Role of activation of myocardial branched-chain 2-oxo acid dehydrogenase complex in the regulation of leucine decarboxylation during cardiac work *in vitro*

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The oxidative decarboxylation of L-[1-¹⁴C]leucine was measured in the isolated perfused rat heart under both working and non-working conditions. Stimulation of decarboxylation of the labelled substrate was observed in working hearts as cardiac work was increased, and in Langendorff hearts upon increasing the coronary flow rate. The rate of L-[1-¹⁴C]leucine decarboxylation was significantly higher ($P < 0.05$) in hearts working against moderate afterload pressure when compared to Langendorff hearts perfused at a matching coronary flow rate. The rate of release of 4-methyl-2-oxo[1-¹⁴C]pentanoate to the perfusate was high in Langendorff hearts, and was unaffected by changes in coronary flow. In contrast, perfusate levels of ¹⁴C-labelled 4-methyl-2-oxopentanoate decreased significantly upon the establishment of the working condition ($P < 0.05$). These findings suggested an enhancement in the efficiency of the decarboxylation of the 2-oxo acid in response to cardiac work. The amount of branched-chain 2-oxo acid dehydrogenase complex present in the active form was measured in freeze-clamped hearts. Cardiac work resulted in a rapid activation of the complex ($P < 0.02$) within 5 min of work when compared to control Langendorff hearts perfused at matching coronary flow rates. To a lesser extent, increasing the coronary flow rate in Langendorff-perfused hearts also led to activation of the enzyme complex. These studies suggest the following: a) L-leucine oxidation in myocardial tissue can be accelerated by exercise as it is in other tissues; b) this regulatory response can be evoked by the contractile activity of the heart itself, independent of contributions by circulating factors or nervous stimuli; and c) regulation of the activity state of the branched-chain 2-oxo acid dehydrogenase complex is involved in the mechanism by which metabolic flux through this pathway is controlled during cardiac work.

INTRODUCTION

Recent experimental activity in the area of amino acid metabolism has brought about the realization that catabolism of protein contributes significantly to the energy required during exercise, even in dietary sufficiency. Through tracer-infusion studies and measurement of the excretion of nitrogenous compounds, it has been determined that some 5–20% of the additional energy requirement during exercise may be met through the catabolism of plasma amino acids and intracellular protein (Lemon & Mullin, 1980; Dohm *et al.*, 1982; Wolfe *et al.*, 1982; Henderson *et al.*, 1985). The branched-chain amino acids are oxidized readily in muscle, and several laboratories have reported the stimulation of whole-body leucine oxidation during exercise in rats (White & Brooks, 1981; Lemon *et al.*, 1982; Henderson *et al.*, 1985) as well as in humans (Rennie *et al.*, 1981; Hagg *et al.*, 1982; Wolfe *et al.*, 1982). In distinct contrast with such whole-body results, Morgan *et al.* (1980) found that work *in vitro* led to a decrease in the rate of protein degradation in the perfused rat heart. Factors controlling protein/amino acid metabolism in the heart during exercise may then differ from those governing these processes in skeletal muscle and other tissues.

Metabolism of the branched-chain amino acids in most mammalian tissues involves reversible transamin-

ation to the 2-oxo acid, followed by an irreversible oxidative-decarboxylation step, catalysed by the branched-chain 2-oxo acid dehydrogenase multienzyme complex. The resultant acyl-CoA derivatives are further metabolized in reactions similar to the β -oxidation of fatty acids. On the basis of measurements of the maximal activities of enzymes of the pathway in homogenates of extrahepatic tissues, it appeared that the decarboxylation step could be rate-limiting for the overall pathway (Shinnick & Harper, 1976; Odessey & Goldberg, 1979). It is clear that the branched-chain 2-oxo acid dehydrogenase complex catalyses a regulatory step. The enzyme complex is subject not only to allosteric feedback regulation (Parker & Randle, 1978a,b; Pettit *et al.*, 1978), but also to covalent interconversion between active (dephosphorylated) and inactive (phosphorylated) forms (Odessey, 1980; Parker & Randle, 1980; Hughes & Halestrap, 1981; Buxton & Olson, 1982). The protein kinase and phosphoprotein phosphatase associated with the complex also can be regulated (Lau *et al.*, 1982; Damuni *et al.*, 1984; Paxton & Harris, 1984).

