SHORT COMMUNICATION

Protein synthesis in isolated human skeletal muscle tissue: evaluation of an experimental model

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Summary
Amino acids were incorporated into soluble proteins, myosin and myoglobin at constant rates for at least 4 h on incubation of isolated human skeletal muscle fibres. The time course of incorporation of leucine into proteins not attached to ribosomes was approximately constant, suggesting a continuous termination of proteins. A comparison between estimated pool size of ribosomes and incorporation rate of leucine into proteins indicated that initiation of peptides occurred even in the absence of insulin.

Key words: amino acids, initiation of peptides, insulin, protein synthesis, skeletal muscle.

Introduction
Isolated human skeletal muscle-fibre bundles incorporate amino acids into proteins at a constant rate and the synthesis of peptides in vitro is regulated by insulin and amino acids (Lundholm & Scherstén, 1975a, b). However, it is unclear to what extent the incorporation of amino acids into proteins indicates synthesis of proteins in vivo, and whether cut isolated human muscle fibres can translate genetic information in vitro and terminate complete proteins at a constant rate. In short-time experiments, paradoxically a constant peptide synthesis rate could be achieved if the pool of nascent peptides in the cells is large and the protein metabolism is low. Amino acids could be taken up mainly by open sites of nascent peptides and thus not indicate continuous movement of ribosomes on and off the messenger. A constant incorporation rate of amino acids into proteins in vitro would then be possible without initiation or termination of proteins.

The aim of the present study was to evaluate the isolated human skeletal muscle fibres in the above respects.

Materials and methods
Isotopes were from NEN Chemicals, Langen, West Germany, reagents from Sigma Chemical Company, St Louis, U.S.A., and Sephadex from Pharmacia, Uppsala, Sweden.

Muscle biopsies
Biopsies were taken from the rectus abdominis muscle in patients (n = 34) operated on for uncomplicated gallstone disease. The biopsies were transported and prepared in Krebs-Ringer bicarbonate buffer solution (pH 7-4) at 22°C. Preparation and incubation of muscle fibres and determination of proteins and counting of radioactivity were as reported previously (Lundholm & Scherstén, 1977). The specific radioactivity of the incubation medium was used for the calculation of the incorporation rate of amino acids into proteins, since this pool has been shown as the best approximation (Lundholm & Scherstén, 1975b).

Purification and separation of proteins
Soluble tissue proteins and myosin were extracted as described by Perry (1955). Myoglobin was isolated as described in the legend to Fig. 1. The
relative incorporation rates of amino acids into muscle proteins of various molecular sizes were determined as described by Dice, Dehlinger & Schimke (1973).

RNA fractionation

The release rate of labelled proteins from ribosomes was estimated by the determination of the time course of incorporation of amino acids into proteins drawn from the top fraction of a continuous ribonuclease-free sucrose gradient, in which polysome profiles had been analysed (Morgan, Jefferson, Wolpert & Rannels, 1971). The top fraction contained no RNA and the significance of contamination of labelled t-RNA was minimized by heating to 90°C. RNA, sodium, potassium and water content were determined as previously described (Lundholm, Bylund, Holm & Schersten, 1976).

Nonparametric statistical analysis (Wilcoxon's and Walsh test) was applied (Siegel, 1956).

Results

Leucine, phenylalanine and tyrosine were linearly incorporated into muscle proteins on incubation for 4 h, irrespective of amino acid concentrations in the incubation medium. Leucine was incorporated at an approximately constant rate into soluble proteins, myosin and highly purified myoglobin (Fig. I). The incorporation rate of leucine into large proteins was higher than into small proteins. This finding probably reflected the relative turnover rate of soluble proteins in vitro and is in accordance with findings in vivo in rat liver (Dice et al., 1973). Leucine was also incorporated at an approximately constant rate into acid-precipitable proteins, which were not attached to ribosomes at the time when the incubation was stopped. These proteins were isolated from the RNA-free top fraction of sucrose gradients prepared for RNA fractionation. This suggests that labelled proteins continuously left the ribosomes within 4 h of incubation.

