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The Effect of Branched Chain Amino Acids on Protein Synthesis in Two Skeletal Muscles of Japanese Quail

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Summary

Two different isolated skeletal muscles of Japanese quail were used. The influence of branched chain amino acids on the fractional rate of protein synthesis (FSR) was evaluated using ^{14}C-tyrosine. The addition of 0.5 mM valine, leucine or isoleucine to the incubation medium significantly decreased ($P<0.05$) the value of FSR in extensor metacarpalis radialis. In the ambiens muscle only the application of leucine increased the FSR significantly while valine and isoleucine were without any effect.

Key words

Fractional protein synthetic rate • Branched chain amino acids • Japanese quail • Isolated skeletal muscles

Introduction

Reports from several laboratories indicate that the branched chain amino acids have a regulatory effect on the turnover of muscle proteins, which depends on the mammalian species studied. Particularly leucine has an anabolic effect on the isolated rat diaphragm (Fulks *et al.* 1975, Buse and Reid 1975, Buse and Weigand 1977, Tischler *et al.* 1982), perfused rat hindquarters (Li and Jefferson 1978) and isolated perfused hearts (Chua *et al.* 1979) in which stimulation of protein synthesis and inhibition of protein degradation were found. Hong and Layman (1984) demonstrated that leucine stimulates protein synthesis, but does not affect protein degradation in soleus and extensor digitorum longus muscles.

Unfortunately, little information is available on the protein turnover in skeletal muscles of birds. Maruyama *et al.* (1978) reported an estimate of protein turnover rates in breast and leg muscles of the chick under various nutritional conditions. Pinchasov and Nir (1988) measured the protein synthesis in breast muscles of chickens during intermitted feeding. Klasing and Jarrell (1985) reported the effect of various regulatory agents on the rate of protein degradation in two isolated chick skeletal muscles. Since, the results were

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contradictory, the aim of our study was to evaluate the effect of branched chain amino acids (valine, leucine, isoleucine) on the protein synthesis in two different skeletal muscles of the Japanese quail.

Methods

Birds

Six 28-day-old cockerels of the Japanese quail (*Coturnix coturnix japonica*), weighing 55-75 g, were used for the experiments.

Experimental design

Quails were fed with commercial feed which was withdrawn 48 h prior to decapitation. The extensor metacarpalis radialis muscle and ambiens muscle were excised, weighed and used for subsequent examination according to Li *et al.* (1973).

Muscles were preincubated at 41 °C (body temperature of birds) for 60 min in a medium consisting of 3 ml of Krebs-Ringer buffer with 10 mM glucose, chloramphenicol 0.3 μg/ml and trichloracetic acid-soluble fraction of the plasma from birds fasted for 48 h and bubbled with CO_2/O_2 (1:19) mixture. The muscles were than placed into a fresh incubation medium of the same composition, containing 0.21 μCi/ml ^{14}C-tyrosine in the control group or 0.21 μCi/ml ^{14}C-tyrosine with 0.5 mM leucine, isoleucine or valine added in the experimental groups. After the incubation at 41 °C for 90 min, the muscles were washed with Krebs-Ringer buffer, frozen in liquid nitrogen and pulverized.

The muscle samples were then homogenized in 10 % trichloracetic acid (TCA) with Janke-Kunkel homogenizer at 4 °C for 2 min. The homogenates were centrifuged for 20 min at 4000 rpm at 4 °C and the acid soluble supernatants were extracted 3 times with ether. Radioactivity was assessed in the aliquots by liquid scintillation counting (Beckman LS 6000 SE) and free tyrosine was determined by fluorometry (Udenfried and Cooper 1952). For the measurement of ^{14}C-tyrosine incorporation into proteins, the acid-precipitated material was washed with ether (3 times) and then solubilized with 0.5 M NaOH at 80 °C for 20 min. The radioactivity and tyrosine content were assessed in the aliquots as above and proteins were determined according to Lowry *et al.* (1951).

The fractional rate of protein synthesis (FSR) was calculated from the specific radioactivity of protein-bound tyrosine (S_b) and the specific radioactivity of free tyrosine (S_a) according to McNurlan *et al.* (1979)

$$FSR = S_b \times 100 / S_a \times t$$

t = time of incubation [day]

Reagents

All commercially available chemicals were of the highest purity. ^{14}C-tyrosine was purchased from ÚVVR, Prague, L-amino acids from Serva and chloramphenicol from Calbiochem.

Statistical analysis

Differences between experimental groups were evaluated using paired Student's t-test ($P<0.05$).

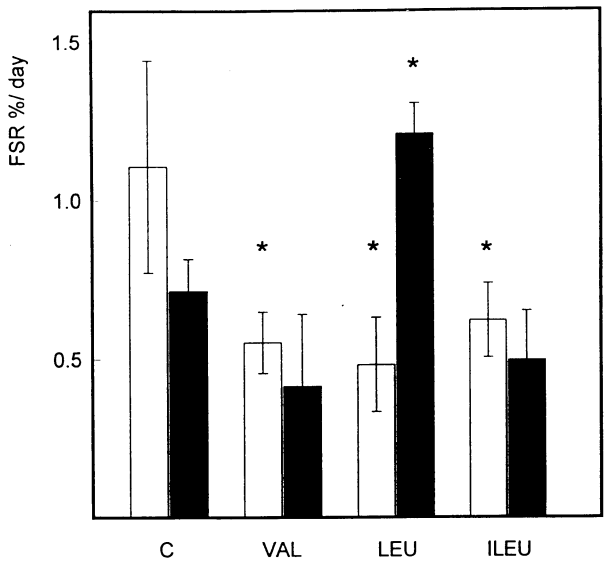

Fig. 1. *Fractional rate of protein synthesis in m. extensor metacarpalis radialis (open bars) and m. ambiens (full bars). C – control group, VAL, LEU, ILEU – experimental groups with addition of 0.5 mM valine, leucine or isoleucine to the incubation medium. * P<0.05 vs. control group*

