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A comparison of ^{15}N proline and ^{13}C leucine for monitoring protein biosynthesis in the skin

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Summary. The tracers L ^{15}N-proline and L(1-^{13}C)-leucine were used to explore the synthesis of skin proteins in vivo in rabbits. They orally received a single dose containing an equimolecular mixture of L(1-^{13}C)-leucine and L ^{15}N-proline. The changes in the amounts of these tracers in blood and skin were monitored for a total of 8 h. The data showed the appearance of the two tracers in blood within 15 min and their clearance in 8 h. They were both rapidly (15 min) incorporated into skin proteins, but more proline was incorporated than leucine. We therefore consider L ^{15}N-proline to be a better tracer than L(1-^{13}C)-leucine for studying protein metabolism in the skin.

Keywords: Amino acids – Tissue protein synthesis – Stable isotope amino acids

Introduction

In vivo protein metabolism was explored using amino acids labelled with stable isotopes. The essential amino acid leucine, as L(1-^{13}C)-leucine, has been used to monitor the metabolism of plasma amino acids in humans (Matthews et al., 1980; Motil et al., 1981; Bier and Matthews, 1982; Beaufrère et al., 1990; Carraro et al., 1991; Denne et al., 1992; Krempf et al., 1993; Castaneda et al., 1995) and the metabolism of the proteins in skeletal muscle (Rennie et al., 1982, 1983). Collagen metabolism has been explored in animals using the amino acid proline labelled with tritium (Jackson et al., 1975; Sodek 1977; Spanheimer et al., 1991). Proline is required for optimal growth in some animals such as pig, and should therefore considered as a conditionally indispensable amino acid (Ball et al., 1986; Jaksic et al., 1991; Berthold et al., 1995; Miller et al., 1995). This amino acid represents one fifth of skin collagen and is the metabolic precursor of hydroxyproline. Although endogenous synthesis of proline occurs in human (Adams and Franck, 1980), stable isotope experiments have suggested that rate of proline biosynthesis is under 7% (Berthold et al., 1995). Moreover, orally dispensed proline is not metabolized in the liver

under physiologic conditions (Jaksic et al., 1987). The presented study therefore compares the metabolic course of leucine and proline in two compartiments, the blood and the skin.

Materials and methods

Natural L-leucine, natural L-proline and cycloleucine were purchased from SIGMA. L ^{15}N-proline (99% atom ^{15}N) and L(1-^{13}C)-leucine (99% atom ^{13}C) were obtained from EURISOTOP (Saclay France). Isotopic and chemical purities were improved by gas chromatography-mass spectrometry. The amino acids were dissolved in water and sterilized by filtration through a 0.22 μm filter (Millipore), before oral administration.

All experiments were performed on 8 week old, New Zealand white rabbits weighing 2300–2400 g. The rabbits were individually housed and allowed free access to water and food (U.A.R. Ref 112, Epinay sur Orge, France). A catheter for blood sampling was inserted into an ear vein on the day of experiment. An initial blood sample (2 ml) and punch biopsy (4 mm diameter) of back skin were collected at the start of each experiment for measuring the natural isotopic abundances of leucine, proline, glutamic acid. Each animal orally received a single dose of an equimolecular mixture of L(1-^{13}C)-leucine and L ^{15}N-proline (172 μmol/kg). Blood and punch biopsy samples were collected 15, 30, 60, 90, 120 and 480 minutes after ingestion of the amino acid mixture. The isotopic enrichment and the total concentrations of leucine, proline, glutamic acid were measured. The test group contained 5 rabbits.

Blood samples were collected in heparinized tubes, centrifuged for 10 minutes at 1400 $\times$ g at 4°C, the plasma deproteinized with sulfo-5-salicylic acid (50 mg/ml). The resulting supernatant was freezed. Punch biopsy specimens were stored at −20°C. These samples were dehydrated and delipidated in methylal/methanol (4:1) for 48 hours, and hydrolysed in 6 M HCl for 24 h at 110°C. The amino acids were purified from 0.5 ml plasma and from 0.5 ml biopsy hydrolysate using a column (0.5 cm internal diameter) filled by resin (2 cm height) Dowex AG 50 W X 8, 200–400 mesh (Biorad Laboratories). The resin was washed with deionized water (5 ml) and free amino acids were eluted with NH_4OH 4 M (2 ml). The eluates were evaporated to dryness and N-heptafluorobutyryl isobutyl ester derivatives were prepared (MacKenzie and Hogge, 1977; Desgres et al., 1979). The dried amino acids samples were first esterified with isobutanol, HCl (500 μl) at 100°C for 40 minutes, evaporated to dryness under nitrogen, and then reacted with ethylacetate/heptafluorobutyric anhydride (80 μl:20 μl) at 110°C for 30 minutes. Excess reagents were removed by evaporation at room temperature under nitrogen. The derivatives were dissolved in 100 μl ethyl acetate and analysed by gas chromatography-mass spectrometry (GC-MS). Isotope enrichment was determined by selected ion monitoring gas chromatography-mass spectrometry (GC-MS: Hewlett Packard model 5890 gas chromatograph, model 5989 mass spectrometer). The analytical capillary column was HP1 (25 m 0.2 mm I.D., 0.33 μm film thickness). The analytical conditions were: carrier gas helium flow rate 1.5 ml/min, injection port temperature 250°C, detector 260°C, temperature programming 3°C/min from 90°C to 240°C. Source temperature was 240°C and quadrupole 100°C. The ioniser voltage was 70 eV. The select ion monitoring mode allows to monitor ions at m/z (327; 328) natural leucine, L(1-^{13}C)-leucine (Périer et al., 1996), (367; 368) natural L-proline, L ^{15}N-proline and (280; 281) natural L-glutamic acid and L ^{15}N-glutamic acid. The total purified and derivatized amino acids in plasma and skin were also measured by gas chromatography (Packard model 428; analytical column was filled with phase OV 1% on chromosorb WAW 100–200 mesh) using cycloleucine as internal standard.

