unoperated rats. At this time (16 days) T₃ rats were divided into three groups, i.e. given tri-iodothyronine and fed ad libitum, given tri-iodothyronine and fed a restricted amount of food (14 g per day, slightly less than that eaten by the T₃ rats) or left untreated. In this way the effects of tri-iodothyronine replacement could be measured in rats in which muscle growth was unrestricted or restricted. Measurements were made after 7 days of these regimes.

The results are shown in the table. At 5 days, although growth was unchanged in the T₃ rats, protein synthesis and degradation were decreased. No further decrease in the fractional degradation rate was observed in the T₃ rats, but the fractional synthesis rate did continue to decrease, so that at 16 days growth was markedly slowed compared with unoperated rats and the decreased turnover was maintained throughout the 7 day treatment period. In the tri-iodothyronine-treated groups muscle growth was increased and this involved increases in both the fractional synthesis rate and the fractional degradation rate. In the food-restricted treated group growth was not stimulated (above the slow rate in the untreated T₃ rats), but nevertheless the fractional synthesis rate and the fractional degradation rate were increased. The degradation rate in the tri-iodothyronine-treated groups was higher than in unoperated controls (16 days) and is certainly as high as in young controls (sham-operated). This may represent a certain elevation above the true normal rate of degradation for these rats.

It is apparent that the changes in the fractional synthesis rate involved mainly changes in protein synthetic capacity (i.e. RNA/protein ratios) rather than RNA activity (rates of synthesis per unit of RNA). This would indicate that there is not a requirement for tri-iodothyronine for normal translation. Measurements of circulating insulin concentrations in these rats, a hormone that has been implicated in the regulation of the fractional protein synthesis rate, indicated normal values in all groups. These changes in RNA capacity without marked changes in RNA activity are unusual in our experience. As far as the changes in protein degradation are concerned, measurements in this laboratory of lysosomal proteinase activity show that thyroid deficiency decreases cathepsin D activity, but does not change pepstatin-insensitive autolysis, whereas the tri-iodothyronine treatment regime used in this experiment normalizes cathepsin D and causes elevation of pepstatin-insensitive autolysis (S. J. Rosochacki, J. G. Brown & D. J. Millward, unpublished work).

It is clear that thyroid status is an important determinant of muscle protein metabolism. The impact on synthesis is largely through changes in RNA concentration without much change in activity. Generally (as for example in nutritionally induced synthesis changes) RNA capacity and activity change in parallel. The mechanism of breakdown rate changes is not well understood, but lysosomal activities seem to change in parallel with breakdown rate.

Supported by the M.R.C. and the Muscular Dystrophy Group of Great Britain.


The effect of glucagon on muscle protein synthesis in the perfused rat hemicorpus

VICTOR R. PREEDY, VIRGINIA M. PAYN and PETER J. GARLICK

Department of Human Nutrition, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

The notion that muscle is not a target tissue for glucagon is a statement that has been perpetuated throughout the literature, despite studies by Beaty et al. (1963) and Peterson et al. (1963). These authors suggested that glucagon acted directly to diminish muscle protein synthesis in vitro. Their results were inconclusive, because accurate rates of protein synthesis cannot be determined without measuring the specific radioactivity of the precursor pool. We therefore attempted to test the hypothesis that glucagon acts directly to alter muscle protein metabolism in vitro. For this we measured rates of protein synthesis in the perfused rat hemicorpus, which overcomes some of the problems associated with incubated muscle preparations. Perfused hemicorpuses were prepared from male rats (COBS Wistar, Charles Rivers) weighing 150–190 g by the method of...
Table 1. Synthesis rates of mixed muscle protein in plantaris muscles of fed and starved rats in response to glucagon and insulin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control (no hormones)</th>
<th>Glucagon</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed</td>
<td>15.0 ± 0.7 (6)*</td>
<td>11.9 ± 0.9 (6)*</td>
<td></td>
</tr>
<tr>
<td>Starved (25h)</td>
<td>10.3 ± 1.0 (5)††</td>
<td>7.3 ± 0.5 (4)†</td>
<td>14.9 ± 0.9 (4)†</td>
</tr>
</tbody>
</table>

Jefferson (1975). Food pots were removed from the animals in the starved group 25h before measurement of rates of protein synthesis in vitro. Details of the perfusate and the method of measuring synthesis rates from the incorporation of [U-14C]tyrosine were as previously described (Preedy et al., 1979). Where applicable, insulin and glucagon (gifts from Novo Laboratories, Basingstoke, Hants, U.K.) were added to the perfusate so that the initial concentrations were 25m-μ/l/ml and 1μg/ml respectively. [U-14C]Tyrosine was added to the perfusate 15min after the start of the perfusion. Fractional synthesis rates were calculated from the specific radioactivities of intracellular and protein-bound tyrosine in the plantaris muscle taken at the end of 50min perfusion.

