Vol. 172, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 15, 1990 Pages 243-248

REGULATION BY INDUCTION OF BRANCHED-CHAIN 2-OXO ACID DEHYDROGENASE COMPLEX IN CLOFIBRATE-FED RAT LIVER

Kazuo Ono, Hiroshi Shioya, Masatoshi Hakozaki, Kazuyuki Honda, Tsutomu Mori and Hideo Kochi[1]

Department of Biochemistry, Fukushima Medical College, Hikari-ga-oka, Fukushima 960-12, Japan

Received September 1, 1990

Summary: The activity of branched-chain 2-oxo acid dehycrogenase complex increased 3.0-fold in liver of rats fed on 0.1%(w/w) clofibrate. Immunotitration experiments with antibodies against the constituent enzymes of the complex revealed that this increase resulted mainly from the increased amounts of only two(a decarboxylase and a lipoate acyltransferase) of three components of the complex and that the other component(dihydrolipoamide dehydrogenase) remained unchanged in its content, irrespective of clofibrate administration. The increases of both enzyme components were associated with increases in their mRNA levels which were estimated by in vitro translation with poly(A)$^+$ RNA.

Branched-chain 2-oxo acid dehydrogenase(BCKADH)[EC 1.2.4.4] is an intramitochondrial multi-enzyme complex comprising three enzyme components, a decarboxylase(E_1), a dihydrolipoyl transacylase (E_2) and dihydrolipoamide dehydrogenase(E_3)[EC 1.6.4.3]. The enzyme complex catalyzes the oxidative decarboxylation of branched-chain 2-oxo acids, the first committed step for the flux of branched-chain amino acid catabolism. Based on the evidences reported so far, liver has the highest BCKADH activity among animal tissues and organs(1) and is considered to be the principle organ for catabolism of branched-chain 2-oxo acids(2).

It is known that the activity state of BCKADH complex is regulated by phosphorylation(inactivation)/dephosphorylation(activation) of E_1 of the complex(3). A protein kinase intrinsic to the complex(4) and BCKADH-specific phosphatase(5) is considered to participate in the conversion of the activity state each other. This regulatory mechanism appears usually to occur in both skeletal muscle(1) and adipose tissue(6) where BCKADH content is extremely low as compared with liver, but not to be the case in liver where most of BCKADH exists as the active form(7). Moreover, no other mode of regulation for the enzyme complex is known.

[1] To whom correspondence should be addressed.

Abbreviations: BCKADH, branched-chain 2-oxo acid dehydrogenase complex; E_1, decarboxylase component of BCKADH; E_2, acyltransferase component of BCKADH; E_3, dihydrolipoamide dehydrogenase; SDS-PAGE, sodium dodesylsulfate-polyacrylamide gel electrophoresis.

0006-291X/90 $1.50

It is previously reported that clofibrate feeding to rat causes hepatomegaly accompanied by the increase in the ratio of mitochondria to cells(8), thereby increasing liver BCKADH activity(9). However, this increase in liver BCKADH activity can not be explained either by the change in the activity state of the complex or by hepatomegaly, or both of them.

In the present study, we describe that the increase in liver BCKADH activity of clofibrate-fed rat was basically due to the increase in the contents of both E_1 and E_2, but not of E_3, of the complex, which resulted from elevation of the mRNA levels for both enzyme components.

MATERIALS AND METHODS

Materials. Radioactive compounds and rabbit reticulocyte lysate were the products of Amersham. Oligo(dT)-cellulose(type 2) was purchased from Collaborative research. Clofibrate was a gift from ICI. Na-2-chloroisocaproate was made from D,L-leucine by the method of Harris et al.(10). Other chemicals used were of analytical grade. A broad spectrum phosphatase was partially purified from rabbit liver(11).

Treatment of animals and preparation of enzyme samples. Male Wistar rats weighing about 200 g were used. Standard rat chow pellets(CE-2, Clea Japan Inc.) were mixed with acetone(for the control diet) or clofibrate diluted with acetone to a final clofibrate concentration in the chow of 0.1 %(w/w). The chow was dried completely to remove acetone. Liver homogenate, mitochondria and mitochondrial extract were prepared as described(12). Phosphatase treatment was done by incubating mitochondrial extracts(containing HEPES instead of phosphate buffer) with a broad spectrum phosphatase in the presence of 0.1mM Na-2-chloroisocaproate, a BCKADH kinase inhibitor, for 40 min at 30°.

