Rates of protein turnover in vivo and in vitro in ventricular muscle of hearts from fed and starved rats

Victor R. PREEDY, David M. SMITH, Nessa F. KEARNEY and Peter H. SUGDEN
Department of Cardiac Medicine, Cardiothoracic Institute, 2 Beaumont Street, London W1N 2DX, U.K.

(Received 28 February 1984/Accepted 24 May 1984)

Starvation of 300g rats for 3 days decreased ventricular-muscle total protein content and total RNA content by 15 and 22% respectively. Loss of body weight was about 15%. In glucose-perfused working rat hearts in vitro, 3 days of starvation inhibited rates of protein synthesis in ventricles by about 40–50% compared with fed controls. Although the RNA/protein ratio was decreased by about 10%, the major effect of starvation was to decrease the efficiency of protein synthesis (rate of protein synthesis relative to RNA). Insulin stimulated protein synthesis in ventricles of perfused hearts from fed rats by increasing the efficiency of protein synthesis. In vivo, protein-synthesis rates and efficiencies in ventricles from 3-day-starved rats were decreased by about 40% compared with fed controls. Protein-synthesis rates and efficiencies in ventricles from fed rats in vivo were similar to values in vitro when insulin was present in perfusates. In vivo, starvation increased the rate of protein degradation, but decreased it in the glucose-perfused heart in vitro. This contradiction can be rationalized when the effects of insulin are considered. Rates of protein degradation are similar in hearts of fed animals in vivo and in glucose/insulin-perfused hearts. Degradation rates are similar in hearts of starved animals in vivo and in hearts perfused with glucose alone. We conclude that the rates of protein turnover in the anterogradely perfused rat heart in vitro closely approximate to the rates in vivo in absolute terms, and that the effects of starvation in vivo are mirrored in vitro.

The perfused rat heart in vitro has been used extensively in studies of protein synthesis and degradation. It is not known how closely rates of these processes in vitro match those in vivo. It is important before extrapolating from results obtained in vitro to the heart in vivo that rates of protein turnover should be comparable. Studies on other tissues have given conflicting results. In rabbit forelimb muscles, protein-synthesis rates in vitro were 22–38% of rates in vivo, depending on whether muscles were stretched (Palmer et al., 1981). In rat soleus muscle, synthesis rates in vitro were only 50–70% of those in vivo, whereas degradation rates in vitro were 16–112% greater than those in vivo, depending on pretreatment (overloading, denervation) of the muscle in vivo (Goldspink et al., 1983). The preparation in vitro was always in negative nitrogen balance, a situation common in preparations in vitro. Studies in two other systems have shown increases in protein synthesis in vitro. In the perfused hemi-corpus, protein-synthesis rates in the soleus were elevated as much as 2-fold over those in vivo, but in the gastrocnemius, rates of protein synthesis were similar in vivo and in vitro (Preedy & Garlick, 1983). In rabbit alveolar macrophages protein synthesis was 5 times more rapid in vitro than in vivo (Oliver et al., 1984).

It is also important to know to what extent interventions in vivo are reflected in vitro, and vice versa. There has been considerable confusion concerning the effects of starvation on cardiac protein synthesis. In vivo, some experiments have suggested that cardiac protein is relatively slowly lost after 2 days of starvation compared with, for example, liver (Garlick et al., 1975; Goodman & Ruderman, 1980), whereas others indicated a large loss of cardiac mass on more prolonged starvation (Li & Goldberg, 1976; Griffin & Wildenthal, 1978). In vivo, 2 days of starvation decreased

Abbreviations used: $k_s$, fractional rate of protein synthesis; $k_d$, fractional rate of protein degradation; $S_t$, tissue specific radioactivity of free [4-3H]phenylalanine.
protein synthesis by about 50% (Garlick et al., 1975) in rats of about 100 g body wt. However, in perfused hearts taken from 250–350 g rats, protein-synthesis rates were depressed by only 16% compared with fed rats, whereas in skeletal muscle (gastrocnemius) rates were decreased by 46% (Rannels et al., 1978). Inhibition of heart protein synthesis was entirely attributable to loss of RNA (Rannels et al., 1978). Some of the confusion may have resulted because different workers have used rats of different ages, periods of starvation have varied and animal groups may not have been accurately weight-matched. Thus there has been no single study on loss of heart protein on starvation and protein turnover rates in vivo and in vitro.

