Regulation of Protein Synthesis in Heart Muscle

I. EFFECT OF AMINO ACID LEVELS ON PROTEIN SYNTHESIS*

(Received for publication, August 17, 1970)

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SUMMARY

Protein synthesis in heart muscle has been studied in the isolated rat heart perfused with buffer containing glucose and various amino acid mixtures. The rate of synthesis was evaluated by measuring incorporation of 14C-phenylalanine into whole heart protein and myosin. Protein degradation was estimated by measuring the fall in the specific activity of the free phenylalanine pool and the release of phenylalanine from the heart. 14C-Phenylalanine was suitable for these studies since it equilibrated rapidly with an intracellular pool of stable size and was not converted to other amino acids. Participation of the intracellular free amino acid pool as an intermediate in the pathway of protein synthesis was established. Synthesis of whole heart protein and myosin was increased about 40% when amino acid levels were increased from 1 to 5 times normal plasma levels. A group of 12 amino acids substituted for the complete mixture in producing this effect. Protein degradation appeared to occur at about twice the rate of protein synthesis during perfusion with normal plasma levels of amino acid. When perfusate levels of amino acid were raised, cellular levels of 12 amino acids increased. In general, intracellular amino acid levels reached stable values within 30 min. These studies indicated that the free amino acid pool was an intermediate in the pathway of protein synthesis could be stimulated by raising perfusate amino acid levels above those normally found in rat plasma. Although net uptake of isoleucine and leucine was observed, suggesting that these amino acids were used as oxidative substrate, the increased rate of synthesis appeared to be due to a direct effect on the pathway of protein synthesis rather than an indirect effect mediated by changes in energy levels of the cell.

Experimental Procedure

Perfusion of Hearts for Estimation of Amino Acid Levels and Protein Synthesis—Hearts were removed from Sprague-Dawley rats (200 to 350 g) that had been fasted overnight, heparinized, and anesthetized with Nembutal. Hearts were perfused by a modified Langendorff method as described earlier (18). A preliminary perfusion (10 min) was carried out using Krebs-Henseleit bicarbonate buffer (19) gassed with O2 : CO2 (95:5%) and containing heparin (1 mg per ml). 14C-amino acids, and glucose (15 mM). This buffer passed through the heart a single time and was discarded. Recirculation of a measured volume of buffer containing 14C-amino acids, other 14C-amino acids, and glucose followed the preliminary perfusion. Of the radioactive buffer, 10 ml usually were washed through the heart to reduce dilution of the specific activity of 14C-amino acid. Recirculation was con...
Changes in perfusate radioactivity were determined by calorimetric determination of specific radioactivity of phenylalanine (dpm/mg) - incorporation (dpm/mg).-1000

specific radioactivity of heart phenylalanine (dpm/mole)

Perfusion of Hearts for Estimation of Extracellular Space and Total Water Content—Hearts were removed and perfusion was begun as described above. Buffer used during the recirculation period contained 14C-D-sorbitol (2.5 mm) and 14C-amino acids at the concentrations normally found in rat serum, and glucose (15 mm). At the end of perfusion, hearts were rinsed in 0.15 NaCl, blotted, and frozen. 14C-Sorbitol space was determined as described for 14C-amino acid space. Total water was estimated by drying a sample of heart powder to constant weight in a vacuum desiccator (26).

Perfusion of Hearts for Estimation of ATP, Creatine-P, and Glycogen—Buffer used during the recirculation period contained glucose (15 mm) and 14C-amino acids at the concentrations indicated in Table X. Hearts were frozen with a Wollenberger clamp (23) while still being perfused. For estimation of ATP and creatine-P, heart powder (approximately 1 g) was extracted with 1 N perchloric acid. The extract was neutralized with KOH and assayed by an enzymatic method involving glucose 6-phosphate dehydrogenase, hexokinase, and creatine kinase (27). Glycogen was determined on samples of heart powder as described earlier (24).

Preparation and Amino Acid Composition of Heart Myosin—Samples of heart powder (2 to 3 g) were used for preparation of myosin by the procedure of Weeks and Hartlcy (28). When working with small amounts of protein, it was important to determine volumes of myosin pellets and solutions by weighing the tubes and beakers before and after addition of myosin. In this way, toxicity of myosin solutions could be closely controlled. The yield of rat heart myosin was 4 to 6 mg per g. Quantities of myosin were estimated by the absorption at 280 and 260 nm (29) and by the method of Lowry et al. (30). Samples of myosin were prepared for estimation of radioactivity as described above except that trichloroacetic acid was substituted for perchloric acid. Amino acid composition of myosin was estimated after hydrolysis in 6 N HCl at 105° for 24 hours. Noreleucine (1 μmole per mg of protein) was added as standard. Following hydrolysis, the sample was lyophilized and the residue dissolved in citric acid buffer (pH 2.2) for application to the column of the amino acid analyzer.

