Effects of Dicarboxylic Acids on Fatty Acid-Metabolizing Enzymes in Cultured Rat Hepatocytes

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A clear chain-length dependent effect was observed for peroxisomal fatty acid β -oxidation and carnitine acetyltransferase and also for mitochondrial carnitine palmitoyltransferase in primary cultures of rat hepatocytes. The extent of modulation of peroxisomal β -oxidation was higher with even-carbon numbered dicarboxylic acids than with odd-carbon numbered ones, although such a tendency was not detected in the mitochondrial reactions. These results indicate difference in the effect of fatty acid-derived dicarboxylates on peroxisomal and mitochondrial functions.

Key words dicarboxylic acid; fatty acid metabolism; hepatocyte culture

Increased excretion of even- and odd-numbered saturated dicarboxylic acids (DCAs) in urine has been reported for rats following starvation and with diabetes where fatty acid oxidation is increased.¹⁾ DCAs are formed from fatty acids through microsomal cytochrome P4504A (CYP4A)-mediated ω -hydroxylation in liver or kidney.²⁾ The physiological importance of DCA formation remains unclear. It seems, however, that overproduction of DCAs affects fatty acid metabolism in the liver. In the present study, we tested the effects of several different dicarboxylic acids on the induction of microsomal, peroxisomal, and mitochondrial fatty acid-metabolizing enzymes using primary cultures of rat hepatocytes.

MATERIALS AND METHODS

Dodecanoic acid (DCA-12) and clofibrate (CLF) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Anti-rat CYP4A serum was obtained from Daiichi Pure Chemicals (Tokyo, Japan). Dicarboxylic acids (DCA-13—DCA-18) (DCA-*n* indicates a dicarboxylic acid with *n* carbon atoms) were gifts from Japan Energy Co. (Saitama, Japan).

Hepatocytes isolated from normal male Sprague-Dawley rats (Clea Japan) were plated on a dish coated with matri-gel at a density of 1×10^7 cells/10 cm dish and cultured in 8 ml Waymouth 752/1 modified medium.³⁾

CLF and DCAs were dissolved in dimethyl sulfoxide (DMSO). Addition of 100 μ M CLF or DCA was normally started 3 d after plating and continued until Day 5. Control cultures were exposed to 0.2% DMSO alone. Culture media were changed every 24 h. Cyanide-insensitive palmitoyl-CoA oxidase⁴⁾ and carnitine acetyltransferase⁵⁾ activities were measured in the $9000 \times g$ supernatant of the cell homogenate. The activities of carnitine palmitoyltransferase⁵⁾ and succinate dehydrogenase⁶⁾ were assayed in the mitochondrial fraction. The total CYP4A content and laurate ω -hydroxylase activity⁷⁾ were quantitated in the microsomal fraction. Protein was determined by the method of Lowry *et al.*⁸⁾ For all these assays, samples were prepared from the same culture dish in each experiment. The results are expressed as means $\pm$ S.D. Statistical significance was assessed by Student's *t*-test.

RESULTS AND DISCUSSION

In this culture system, microsomal total CYP4A content (2.8-fold) and laurate ω -hydroxylase activity (5.1-fold) were increased following treatment of hepatocytes with 100 μ M CLF (Table 1). Peroxisomal palmitoyl-CoA oxidase and carnitine acetyltransferase activities were increased 1.6-fold and 1.5-fold, respectively, although there were no marked changes in mitochondrial carnitine palmitoyltransferase and succinate dehydrogenase activities.

Table 1. Effects of CLF and DCAs on Microsomal, Peroxisomal and Mitochondrial Enzyme Levels in Cultured Hepatocytes

