Modulations of Muscle Protein Metabolism by Branched-Chain Amino Acids in Normal and Muscle-Atrophying Rats

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ABSTRACT It has been shown that BCAAs, especially leucine, regulate skeletal muscle protein metabolism. However, it remains unclear how BCAAs regulate muscle protein metabolism and lead to anabolism in vivo. We examined muscle protein synthesis rate and breakdown rate simultaneously during BCAA infusion in muscle atrophy models as well as in normal healthy rats. Corticosterone-treated rats and hindlimb-immobilized rats were used as muscle atrophy models. Muscle protein synthesis rate and breakdown rate were measured as phenylalanine kinetics across the hindlimb. In anesthetized normal rats, BCAAs stimulated muscle protein synthesis despite low insulin concentration and did not suppress muscle protein breakdown. In corticosterone-treated rats, BCAAs failed to restore inhibited muscle protein synthesis, but reduced muscle protein breakdown. Immobilization of hindlimb increased muscle protein breakdown within a day. BCAAs did not change muscle protein metabolism, although essential amino acids (EAA) suppressed muscle protein breakdown in hindlimb-immobilized rats. We also evaluated changes of fractional synthesis rate (FSR) of skeletal muscle protein during infusion of leucine alone or EAA for 4 h in anesthetized normal rats. FSR showed a transient increase at 15–30 min of leucine infusion and then declined, whereas FSR stayed elevated throughout EAA infusion. We concluded that 1) BCAAs primarily stimulate muscle protein synthesis in normal rats independently of insulin; 2) EAA is required to maintain the BCAA stimulation of muscle protein synthesis; and 3) The effects of BCAAs on muscle protein metabolism differ between atrophy models. J. Nutr. 136: 234S–236S, 2006.

KEY WORDS: branched-chain amino acids • leucine • essential amino acids • muscle protein metabolism • muscle atrophy
by the tracer incorporation technique, was observed within 90 min after a single bolus injection of leucine (20). Overall, it remains unclear how BCAAs regulate muscle protein metabolism and lead to anabolism in vivo, whether through stimulation of protein synthesis or inhibition of proteolysis.

**Effect of BCAA on muscle protein metabolism in normal rats**

Buse (6) intravenously infused BCAAs with glucose and isotope tracer into normal food-deprived rats for 6 h and observed increased tracer incorporation into muscle protein, which suggests increased muscle protein synthesis. In normal healthy rats, 1 h of intravenous infusion of BCAAs with glucose or with insulin stimulates muscle protein synthesis (7). A leucine-supplemented meal enhances the postprandial (90–120 min after meal) muscle protein synthesis rate in adult rats as well as old rats (14) and its anabolic effect persists at least 10 d (15). However, there was no stimulation of muscle protein synthesis either within 10 min after intravenous injection of leucine alone or 1 h after intraperitoneal injection of leucine alone (25). Oral gavage of leucine alone, however, increases the muscle protein synthesis rate 20 to 60 min after gavage in normal rats (8–10,12,16). As just described, leucine/BCAA stimulation of muscle protein synthesis in rats is reported in many papers, but there is no information on muscle protein breakdown because only isotope tracer incorporation techniques were used in all these studies. Therefore, we examined muscle protein synthesis rate and breakdown rate simultaneously during BCAA infusion in normal healthy rats under ketamine-xylazine general anesthesia, which blunts insulin secretion (26,27). Muscle protein synthesis rate and breakdown rate were measured as phenylalanine kinetics across the hindlimb, determined by the three-pool model (28), which used intramuscular phenylalanine enrichment combined with the arterial-venous balance method using stable isotope tracers in arterial-venous balance method using stable isotope tracers in normal rats. The stimulation is transient and independent of insulin in normal rats. The latter is comparable to the results of Crozier et al. (16), who found that oral administration of leucine as low as 135 mg/kg enhanced skeletal muscle protein synthesis, whereas it did not affect serum insulin concentration.