Estimation of Quantity and Radioactivity of Phenylalanine in Heart Protein—Samples of heart protein that had been prepared for estimation of radioactivity were hydrolyzed in 6 N HCl as described above. Radioactivity in the phenylalanine peak was estimated by collecting the eluate directly from the column of the amino acid analyzer into scintillation vials. One-minute samples were collected in the area of the chromatogram where phenylalanine was expected. A single peak of radioactivity appearing at the time expected for phenylalanine was found. Radioactivity in this peak was determined by summing the disintegrations appearing in each sample. The quantity of phenylalanine in heart protein (micromoles per mg) was determined by the usual amino acid analysis procedure.
Regulation of Protein Synthesis in Heart Muscle. I

Vol. 246, No. 7

RESULTS

Intracellular Amino Acid Pool as Intermediate in Protein Synthesis—Studies of protein synthesis in kidney cortex slices (15) and in skeletal muscle (16) have indicated that amino acid may go directly from the transport system in the cell membrane to protein without mixing with the intracellular pool of free amino acid. Studies in the isolated rat diaphragm (14) have suggested that the intracellular pool of free amino acid was compartmentalized and that not all of these compartments were on the synthetic pathway. Before designing experiments to study regulation of protein synthesis, the involvement of the intracellular pool in protein synthesis was evaluated. If equilibration of the intracellular pool with \(^{14}\)C-amino acid preceded incorporation into protein, a lag in the incorporation of radioactivity into protein would be expected. As seen in Fig. 1, a lag in incorporation of \(^{14}\)C-lysine and \(^{14}\)C-glycine was observed during the period when radioactivity of the intracellular water was rising. This lag was more apparent with glycine, for which the radioactivity of the intracellular water increased linearly throughout the experiment. No lag was seen with phenylalanine, for which the radioactivity of intracellular water rapidly reached a plateau.

When extracellular \(^{14}\)C-amino acid was washed from the heart after 10 min of recirculation, radioactivity of the intracellular water due to lysine and glycine was well maintained and incorporation continued at the initial rate. Phenylalanine was rapidly lost from the heart during perfusion with buffer free of amino acid and the rate of incorporation fell below that of the first 10 min. After 2 min of washout, radioactivity of the effluent buffer had fallen to less than 1% of that present during the recirculation period. A rapid washout of sorbitol from the extracellular space has been reported (26). These experiments indicated that the intracellular pools of glycine and lysine were on the pathway of protein synthesis. The intracellular pool of phenylalanine equilibrated so rapidly that its role as an intermediate in the synthetic pathway could not be evaluated. Additional experiments to determine the specific activity and size of the \(^{14}\)C-amino acid pool, conversion of the \(^{14}\)C-amino acids into other radioactive products, and the stability of the rate of protein synthesis under these conditions would be required to decide whether the glycine and lysine pools were compartmentalized.

Selection of \(^{14}\)C-Amino Acid for Estimation of Rate of Protein Synthesis—If a radioactive amino acid is to be used to investigate the rate of protein synthesis in cardiac muscle cells, it should have (a) a stable intracellular concentration, (b) a rapid rate of transport or exchange diffusion, or both, (c) no possibility of conversion to other amino acids, and (d) a measurable rate of incorporation.
into a specific muscle protein. As shown in Fig. 2, $^{14}C$-phenylalanine rapidly reached a stable distribution within the heart. This distribution was affected both by the perfusate concentration of phenylalanine and by the concentration of other amino acids. When the intracellular phenylalanine concentration was calculated from amino acid analyses to be reported later in this paper, the concentration was equal to that calculated from the distribution of $^{14}C$-phenylalanine. These results indicated that the entire phenylalanine pool rapidly equilibrated. As seen in Table I, the rate of exchange of labeled phenylalanine between the intracellular and extracellular pools was sufficiently rapid to maintain equal specific activities in heart and perfusate phenylalanine. Since these specific activities were equal, any intracellular compartments of phenylalanine also appeared to have reached the specific activity of perfusate phenylalanine. In addition, phenylalanine did not appear to be converted to other amino acids in heart muscle. Phenylalanine hydroxylase has been reported to be lacking in muscle (31) and conversion to other amino acids could not be detected by analysis of either picric acid extracts of heart or perfusate or by analysis of acid hydrolysates of heart protein. Of the radioactivity of perfusate or heart extracts, 97% could be recovered in the phenylalanine peak after 90 to 180 min of perfusion. After 3 hours of perfusion, 98% of the radioactivity incorporated into heart protein could be recovered in the phenylalanine peak. These findings, together with measurable rates of incorporation into a specific heart protein, as described later in the paper, indicated that phenylalanine satisfied the criteria for measurements of protein synthesis.