**Experimental**

**Materials**

Radiochemicals were from Amersham International, Amersham, Bucks., U.K. Chemicals were from BDH Chemicals, Dagenham, Essex, U.K. Biochemicals were from Sigma Chemical Co., Poole, Dorset, U.K., except for tRNA, which was from BCL, Lewes, East Sussex, U.K. Novo Industries Actrapid mono-component pig insulin (100 units/ml) was from the National Heart Hospital pharmacy.

**Animals**

Male Sprague-Dawley rats were from Bantin and Kingman, Hull, Humberside, U.K. They were kept in the animal house at 22–24°C for at least 1 week before use, during which time they had free access to water and food (Diet 86A; E. Dixon and Sons, Ware, Herts., U.K.). Animal-house lighting was set for a 12h-light/12h-dark cycle, with the light phase starting at 08:00h. All rats used in this study were about 300 g body wt. (see the Table legends for precise weights). Rats were starved in grid-bottomed cages.

**Measurement of protein synthesis in vitro and in vivo**

For measurements in vitro, hearts were perfused as anterograde (working) preparations by the method of Taegtmeyer et al. (1980) as described in detail previously (Sugden & Smith, 1982b). Hearts were initially perfused retrogradely at 10 kPa pressure as non-recirculating preparations with Krebs & Henseleit (1932) bicarbonate-buffered saline containing 5 mM-glucose and equilibrated with O₂/CO₂ (19:1). After cannulation of the left atrium (which was done as quickly as possible), the preparation was switched to an anterogradely perfused preparation in which medium was recirculated. The filling pressure was 0.5 kPa and the aortic pressure was 7 kPa. The medium was the same as in the retrograde perfusion, but contained additionally 0.4 mM-[4-³H]phenylalanine (sp. radioactivity 0.75 Ci/mol) and the remaining plasma amino acids each at a concentration of 0.2 mM. Insulin, when present, was added to media used for both the retrograde and anterograde perfusions at a concentration of 50 munits/ml (0.35 μM). After 2 h of perfusion (or earlier when the time course of protein synthesis was studied), hearts were removed from cannulae, dissected, frozen in liquid N₂ and stored at −80°C. The kₙ values were determined as in Garlick et al. (1980), with HClO₄ (final concn. 0.625 M) as the protein precipitant. Hearts from fed animals were perfused in the morning starting at 09:30h, and from starved animals in the afternoon starting at 14:00h. Preliminary work had established, however, that there was no significant difference between protein-synthesis rates of hearts from fed animals perfused in the morning or afternoon.

For measurements of protein synthesis in vivo, the 'flooding dose' method of Garlick et al. (1980) was used. [4-³H]Phenylalanine (150 mM; sp. radioactivity 0.7 Ci/mol) was administered via a lateral tail vein at a dose of 1 ml/100 g body wt. Injections of fed animals were started at 08:45h and finished by 10:00h. Starved animals were injected subsequently, finishing by 11:00h. Animals were beheaded at 2 or 10 min after injection, blood was collected and hearts were removed into an ice/water mixture as quickly as possible (about 45 s after decapitation) for subsequent dissection. Ventricles were frozen in liquid N₂ and stored at −80°C. The kₙ values were determined as in Garlick et al. (1980). Plasma specific radioactivities were also measured, and in all cases Sₛ values were equilibrated with plasma specific radioactivities at 2 and 10 min.

For experiments in vitro only, kₙ was also calculated from the incorporation of phenylalanine in terms of pmol/mg of protein determined as in Smith & Sugden (1983b, c) and the phenylalanine content (0.276 ± 0.020 μmol/mg of protein; 19 observations) of protein [purified as in Smith & Sugden (1983b)] hydrolysed in 6 M HCl for 48 h (which produced maximal phenylalanine release). Perfusate [4-³H]phenylalanine specific radioactivity was measured as in Smith & Sugden (1983b, c). In all these determinations phenylalanine was assayed as in Rubin & Goldstein (1970) as modified by Sugden & Smith (1982a).

**Measurement of protein degradation in vitro and in vivo**

Hearts were perfused essentially as in protein-synthesis experiments, except that amino acids were omitted from perfusates and 0.02 mM-cycloheximide was included. Methodology has been described in detail previously (Sugden & Smith, 1984).
of release of phenylalanine into the perfusate (in units of pmol/h per g dry wt.), the heart protein/dry wt. ratio (0.8) and the phenylalanine content of heart protein. In vivo, \( k_d \) values were calculated from the difference between \( k_d \) and the fractional rate of protein deposition.

