 123 (1974).
3. Griggs, D. M., Jr., Tchokoev, V. V., and Chen, C. C., Amer. J. Physiol. **222,** 705 (1972).
4. Allison, T. B., Ramsey, C. A., and Holsinger, J. W., Jr., J. Mol. Cell. Cardiol. **9,** 837 (1977).
5. Dowell, R. T., Proc. Soc. Exp. Biol. Med. **154,** 423 (1977).
6. Malik, A. B., Abe, T., O'Kane, H., and Geha, A. S., Amer. J. Physiol. **225,** 186 (1973).
7. Gamble, W. J., Phornphutkul, C., Kumar, A. E., Sanders, G. L., Manasek, F. J., and Monroe, R. G., Amer. J. Physiol. **224,** 877 (1973).
8. Srere, P., Methods Enzymol. **13,** 3 (1969).
9. Pesce, A., McKay, R. H., Stolzenbach, F., Cahn, R. D., and Daplan, N. O., J. Biol. Chem. **239,** 1753 (1964).
10. Page, E., Polimeni, P. I., Zak, R., Earley, J., and Johnson, M., Circ. Res. **30,** 430 (1972).
11. Albin, R., Dowell, R. T., Zak, R., and Rabinowitz, M., Biochem. J. **136,** 629 (1973).
12. Penney, D., Biochem. Biophys. Acta **358,** 21 (1974).
13. Penney, D., Dunham, E., and Benjamin, M., Toxicol. Appl. Pharmacol. **28,** 493 (1974).
14. Bishop, S. P., and Altschuld, R. A., Amer. J. Physiol. **218,** 153 (1970).
15. York, J. W., Penney, D. G., Weeks, T. A., and Stagni, P. A., J. Appl. Physiol. **40,** 923 (1976).
16. Spann, J. F., Jr., Buccino, R. A., Sonnenblick, E. H., and Braunwald, E., Circ. Res. **21,** 341 (1967).
17. Bing, O. H. L., Matushita, S., Fanburg, B. L., and Levine, H. J. Circ. Res. **28,** 234 (1971).
18. Mehmel, H. C., Mazzoni, S., and Krayenbuehl, H. P., Amer. Heart J. **90,** 236 (1975).

Received February 14, 1978. P.S.E.B.M. 1978, Vol. 158.