**Effect of Perfusate Amino Acid Concentration on Incorporation of $^{14}C$-Amino Acid into Heart Protein—Synthesis of whole heart protein was increased as perfusate amino acid concentration was raised (Table II). In these experiments, incorporation of

### Table I

**Equilibration of phenylalanine specific activity between perfusate and heart muscle**

Hearts were removed and perfused with various amino acid mixtures as described under "Experimental Procedure" and in Table VII. Heart and perfusate samples used for estimation of phenylalanine specific activity were the same as those used for amino acid analysis (Table VII). Three pools of three to eight hearts were analyzed for each condition. In these experiments, the heart was shifted directly from perfusate used for preliminary perfusion to 25 ml of perfusate used for recirculation. Carryover of $^{14}C$-phenylalanine into the recirculation buffer accounted for the drop in specific activity of perfusate phenylalanine between the initial and 30-min values.

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>Period of perfusion (min)</th>
<th>Specific activity of $^{14}C$-phenylalanine in heart</th>
<th>Specific activity of $^{14}C$-phenylalanine in perfusate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Perfusate</td>
<td>Heart</td>
</tr>
<tr>
<td>Only $^{14}C$-phenylalanine, 0.08 mM</td>
<td>0</td>
<td>722 ± 52</td>
<td>637 ± 52</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>613 ± 25</td>
<td>476 ± 28</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>537 ± 16</td>
<td>409 ± 22</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>455 ± 35</td>
<td>409 ± 22</td>
</tr>
<tr>
<td>All amino acids at normal plasma levels, including $^{14}C$-phenylalanine, 0.08 mM</td>
<td>0</td>
<td>646 ± 17</td>
<td>509 ± 50</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>599 ± 14</td>
<td>475 ± 27</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>547 ± 22</td>
<td>423 ± 38</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>414 ± 16</td>
<td>423 ± 38</td>
</tr>
<tr>
<td>All amino acids at 5 times normal plasma levels, including $^{14}C$-phenylalanine, 0.4 mM</td>
<td>0</td>
<td>676 ± 33</td>
<td>667 ± 35</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>616 ± 25</td>
<td>586 ± 14</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>624 ± 19</td>
<td>581 ± 27</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>596 ± 8</td>
<td>581 ± 27</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

### Table II

**Effect of perfusate amino acid concentration on incorporation of phenylalanine into heart protein**

Hearts were removed and perfused as described under "Experimental Procedure" and in Table VII. Samples used for estimation of phenylalanine incorporation were the same as those used for amino acid analysis (Table VII). The micromoles of phenylalanine incorporated per g of heart protein were calculated using the specific activities of heart phenylalanine reported in Table I. The rate of synthesis during the first 30 min was calculated using the specific activity of heart phenylalanine at 30 min. Rates of synthesis between 30 and 180 min were calculated using the average specific activity of heart phenylalanine for the period.

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>Period of perfusion (min)</th>
<th>dpm/mg protein</th>
<th>pmol/g protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Only $^{14}C$-phenylalanine, 0.08 mM</td>
<td>30</td>
<td>331 ± 15</td>
<td>0.52 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>658 ± 37</td>
<td>1.14 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>1026 ± 42</td>
<td>1.97 ± 0.06</td>
</tr>
<tr>
<td>All amino acids at normal plasma levels, including $^{14}C$-phenylalanine, 0.08 mM</td>
<td>30</td>
<td>358 ± 26</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>694 ± 31</td>
<td>1.24 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>1130 ± 37</td>
<td>2.22 ± 0.08</td>
</tr>
<tr>
<td>All amino acids at 5 times normal plasma levels, including $^{14}C$-phenylalanine, 0.4 mM</td>
<td>30</td>
<td>458 ± 42</td>
<td>0.69 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>836 ± 21</td>
<td>1.29 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>1088 ± 147</td>
<td>2.75 ± 0.23</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

$^a$ p < 0.05 versus only phenylalanine.