Enzyme assays. BCKADH activity was assayed as previously described(12). 2-Oxo acid substrate employed for the assay was either [1-^{14}C]-labeled 2-oxoisovalerate at a concentration of 5 mM(with homogenate or intact mitochondria) or non-labeled 2-oxoisovalerate at a concentration of 0.2 mM(with mitochondrial extracts). Pyruvate and 2-oxoglutarate dehydrogenase complexes were assayed by measuring $^{14}CO_2$ formed from respective [1-^{14}C]-labeled substrates(13). E_3 activity was determined by monitoring lipoamide-dependent NADH oxidation at 340nm(14). The assay for branched-chain amino acid aminotransferase was performed by the method of Ichihara et al.(15) with leucine as substrate. One unit of enzyme activity was defined as the enzyme amount either forming one μmol of product or transforming one μmol of substrate per min.

Immunological methods. Antibodies were raised in rabbits with purified E_1, E_2, E_3 or E_1-E_2 subcomplex of rat liver BCKADH(12) as antigen and purified by DE-23 column chromatography(16). For immunotitration, mitochondrial extract(0.4 mg of protein) was incubated with varied amounts of rabbit IgG for 12 hr at 4°. The mixture was centrifuged and the resulting supernatant was used for enzyme assay.

Preparation of poly(A)$^+$RNA. Total RNA was extracted from rat liver by the method of Chirgwin et al.(17). Poly(A)$^+$RNA was isolated from total RNA according to the manufacturer's instructions. The yields were 70-80 μg poly(A)$^+$RNA/g liver and practically the same between clofibrate-fed and control rats.

In vitro translation and fluorography. Translation assay was carried out in the mixture containing 2 μg of poly(A)$^+$RNA and 20 μCi of [^{35}S]methionine in a total volume of 25 μl in rabbit reticulocyte lysate system. After incuvation at 30° for 60 min, the translation mixture was diluted with 9 volume of ice cold 0.15 M NaCl in 10 mM Tris-HCl(pH7.4) containing 0.1 % Triton X-100, 0.1 % SDS and 5 mM EDTA. To the diluted mixture was added 5 μg of IgG followed by incubation at 4° overnight. The nixture was then treated with 1 mg of Protein A-Sepharose(7 μg as Protein A) for 60 min by gentle agitation. The Sepharose gell was extensively washed with the above buffer containing 1 mM methionine by means of repeating centrifugation. Proteins were dissociated from the gel by addition of the SDS-PAGE buffer of Laemli(18). The extracted proteins were heated for 2 min in boiling water, then

subjected to 10 % slab-gel electrophoresis(12). Radioactive proteins were visualized by fluorography and quantitated by densitometric scanning of the fluorograms with HITACHI 557 spectrophotometer.

RESULTS

When rats were fed on 0.1% clofibrate-containing diet for 14 days the specific BCKADH activity of liver increased 3.0-fold(Table 1). The activity calculated on g liver basis was a 4.3-fold increase(see below) since clofibrate administration caused the increase in mitochondria content of cells(9). Catalase activity also increased undoubtedly due to peroxisome proliferation(19). No activity increase was observed for E_3, and for pyruvate and 2-oxoglutarate dehydrogenase complexes as well.

This increase in BCKADH activity must have resulted mainly from the increase of enzyme component(s) of the complex since most BCKADH in liver is thought to be in the active state(8), but E_3 is unlikely to be the one. This was examined by the immunotitration experiments with anti-E_1-E_2 and anti-E_3 IgG.

As shown in Fig. 1A, clofibrate administration caused a 2.8-fold increase in the specific BCKADH activity, and the phosphatase treatment increased only the activity of control rat by 16 %. The immunotitration experiments showed a 2.4-fold increase in E_1-E_2 content of clofibrate-fed rat(Fig. 1A), but no increase in E_3(Fig. 1B). Similar experiment employing anti-E_1 and anti-E_2 IgG suggested that both E_1 and E_2 contents increased in clofibrate-fed rat liver(data not shown).