Studies of the catabolism of low, physiological, concentrations of branched-chain amino acids (0.05–0.5 mM) in intact tissue preparations have indicated that transamination may be rate-limiting under some circumstances (Aftring *et al.*, 1985; Hildebrandt *et al.*, 1985). As such, careful selection of experimental conditions is

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necessary in studying the rather complex regulation of this pathway.

Treadmill exercise results in the activation of the branched-chain 2-oxo acid dehydrogenase complex in rat heart tissue as well as in skeletal muscle (Wagenmakers *et al.*, 1984; Kasperek *et al.*, 1985). In such studies it is unclear whether the effects of exercise on myocardial metabolism represent a direct response of the heart to contractile activity or whether such effects arise secondarily through neuronal and/or hormonal stimuli. Moreover, the studies employed untrained animals, raising the distinct possibility of stress involvement. Stress hormones influence the rate of leucine oxidation in the heart *in vitro* (Buse *et al.*, 1973; Hildebrandt *et al.*, 1985).

Ichihara *et al.* (1980) investigated the metabolism of leucine in the isolated perfused rat heart, which can perform pressure-volume work *in vitro*. They found that the rate of decarboxylation of leucine supplied at 1 mM or 5 mM was greater in working hearts than in hearts perfused by the Langendorff (1895) method; the mechanism of the stimulation was not determined. In the present report, we have examined the influence of cardiac work *in vitro* on the rate of oxidative decarboxylation of leucine supplied at a low, physiological, concentration (0.1 mM). Furthermore, the involvement of activation/inactivation of the branched-chain dehydrogenase complex in such work-related changes in metabolic flux was investigated. In addition, we have attempted to distinguish the effects of workload from effects related to the changes in coronary flow rate which occur spontaneously upon imposition of the workload.

MATERIALS AND METHODS

Male rats (200–300 g) (Houston Facility of Harlan Sprague-Dawley, Indianapolis, IN, U.S.A.) were pro-

Rate of metabolite formation (nmol/min per g) =

$$\left[\frac{{}^{14}\text{CO}_2 \text{ trapped (d.p.m.)}}{\text{sample size (3.5 ml)}} \times \frac{1}{\text{sp. radioactivity (d.p.m./nmol)}} - \text{blank correction (nmol/ml)} \right] \times \frac{\text{coronary flow rate (ml/min)}}{\text{heart wt. (g)}}$$

vided with autoclaved Teklad rat diet LM485 (Teklad, Winfield, IN, U.S.A.) *ad libitum* and maintained on a 07:00–21:00 light cycle for 2–4 weeks. Chow was removed from the cages 2 days before rats were killed. Rats were anaesthetized and heparinized a few minutes before thoracotomy. The hearts were excised rapidly and chilled in ice-cold iso-osmotic saline. The aorta was dissected free, then cannulated to establish non-recirculating Langendorff perfusion at a constant coronary flow rate of 16 ml/min per g heart wt. (Langendorff, 1895). Incision of the right ventricle and cannulation of the left atrium were completed during the first few minutes of perfusion. For working heart preparations, the Langendorff circuit was closed after 10 min of perfusion, and hearts were allowed to begin self-perfusion and pumping against hydrostatic pressure, in accordance with the technique of Neely *et al.* (1967). Filling pressure and afterload for each of the experimental groups are specified in Table 1. Aortic flow and coronary flow were collected for periodic measurement of flow rates, and the ratio between the two rates provided a criterion of the quality of the preparation.