$^b$ p < 0.05 versus normal plasma levels.
phenylalanine into protein was calculated using the specific activity of heart phenylalanine (Table I). The effect of perfusate amino acid concentration became significant after 90 min of perfusion and was more marked after 3 hours. Since the specific activities of perfusate and heart phenylalanine did not fall significantly when 5 times the normal plasma level was present (Table I), an experimental design was adopted to maintain constant phenylalanine specific activities when normal plasma levels were added (Fig. 3). The volume of perfusate was increased to maintain the same quantity of phenylalanine in the extracellular pool with normal plasma levels as when 5 times the normal plasma level was present. An increase in protein synthesis with higher perfusate concentrations of amino acids was confirmed in these experiments. The effect was particularly apparent after 3 hours of perfusion.

The effect of a wider range of perfusate amino acid concentrations on incorporation of phenylalanine and isoleucine into heart protein indicated that the maximal effect was obtained when 5 times normal plasma levels were added (Table III). The magnitude of the increase of \(^{14}C\)-isoleucine incorporation was comparable to that found with \(^{14}C\)-phenylalanine. The effect of perfusate amino acid concentration was also examined in experiments in which the concentration of phenylalanine was maintained at normal plasma levels (Fig. 4). Incorporation of phenylalanine increased as the concentration of other amino acids was raised from 0 to 2 times normal plasma levels. When \(^{14}C\)-amino acids were added, intracellular phenylalanine concentrations fell and incorporation reached a plateau. The fall in intracellular phenylalanine concentration was presumed to be due to competitive inhibitory effect.

**Figure 3.** Effect of perfusate amino acid concentration on incorporation of phenylalanine into heart protein. Hearts were perfused as described under “Experimental Procedure” and Fig. 2. When only Phe or all amino acids at normal plasma level were added to the perfusate, 125 ml of buffer were recirculated. When 5 times normal plasma levels of all amino acids were added, 25 ml of buffer were recirculated. Ten milliliters of buffer containing radioactive amino acid were washed through the heart before recirculation was begun. Six to 25 hearts were perfused for each condition indicated. The mean ± S.E. is shown on the figure.

**Figure 4.** Effect of the perfusate concentration of other amino acids on the incorporation of phenylalanine into heart protein. Hearts were perfused as described under “Experimental Procedure.” The concentrations of various amino acids used to simulate normal plasma levels are given in Table VII. Perfusate phenylalanine concentration was 0.08 mM in all experiments. Buffer, 125 ml, was recirculated for 3 hours. Six hearts were perfused for each condition. The mean ± S.E. is shown on the figure.

**Table III**

<table>
<thead>
<tr>
<th>Amino acid mixture</th>
<th>(^{14}C)-Amino acid</th>
<th>Per fusate radioactivity</th>
<th>(^{14}C)-Amino acid incorporation</th>
<th>(^{14}C)-Amino acid space</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 min</td>
<td>60 min</td>
<td>60 min</td>
</tr>
<tr>
<td>All amino acids at normal plasma level</td>
<td>Phe</td>
<td>122 ± 2</td>
<td>110 ± 2</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>All amino acids at 2 times normal plasma levels</td>
<td>Phe</td>
<td>123 ± 1</td>
<td>108 ± 1</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>All amino acids at 5 times normal plasma levels</td>
<td>Phe</td>
<td>592 ± 12</td>
<td>572 ± 8</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>All amino acids at 10 times normal plasma levels</td>
<td>Phe</td>
<td>1104 ± 12</td>
<td>1132 ± 12</td>
<td>74 ± 3</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.
Phenylalanine 5 2.03 ± 0.06

Issue was 593 ± 10 spm per n mole.

Phenylalanine, threonine, serine, 5 2.43 ± 0.07

mental Procedure." Concentrations of various amino acids used to simulate normal plasma levels are given in Table VII. Buffer (25 ml) was recirculated for 3 hours. Radioactive buffer (10 ml) was washed through the heart before recirculation was begun. Six hearts were perfused for each condition.