To elucidate the mechanism of BCKADH increase, we measured E_1 and E_2 mRNA activities by analyzing the in vitro translation products of poly(A)$^+$ RNA(Fig. 2). When translation products were immunoselected with anti-E_1 IgG, two main protein bands were detected on SDS-PAGE(lane 1 in Fig. 2A). These proteins were identified as in vitro translated α(45 kDa) and β(35 kDa) subunits of E_1(12) since the addition

Table 1. Effect of clofibrate administration on various enzyme activities of rat liver

Enzyme	Enzyme activity(munit/mg protein)[a]	
	Control	Clofibrate-fed
Lactate dehydrogenase	1780±270	2010±320
Catalase	528±36	1152±247
Succinate dehydrogenase	11.3±4.1	8.8±1.3
Glutamate dehydrogenase	85.3±18.2	85.5±10.2
Branched-chain amino acid aminotransferase	0.52±0.07	0.52±0.01
Branched-chain 2-oxo acid dehydrogenase	5.31±0.63	16.14±2.63
Dihydrolipoamide dehydrogenase	543±33	570±32
Pyruvate dehydrogenase	28.2±4.0	28.6±7.2
2-Oxoglutarate dehydrogenase	24.7±2.1	25.8±3.3

[a] Rats were fed on control or 0.1% clofibrate-containing diet for 14 days. Enzyme assays were done with intact mitochondria except lactate dehydrogenase, catalase and branched-chain amino acid aminotransferase which were assayed with homogenate as described in Materials and Methods. Values are expressed as means±S.D. for five rats.

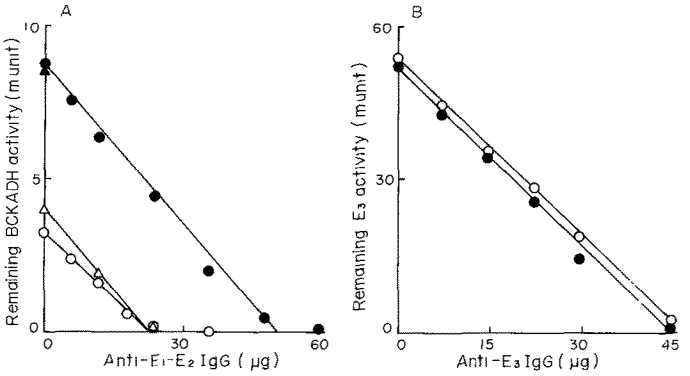

Fig. 1. Quantitative measurement of E_1-E_2 and E_3 in control and clofibrate-fed rat liver by immunotitration. Varied amounts of either anti-E_1-E_2 IgG or anti-E_3 IgG were added to phosphatase-pretreated (triangle) or -untreated(circle) mitochondrial extracts(0.4 mg of protein) of rats fed on control(open symbols) and 0.1% clofibrate-containing diet for 14 days(closed symbols). Immunoprecipitates formed were removed by centrifugation and the resulting supernatants were assayed for BCKADH(A) and E_3(B) activities as described in Materials and Methods.

of purified non-radioactive E_1 specifically excluded both the radioactive bands by competition. Similarly, the 53 kDa protein was found to be the product of E_2 mRNA(12). Hence, mRNA activities for $E_1\alpha$, $E_1\beta$ and E_2 could be measured by scanning autoradiograms(lane 6-10).

Figure 2B shows the increases in respective mRNA levels as well as in BCKADH activity during clofibrate administration. In the experiment, BCKADH activity was calculated on g liver basis since poly(A)$^+$RNA yield per g liver was practically the same between clofibrate-fed and control rat. All $E_1\alpha$, $E_1\beta$ and E_2 mRNA activities were slightly increased on day 3 though $E_1\alpha$ and $E_1\beta$ mRNA increases appeared to be preceded by E_2 mRNA increase. Maximum increases were observed on day 5 of clofibrate administration for all $E_1\alpha$, $E_1\beta$ and E2 mRNAs, and were 3.1-, 4.6- and 3.5-fold, respectively. On the contrary, BCKADH activity increase was evident on day 3 and as high as 2.0-fold. This value was equal to a 1.4-fold increase as calculated in terms of the specific activity for mitochondrial proteins. Maximum BCKADH activity(a 4.2-fold increase) was observed on day 8 of clofibrate administration.