Transportation and preparation of tissue in ice-chilled buffer (4°C) (20–30 min) resulted in a lower incorporation rate of amino acids into proteins than transportation and preparation at 22°C. This effect of low temperature could be further accentuated by ouabain (10⁻⁴ mol/l). However, the degree of insulin stimulation of the incorporation rate of leucine into proteins was unaltered, irrespective of the temperatures of the buffer for transportation and preparation. This suggests that basic hormonal regulation mechanisms for protein synthesis can be operating even though the cellular milieu is disturbed. Potassium concentration of muscle fibres (330 μmol/g dry weight) decreased (P < 0.05) and sodium and water content increased immediately after the biopsy was taken, which was accentuated only slightly by tissue preparation. The potassium (275 μmol/g dry weight) and sodium content remained unchanged during 4 h incubation. Ouabain (10⁻⁴ mol/l) decreased tissue potassium and increased tissue sodium content by about 75%.

[¹⁴C]Uridine was incorporated into RNA at a constant rate, and the tissue RNA content did not decrease during transportation, preparation and incubation for 4 h at 37°C. Actinomycin D (0-039 μg/ml) inhibited 90% of uridine incorporation into RNA, but leucine was still consistently incorporated into proteins, implying that the pool size of messenger RNA at the time of tissue sampling may be an important factor for the estimated rate of protein synthesis in this system in vitro. Cycloheximide (10 μg/ml) almost extinguished the incorporation of phenylalanine into proteins.

Aurintricarboxylic acid (5 × 10⁻⁵ mol/l) significantly decreased the stimulation by insulin (P < 0-025), but not that by amino acids, of the incorporation rate of leucine into proteins. This suggests different mechanisms for insulin and
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incubation. Puromycin (2.6 nmol/g of protein) was completely distributed in the tissue within 30 min of incubation, after which time the incorporation of puromycin into proteins had also ceased. Tyrosine and all the other amino acids at equimolar concentrations with puromycin were also completely distributed in the fibres within 30 min of incubation. After 4 h incubation the estimated amount of amino acids incorporated into proteins, calculated from the mean specific radioactivity of 14C-labelled amino acids in the incubation medium, was approximately 90 times that of puromycin (5–8 nmol/g of protein). Accepting that every incorporated molecule of puromycin represents one un-finished nascent peptide, this suggests that the muscle tissue contained approximately 4 × 10^15 nascent peptides/g of protein (calculated from the Avogadro number).

Discussion

Morgan, Rannels, Wolpert, Giger, Robertson & Jefferson (1972) reported that the perfused rat heart, one of the most intact experimental models, showed signs of decreasing performance during short-time perfusions at 37°C. Likewise it has been reported that the content of polysomes of liver and muscle tissue decreased rapidly during experiments in vitro (Jefferson & Körner, 1969; Manchester, 1974). These findings may be dependent on a block of initiation or unspecific disintegrations of the polysomes. It is likely that corresponding problems are associated with this isolated muscle preparation. Therefore it was difficult to interpret the biological significance of the constant incorporation rate of amino acids into proteins for as long a time as 4 h. The explanation could be that the incorporated amino acids were taken up predominantly by open sites on nascent polypeptides and that elongation and termination of peptides were poor. However, our experimental results provide circumstantial evidence for initiation of protein synthesis in vitro even in the absence of insulin stimulation. Muscle tissue contained about 5 mg of RNA/g of protein, which corresponded to about 1–2 nmol of ribosomes/g of protein, if the molecular weight of muscle ribosomes is accepted to be 2.5 × 10^6. The incorporation rate of leucine was estimated to be 250 nmol/h, which means that each ribosome incorporated 125–250 leucine molecules/h. This rate implies that initiation of peptide formation must have occurred and probably also terminated, since labelled proteins probably left ribosomes continuously during 4 h incubation.

Our results suggest that this muscle preparation may be useful for qualitative evaluation of protein synthesis in human skeletal muscles.

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References


