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COMPARISON OF THE INDUCING EFFECT OF DEHYDROEPIANDROSTERONE ON HEPATIC PEROXISOME PROLIFERATION-ASSOCIATED ENZYMES IN SEVERAL RODENT SPECIES

A SHORT-TERM ADMINISTRATION STUDY

MITSUHIRO SAKUMA, JUNJI YAMADA* and TETSUYA SUGA
Department of Clinical Biochemistry, Tokyo College of Pharmacy, Tokyo, Japan

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Abstract—The *in-vivo* effect of dehydroepiandrosterone (DHEA) on hepatic enzyme activities of rats, mice, hamsters and guinea pigs was investigated. After DHEA treatment (300 mg/kg body weight, *per os*, 14 days), the activities of peroxisomal β-oxidation, catalase, carnitine acetyltransferase, carnitine palmitoyltransferase, lauric acid ω-hydroxylation, 1-acylglycerophosphocholine acyltransferase, malic enzyme and cytosolic palmitoyl-CoA hydrolase were increased in rats and in mice although to a smaller extent in the latter. These enzyme activities, however, were unchanged in hamsters with the exception of ω-hydroxylation (2.5-fold increase) and 1-acylglycerophosphocholine acyltransferase (2.0-fold increase). No significant changes were observed in any of these enzyme activities in guinea pigs. Immunoblot analysis confirmed the induction of peroxisomal acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme in rats and mice. These results indicate that there are species differences in the inducing effect of DHEA on hepatic peroxisome proliferation-associated enzymes, which correlates well with the enzyme induction observed with other peroxisome proliferators.

Dehydroepiandrosterone (DHEA†) is a naturally occurring C_{19} steroid in mammals, and has been shown recently to be a peroxisome proliferating agent [1–4]. The first report on the inducing effect of DHEA on peroxisome proliferation appeared in 1987 [1]. In that study, Leighton *et al.* found marked induction of peroxisomal acyl-CoA oxidase in the liver of rats treated with DHEA. Subsequently, Wu *et al.* [2], Frenkel *et al.* [3] and Yamada *et al.* [4] demonstrated DHEA-induced peroxisome proliferation in the liver of rats or mice based on morphological examination; co-induction of peroxisomal and extra-peroxisomal enzymes which are associated with fatty acid metabolism also has been revealed. In addition, our previous studies [4, 5] showed that the magnitude of the enzyme induction varies in different sexes and tissues. Thus, it appears that DHEA is a typical peroxisome proliferator having properties common to the other known peroxisome proliferators.

The present study was designed to further characterize DHEA as a peroxisome proliferator by investigating the effect of DHEA on hepatic peroxisome proliferation-associated enzymes in several rodent species. The inducing effect of peroxisome proliferators is manifested in the liver of various animals [6–8]. However, the magnitude of peroxisome proliferation and enzyme induction varies in different species and is maximal in rats and/or mice [6–13]. To examine whether this is also the case for DHEA, we compared hepatic responses to a short-term administration of DHEA in rats, mice, hamsters and guinea pigs.

* Corresponding author: Junji Yamada, Ph.D., Department of Clinical Biochemistry, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan. Tel. (426) 76-5111; FAX (426) 75-2605.

† Abbreviations: DHEA, dehydroepiandrosterone; and GPC, glycerophosphocholine.

MATERIALS AND METHODS

Materials. DHEA was purchased from the Tokyo Kasei Kogyo Co. (Tokyo, Japan). L-Carnitine was donated by the Earth Pharmaceutical Co. (Akoh, Japan). All other chemicals were obtained from commercial sources and were of the highest purity available.

Animals and treatment. Male Fischer 344 rats, B6C3F1 mice, golden Syrian hamsters and albino Hartley guinea pigs, 7 weeks of age, were used. DHEA was suspended in 0.5% carboxymethylcellulose and administered by gastric intubation once a day for 14 days, at a dose of 300 mg/kg body weight. Control animals were similarly given the vehicle alone (5 mL/kg body wt).

In our previous study [4], it was found that a dose level of 300 mg of DHEA/kg body weight and a time period of 14 days are sufficient to elicit maximal induction of enzymes in the liver of rats; the enzyme induction occurs in a dose-dependent manner up to about 200 mg/kg. Therefore, the dosage of 300 mg/kg was employed in this study; over 300 mg/kg was practically difficult to administer by gastric intubation due to the insolubility of DHEA in aqueous solution.