The amounts of L(1-^{13}C)-leucine and L ^{15}N-proline in blood and skin were deduced from the isotopic enrichment factor and the amounts of total amino acids (Campbell, 1974). The differences in leucine and proline in blood and skin were assessed by Student's paired t-test and the correlation coefficient. The results were considered significant when $p < 0.05$.

Results

The changes in the concentrations of L(1-^{13}C)-leucine and L ^{15}N-proline in the blood are shown in Fig. 1. The two tracers were present in the blood 15 min after ingestion. The mean concentration of L(1-^{13}C)-leucine was 20 nmol/ml blood and that of L ^{15}N proline was 59 nmol/ml blood. The two tracers were completely cleaned from the plasma within 8 hours after oral administration. They began to be incorporated into skin proteins 15 minutes after ingestion. L ^{15}N-proline was incorporated more extensively into tissue protein than was L(1-^{13}C)-leucine. Their incorporative profiles were also different, since L(1-^{13}C)-leucine was incorporated more later. The mean values of L(1-^{13}C)-leucine and L ^{15}N-proline in blood (15 min) $p = 0.022$ and in skin (2 hours) $p = 0.009$ were different. Finally the amounts proline and leucine in blood and in skin changed independantly since they were not related (Table 1).

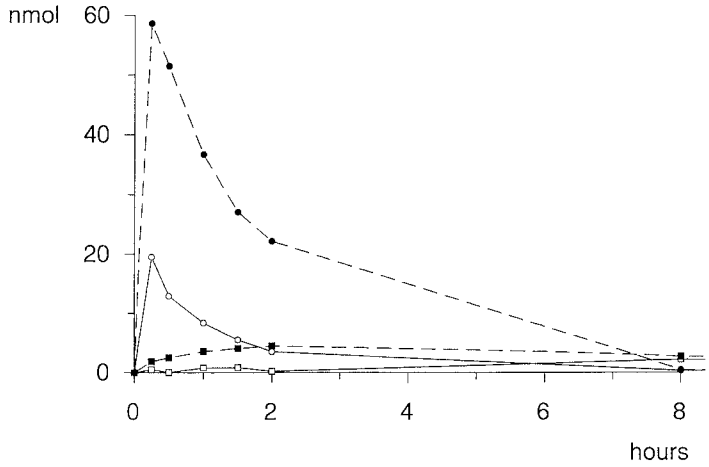

Fig. 1. Evolutionary changes in L(1-^{13}C)-leucine (○), L ^{15}N-proline (●) amounts in the blood (nmol/0.5 ml plasma); L(1-^{13}C)-leucine (□), L ^{15}N-proline (■) amounts in the skin (nmol/mg)

Table 1. Amounts of L ^{15}N-proline and L(1-^{13}C)-leucine in the blood (15 minutes after oral administration) and in the skin (2 hours after oral administration)

Compartment / Experimental data	Blood (15 minutes)		Skin (2 hours)	
L ^{15}N-proline amount	Mean	SD	Mean	SD
nmol/ml (blood)				
nmol/l/mg (skin)	58.6	23.8	4.5	2.0
L(1-^{13}C)-leucine amount	Mean	SD	Mean	SD
nmol/ml (blood)				
nmol/mg (skin)	19.5	4.7	0.2	0.45
P (t-test)	0.022		0.009	
r (correlation)	0.772 ($p > 0.05$)		0.839 ($p > 0.05$)	

Discussion

This study explores the dynamic process of protein synthesis in the skin using two amino acid tracers L(1-^{13}C)-leucine and L ^{15}N-proline. The tracers have different metabolic courses, since leucine is an essential amino acids and proline is only a conditionally essential amino-acid. The two tracers were found in the blood and the skin 15 minutes after their ingestion and began to be incorporated into skin proteins at about the same time. However, proline was incorporated faster and to a greater extent than leucine (Fig. 1). In blood, the course of L(1-^{13}C)-leucine and L ^{15}N-proline were similar and both tracers were depleted 8 hours after their ingestion. Moreover despite their equimolecular concentrations in the tracer dose, their blood concentrations were quite different; L ^{15}N-proline concentration was higher than that of L(1-^{13}C)-leucine amounts. This difference could be due to the metabolic transformation of leucine, its sequestration in splanchnic area or a greater incorporation into tissue proteins. The most likely explanation is splanchnic sequestration since the amount of leucine incorporated into tissue proteins was lower than that of proline. Hence, proline seems to be a better tracer than leucine for exploring the dynamics of tissue protein synthesis. Proline was not metabolized into glutamic acid, since no L ^{15}N-glutamic acid was found in the blood or in the skin during the experiment. There was a statistical difference between the time courses of proline and leucine in the blood or in the skin. This is valuable for ascertaining that proline should be considered to be a conditionally essential amino acid. This amino acid is also the metabolic precursor of hydroxyproline, the distinctive amino acid of collagen. Consequently we recommend that L ^{15}N-proline be used as tracer for exploring tissue protein metabolism in vivo, rather than L(1-^{13}C)-leucine.

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