The Conversion of [1-¹⁴C]Pyruvate into [¹⁴C]Alanine in Rat Skeletal Muscle: Its Relevance to the Effect of Insulin on Protein Synthesis

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It has been postulated that accelerated amino acid transport is responsible for the insulin-mediated stimulation of the incorporation of amino acids into protein (Guroff & Udenfriend, 1961; Akedo & Christensen, 1962). Manchester & Krah1 (1959) and Wool & Krah1 (1964) have, however, shown that insulin increases the incorporation of ¹⁴C-labelled amino acids into protein when rat diaphragm is incubated with ¹⁴C-labelled carboxylic acid precursors of amino acids. On the basis of an assumption that amino acids are formed intracellularly, these workers concluded that enhanced incorporation of radioactivity into muscle protein in the presence of insulin was not a result of a stimulation of amino acid transport. To test the validity of this assumption we have studied the conversion of pyruvate into alanine in rat extensor digitorum longus muscle and hemidiaphragm.

When extensor digitorum longus muscles were incubated in Krebs–Ringer bicarbonate buffer (Hider *et al.*, 1971) containing [1-¹⁴C]pyruvate (0.1 mM) (sp. radioactivity 14 Ci/mol; The Radiochemical Centre, Amersham, Bucks., U.K.) and [¹²C]alanine (0.1–10 mM) for 1 h, the intracellular concentration of alanine increased linearly with the increase in alanine concentration in the medium (Fig. 1). Intracellular extracts and incubation media were prepared for scintillation counting and amino acid analysis as described previously (Hider *et al.*, 1971). The effluent from the ion-exchange column was divided to enable the determination of the amino acid by the usual colorimetric method together with the measurement of ¹⁴C radioactivity. The amount of [¹⁴C]alanine present in the medium increased with the alanine concentration. In contrast, the intracellular [¹⁴C]alanine concentration reached a plateau at a concentration in medium of approx. 1 mM (Fig. 1). The specific radioactivity of the alanine in the medium at the end of the incubation only varied by a factor of 5 even though the alanine concentration in the medium at the start of the incubation differed by a factor of 100. Experiments with diaphragm exhibited the same tendency.

The increase in intracellular alanine concentrations would lead to an increase in the intracellular pyruvate

concentration as a result of transaminase activity. Such an increase in liver pyruvate has indeed been observed during perfusion with alanine (Haeckel & Haeckel, 1970). If the rate of pyruvate utilization by muscle is independent of the alanine concentrations in the medium used in this study, then proportionately less pyruvate will be oxidized when the muscle is incubated in high alanine concentrations. As a result the pool of [¹⁴C]pyruvate + [¹⁴C]alanine would be larger, which explains the association of increasing conversion of [¹⁴C]pyruvate into [¹⁴C]alanine with increasing alanine concentration in the medium (Fig. 1).

There are two possible explanations for the finding that the concentration of [¹⁴C]alanine in the medium increases with increasing concentrations of [¹²C]alanine in the medium: either there is appreciable extracellular conversion of pyruvate into alanine, or intracellular and extracellular alanine are in rapid equilibrium via exchange diffusion. To estimate the extent of extracellular conversion, paired muscles were preincubated for 30 min each in Krebs–Ringer bicarbonate buffer containing pyruvate (0.1 mM) and alanine (1 mM). After preincubation one of the muscles was removed from its flask and [1-¹⁴C]pyruvate was subsequently added to the remaining medium. The incubation was continued for a further 30 min. In contrast, the control muscle was not removed from its flask but [1-¹⁴C]pyruvate was added to the medium and the muscle was incubated for a further 30 min. After incubation the media were prepared for amino acid analysis and scintillation counting of radioactivity.

Appreciable conversion of [1-¹⁴C]pyruvate into [¹⁴C]alanine occurred when pyruvate was incubated with solutions obtained from preincubations with diaphragm; for instance, a 10% conversion was observed in the absence of muscle compared with a 25% conversion in the presence of the muscle. This contrasted markedly with extensor digitorum longus muscle, where there was an extremely small extracellular contribution to this conversion.

Thus it would appear that the accumulation of [¹⁴C]alanine in the medium results largely from exchange diffusion, although with hemidiaphragm there is an appreciable extracellular transamination.

Wool & Krah1 (1964) attempted to demonstrate that, when diaphragm muscle is incubated

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Fig. 1. Conversion of pyruvate into alanine by extensor digitorum longus muscle

Extensor digitorum longus muscles were incubated in Krebs–Ringer bicarbonate buffer containing [1-¹⁴C]-pyruvate (100 μM) and various concentrations of alanine for 60 min. Each value is the mean of three determinations. □, Concn. of alanine in tissue; ●, [¹⁴C]alanine in medium (c.p.m.); ○, [¹⁴C]alanine in tissue (c.p.m.).

Table 1. Accumulation of alanine, aspartate and glutamate in media containing diaphragm muscle during 90 min of incubation

The left-hand values refer to the initial amino acid concentrations and the right-hand values to the final concentrations. Glutamine and asparagine were hydrolysed during the isolation procedure and hence appeared with glutamate and aspartate on the chromatogram. The diaphragms used in this study were from larger rats (110–130 g), and were incubated in Krebs–Ringer bicarbonate solution (1 ml), under the conditions used by Wool & Krahl (1964). The results are the means of three determinations.

Initial amino acid concentration (mM)			Final amino acid concentration (mM)		
Aspartate	Glutamate	Alanine	Aspartate	Glutamate	Alanine
1000	1500	7500	985	1510	7500
100	150	750	155	370	740
10	15	75	50	250	290
0	0	0	30	250	210

with [¹⁴C]carboxylic acids, the insulin-induced enhancement of the incorporation of ¹⁴C radioactivity into protein did not result from the tissue reutilizing effluxed amino acids. By incubating diaphragm muscle with [¹⁴C]pyruvate and high concentrations of unlabelled amino acids Wool & Krahl (1964) intended to dilute any effluxed ¹⁴C-labelled amino acids appearing in the medium, to prevent the reutilization of the label. Experiments of this kind are extremely difficult to design, because the amino acid concentration at the muscle membrane is different from that of the incubation medium (Hider *et al.*,

1971). When diaphragm muscles were incubated in Krebs–Ringer bicarbonate buffer under the conditions used by Wool & Krahl (1964), there was appreciable efflux of amino acids (Table 1). As a consequence amino acid concentrations in the medium that had originally been widely different finally approximated to one another. Similar efflux rates have been reported for extensor digitorum longus muscle (Hider *et al.*, 1969).

Thus under the incubation conditions used by Wool & Krahl (1964) there is appreciable efflux of amino acids, both labelled and unlabelled. Further,

with diaphragm there is extensive extracellular transamination, presumably owing to damaged tissue. A further complication with this experiment arises from the fact that there is an increase in the conversion of [^{14}C]pyruvate into [^{14}C]alanine when the alanine concentration in the medium is increased (Fig. 1).

The specific radioactivity of [^{14}C]alanine at the membrane surface is likely to be much higher than that in the bulk incubation medium, owing to the net efflux of [^{14}C]alanine.

Consideration of the present findings allows the results obtained by Wool & Krahl (1964) to be readily explained in terms of reutilization of effluxed amino acids.

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