In Langendorff-perfusion experiments, the same incisions and cannulations were performed and the coronary flow rate was adjusted after 10 min of perfusion as described in the Table and Figure legends. The perfusate was a modified Krebs–Henseleit (1932) buffer, containing 1.75 mM-CaCl₂, 0.5 mM-EDTA, 15 mM-D-glucose and 0.1 mM-L-leucine. The buffer was filtered through a 0.4 µm-pore-size filter and saturated with O₂/CO₂ (19:1) at 36 °C.

For measurement of metabolic flux rates, L-[1-¹⁴C]-leucine (50 Ci/mol; purchased from ICN, Irvine, CA, U.S.A.) was infused at a constant rate (about 10⁸ d.p.m./min) into the aortic cannula during Langendorff perfusion, or into the left-atrial cannula during the working-heart phase. With this design the specific radioactivity of leucine in the perfusate varied inversely with cardiac output (aortic flow rate plus coronary flow rate), but greater accuracy was obtained by measuring ¹⁴C radioactivity per ml in the coronary effluent, and dividing by the substrate concentration. Equilibration of the label between the extra- and intra-cellular pools was essentially complete within about 3 min. A second portion (3.5 ml) was acidified to release ¹⁴CO₂, which was trapped in phenethylamine (Waymack *et al.*, 1980; Buffington *et al.*, 1979). The sample was then treated with H₂O₂ to decarboxylate the radioactive 2-oxo acid quantitatively (Odessey & Goldberg, 1979; Schadevaldt *et al.*, 1984), and liberated ¹⁴CO₂ was collected in the same way. Blank corrections (in nmol/ml) were determined by measuring the amount of ¹⁴C label trapped after acidification or H₂O₂ treatment of perfusate samples obtained through the aortic cannula while buffer flowed through the Langendorff circuit at various flow rates. This molar concentration was essentially constant over the range of flow rates encountered in these experiments. Metabolic rates, expressed per g wet wt. of the well-blotted tissue, could then be calculated as follows:

These calculated rates may represent underestimates, as the specific radioactivity of the substrate was uncorrected for the mixing of [¹⁴C]leucine with endogenous pools; however, the outcome of the experiments was such that overall conclusions were unaffected (e.g., see the Results and discussion section).

To determine the amount of active branched-chain 2-oxo acid dehydrogenase complex in the cardiac tissue, hearts were freeze-clamped during perfusion, then assayed under conditions designed to block the inter-conversion between active and inactive forms. The frozen pulverized tissue was homogenized in 50 mM-potassium phosphate, pH 7.4, containing 5 mM-dithiothreitol and 1 mM-benzamidine, plus 10 mM-EDTA, 10 mM-dichloroacetate and 50 mM-NaF to inhibit kinase and phosphatase activities. The enzyme activity was assayed in 1 ml incubation mixtures containing the following components: 0.5 ml of homogenate (3–4 mg of protein), 5 mM-Mg²⁺, 0.1 % (v/v) Triton X-100, 10 mM-NAD⁺, 2 mM-thiamin pyrophosphate, 1 mM-CoA and 5 mM-4-methyl-2-oxo[1-¹⁴C]pentanoate (synthesized by the method of Rudiger *et al.*, 1972). Incubations were

Table 1. Mechanical performance of working heart preparations

Rat hearts were Langendorff-perfused for 10 min before the initiation of work, as described in the Materials and methods section. Mean values $\pm$ S.E.M. are listed.