Results

The addition of valine, leucine or isoleucine to the extensor metacarpalis radialis muscle significantly decreased (by 50 %) the fractional rate of protein

synthesis (FSR) (Fig. 1). The fractional rate of protein synthesis in the ambiens muscle was only changed after the application of leucine. This amino acid significantly increased the value of FSR (Fig. 1). The addition of any of the three amino acids did not affect the protein content in both muscles (Table 1).

Table 1. Protein content (mg/100 mg tissue) in m. extensor metacarpalis radialis and m. ambiens

Group	M. extensor metacarpalis radialis	M. ambiens
C	8.32±0.43	7.77±0.13
VAL	7.33±0.69	6.11±1.01
LEU	8.04±0.72	8.51±1.11
ILEU	7.27±0.85	6.84±0.20

Means ± S.D., C – control group, VAL, LEU, ILEU – experimental groups with addition of 0.5 mM valine, leucine or isoleucine to the incubation medium.

Discussion

The aim of this study was to compare the influence of valine, leucine and isoleucine on skeletal muscle protein synthesis in two different skeletal muscles of Japanese quail: extensor metacarpalis radialis muscle (EMR), a wing muscle, and ambiens muscle (AM) which is a leg muscle.

It is generally recognized that the rate of protein synthesis and breakdown in different skeletal muscles vary according to their fiber type composition. Muscles with dark (slow-twitch) fibers synthesize proteins more rapidly than those with predominantly white (fast-twitch) fibers (Goldberg 1967, Flaim *et al.* 1980). The relationship between protein synthesis and fiber type composition should be made on the basis of the functional area of the muscle occupied by each fiber type. The most important factor determining protein turnover rates is the speed of contraction (Garlick *et al.* 1989). ATPase activity of muscle myosin varies according to the speed of contraction. However, the muscles used in our experiment (EMR and AM) are probably white muscles, because wing and leg muscles of birds consist of white fibers mostly (Kolda and Komárek 1958). However, the ATPase activity in these two muscles is different (unpublished results). The extensor metacarpalis radialis muscle is a slower muscle (lower ATPase activity) with higher rate of protein synthesis than the ambiens muscle. This was confirmed by FSR in both muscles of control quails.

The influence of branched chain amino acids on the protein synthesis and degradation was observed in several laboratories (Tischler *et al.* 1982, Buse 1981, Hong and Layman 1984). Especially the effect of leucine seems to be controversial. According to Buse and Reid (1975), the anabolic effect of leucine entails the stimulation of proteosynthesis accompanied by the inhibition of degradation, while Hong and Layman (1984) reported enhanced protein synthesis without any effect on protein degradation. However, the influence of leucine was highly variable in different muscles. While leucine acts as an inhibitor of protein degradation in the isolated rat diaphragm (Tischler *et al.* 1982, Buse and Weigand 1977), it is without any effect on protein degradation in the soleus and extensor digitorum longus muscles (Hong and Layman 1984). The different effect of branched chain amino acids on various muscles may be caused by a different protein turnover in individual fiber types. Muscles with a faster rate of protein synthesis responded more to addition of leucine (Hong and Layman 1984) than muscles with slower rate. In our experiments we observed higher values of the fractional rate of protein synthesis in EMR than in AM in a control group of quails. In agreement with the above observation, EMR seems to be more sensitive to the influence of branched chain amino acids than AM.

The effect of valine, leucine and isoleucine on these two muscles was different, especially leucine influenced the value of FSR in AM and EMR quite differently. It seems that while leucine stimulates proteosynthesis in AM, which is in agreement with recent findings in mammals, it probably inhibits this process in EMR. In the case of isoleucine and valine, the results obtained in the ambiens muscle are in agreement with those reported in rats, namely that these two amino acids have little or no effect on proteosynthesis (Buse and Weigand 1977, Li and Goldberg 1976). Contrary to this observation are the results in EMR, where isoleucine and valine significantly decreased FSR. It thus seems that branched chain amino acids inhibit proteosynthesis in EMR.

Differences between mammals and Japanese quails were also found in the metabolism of branched chain amino acids. A dietary excess of leucine stimulates the oxidation of valine and isoleucine and contributes to

the reduction of their pools (Harper 1956). This amino acid antagonism observed in rats (Torres *et al.* 1993) and chickens (Smith and Austic 1978) was not confirmed in Japanese quails (Mason and Ward 1981).

While the protein content in both muscles did not change in spite of the increasing or decreasing FSR, it can be assumed that branched chain amino acids influenced not only proteosynthesis, but also degradation of muscle proteins. However, to confirm this assumption it is also necessary to evaluate the fractional rate of degradation in the same muscles.

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