Perfusate amino acid Times normal plasma level Phenylalanine incorporation µ moles/g protein

Phenylalanine 5 2.03 ± 0.06

Pheylalanine, threonine, serine, valine, methionine, isoleucine, leucine, tyrosine, histidine, proline, tryptophan, cysteine 5 2.44 ± 0.07

* Mean ± standard error of the mean.

TABLE V

Effect of perfusate amino acid concentrations on incorporation of 14C-phenylalanine into whole heart protein and myosin

Hearts were removed and perfused for 3 hours as described under "Experimental Procedure." Per fusate (125 ml) was recirculated when the normal plasma level of phenylalanine was present and 25 ml of perfusate were recirculated when 5 times the normal plasma level was added. Twelve hearts were perfused under each condition and analyzed individually for incorporation into heart protein. Pools of three hearts were used for preparation of myosin. The specific activity of perfusate phenylalanine was 593 ± 10 spm per µ mole.

ATPase activity of heart myosin averaged 0.87 ± 0.04 µ moles of P1 released per mg per min when assayed at 25° in a buffer containing 0.25 M KCl, 0.05 M Tris, 5 mM ATP, 5 mM CaCl2, pH 7.5. When CaCl2 was replaced by MgCl2, ATPase activity was 0; when CaCl2 was omitted, 0.14 ± 0.01 µ moles of P1 were released per mg per min.

Amino acid mixture 14C-Phenylalanine incorporation

Heart protein µ moles/g protein

Only 14C-phenylalanine, 0.08 mM 1.63 ± 0.06a 0.81 ± 0.04

All amino acids at normal plasma levels, including 14C-phenylalanine, 0.08 mM 1.70 ± 0.07 0.89 ± 0.02

All amino acids at 5 times normal plasma levels, including 14C-phenylalanine, 0.4 mM 2.36 ± 0.12 1.35 ± 0.09

* Mean ± standard error of the mean.

inhibition of membrane transport. As shown in Table VII, addition of 5 times normal plasma levels of phenylalanine along with 5 times normal plasma levels of other amino acids resulted in intracellular phenylalanine levels approximately 4-fold greater than shown in Fig. 4. Incorporation of phenylalanine was also 40% greater (Table II). These results suggested that intracellular phenylalanine levels restricted protein synthesis when high perfusate levels of other amino acids were added.

Myosin was prepared as described under "Experimental Procedure." Results are expressed as a ratio of various amino acids to the content of phenylalanine. Phenylalanine content of rat heart myosin was 0.23 ± 0.01 µ moles per mg. Amino acid composition of skeletal muscle myosin was taken from the data of Weeds and Hartley (28). Four preparations of rat heart myosin were analyzed.

A group of 12 amino acids was found to have the same effect on phenylalanine incorporation as the complete amino acid mixture (Table IV). Intracellular concentrations of 9 of this group were below 0.35 mM in unperfused heart (Table VII). Intracellular concentrations of proline, cystine, and tryptophan were too low for reliable measurements by the procedures that were employed.

The effect of amino acid concentration on protein synthesis in heart muscle cells was established by measuring the rate of synthesis of myosin. As shown in Table V, myosin synthesis occurred at about 50% of the rate of whole heart protein. Myosin synthesis was increased approximately 50% when perfusate amino acid levels were raised from 1 to 5 times normal plasma levels. The myosin used for these studies was characterized by possessing (a) a Ca++-activated ATPase activity (Table V) and (b) amino acid composition similar to that of skeletal muscle myosin (Table VI).

These results indicated that protein synthesis in heart muscle became dependent upon the intracellular concentrations of several amino acids during perfusion in vitro. The effect could be demonstrated by studying either synthesis of whole heart protein or myosin. The remainder of the experiments in this paper were carried out to describe (a) changes in intracellular amino acid levels when perfusate concentrations were changed, (b) uptake and release of amino acids by the heart, and (c) effects of perfusate amino acid concentrations on levels of high energy phosphates and glycogen in heart muscle.