Experimental condition ...	A	B	C
No. of hearts (n)	12	8	9
Filling pressure (cm water)	8	15	15
Afterload (cm water)	80	80	120
Coronary flow (ml/min per g)			
15 min work	22 $\pm$ 1	22 $\pm$ 1	30 $\pm$ 2
30 min work	22 $\pm$ 1	23 $\pm$ 1	32 $\pm$ 2
Aortic flow (ml/min per g)			
15 min work	46 $\pm$ 3	59 $\pm$ 3	35 $\pm$ 3
30 min work	39 $\pm$ 2	51 $\pm$ 3	27 $\pm$ 3
Cardiac output (ml/min per g)			
15 min work	68 $\pm$ 4	81 $\pm$ 3	65 $\pm$ 5
30 min work	61 $\pm$ 3	74 $\pm$ 3	59 $\pm$ 4
Flow ratio, aortic/coronary			
15 min work	2.2 $\pm$ 0.1	2.6 $\pm$ 0.1	1.1 $\pm$ 0.1
30 min work	1.8 $\pm$ 0.1	2.2 $\pm$ 0.1	0.9 $\pm$ 0.1

terminated after 5 min at 37. °C by addition of 0.5 ml of 3 % (w/v) SDS/2 M-sodium acetate, pH 3.3, and $^{14}\text{CO}_2$ was collected in phenethylamine.

Statistical comparisons employed the two-tailed Student *t* test in all cases.

RESULTS AND DISCUSSION

The pressure-volume work performed by the isolated perfused rat heart can be controlled experimentally by varying the left-atrial filling pressure, which determines the volume of fluid delivered per stroke, and the aortic afterload, or resistance pressure. Three combinations of filling pressure and afterload, designated A, B and C, were employed in the present series of experiments. The mechanical performance of perfused hearts under these work conditions is summarized in Table 1. In each of the experiments described below, standard Langendorff perfusions (group L_s) were performed, as well as Langendorff perfusions in which the coronary flow rate was adjusted at 10 min to match the coronary flow rates attained in the working heart preparation. These two flow-matched control groups are designated L_{ab} (flow raised to 22 ml/min per g) and L_c (flow raised to 31 ml/min per g). The aortic pressure was stable and proportional to the coronary flow rate in these hearts throughout the perfusion period. At the end of this period, the viability of the Langendorff preparation was demonstrated by the retention of the ability to perform work, with acceptable cardiac outputs and flow ratios as indicated in Table 1.

When L-[1- ^{14}C]leucine was provided at 0.1 mM, a concentration comparable with its plasma value in the post-absorptive state, the transamination and subsequent decarboxylation of the labelled amino acid could be assessed by measuring the appearance of 4-methyl-2-oxo[^{14}C]pentanoate and $^{14}\text{CO}_2$ in the coronary effluent (Figs. 1 and 2). During standard Langendorff perfusion a low initial rate of leucine decarboxylation was observed. With continued perfusion, the rate of decarboxylation

Fig. 1. Effect of coronary flow and cardiac work on rates of oxidative decarboxylation of L-[1- ^{14}C]leucine

Decarboxylation of L-[1- ^{14}C]leucine was calculated as described in the Materials and methods section from $^{14}\text{CO}_2$ measured in coronary effluent at various times after the change in flow rate or initiation of work; starting values (shown at 0 min) were measured before the change in perfusion. Each point represents the mean $\pm$ S.E.M. for a group of six to ten similarly treated hearts. Experimental groups are denoted as follows: working hearts perfused under conditions A ($\circ$), B ($\bullet$), or C ($\square$) as detailed in Table 1; Langendorff hearts subjected to an increase in flow rate to 22 ml/min per g ($\blacktriangle$, L_{ab}) or 31 ml/min per g ($\blacksquare$, L_c). Results obtained in standard Langendorff perfusions were indistinguishable from those of group L_{ab} , and have been omitted for clarity. *Significantly different from group L_{ab} ($P < 0.05$).

gradually increased; however, the rate of release of ^{14}C -labelled 2-oxo acid to the perfusate remained relatively high throughout the perfusion period. The results of these standard Langendorff perfusions were virtually identical with those obtained in the group- L_{ab} hearts, in which the flow rate was increased to 22 ml/min per g. On comparison of the metabolic flux data for working hearts in groups A and B with this flow-matched Langendorff control, it was found that the rate of decarboxylation of L-[1- ^{14}C]leucine was stimulated after the initiation of work, and that the degree of stimulation was dependent on the amount of work performed. Formation of $^{14}\text{CO}_2$ was significantly higher ($P < 0.01$) in the group-B hearts compared with the control group L_{ab} . In the group-A perfusion condition, an intermediate degree of stimulation was apparent at all time points, but the effect was not large enough to be considered statistically significant.