**Other methods**

RNA was measured by the method of Munro & Fleck (1969) and protein by the method of Gornall et al. (1949), by using the standardization procedure described by Smith & Sugden (1983b).

**Statistical methods**

Results are expressed as means ± S.E.M. with the numbers of observations in parentheses where applicable. Statistical significance was determined by a two-tailed unpaired Student’s t test. Values of \( P < 0.05 \) were taken as being statistically significant.

**Results**

**Effects of starvation on ventricular weight, protein content and RNA content**

After 3 days of starvation, rats had lost 22\% of their body weight and 19\% of their ventricular wet weight (Table 1). Cardiac protein concentration did not alter significantly. The decrease in total ventricular protein content was less than the decrease in body weight, resulting in a significant increase in the ventricular-protein-content/body-weight ratio. Thus the heart loses protein less rapidly than the whole body loses weight during starvation. Starvation decreased the ventricular RNA/protein ratio and RNA content, as shown previously (Garlick et al., 1975; Rannels et al., 1978; Goodman & Ruderman, 1980). From these results, starvation should cause alterations in rates of both protein and RNA turnover.

**Rates of protein synthesis in vitro and in vivo**

Rates of protein synthesis in ventricles of hearts perfused in vitro from fed or 3-day-starved rats were linear with time for at least 2 h of perfusion (results not shown). After 2 h of perfusion, free tissue [4\(^{-}\)H]phenylalanine specific radioactivities (measured as in Garlick et al., 1980) were 97\% of perfuse values (measured as in Smith & Sugden, 1983b,c). Effects of starvation and insulin on ventricular protein-synthesis rates in vitro are shown in Table 2(a). Measurement of \( k_s \) in the same ventricles by the method of Smith & Sugden (1983b,c) and protein phenylalanine content (see the Experimental section) gave values of 5.28 ± 0.22%/day for fed rats, 2.81 ± 0.19%/day for starved rats and 8.04 ± 0.43%/day for perfusions in the presence of insulin (\( P < 0.001 \) for fed group versus starved or insulin-perfused groups), confirming that the experimentally determined value for the phenylalanine content of ventricular protein is correct. Insulin (50 munits/ml) stimulated protein synthesis in vitro by 52–70\%, whereas starvation decreased it by 38–47\%. Although a decrease in the RNA/protein ratio (i.e. a loss of protein-synthesis capacity) caused a 15\% inhibition of protein synthesis during starvation, 63\% of the inhibition was attributable to a decrease in efficiency of protein synthesis. Rannels et al. (1978) reported that the 16\% inhibition of cardiac protein synthesis induced by 2 days of starvation was wholly attributable to a decrease in the RNA/protein ratio and not to any change in the efficiency of protein synthesis. Our results contradict the conclusions of Rannels et al. (1978). Variations in experimental design or technique could have caused these differences. Rannels et al. (1978) perfused hearts retrogradely with 15mM-glucose, apparently in the absence of insulin, whereas we perfused anterogradely with 5mM-glucose. Rat weights were similar. We used rats starved for 3 days rather than 2 days, although we have shown that the efficiency of ventricular

---

**Table 1. Body weights, ventricular weights, protein and RNA contents of fed and 3-day-starved rats**

<table>
<thead>
<tr>
<th></th>
<th>Fed</th>
<th>3-day-starved</th>
<th>Change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body wt. (g)</td>
<td>—</td>
<td>283 ± 3</td>
<td>—</td>
</tr>
<tr>
<td>Body wt. on decapitation (g)</td>
<td>286 ± 2</td>
<td>222 ± 3**</td>
<td>−22</td>
</tr>
<tr>
<td>Ventricular wet wt. (g)</td>
<td>893 ± 14</td>
<td>723 ± 19**</td>
<td>−19</td>
</tr>
<tr>
<td>Total ventricular protein content (mg)</td>
<td>142 ± 2</td>
<td>120 ± 4**</td>
<td>−15</td>
</tr>
<tr>
<td>Total ventricular protein content/body wt. (mg/g)</td>
<td>0.497 ± 0.008</td>
<td>0.544 ± 0.016*</td>
<td>+9</td>
</tr>
<tr>
<td>Ventricular RNA/protein ratio (µg/µg)</td>
<td>6.85 ± 0.13</td>
<td>5.86 ± 0.16**</td>
<td>−15</td>
</tr>
<tr>
<td>Total ventricular RNA content (µg)</td>
<td>975 ± 25</td>
<td>705 ± 13**</td>
<td>−28</td>
</tr>
</tbody>
</table>
Table 2. Ventricular protein-synthesis rates in vitro and in vivo

