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Clofibrate treatment promotes branched-chain amino acid catabolism and decreases the phosphorylation state of mTOR, eIF4E-BP1, and S6K1 in rat liver

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Abstract

Leucine stimulates protein synthesis by modulating the mammalian target of rapamycin (mTOR) signaling pathway. We hypothesized that promotion of the branched-chain amino acid (BCAA) catabolism might influence the leucine-induced protein synthesis. Clofibric acid (an active metabolite of clofibrate) is known to promote the BCAA catabolism by activation of branched-chain α-keto acid dehydrogenase complex (BCKDC), the rate-limiting enzyme of the BCAA catabolism. In the present study, we examined the phosphorylation state of mTOR, eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), and ribosomal protein S6 kinase 1 (S6K1) in liver of rats with or without activation of the BCKDC by clofibrate treatment. Clofibrate-treated rats were prepared by oral administration of clofibrate 5 h before sacrifice. In order to stimulate phosphorylation of components in the mTOR signaling pathway, rats were orally administered with leucine 1 h before sacrifice. Clofibrate treatment almost fully activated hepatic BCKDC and significantly decreased the plasma leucine concentration in rats without leucine administration, resulting in decreased mTOR and 4E-BP1 phosphorylation. Similarly, in rats administered with leucine, clofibrate treatment attenuated the predicted increase in plasma leucine concentration as well as the phosphorylation of mTOR, 4E-BP1, and S6K1. These results suggest that BCAA catabolism enhanced by clofibrate treatment has significant influences on the leucine-induced activation of translation initiation processes.

Keywords: Clofibrate; Branched-chain α-keto acid dehydrogenase complex; mTOR; eIF; S6K1; 4E-BP1; Leucine

Introduction

The translation phase in protein synthesis comprises four sequential steps, namely initiation, elongation, termination, and reuse of ribosome. Initiation is the most important step in the regulation of translation (Pain, 1996). It has been reported that, after starvation, food intake stimulates protein synthesis in the liver and skeletal muscle of rats (Yoshizawa et al., 1998) and that food intake is critical for stimulation of translation initiation (Yoshizawa et al., 1997). The amino acids from dietary protein, especially leucine, are known activators of translation initiation factors (Anthony et al., 2000; Hong and Layman, 1984; Kimball et al., 1998). It has also been reported that branched-chain amino acids (BCAAs) promote albumin synthesis in rat primary hepatocytes through the mammalian target of rapamycin (mTOR) signaling pathway with no effect on the synthesis of intracellular proteins and the amount of albumin mRNA (Ijichi et al., 2003).

Regulation of translation initiation occurs principally by changes in the phosphorylation state of eukaryotic initiation factors (eIFs) (Proud, 2002). Phosphorylation of eIF4E-binding protein-1 (4E-BP1) releases eIF4E, which is then free to bind with eIF4G to form eIF4F, the complex required for translation initiation. This occurs because the binding site for 4E-BP1 on eIF4E overlaps with the eIF4G binding site. Thus, 4E-BP1 plays a key role in the regulation of mRNA translation in eukaryotic cytoplasm (Flynn and Proud, 1996; Gingras et al.,

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1999). In addition, leucine has been reported to stimulate phosphorylation of 4E-BP1 in various organs (Fox et al., 1998; Kimball et al., 1998; Xu et al., 1998).

Phosphorylation of the ribosomal protein S6 catalyzed by specific kinases, especially S6 kinase 1 (S6K1), results in preferential translation of mRNAs containing an oligopyrimidine tract at the 5′-end of the message (TOPS mRNAs), which include some elongation factors such as eEF1A and eEF2 (Kimball et al., 1999). Therefore, the S6K1 is suggested to play a key role in the regulation of protein synthesis by controlling the biosynthesis of translational components (Dufner and Thomas, 1999). The mTOR catalyzes phosphorylation of three sites on S6K1, namely Thr229, Thr389, and Ser404. Among these sites, phosphorylation of Thr389 plays the most important role in activation of S6K1 (Pearson et al., 1995).

Recent studies have demonstrated that mTOR mediates the activating effects of amino acids on 4E-BP1 and S6K1 (Kimball et al., 1999; Patti et al., 1998) and that mTOR Ser2448 phosphorylation is involved in the regulatory mechanism (Bolster et al., 2002; Peterson et al., 2000). Leucine is known to bring about activation of mTOR, which in turn signals phosphorylation of 4E-BP1 and S6K1. Time course studies on the changes in the phosphorylation state of 4E-BP1 and S6K1 induced by administration of leucine demonstrated that phosphorylation of these compounds in rat liver and muscle was at maximum levels 1 h after leucine loading (Yoshizawa et al., 2001).