Performance of work under experimental condition C, where a greater afterload was imposed, resulted in the highest rate of $^{14}\text{CO}_2$ formation. This degree of stimulation of decarboxylation was, however, not distinguishable from that observed in the Langendorff hearts in the

Fig. 2. Effect of coronary flow and cardiac work on rates of release of 4-methyl-2-oxo[1- 14 C]pentanoate by the perfused heart

Release of 4-methyl-2-oxo[1- 14 C]pentanoate was calculated as described in the Materials and methods section from the concentrations measured in coronary effluent, in the same samples from which the data shown in Fig. 1 were obtained. The time scale, denotations of symbols, and numbers of observations are as described in the legend to Fig. 1. Results obtained in standard Langendorff perfusion were indistinguishable from those of group L_{ab} and have been omitted for clarity. *Significantly different from group L_{ab} ($P < 0.05$); †significantly different from group L_c ($P < 0.01$).

flow-matched group L_c . No change occurred in the rate of radioactive metabolite formation upon switching back to the standard Langendorff mode of perfusion after 30 min of work under experimental conditions A, B or C.

The influence of cardiac work and coronary flow rate on the release of 4-methyl-2-oxo[14 C]pentanoate from the heart was less complicated (Fig. 2). The rate of release of the 14 C-labelled 2-oxo acid to the perfusate remained high throughout the entire 30 min observation period, regardless of coronary flow, in all Langendorff perfusions, but decreased when work was initiated ($P < 0.05$). The magnitude of the effect was comparable, regardless of the filling pressure and afterload.

These metabolic rates were calculated without correction for intracellular isotopic dilution of the radioactive substrate, and as such probably represent underestimates. The changes in metabolic flux in response to work or variation of coronary flow should not be regarded as absolute values. In this regard, Morgan *et al.* (1980) observed decreased tissue L-leucine concentrations in working rat hearts, corresponding to an increase in the rate of myocardial protein synthesis and a decrease in degradation. Changes in protein metabolism leading to positive nitrogen balance also were noted in Langendorff preparations upon doubling of the coronary flow rate, although the effect did not appear until the second hour of perfusion (Gordon *et al.*, 1985). In the present study, then, it is reasonable to expect that changes in isotopic

Table 2. Effect of cardiac work and coronary flow on activity of the branched-chain 2-oxo acid dehydrogenase complex in the perfused heart

Hearts were Langendorff-perfused for 10 min before the initiation of work under experimental condition A, B or C (as detailed in Table 1), or before increasing the coronary flow rate to 22 ml/min per g (L_{ab}) or 31 ml/min per g (L_c). The hearts were freeze-clamped 5 or 20 min after the change in perfusion, and the amount of active branched-chain 2-oxo acid dehydrogenase complex present in the tissue was determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for the numbers of observations in parentheses. *Significantly different from group L_{ab} ($P < 0.02$); †significantly different from group L_c ($P < 0.02$).