<table>
<thead>
<tr>
<th></th>
<th>$S_i$ (d.p.m./nmol)</th>
<th>$k_s$ (%/day)</th>
<th>RNA/protein ratio (µg/mg)</th>
<th>Efficiency of protein synthesis (g of protein synthesized/day per g of RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) In vitro</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>1458 ± 11</td>
<td>5.05 ± 0.41</td>
<td>6.81 ± 0.22</td>
<td>7.48 ± 0.67</td>
</tr>
<tr>
<td>3-day-starved</td>
<td>1408 ± 13</td>
<td>3.12 ± 0.26**</td>
<td>5.86 ± 0.16*</td>
<td>5.32 ± 0.41*</td>
</tr>
<tr>
<td>Fed + insulin</td>
<td>1428 ± 8</td>
<td>8.56 ± 1.27**</td>
<td>6.92 ± 0.06</td>
<td>12.33 ± 1.88*</td>
</tr>
<tr>
<td>(b) In vivo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed</td>
<td>1358 ± 25</td>
<td>8.95 ± 0.74</td>
<td>6.23 ± 0.20</td>
<td>14.54 ± 1.01</td>
</tr>
<tr>
<td>Starved</td>
<td>1487 ± 6</td>
<td>5.35 ± 0.76**</td>
<td>5.84 ± 0.17</td>
<td>8.93 ± 1.33**</td>
</tr>
</tbody>
</table>

Protein synthesis is decreased in vitro by 43% in 2-day-starved rats (D. M. Smith & P. H. Sugden, unpublished work). We suggest that cardiac protein synthesis is much more sensitive to starvation than was suggested by Rannells et al. (1978).

In vivo, preliminary experiments established that there was only a 3-5% decline in plasma [4-3H]phenylalanine specific radioactivity between 2 and 10 min after injection of radiolabel. The mean decrease in ventricular free [4-3H]phenylalanine specific radioactivity between 2 and 10 min was 5.0 ± 1.4% (14 observations). Starvation for 3 days inhibited protein synthesis (Table 2b). In vivo, efficiencies of protein synthesis were significantly decreased by starvation. We conclude that cardiac protein synthesis is inhibited by 3 days of starvation, as was suggested by experiments in vitro (Table 2a).

Rates of protein synthesis and the effects of starvation in vitro and in vivo may be compared. The $k_s$ values in ventricles of rats perfused in vitro with media containing insulin were 96% of those for fed rats in vivo. The efficiency of protein synthesis in vitro was 85% of values in vivo, reflecting the contribution of blood to the total heart protein in vivo. Rates of protein synthesis in the perfused heart are thus very similar to rates in vivo. The comparison between insulin-perfused hearts and hearts from fed rats in vivo is justified, since in these post-prandial rats insulin concentrations are likely to be high. Infusion of insulin into fed rats in vivo to very high plasma concentrations (>2500 µunits/ml) did not stimulate skeletal muscle protein synthesis, although much lower concentrations (about 70 µunits/ml) stimulated protein synthesis in post-absorptive animals (Garlick et al., 1983). To our knowledge, analogous experiments have not been reported for the heart. Goldspink et al. (1983) also included insulin in media for their experiments in vitro. The $k_s$ values and efficiencies of protein synthesis in vivo in ventricles of 3-day-starved rats were similar to rates in vitro for glucose-perfused hearts taken from fed animals, and were greater ($P<0.05$) than rates in vitro for glucose-perfused hearts taken from 3-day-starved animals. This finding may indicate the importance of non-carbohydrate fuels (Rannells et al., 1974) or other factors in the partial maintenance of cardiac protein-synthesis rates during starvation. These results also show a good qualitative correlation of the effects of starvation between experiments in vitro and in vivo.