Table 1 shows an increase in synthesis rates after addition of insulin to preparations from starved rats. High concentrations of glucagon, however, suppressed the synthesis of mixed muscle protein in plantaris muscle from both fed and starved groups. As there is some doubt as to whether the specific radioactivity of the free [U-14C]tyrosine at the site of protein synthesis is represented by tissue intracellular or perfusate plasma values, calculations of synthesis rates were also made by using perfusate and protein-bound specific radioactivities. The same results were obtained, i.e. an increase with insulin and a decrease with glucagon. Additional evidence for an effect of glucagon was obtained by the observation of a decrease in the intracellular concentration of branched-chain amino acids in gastrocnemius muscles from the fed group (V. R. Preedy, P. G. Broadbent & P. J. Garlick, unpublished work).

These results may have important physiological implications. If glucagon were to behave similarly at normal physiological concentrations in vivo one could postulate that the increased glucagon concentrations observed in various pathological states could be partly responsible for a decreased synthesis of protein in muscle. For example, hyperglucagonaemia is often observed in experimental and non-experimental diabetes. Thus the concept that the decrease in muscle protein synthesis seen in experimental diabetes (e.g. Pain & Garlick, 1974) is largely due to a deficiency of plasma insulin has to be re-examined in the light of these results.

In conclusion, addition of glucagon to the perfusate of rat hemispheric preparations suppresses the synthesis of plantaris muscle protein from both fed and starved rats. It is therefore possible that this effect may be important in the regulation of protein metabolism in the whole animal.


High activity for triacylglycerol formation and hydrolysis in isolated oligodendroglia from myelinating rat brain

ERIC M. CAREY,† ULRICH STOLL† and ANGELA CARRUTHERS* 

*Department of Biochemistry, The University of Sheffield, Sheffield S10 2TN, U.K. and †The Institute for Biochemistry, The Medical High School, Hanover, Federal Republic of Germany

The presence of fat droplets in myelinating glia before the commencement of myelination has frequently been observed in certain regions of the central nervous system (Gilles, 1976). Such 'fatty' glial cells are no longer apparent during and after the active phase of myelination. 'Fatty' glial cells may arise as a result of active lipid biosynthesis preceding myelin membrane formation. This is suggested by previous results where the maximal activity of fatty acid synthesis in cerebral cortical tissue occurs before myelination in the rabbit and rat (Cantrill & Carey, 1975), whereas other lipogenic activities implicated in the formation of specific myelin components, such as fatty acid elongation (Carey & Parkin, 1975) and cerebrosides formation by UDP-galactose-ceramide galactosyltransferase (Constantino- Ceccarini & Suzuki, 1975), are maximal at the time of maximum myelin deposition. Therefore 'fatty' glia could originate from enhanced lipid deposition before membrane proliferation. The lipid may act as a precursor pool for myelin acyl groups after further transformations. Fat-laden cells are also common in human infant brain in pathological conditions such as congenital heart defects with impaired respiration. Neutral lipids (triaclyglycerol and cholesterol ester) are absent or present in very small amounts in the mature central nervous system, but there are no data on the late foetal or early neonatal brain. Tissue slices of cerebral cortex synthesize triacylglycerol from glucose at the early neonatal stage (Carey, 1975). Therefore it was of interest to examine the metabolic activity for triacylglycerol synthesis and hydrolysis in a more defined cell fraction.

Cells were isolated from 17-day-old rat brain by a method described previously (Carruthers & Carey, 1979). Oligodendroglial and neuronal cell bodies, devoid of membrane processes, were characterized by light and electron microscopy, anti-cerebroside antiserum, 'marker' enzymes and protein profiles on sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. For the incorporation of fatty acids into glycerolipid, intact or disrupted cells were incubated with 0.5 mM [3-14C]palmitate or [1-14C]oleate bound to bovine serum albumin for periods up to 4h in a phosphate-buffered medium containing glucose (Carruthers & Carey, 1979). Lipids were extracted and