Effect of Perfusate Amino Acid Concentrations on Intracellular Concentrations of Various Amino Acids—When the heart was perfused in vitro with normal plasma levels of amino acid, intra-
were much higher than the present values. continued to increase. These results indicated that stable intracellular levels of aspartic acid, asparagine, and glutamine and taurine decreased as perfusion was continued, whereas glycine and serine ever, intracellular levels of aspartic acid, asparagine, and glutamine were lower in the present experiments. Serine amino acids were present. The intracellular concentrations reported in this laboratory and the values reported by others (21, 32, 33). The serum levels reported in this paper were determined in intracellular concentrations of all amino acids that were measured were maintained at or above the concentrations found in vivo with the exception of glutamic acid (Table VII). Glutamic acid levels fell even when 5 times normal plasma levels of all amino acids were present. The intracellular concentrations reported in this paper generally agreed with those found by Scharff and Wool (33). The serum concentrations (0.05 mM) were added to the perfusate, but only 50% of this value was obtained by analyzing the perfusion medium. Oxidation of methionine may have accounted for this loss. In addition to the amino acids that are reported, tryptophan (0.05 mMr) was included in the normal amino acid mixtures. Perfusate (25 ml) was recirculated in each case. In addition to the 90-min values, intracellular concentrations were measured after 30 and 180 min. After 30 min of perfusion, the following concentrations (10% mM) were significantly different from the 90-min value: (a) aspartic acid, 5 X, 348 ± 38; (b) asparagine, 1 X, 121 ± 22; (c) glutamic acid, PHE, 570 ± 43; (d) glutamic acid, 1 X, 784 ± 16; (e) glutamic acid, 5 X, 1106 ± 97; (f) serine, 5 X, 39 ± 5; (g) glycine, PHE, 64 ± 2; (h) glycine, 1 X, 77 ± 6; (i) value, 5 X, 21 ± 3. After 180 min of perfusion, the following concentrations (10% mM) were significantly different from the 90-min value: (a) aspartic acid, PHE, 51 ± 7; (b) aspartic acid, 1 X, 77 ± 20; (c) glutamic acid, PHE, 487 ± 1; (d) glutamine, PHE, 770 ± 61; (e) leucine, 5 X, 12 ± 4.

Intracellular amino acid concentrations of unperfused hearts were calculated assuming that extracellular volume was 22 ml/100 g and the intracellular volume was 56 ml/100 g (33). Extracellular water of perfused hearts was estimated by measuring sorbitol space during periods ranging from 10 to 180 min. Sorbitol space was 56 ml/100 g at all time periods. Total water content of these hearts was 80 ml/100 g at all time periods. Five pools of six hearts were analyzed to obtain serum and levels in vitro. Three pools of heart and perfusion medium were analyzed to obtain levels in vitro. When only phenylalanine was added to the buffer, eight hearts were included in the pool. When normal serum levels of amino acid were present, six hearts were pooled, and when 5 times normal levels were added, three hearts were pooled. These quantities of heart muscle were needed to obtain reliable analyses.
The absolute rate of proteolysis would have been about 1.1 mg of heart protein per g per hour. Release of tyrosine, methionine, and arginine occurred at about the same rate as phenylalanine.

Since whole heart protein contains about 0.28 ± 0.02 pmol of phenylalanine was not produced or degraded in heart muscle, release could be equivalent to 0.4 mg of heart protein per g per hour. The net degradation of 0.7 mg of heart protein per g per hour would have been required to account for the phenylalanine that was released. Incorporation of [14C]-phenylalanine into the protein of 1 g of heart (160 mg) amounted to about 0.12 pmol per mg, net degradation of 0.7 mg of heart protein per g would have been equivalent to 0.4 mg of heart protein per g per hour. The absolute rate of proteolysis would have been about 1.1 mg of heart protein per g per hour. Release of tyrosine, methionine, and arginine occurred at about the same rate as phenylalanine.

Incorporation of [14C]-phenylalanine into the protein of 1 g of heart (160 mg) amounted to about 0.12 pmol of phenylalanine per g per hour (Table II). This would be equivalent to 0.4 mg of heart protein per g per hour. The absolute rate of proteolysis would have been about 1.1 mg of heart protein per g per hour. Release of tyrosine, methionine, and arginine occurred at about the same rate as phenylalanine.

Efflux of amino acids from the cell was estimated in experiments in which only phenylalanine was added to the perfusate (Table IX). Since efflux of amino acid appears to be a non-saturable process (34), rates were expressed as micromoles transferred per mmole of concentration gradient. Rates of efflux varied as much as 150-fold among the amino acids. The lowest permeabilities were observed with aspartic and glutamic acid. Permeability of the neutral amino acids, threonine, serine, glycine, and alanine, was about 0.7 in each case. These amino acids...
Table X

Effect of amino acid concentration on heart content of ATP, creatine-P, and glycogen

Hearts were perfused as described under “Experimental Procedure.” The buffer used for the preliminary perfusion of 10 min contained neither glucose nor amino acids. Buffer used for the recirculation period lasting either 30 or 180 min contained 15 mM glucose in addition to the amino acids listed above. Results are expressed per g of dry heart weight. The number of hearts that were perfused is indicated by figure in parentheses.