DISCUSSION

Above results show that the increase in liver BCKADH activity by clofibrate administration resulted from both enzyme activation, probably due to dephosphorylation of $E_1\alpha$ subunit(3), and induction of E_1 and E_2 due to the increase in respective mRNA levels. However, the enzyme induction was much more dominant for the activity increase than the enzyme activation. It should be noted that E_3, which is also a constituent enzyme not only of pyruvate and 2-oxoglutarate dehydrogenase complexes analogous to BCKADH but also of glycine cleavage system in common(13), remained unchanged in its activity during clofibrate administration.

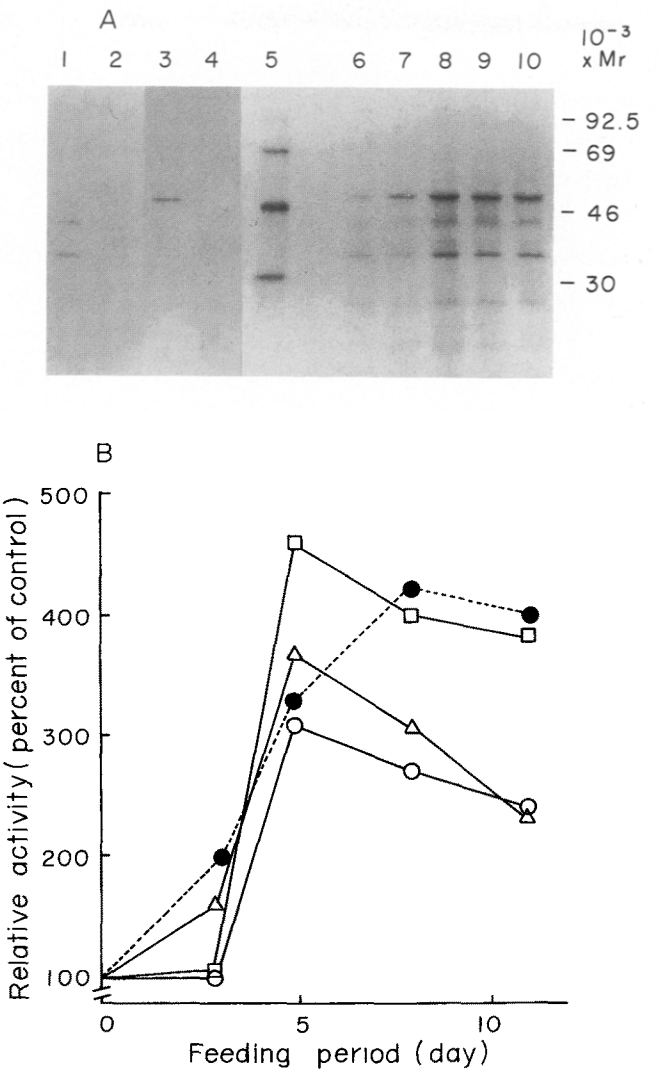

Fig. 2. Effect of clofibrate administration on mRNA levels for $E_1\alpha$, $E_1\beta$ and E_2 as well as on BCKADH activity. A, Quantitative measurement of mRNA levels by analysis of in vitro translation products. In vitro translation products of poly(A)+RNA(2 μg) were treated with 5 μg of anti-E_1(lane 1-2), anti-E_2(lane 3-4) or anti-E_1-E_2(lane 6-10) IgG and Protein A Sepharose(1 mg), then subjected to 10% SDS-PAGE followed by fluorography as described in Materials and Methods. Lane 1-4; with poly(A)+RNA from rat fed on 0.1% clofibrate-containing diet for 14 days. In lanes 2 and 4 were added purified E_1(7.2 μg) and E_2(4 μg), respectively, at immunoreaction step. Lane 5; ^{14}C-labeled molecular markers. Lane 6; with poly(A)+RNA of control rat. Lane 7-10; with poly(A)+RNA of rat fed on 0.1% clofibrate-containing diet for 3, 5, 8 and 11 days. B, Time course of mRNA levels and of BCKADH activity. Messenger RNA activities for α(○) and β(□) subunits for E_1 and for E_2(△) were assigned into an arbitrary unit by scanning fluorogram in A(lane 6-10) and expressed as percent of control mRNA activity. BCKADH activities(per g wet weight liver, ●) were determined with homogenate at the indicated time and expressed as relative ratio to control activity.

