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Octanoic acid promotes branched-chain amino acid catabolisms via the inhibition of hepatic branched-chain alpha-keto acid dehydrogenase kinase in rats

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ABSTRACT

Objective. It has been reported that administration of octanoic acid, one of medium-chain fatty acids (MCFAs), promoted leucine oxidation *in vitro* and *in vivo*, but it remained unclear how octanoic acid stimulated leucine oxidation. Therefore, the aim of this study was to elucidate the mechanism that octanoic acid facilitates branched-chain amino acid (BCAA) catabolism.

Materials/Methods. In *in vivo* experiments, male rats were orally administered MCFAs as free fatty acids or triacylglycerol (trioctanoin), and then activities of hepatic branched-chain α-ketoacid dehydrogenase (BCKDH) complex (BCKDC) and BCKDH kinase (BDK) and alterations in the concentration of blood components were analyzed. In *in vitro* experiments, purified BCKDC associated with BDK (BCKDH–BDK complex) was reacted with various concentrations of hexanoic, octanoic, and decanoic acids.

Results. Oral administration of trioctanoin in rats activated hepatic BCKDC via down-regulation of BDK activity in association with a decrease in plasma BCAA concentration and an increase in serum ketone body concentration. *In vitro* experiments using purified BCKDH–BDK complex showed that MCFAs (hexanoic, octanoic, and decanoic acids) inhibited BDK activity and that this inhibition was higher in hexanoic and octanoic acids than in decanoic acid. Oral administration of octanoic acid, but not decanoic acid, in rats activated hepatic BCKDC via down-regulation of BDK activity by decreasing the amount of BDK bound to the complex. The serum ketone body level was elevated by both administration of octanoic acid and decanoic acid.

Conclusion. These results suggest that octanoic acid promotes BCAA catabolism *in vivo* by activation of BCKDC via decreasing the bound form of BDK.

1. Introduction

Branched-chain amino acid (BCAA) catabolism is potently regulated by mitochondrial branched-chain α-ketoacid dehydrogenase (BCKDH) complex (BCKDC), which catalyzes the irreversible oxidative decarboxylation of the three branched-chain α-ketoacids (BCKAs) generated by reversible transamination of BCAAs in the BCAA catabolic pathway [1]. BCKDC is a

Abbreviations: MCFA, medium-chain fatty acid; BCAA, branched-chain amino acid; BCKDH, branched-chain α-ketoacid dehydrogenase; BCKDC, BCKDH complex; BDK, BCKDH kinase; BCKA, branched-chain α-ketoacid; MCT, medium-chain triacylglycerol; CMC, carboxymethylcellulose; FFA, free fatty acid.

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multienzyme complex composed of E1 (consisting of α and β components), E2 and E3 [1], and its activity is subject to regulation through reversible phosphorylation (inactivation) and dephosphorylation (activation) of the E1α component by a specific kinase (BCKDH kinase: BDK) and a specific phosphatase (BCKDH phosphatase), respectively [1]. The BDK bound to the complex (bound form) plays a particularly important role in the regulation of BCKDC activity, as the amount of bound BDK is inversely correlated with the complex activity [2]. Known mechanisms for acute control of the activity of BCKDC include direct inhibition of the activity of the complex by NADH and CoA esters derived from the BCAAs [3], and activation of the complex can also be achieved in the short-term by inhibition of BDK activity by α-ketoisocaproate, the transamination product of leucine [4]. Moreover, α-chloroisocaproate [5] and clofibric acid [6–9] also promote activation of BCKDC by inhibition of BDK.

Since total free fatty acid (FFA) and BCAA concentration in blood is increased in type 2 diabetes mellitus [10,11], there are many reports determined the relationship between FFA or FFA oxidation and BCAA catabolism [11,12]. It is known that long-chain fatty acids and their metabolites inhibit BCKDC activity either by affecting redox states or acetyl-CoA concentrations [13–16]. In contrast, it has been demonstrated that administration of octanoic acid, one of the medium-chain fatty acids (MCFAs), promotes leucine oxidation *in vitro* and *in vivo* [16–18]. MCFAs contain 6–10 carbon fatty acids and are found in milk products, chocolate and coconut oil as a medium-chain triacylglycerol (MCT). In the animal system for digestion and absorption, MCFAs generated by a breakdown of MCT are absorbed through the portal system without resynthesis of triacylglycerol and are predominantly subjected to β-oxidation in the liver [19]. Since MCFAs are components of foods and MCT is used as a food additive, it may be important to clarify the potential effects of MCFAs on the metabolic systems of humans and animals, as well as its mechanisms.