Mammals have a characteristic catabolic system for leucine. The first step in this catabolic pathway is reversible transamination to form α-ketoisocaproate, which is catalyzed by the enzyme branched-chain aminotransferase (BCAT). Transamination of leucine occurs out of the liver, mainly in muscle, because BCAT is not found in adult rat liver (Hutson, 1989). The second step in the catabolism of leucine is irreversible oxidative decarboxylation of α-ketoisocaproate, which is catalyzed by the branched-chain α-keto acid dehydrogenase complex (BCKDC). This reaction is the rate-limiting step in the catabolism of leucine (Harper et al., 1984; Harris et al., 1994) and occurs mainly in the liver because the BCKDC activity is markedly higher in liver than in other organs (Harris et al., 1990). This organ specificity of enzymes that regulate BCAA catabolism is typical in rats (Suryawan et al., 1998).

The BCKDC activity is tightly controlled by a specific kinase (Shimomura et al., 1990a) and a specific phosphatase (Damuni and Reed, 1987). The kinase phosphorylates the E_1 subunit of the complex, turning it inactive, and the phosphatase dephosphorylates E_1 and reactivates the complex. Especially, the kinase plays a central role in the regulation of the BCKDC activity (Shimomura et al., 2001).

Clofibrate is a therapeutic agent for patients with hyperlipidemia. It has been reported that clofibric acid, an active metabolite of clofibrate, activates BCKDC and therefore increases the rate of leucine oxidative disposal (Kobayashi et al., 2002; Ono et al., 1990; Paul and Adibi, 1979; Paxton and Harris, 1984a). In addition, clofibric acid has been reported to inhibit protein synthesis (Paul and Adibi, 1980). These facts suggest that enhanced BCAA catabolism by clofibrate treatment might blunt signaling for protein synthesis. In the present study, we have examined this hypothesis using rats, and the liver was chosen as a target organ, since it contains a very high content of BCKDC.

Materials and methods

Materials

Rabbit polyclonal antibodies against phospho-mTOR (Ser2448), total-mTOR, and phospho-S6K1 (Thr389) were purchased from Cell Signaling Technology (Beverly, MA, USA), and goat anti-4E-BP1 and rabbit anti-S6K1 antibodies were purchased from Santa Cruz Biotechnology (CA, USA). Enhanced chemiluminescence (ECL) reagents and Immobilon-P polyvinylidene difluoride (PVDF) membrane were purchased from Amersham Pharmacia Biotech (Little Chalfont, Buckinghamshire, U.K.) and Millipore (Bedford, MA, USA), respectively. Lambda protein phosphatase was obtained from New England BioLabs (Beverly, MA, USA). All other reagents were of biochemical grade.

Animals

Twenty-eight male Sprague-Dawley rats (190–210 SLC Japan, Shizuoka, Japan) were housed in a conventional animal room with temperature at 24±2 °C and 12-h light/dark cycle (lighting beginning at 8:00 h). Semipurified diet [AIN 93 (Reeves et al., 1993) prepared by CLEA Japan, Tokyo, Japan] and water were provided ad libitum for 14 days before the final day of the experiment. The experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of the Nagoya Institute of Technology.

Experimental procedures

On the day of the experiment, the rats were deprived of food for 8 h (from 8:00 to 16:00 h) prior to sacrifice. Three hours after starting the fasting period, rats were randomly assigned to clofibrate-treated and control groups. The former animals were orally administered with 0.2 g/kg body weight (BW) clofibrate suspended in 0.5% methylcellulose (0.02 g clofibrate/ml), and the latter were administered with the same amount of vehicle only. Then, rats were returned to their cages and allowed free access to water only. Four hours later, rats were further divided into leucine and saline subgroups. Leucine was administered suspended in saline (18 g/l; 0.45 g/kg BW) and the same volume of saline only was administered to the remaining rats. One hour after leucine or saline administration, all rats were sacrificed by exsanguination under pentobarbital anesthesia; blood was taken from the inferior vena cava for preparation of plasma. Livers were quickly removed, immediately freeze-clamped at liquid nitrogen temperature, and then stored at −80 °C until analysed.

In many previous studies that examined the effects of leucine or protein ingestion on phosphorylation of the mTOR signaling pathway components, rats were starved for ~18 h before