Experimental group	Branched-chain 2-oxo acid dehydrogenase activity (nmol/min per g)	
	5 min	20 min
L_s	< 5	11 $\pm$ 4 (4)*†
L_{ab}	9 $\pm$ 2 (4)†	59 $\pm$ 9 (6)†
L_c	44 $\pm$ 8 (7)*	106 $\pm$ 13 (4)*
A	44 $\pm$ 10 (4)*	98 $\pm$ 9 (4)*
B	58 $\pm$ 4 (4)*	101 $\pm$ 10 (4)*
C	78 $\pm$ 7 (4)†	96 $\pm$ 7 (4)

dilution of L-leucine upon establishment of work or flow rate changes may have contributed to the observed changes in metabolism of the labelled amino acid. Because of this complication, no assertions can be made solely from the present data regarding the influence of work or flow rate on the rate of transamination of L-leucine. On the other hand, the simultaneous increase in $^{14}\text{CO}_2$ formation and decrease in 4-methyl-2-oxo[14 C]pentanoate formation upon establishment of work cannot both be explained merely in terms of a change in specific radioactivity. If the true intracellular specific radioactivity of the substrate is assumed to have remained constant or decreased, then it may be concluded that cardiac work resulted in an actual increase in the rate of 4-methyl-2-oxopentanoate decarboxylation. Conversely, if one assumes an increase in the specific radioactivity upon working, then a constant flux of the 2-oxo acid through the decarboxylation step was maintained, despite a significant decrease in its tissue concentration. The latter represents the more likely circumstance in light of the aforementioned findings by Morgan *et al.* (1980). The present experiments were conducted at low substrate concentration, where availability of the 2-oxo acid is probably rate-limiting to the decarboxylation reaction. In this situation, the maintenance of constant metabolic flux, despite a substantial decrease in the 4-methyl-2-oxopentanoate concentration, is possible only if an enhancement of the activity of the branched-chain 2-oxo acid dehydrogenase complex occurred during cardiac work. Hence, a real change in the efficiency of the decarboxylation step in response to enhanced work must be inferred, regardless of any changes in the isotopic dilution of L-leucine during the experiments.

Further evidence for this assertion was obtained in the experiment summarized in Table 2. The amount of the branched-chain 2-oxo acid dehydrogenase complex pre-

sent in the active form was assayed in hearts that had been freeze-clamped 5 or 20 min after the change in flow rate or onset of work, or at an equivalent time point in Langendorff perfusions. At all three levels of work, the amount of active complex was significantly higher ($P < 0.02$) than in the corresponding flow-matched Langendorff groups within 5 min, and the mean activity increased according to the level of work performed. The rapidity of the observed increases in assayable activity indicate conversion of inactive enzyme complex into the active form, rather than synthesis *de novo* of enzyme complex. Further activation took place in the working hearts between 5 and 20 min, at which time the amount of enzyme activity measured in the heart tissue was similar for groups A, B and C. To a lesser extent, raising the coronary flow rate of Langendorff-perfused hearts also resulted in activation of the complex. These results demonstrate that conversion of the branched-chain 2-oxo acid dehydrogenase complex into its active form is involved in the work-induced enhancement of metabolic flux through the decarboxylation reaction that was inferred from the data in Figs. 1 and 2. The present findings do not rule out the potential involvement of direct allosteric effects on the complex, which might also play a role in the regulation of the activity of the complex during cardiac work.

During Langendorff heart perfusion, entry of fluid into the ventricles does occur, with the development of left-ventricular pressure approximately equal to the aortic pressure (Neely & Rovetto, 1975). This type of work cannot be controlled readily, but will clearly increase with increasing flow rate, which is directly related to perfusion pressure. Therefore it is quite possible that the activation of the branched-chain dehydrogenase complex observed here in Langendorff hearts in response to increases in flow rate may, at least in part, represent a response to internal cardiac work. The extent to which this effect accounts for the spontaneous activation of the complex seen in standard Langendorff preparations remains to be determined (Waymack *et al.*, 1980; Buxton *et al.*, 1984).