Induction of BCKADH is previously reported to occur during insulin-mediated preadipocyte differentiation(20), but this could be a non-specific phenomenon accompanying differentiation of preadipocytes since pyruvate and 2-oxoglutarate

dehydrogenase complexes as well as all three components of BCKADH were simultaneously induced.

In our preliminary experiments with primary cultured rat hepatocytes, the increase in BCKADH activity through phosphorylation/dephosphorylation mechanism occurred within 6 h in the presence of 0.1 % clofibrate while the increases in E_1 and E_2 contents were observed later on. Furthermore, the increases of both enzyme components by clofibrate administration were completely abolished in the presence of actinomycin D(to be published). Hence, two mechanisms appear to function on the increase in liver BCKADH activity; one is a short-term regulation resulting from change of the activity state of BCKADH and the other is a long-term one resulting from enhancement of the transcription rates of both E_1 and E_2 genes.

REFERENCES

1. Wagenmakers, A.J.M., Schepens, J.T.G. & Veerkamp, J.H.(1984) Biochem. J. 223, 815-821.
2. Harper, A.E., Miller, R.H. & Block, K.P.(1984) Ann. Rev. Nutr. 4, 409-454.
3. Fatania, H.R., Patston, P.A. & Randle, P.J.(1983) FEBS Lett. 158, 234-238.
4. Cook, K.G., Bradford, A.P. & Yeaman, S.J.(1985) Biochem. J. 225, 731-735.
5. Damuni, Z. & Reed. L.J.(1987) J. Biol. Chem. 262, 5129-5132.
6. Frick, G.P. & Goodman, H.M.(1989) Biochem. J. 258, 229-235.
7. Solomon, M., Cook, K.G. & Yeaman, S.J.(1987) Biochim. Biophys. Acta 931, 335-338.
8. Lipsky, N.G. & Pederson, P.L.(1982) J. Biol. Chem. 257, 1473-1481.
9. Wagenmakers, A.J.M., Veerkamp, J.H., Schepenes, J.T.G. & van Moerkerk, H.T.B.(1985) Biochem. Pharmacol. 34, 2169-2173.
10. Harris, R.A., Kuntz, M.J. & Simpson, R.(1988) Methods in Enzymol. 166, 114-123.
11. Paxton, R. & Harris, R.A.(1984) Arch. Biochem. Biophys. 231, 48-57.
12. Ono. K., Hakozaki, M., Kimura, A. & Kochi, H.(1987) J. Biochem. 101, 19-27.
13. Kochi, H., Seino, H. & Ono, K.(1986) Arch. Biochem. Biophys. 249, 263-272.
14. Ide, S., Hayakawa, T., Okabe, K. & Koike, M.(1967) J. Biol. Chem. 242, 54-60.
15. Ichihara, A. & Koyama, E.(1966) J. Biochem. 59, 160-169.
16. Levy, H.B. & Sorber, H.A.(1960) Proc. Soc. Exp. Biol. Med. 103, 250-252.
17. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. & Rutter, W.J.(1979) Biochemistry 18, 5294-5299.
18. Laemmli, U.K.(1970) Nature 227, 680-685.
19. Reddy, J.K., Warren, J.R., Reddy, M.K. & Lalwani, N.D.(1982) Ann. N.Y. Acad. Sci. 386, 81-110.
20. Chuang, D.T., Hu, C.W.C. & Patel, M.S.(1983) Biochem. J. 214, 177-181.