Treatment	Total ^{a)} CYP4A content	Laurate ^{b)} ω -hydroxylase	Palmitoyl-CoA ^{c)} oxidase	Carnitine ^{c)} acetyltransferase	Carnitine ^{c)} palmitoyltransferase	Succinate ^{c)} dehydrogenase
Control (DMSO)	4.65 $\pm$ 0.66	10.0 $\pm$ 1.5	2.19 $\pm$ 0.06	10.87 $\pm$ 0.83	3.50 $\pm$ 0.79	2.69 $\pm$ 0.69
CLF	12.98 $\pm$ 0.54*	51.0 $\pm$ 1.9*	3.54 $\pm$ 0.40*	16.74 $\pm$ 0.95*	3.44 $\pm$ 0.37	2.29 $\pm$ 0.71
DCA-12	4.82 $\pm$ 0.20	9.0 $\pm$ 1.7	3.44 $\pm$ 0.18*	13.37 $\pm$ 0.67	3.01 $\pm$ 0.65	2.97 $\pm$ 0.34
DCA-13	5.05 $\pm$ 0.56	13.2 $\pm$ 0.9	2.54 $\pm$ 0.28	10.63 $\pm$ 0.70	2.64 $\pm$ 0.66	2.22 $\pm$ 0.40
DCA-14	6.27 $\pm$ 0.61	17.0 $\pm$ 4.3	2.93 $\pm$ 0.20*	13.50 $\pm$ 0.85	2.61 $\pm$ 0.87	2.08 $\pm$ 0.70
DCA-15	4.60 $\pm$ 0.42	11.7 $\pm$ 1.7	2.25 $\pm$ 0.20	11.81 $\pm$ 0.56	2.64 $\pm$ 0.60	1.61 $\pm$ 0.22
DCA-16	6.84 $\pm$ 0.27*	18.0 $\pm$ 3.1*	2.94 $\pm$ 0.24*	14.74 $\pm$ 0.94*	2.91 $\pm$ 0.81	1.49 $\pm$ 0.26*
DCA-17	4.58 $\pm$ 0.29	14.9 $\pm$ 3.9	2.54 $\pm$ 0.24	10.84 $\pm$ 0.63	2.60 $\pm$ 0.18	1.24 $\pm$ 0.09*
DCA-18	4.38 $\pm$ 0.42	11.5 $\pm$ 1.2	2.47 $\pm$ 0.30	12.30 $\pm$ 0.67	1.82 $\pm$ 0.27*	1.40 $\pm$ 0.32*

Rat hepatocytes were cultured as described in the Materials and Methods section. Each value represents the mean $\pm$ S.D. a) pmol/mg protein/min. b) pmol/mg protein/min. c) nmol/mg protein/min. * Significantly different from control, $p < 0.05$.

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Since the increase in peroxisomal fatty acid β -oxidation following treatment with CLF and other peroxisome proliferators is accompanied by induction of microsomal fatty acid ω -hydroxylation *in vivo* and *in vitro*,^{9,10} the DCAs formed *via* ω -hydroxylation of fatty acids may induce peroxisomal acyl-CoA oxidase and modify fatty acid metabolism. Therefore, DCAs with different chain lengths were added to the serum- and fatty acid-free medium to evaluate their actions on fatty acid metabolism in rat hepatocytes. The highest concentrations of DCAs that were completely soluble in the culture medium were used in this study. Peroxisomal palmitoyl-CoA oxidase activity was significantly increased by the addition of DCA-12 (1.6-fold), DCA-14 (1.3-fold), or DCA-16 (1.3-fold) in cultured hepatocytes, whereas DCA-13, DCA-15, DCA-17, and DCA-18 had no significant effect on enzyme activity. Carnitine acetyltransferase activity was also increased in a pattern similar to palmitoyl-CoA oxidase, suggesting that DCAs with an even number of carbon atoms act as better inducers of peroxisomal fatty acid β -oxidation than DCAs with odd numbers of carbon atoms. Prior to further metabolism of DCAs, formation of the corresponding dicarboxylic acyl-CoA esters is necessary. Thus, we examined the formation of CoA-derivatives of DCAs in liver. The formation was chain-length dependent and was optimal for DCA-12 in rat liver microsomes, consistent with the report by Vamecq, *et al.*¹¹ The extent of formation of DCA-CoA derivatives was not significantly different between DCA-12 and DCA-13, between DCA-14 and DCA-15, or between DCA-16 and DCA-17 (data not shown). Thus, the changes in peroxisomal β -oxidation in DCA-treated hepatocytes cannot be attributed to the extent of DCA-CoA derivative formation. In addition, the activity of mitochondrial carnitine palmitoyltransferase was slightly decreased by treatment of hepatocytes with DCAs in primary culture. A significant difference was observed in the carnitine palmitoyltransferase activity in cells treated with DCA-18. This finding suggests that added DCAs do not stimulate

fatty acid β -oxidation in the mitochondria of hepatocytes. Succinate dehydrogenase activity was significantly decreased by the addition of DCA-16, DCA-17, or DCA-18, suggesting that direct addition of excess amounts of longer chain DCAs impairs mitochondrial function in cultured hepatocytes.

In summary, we have shown that long-chain DCAs evoke diverse responses in the fatty acid-oxidizing enzyme functions of liver microsomes, peroxisomes, and mitochondria. Added long-chain DCAs with even numbers of carbon atoms, but not odd numbered ones, increase fatty acid β -oxidation in peroxisomes in primary cultures of rat hepatocytes. Endogenous DCAs accumulate in starved or diabetic rats and these results suggest the possible effect of DCAs on fatty acid-metabolizing enzymes in the liver although, of course, further detailed studies are required.

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