**Effect of BCAA on muscle protein metabolism in muscle atrophying rats**

The catabolic effects of glucocorticoids on muscle protein metabolism are well known. Glucocorticoid administration results in muscle atrophy. It is generally agreed that glucocorticoids inhibit muscle protein synthesis and stimulate muscle protein breakdown (29–34). We evaluated the effects of amino acid infusion on muscle protein synthesis and breakdown by an arterial-venous balance method using stable isotope tracers in corticosterone-treated (100 mg·kg⁻¹·day⁻¹ subcutaneously for 3 d) rats. When BCAAs (246 mg·kg⁻¹·h⁻¹ for 2 h; prime: 164 mg/kg) were infused into corticosterone-treated rats, improved net balance and reduced muscle protein breakdown were observed at the last 20 min of the infusion (H. Kobayashi, H. Suzuki, unpublished results). BCAA stimulation of muscle protein synthesis seen in normal rats was not observed in corticosterone-treated rats. Rieu et al. (35) reported that muscles from adult and old rats treated with dexamethasone became leucine resistant to protein synthesis. It has already been shown that glucocorticoids inhibit amino acid–mediated signaling pathways involved in control of muscle protein synthesis in rats (36) as well as in humans (37,38).

Muscle protein metabolism in disuse-atrophy model rats was also examined. Although it has been reported that muscle protein synthesis significantly declined during the first 6 h of immobilization (39), we found that immobilization of hindlimb by cast fixation for 1 d increased muscle protein breakdown. Meanwhile, muscle protein synthesis remained unchanged (H. Kobayashi, H. Suzuki, unpublished results). BCAA infusion (246 mg·kg⁻¹·h⁻¹ for 105 min; prime: 164 mg/kg) affected neither muscle protein synthesis nor breakdown in hindlimb-immobilized rats. However, essential amino acid (EAA) infusion (600 mg·kg⁻¹·h⁻¹ for 105 min; prime: 400 mg/kg) suppressed muscle protein breakdown in hindlimb-immobilized rats (H. Kobayashi, H. Suzuki, unpublished results). The difference in BCAA effects on muscle protein metabolism between rat models should be further studied.

**Time-dependent changes in muscle protein synthesis during amino acid infusion**

It has been reported that the positive effect of leucine infusion on muscle protein metabolism is transient in humans (40) and neonatal pigs (41). Also, it is suggested that availability of all amino acids (40,42) or all EAAAs (41) is necessary to sustain protein synthesis increased by leucine infusion. We evaluated changes of arterial plasma amino acid concentrations and fractional synthesis rate (FSR) of skeletal muscle protein during primed and constant infusion of leucine alone (100 mg·kg⁻¹·h⁻¹; prime: 66.7 mg/kg) or the same amount of leucine with other essential amino acids (EAA, 600 mg·kg⁻¹·h⁻¹; prime: 400 mg/kg) for 4 h in ketamine-xylazine anesthetized normal rats. FSR was determined by the flooding-dose method using L-[ring-2H₅]phenylalanine. During the infusion of leucine alone, plasma leucine concentration increased 3- to 4-fold and plasma concentrations of all other EAAAs were reduced. During the EAA infusion, plasma concentrations of EAAAs were increased 2- to 5-fold. FSR showed transient increase at 15–30 min of leucine infusion and then declined, whereas FSR stayed elevated throughout EAA infusion as expected (H. Kobayashi, H. Suzuki, et al., unpublished results). Leucine stimulation of muscle protein synthesis is transient and EAAAs are needed to be infused concomitantly to maintain an increased muscle protein synthesis rate as suggested. Increased muscle protein synthesis might not have been observed in human infusion studies (21–23) because leucine or BCAA was infused for 3 h or longer and muscle protein turnover rates were determined at the end of infusion.

**Conclusions**

Leucine primarily stimulates muscle protein synthesis in normal rats. The stimulation is transient and independent of insulin. Increased muscle protein synthesis is maintained by EAA infusion. Because different types of injury induce different protein metabolic responses (i.e., variations in synthesis versus
breakdown), it is not surprising that the BCAA effect differs according to the model. Determination of the profile of protein metabolism response in a given stress situation forms the rationale for providing or not providing pharmacological amounts of BCAAs.

ACKNOWLEDGMENT

We thank Kazutaka Shimbo, Shinich Ozawa, and Hiroshi Miyano for help in analysis, and Yoshiro Kitahara for helpful advice on atrophy model rats.

LITERATURE CITED