The work-induced stimulation of the decarboxylation *in vitro* of L-[1-¹⁴C]leucine, determined in the present studies with a physiological amino acid concentration (0.1 mM), is in basic agreement with similar findings obtained by Ichihara *et al.* (1980) in experiments conducted at much higher perfusate L-leucine concentrations (1–5 mM). It has been further demonstrated here that the activation of the branched-chain 2-oxo acid dehydrogenase complex plays an important role in the regulation of L-leucine metabolism during cardiac work. The latter result can be compared with the corresponding activation of the heart complex *in vivo* seen in rigorously exercised animals (Wagenmakers *et al.*, 1984; Kasperek *et al.*, 1985). Kasperek and co-workers were able to show a rapid inactivation of the branched-chain complex in the heart after cessation of treadmill running, whereas no reversal of the effect of work was seen in the experiments reported here. It may be the case that this reversal requires the participation of an extra-myocardial component, for instance a circulating factor or neuronal stimulus.

Although the myocardial concentrations of branched-chain amino acid decrease during work (Morgan *et al.*, 1980) the results of the present experiments suggest that it should be possible for the rate of oxidative decarboxyl-

ation of L-leucine to be maintained despite the lower availability of substrate, through activation of the branched-chain complex. Under these circumstances, a shift from the metabolism of endogenous substrate to the utilization of exogenous branched-chain compounds might also be expected. Such a situation would be consistent with the overall tendency to conserve myocardial protein under stressful conditions, at the expense of the protein stored in liver and skeletal muscle.

Although heart mitochondria contain a considerable amount of branched-chain 2-oxo acid dehydrogenase complex, it is normally almost completely in the inactive form (Odessey, 1980; Waymack *et al.*, 1980; Patston *et al.*, 1984; Wagenmakers *et al.*, 1984). A variety of mediators, including fatty acids, ketone bodies, glucose and pyruvate, have been shown to influence the regulation of the complex; however, all of these effectors are inhibitory (Buse *et al.*, 1972, 1973; Buffington *et al.*, 1979; Sans *et al.*, 1980; Waymack *et al.*, 1980; Buxton *et al.*, 1984). As a result, the purpose served by maintaining this multienzyme complex in largely the inactive form has been somewhat unclear. The demonstrations of activation of the branched-chain 2-oxo acid dehydrogenase complex in heart and skeletal muscle in response to exercise (Wagenmakers *et al.*, 1984; Kasperek *et al.*, 1985), together with the results presented here, support the hypothesis that the large amount of phosphorylated complex in the heart serves as a reserve which can be activated rapidly, to meet the metabolic demands of cardiac work. An understanding of the regulation of this key step in branched-chain amino acid metabolism during contractile activity, and its relationship to other work-related changes in myocardial protein/amino acid metabolism, should be of value in determining the processes involved in the cardiac hypertrophy associated with subjection of the heart to increased pressure loads *in vivo*.

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REFERENCES

- Aftring, R. P., Manos, P. N. & Buse, M. G. (1985) *Metab. Clin. Exp.* **34**, 702–711
- Buffington, C. K., DeBuysere, M. S. & Olson, M. S. (1979) *J. Biol. Chem.* **254**, 10453–10458
- Buse, M. G., Biggers, J. F., Friderici, K. H. & Buse, J. F. (1972) *J. Biol. Chem.* **247**, 8085–8096
- Buse, M. G., Biggers, J. F., Drier, C. & Buse, J. F. (1973) *J. Biol. Chem.* **248**, 697–706
- Buxton, D. B. & Olson, M. S. (1982) *J. Biol. Chem.* **257**, 15026–15029
- Buxton, D. B., Barron, L. L., Taylor, M. K. & Olson, M. S. (1984) *Biochem. J.* **221**, 593–599
- Damuni, Z., Merryfield, M. L., Humphreys, J. S. & Reed, L. J. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4335–4338
- Dohm, G. L., Williams, R. T., Kasperek, G. J. & van Rij, A. M. (1982) *J. Appl. Physiol.* **52**, 27–33.
- Gordon, E. E., Kira, Y. & Morgan, H. E. (1985) in *Intracellular Protein Catabolism* (Khairallah, E. A., Bond, J. S. & Bird, J. W. C., eds.), pp. 521–530, Alan R. Liss, New York
- Hagg, S. A., Morse, E. L. & Adibi, S. A. (1982) *Am. J. Physiol.* **242**, E407–E410
- Henderson, S. A., Black, A. L. & Brooks, G. A. (1985) *Am. J. Physiol.* **249**, E137–E144