As described above, octanoic acid is known to promote BCAA oxidation, which has been attributed to the activation of BCKDC [9,16–18]. This activation of BCKDC may be due to the inhibition of BDK by octanoic acid, but clear evidence of the effects of MCFAs on BDK is not yet available. In order to clarify the mechanism responsible for the activation of BCKDC by MCFAs including octanoic acid, we first examined the effect of trioctanoin administration in rats on BCAA catabolism, in which hepatic BCKDC and BDK activities were measured. Secondary, we examined the influence of MCFAs on hepatic BDK activity in both *in vitro* and *in vivo* experiments.

2. Materials and Methods

2.1. Materials

Trioctanoin, α-ketoisovaleric acid and pyruvate dehydrogenase were from Sigma Aldrich Japan (Tokyo, Japan). Butanoic acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, 0.5% carboxymethylcellulose (CMC), and 0.5% methylcellulose were purchased from Wako Pure Chemical Industries (Osaka, Japan), lambda protein phosphatase was from New England BioLabs (Beverly, MA), and heparin was from Mochida Pharmaceutical (Tokyo, Japan). All other reagents were of biochemical grade.

2.2. Animals

All procedures were approved by the Animal Care Committee of the Nagoya University Graduate School of Bioagricultural Sciences. Female Sprague-Dawley rats aged 8 weeks were obtained from CLEA Japan (Tokyo, Japan) and were housed in a conventional animal room at 22 ± 1 °C, on a 12:12-h light–dark cycle. Animals were fed the CE-2 rodent chow (CLEA Japan) *ad libitum* for 1 week during acclimatization to the animal room, and then used in the experiments.

2.3. Animal Experimental Design

In all animal experiments, rats were deprived of food for about 9.5 h (from 08:00 to 17:30) prior to sacrifice. In the experiments examining MCT dose–response, rats were randomly assigned to six groups, and administered 3 ml of 0.5% CMC or trioctanoin (0.4, 1.0, 1.4, 1.9, or 2.4 mg, diluted with 0.5% CMC to 3 ml) by oral gavage at 12:00. In the following experiment, rats were randomly assigned to control, octanoic acid-treated, and decanoic acid-treated groups. The octanoic acid-treated group was orally administered 1.3 mg of octanoic acid diluted with 0.5% methylcellulose to 3 ml. The decanoic acid-treated group was orally administered 1.5 mg of decanoic acid diluted with 0.5% methylcellulose to 3 ml, and the control group was administered the same volume (3 ml) of vehicle only at 12:00. Exactly 5.5 h after oral administration, the rats were anesthetized with sodium pentobarbital (60 mg/kg body weight) and blood was collected from the inferior vena cava. The liver was collected, freeze-clamped at liquid nitrogen temperature, and then stored at −80 °C until used in the enzyme activity assay.

2.4. Blood Components Assay

The analyses of glucose, total FFA, and total ketone body concentrations in serum were performed by Special Reference Laboratories (SRL) (Tokyo, Japan) using standard clinical methods [20–22]. Plasma BCAA concentration was measured using an amino acid auto-analyzer (Amino Tac, JLC-500/V; Jeol, Tokyo, Japan) with ninhydrin derivatisation as described previously [23]. Plasma BCKA concentration was measured using enzymatic method as described previously [24].

2.5. Assay of BCKDC and BDK Activities

Preparation of liver extracts and assay of BCKDC activity were conducted as described previously [25]. The measurements of actual (the activity *in vivo*; complex partially in active/dephosphorylated state) and total (the activity of the fully active/dephosphorylated enzyme) activities of BCKDC were conducted separately. Dephosphorylation of the complex for measurement of the total activity was accomplished by incubating the enzyme extract with lambda protein phosphatase, as described previously [25]. One unit of the enzyme activity refers to the production of 1 μmol of NADH/min.