<table>
<thead>
<tr>
<th>Amino acid level</th>
<th>Period of perfusion</th>
<th>Tissue content</th>
<th>ATP</th>
<th>Creatine-P</th>
<th>Glycogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal plasma</td>
<td>min (g)</td>
<td>μmoles/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 (9)</td>
<td>24.9 ± 1.4</td>
<td>23.3 ± 1.8</td>
<td>120.3 ± 3.9</td>
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<td></td>
</tr>
<tr>
<td>40 (8)</td>
<td>24.0 ± 1.0</td>
<td>19.9 ± 0.9</td>
<td>97.5 ± 4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>190 (7)</td>
<td>20.4 ± 1.4</td>
<td>18.1 ± 1.6</td>
<td>46.7 ± 4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Times normal</td>
<td>plasma levels</td>
<td>μmoles/g</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 (5)</td>
<td>24.1 ± 1.0</td>
<td>21.5 ± 1.0</td>
<td>96.0 ± 6.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>190 (6)</td>
<td>19.3 ± 1.8</td>
<td>22.3 ± 2.3</td>
<td>96.7 ± 10.3</td>
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* Mean ± standard error of the mean.
  † p < 0.01 versus normal plasma level.

have been reported to share a common transport system (34). The highest permeabilities were encountered with isoleucine, leucine, and histidine. These amino acids also have been reported to share a common transport system (34, 35). In other experiments in heart muscle, rapid equilibration of leucine and isoleucine across the cell membrane has been observed. Permeabilities of lysine and arginine that share the cationic amino acid transport system (36, 37) were low.

Effect of Perfusate Amino Acid on Heart Content of ATP, Creatine-P, and Glycogen—If higher perfusate levels were increasing protein synthesis by providing alternative substrates to the heart, higher levels of ATP and creatine-P might result. As seen in Table X, ATP levels fell about 20% during 3 hours of perfusion in vitro with buffer containing normal plasma amino acid levels. This change was not affected by inclusion of higher levels of amino acid in the perfusate. Creatine-P was unchanged as perfusion was continued with either mixture of amino acid. Sparing of glycogen was demonstrable when 5 times normal plasma levels of amino acid were added. This effect was apparent only after 3 hours of perfusion. Sparing of glycogen by the inclusion of fatty substrates in the buffer has been noted in earlier experiments (38). Since branched chain amino acids are metabolized by formation of fatty-CoA derivatives, uptake and oxidation of these compounds may have accounted for glycogen sparing.

Discussion

Protein synthesis in muscle is regulated by a variety of factors including insulin (39, 40), growth hormone (1, 3, 41, 42), thyroxine (42), steroids (43, 44), anoxia (45), and the mechanical activity of the tissue (46–48). The mechanisms of these effects are incompletely understood at present. The perfused rat heart appears to be a suitable preparation for a study of these problems. The preparations have a stable mechanical performance for periods in excess of 3 hours (18). The level of ventricular pressure development can be closely controlled during perfusion (18). Hormones and substrates reach the cells via the normal capillary bed. The rat heart is sufficiently large to provide adequate material for analysis of free amino acid levels and other metabolites involved in protein synthesis and for preparation of ribosomes and myosin. When compared with the perfused liver, the heart presents a simpler model for studies of mammalian protein synthesis because of slower rates of amino acid oxidation and the absence of conversion of amino acids into glucose. As a result, stable intracellular amino acid levels are much easier to maintain in heart than liver.

Myosin appears to be a suitable protein for investigation of regulation of protein synthesis in cardiac muscle cells. The heart contains approximately 35 μg of myosin per g (49). The yield of purified protein was about 1 to 6 μg per g. Rat heart myosin was found to have a similar amino acid composition to rabbit skeletal muscle myosin. Bárány et al. (50) found less valine and arginine and more leucine and lysine in myosin of rabbit heart than in myosin of white skeletal muscle. The adenosine triphosphatase activity of rat heart myosin was greater than that reported earlier (51) using myosin prepared from dog heart. We have not determined whether this difference is due to species difference or to the method of preparation. In the present studies, myosin was extracted from hearts that had been pulverized in a percussion mortar at liquid nitrogen temperatures. The preparative procedure was completed within 30 hours of the initial extraction and ATPase activity was assayed immediately.