- Hildebrandt, E., Buxton, D. B. & Olson, M. S. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 1212 (Abstr. 4688)
- Hughes, W. A. & Halestrap, A. P. (1981) *Biochem. J.* **196**, 459–469
- Ichihara, K., Neely, J. R., Siehl, D. L. & Morgan, H. E. (1980) *Am. J. Physiol.* **239**, E430–E436
- Kasperek, G. J., Dohm, G. L. & Snider, R. D. (1985) *Am. J. Physiol.* **248**, R166–R171
- Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–36
- Langendorff, O. (1895) *Pfluegers Arch. Ges. Physiol.* **61**, 291–332
- Lau, K. S., Fatania, H. R. & Randle, P. J. (1982) *FEBS Lett.* **144**, 57–62
- Lemon, P. W. R. & Mullin, J. P. (1980) *J. Appl. Physiol.* **48**, 624–629
- Lemon, P. W. R., Nagle, F. J., Mullin, J. P. & Benevenga, N. J. (1982) *J. Appl. Physiol.* **53**, 947–954
- Morgan, H. E., Chua, B. H. L., Fuller, E. O. & Siehl, D. (1980) *Am. J. Physiol.* **238**, E431–E442
- Neely, J. R. & Rovetto, M. J. (1975) *Methods Enzymol.* **39**, 43–60
- Neely, J. R., Liebermeister, H., Battersby, E. J. & Morgan, H. E. (1967) *Am. J. Physiol.* **212**, 804–814
- Odessey, R. (1980) *Biochem. J.* **192**, 155–163
- Odessey, R. & Goldberg, A. L. (1979) *Biochem. J.* **178**, 475–489
- Parker, P. J. & Randle, P. J. (1978a) *Biochem. J.* **171**, 751–757
- Parker, P. J. & Randle, P. J. (1978b) *FEBS Lett.* **90**, 183–186
- Parker, P. J. & Randle, P. J. (1980) *FEBS Lett.* **112**, 186–190
- Patston, P. A., Espinal, J. & Randle, P. J. (1984) *Biochem. J.* **222**, 711–719
- Paxton, R. J. & Harris, R. A. (1984) *Arch. Biochem. Biophys.* **231**, 48–57
- Pettit, F. H., Yeaman, S. J. & Reed, L. J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4881–4885
- Rennie, M. J., Edwards, R. H. T., Krywawych, S., Davies, C. T. M., Halliday, D., Waterlow, J. C. & Millward, D. J. (1981) *Clin. Sci.* **61**, 627–639
- Rudiger, H. W., Langenbeck, U. & Goedde, H. W. (1972) *Biochem. J.* **126**, 445–446
- Sans, R. M., Jolly, W. W. & Harris, R. A. (1980) *Arch. Biochem. Biophys.* **200**, 336–345
- Schadewaldt, P., Oelers, R., Radeck, W. & Staib, W. (1984) *Anal. Biochem.* **143**, 308–315
- Shinnick, F. L. & Harper, A. E. (1976) *Biochim. Biophys. Acta* **437**, 477–486
- Wagenmakers, A. J. M., Schepens, J. T. G. & Veerkamp, J. H. (1984) *Biochem. J.* **223**, 815–821
- Waymack, P. P., DeBuysere, M. S. & Olson, M. S. (1980) *J. Biol. Chem.* **255**, 9773–9781
- White, T. P. & Brooks, G. A. (1981) *Am. J. Physiol.* **240**, E155–E165
- Wolfe, R. R., Goodenough, R. D., Wolfe, M. H., Royle, G. T. & Nadel, E. R. (1982) *J. Appl. Physiol.* **52**, 458–466

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