Rates of protein degradation in vivo and in vitro

In the rats used, body weight increased by 2.5%/day for the week before experimentation. Because rats of 180–350 g body wt. have a constant ventricular-wet-weight/body-weight ratio and ventricular protein concentration (P. H. Sugden & D. M. Smith, unpublished work), the $k_d$ value in fed rats in vivo is 6.45%/day. These calculations may not be entirely reliable, since $k_d$ was measured over 10 min, and thus does not allow for variations occurring during the day. During starvation, the situation is more complex, since rates of protein synthesis and degradation are not in a steady state. From Tables 1 and 2, the average $k_d$ value is 10.35%/day over the 3 days of starvation. Starvation thus increases cardiac protein degradation in vivo. However, for glucose-perfused hearts taken from fed and 2- and 3-day-starved rats (initial body wt. about 300 g), $k_d$ values were 17.9 ± 0.7 (5), 15.5 ± 0.3 (7) and 13.1 ± 0.6 (7)%/day respectively.
Rat heart protein turnover in vivo and in vitro

(P < 0.01 for starved versus fed animals). Crie et al. (1980) and Curfman et al. (1980) have also shown inhibition of cardiac protein degradation in vitro by starvation. This discrepancy can be explained since the inhibition caused by starvation is not as great as that produced by saturating insulin concentrations at physiological work-loads, when the rate of protein degradation can be calculated to be 7.7 ± 0.4%/day (Sugden & Smith, 1982a; Smith & Sugden, 1983a). Cardiac protein-degradation rates in vivo during starvation could thus still be increased above those for fed rats, even though a stable inhibition of protein degradation by starvation is observed in vitro.

Discussion

General methodological considerations

The flooding-dose technique (Henshaw et al., 1971; McNurlan et al., 1979; Garlick et al., 1980) minimizes problems previously associated with measurement of protein-synthesis rates in vivo. Of the previous reports in which protein-synthesis rates in vitro and in vivo were compared, this technique has been used only more recently (Goldspink et al., 1983; Oliver et al., 1984). Other workers have used infusion techniques in vivo where problems of interpretation have arisen because of differing extracellular and intracellular specific radioactivities of amino acid precursor (Palmer et al., 1981; Preedy & Garlick, 1983). Furthermore, in some studies in vitro, the tissue and perfusate amino acid specific radioactivities were not equilibrated after 1h in the hemicorpus (Preedy & Garlick, 1983) or 6h in rabbit skeletal muscles (Palmer et al., 1981). In the anterogradely perfused working rat heart used in our experiments, the perfusate and tissue specific radioactivities of [4-14]Hphenylalanine were equilibrated at the end of 2h of perfusion, and experiments of other workers (McKee et al., 1978) suggest equilibration to be complete in 5–10min. Our results show that, when the flooding-dose technique and a working-heart perfusion model are used, protein-synthesis rates in vitro closely approximate to rates in vivo, and changes in synthesis rates caused by starvation are accurately qualitatively reflected in vitro. Efficiency of protein synthesis may be 15% lower in vitro than in vivo. One possible reason for this is that depletion of cysteine from the perfusate by oxidation may be responsible, and this may be prevented by including mercaptodextran in the medium (Chua et al., 1983). Mercaptodextran was not available to us. In comparing ks values in vivo and in vitro, it should be noted that in fresh rinsed hearts blood comprises about 15% of the total protein.

Insulin and cardiac nitrogen balance

The inclusion of insulin in perfusates containing glucose and amino acids improves cardiac nitrogen balance in vitro. Physiological concentrations of insulin inhibit cardiac protein degradation in vitro (Rannels et al., 1975; Sugden & Smith, 1982a; Smith & Sugden, 1983a). Thus, at physiological work-loads, rates of cardiac protein degradation (Smith & Sugden, 1983a) are 15.7 ± 0.6%/day (13) in the absence of insulin and 7.7 ± 0.4%/day (13) at saturating insulin concentrations. From Table 2(a), hearts in the absence of insulin will be in negative nitrogen balance, but inclusion of insulin induces positive nitrogen balance. If these findings can be extrapolated to the situation in vivo, insulin may be important in regulating cardiac nitrogen balance. Furthermore, cardiac nitrogen balance may be sensitive to variations in plasma insulin concentrations occurring during the day in normal animals. We do not suggest that insulin is the only factor involved: others, such as non-carbohydrate fuels and amino acids, could be important. Although the insulin concentrations used in this study were hyperphysiological, others have shown that physiological insulin concentrations stimulate protein synthesis in the retrogradely perfused heart (Flaim et al., 1983), in the diaphragm (Manchester & Young, 1959; Manchester et al., 1959) and in skeletal muscles (Frayn & Maycock, 1979).

We thank the British Heart Foundation and British Diabetic Association for support, Mr. M. R. Stephens (Department of Biochemistry, National Heart Hospital) for allowing us to use the fluorimeter, and Dr. M. C. Sugden (Department of Chemical Pathology, The London Hospital Medical School) for useful discussion.

References