Synthesis of whole heart protein and myosin can be estimated using the initial rates found during perfusion in vitro. Whole heart protein contains 284 ± 17 μmoles of phenylalanine per g. Since 1.24 μmoles of phenylalanine per g were incorporated in 90 min (Table II), an average of 14.3 days would be required to synthesize the protein in rat heart. Myosin contained 230 μmoles of phenylalanine per g. Since 1.35 μmoles per g were incorporated in 3 hours of perfusion (Table V), 21.3 days would be required to synthesize the myosin found in rat heart. This rate of synthesis may be lower than that found in vitro since synthesis of whole heart protein fell off during this period of perfusion. Myosin has a molecular weight of approximately 500,000 and consists of 2 large subunits with a molecular weight of about 200,000 and 1 or 2 small subunits with a molecular weight of about 20,000 (49). The rate of synthesis estimated in the present experiments was for the whole molecule. In other experiments in which changes in ATPase activity have been measured using myosin prepared from hearts of hypophysectomized rats, ATPase activity was reduced to about 50% of normal 8 days after hypophysectomy (52). This reduction could be due to a change in the small subunits and might suggest that these subunits have a more rapid rate of turnover than the large subunits.

Degradation of heart protein can be estimated from the fall in specific activity of perfusate phenylalanine or from production of phenylalanine by the heart. As discussed earlier, release of phenylalanine into the perfusate suggested that the net rate of protein degradation was 0.7 mg per g of heart per hour. The absolute rate of protein degradation, obtained by the addition of the net rate and the rate of protein synthesis, was about 1.1 mg per g per hour. The fall in specific activity of phenylalanine after 3 hours of perfusion with normal plasma levels of amino acid (Table I) indicated that 0.38 μmoles of phenylalanine had been added for each micromole that was present after 30 min. A total of 2.16 μmoles of free phenylalanine were present in perfusate and heart after 30 min of perfusion. Fall in specific activity of phenylalanine indicated that 2.97 μmoles would have
been present after 3 hours of perfusion if none had been reincorporated into protein. The absolute rate of protein degradation estimated by dilution of phenylalanine specific activity was 1.2 mg per g per hour, in good agreement with the rate estimated from phenylalanine release. Actually found were 2.57 μmol (Table VII), indicating that 0.4 μmol had been reincorporated. The net rate of protein degradation estimated by this method was about 0.6 mg per g of heart per hour. Since the heart contains about 160 mg of protein per g, an average of 6 days would be required to degrade heart protein at the rate observed in vitro. Since an average of 14 days was required to synthesize heart protein, whereas only 6 days were necessary for degradation, perfusion in vitro appeared to have accelerated degradation or to have inhibited protein synthesis. Increased rates of protein synthesis have been observed to reduce the rate (53).

During perfusion of the heart with buffer containing glucose, the rate of protein synthesis was increased about 40% when 5 times normal plasma levels of all amino acids were added as compared with normal plasma levels. With either amino acid mixture, the rate of synthesis decreased during the 2nd and 3rd hours of perfusion to about 60% of the rate observed in the 1st hour. The reduced rates of synthesis observed with these amino acid mixtures were not due to reductions in intracellular amino acid concentrations (Table VII). As shown in the following paper (54), reduced rates of protein synthesis observed in hearts perfused with buffer containing glucose and amino acids were associated with increased numbers of ribosomal subunits and decreased polysomes. When 5 times normal plasma levels of all amino acids were added, smaller numbers of ribosomal subunits and more polysomes were found. A similar effect of amino acid supply on breakdown and reaggregation of liver ribosomes has been found in vitro (55), in the perfused liver (56), and in a cell-free system (57). The effect of amino acid supply on ribosomal aggregation in heart muscle together with comparable ATP and creatine-P levels in hearts perfused with 1 and 5 times normal plasma amino acid levels suggested that higher amino acid levels stimulated protein synthesis directly rather than indirectly by changing the levels of high energy phosphates. The importance of changes in intracellular amino acid levels in accounting for the effects of various factors regulating protein synthesis will be discussed in subsequent papers.

REFERENCES

Regulation of Protein Synthesis in Heart Muscle: I. EFFECT OF AMINO ACID LEVELS ON PROTEIN SYNTHESIS


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