Table 1. Effect of dehydroepiandrosterone (DHEA) on body weight gain, liver weight and hepatic protein content

		Rat	Mouse	Hamster	Guinea pig
Body weight gain (g/14 days)	C	38 ± 4	2.8 ± 0.9	15 ± 7	69 ± 17
	T	30 ± 6* (80)	2.9 ± 0.9 (106)	12 ± 4 (82)	45 ± 13* (65)
Liver weight (% of body weight)	C	3.7 ± 0.4	4.5 ± 0.2	4.7 ± 0.7	3.5 ± 0.6
	T	5.5 ± 0.3† (149)	5.3 ± 0.5* (118)	5.5 ± 0.5 (117)	3.7 ± 0.4 (106)
Hepatic protein content (mg/g liver)	C	218 ± 16	205 ± 10	243 ± 27	239 ± 31
	T	256 ± 4* (118)	229 ± 14† (112)	219 ± 17 (90)	235 ± 21 (98)

Animals were treated with 300 mg of DHEA/kg body weight for 14 days. Values are means ± SD of five animals. Figures in parentheses refer to values expressed as percentage of corresponding control. Abbreviations: C = control; T = treated.

*,† Significantly different (Student's *t*-test) from control data: * $P < 0.05$, and † $P < 0.01$.

All animals were killed 24 hr after the last administration. The animals were anesthetized with diethyl ether and the livers were perfused *in situ* with saline through the portal vein. Liver homogenates were prepared in 0.25 M sucrose containing 1 mM EDTA, 10 mM Tris–HCl (pH 7.5) and 0.1% ethanol, and the microsomal and cytosolic fractions were prepared by ultracentrifugation [4].

Enzyme assays. The activities of peroxisomal β-oxidation (lauroyl-CoA as a substrate) [14, 15], catalase [16] and carnitine acyltransferases [17] were measured in liver homogenates. Microsomal lauric acid ω-hydroxylation was assayed as previously described [5]. The activity of microsomal 1-acyl-GPC acyltransferase [18, 19] was measured with oleoyl-CoA and 1-palmitoyl-GPC. The activities of malic enzyme (decarboxylating) [20] and palmitoyl-CoA hydrolase [21] were measured in the cytosolic fraction. Protein was determined by the method of Lowry *et al.* [22].

Immunoblotting. Liver homogenates (10 μg protein/lane) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (10% gel) [23], and the proteins separated were transferred to a nitrocellulose sheet and treated with rabbit antiserum against rat liver acyl-CoA oxidase or enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme as described previously [4]. The antigen–antibody complexes were visualized by peroxidase-antiperoxidase staining using 3,3′-diaminobenzidine as reagent.

RESULTS

Changes in body and liver weights of animals following administration of DHEA are summarized in Table 1. In rats and guinea pigs, the body weight gains were decreased to 80 and 65% of the control levels, respectively, while no significant changes were observed in mice and hamsters. It is not known whether the reduced body weight gains in rats and guinea pigs were derived from the antiobesity effect of DHEA [1, 24]. In rats and mice, hepatomegaly was observed as indicated by 49 and 18% increases in the relative liver weight, respectively. The increased value in hamsters was not significant. The protein content per liver weight was also slightly increased in rats and mice.

Table 2 shows changes in hepatic enzyme activities that are known to be increased by peroxisome proliferators in a fashion closely associated with peroxisome proliferation [4, 6–8, 19–21, 25]. DHEA treatment increased the activities of the peroxisome proliferation-associated enzymes in rats and to a smaller extent in mice, although the increase in 1-acyl-GPC acyltransferase activity of mouse liver was not significant; 1.2-fold ($P < 0.05$) and 1.3-fold ($P < 0.01$) increases in the latter enzyme activity were observed in DBA/2 mice and BALB/c mice, respectively (data not shown). In hamsters, these enzyme activities were unchanged, except for lauric acid ω-hydroxylation (2.5-fold increase) and 1-acyl-GPC acyltransferase (2.0-fold increase). No significant changes in the activities were observed in guinea pigs.

In rat liver, increases in the activities of peroxisomal β-oxidation (3.7-fold), carnitine acetyltransferase (22.6-fold), lauric acid ω-hydroxylation (6.6-fold) and malic enzyme (7.5-fold) were relatively great among the enzymes examined (Table 2). Similar results were obtained in our previous study using Wistar rats [4]; however, the magnitude of the induction was about 2-fold greater in Wistar rats than in Fischer rats: 7.9-, 41.3-, 11.2- and 9.2-fold increases in the enzyme activities were observed in Wistar rats treated with DHEA, respectively [4]. Although, in mice (B6C3F1 strain), induction of the above enzymes was modest (2.1- to 3.0-fold, Table 2), essentially the same results (1.5- to 3.2-fold increases in the activities) were obtained with C57BL/6, DBA/2 and BALB/c mice (data not shown).

Induction of peroxisomal β-oxidation enzymes, acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme, was also examined by immunoblotting (Fig. 1). Consistent with the increased activity of peroxisomal β-oxidation (Table 2), the specific contents of these enzyme proteins were increased in the livers of rats and mice treated with DHEA (Fig.

Table 2. Effect of dehydroepiandrosterone (DHEA) on the activities of hepatic peroxisome proliferation-associated enzymes

		Rat	Mouse	Hamster	Guinea pig
Peroxisomal β-oxidation (μmol/min/g liver)	C	1.53 ± 0.08	1.10 ± 0.11	3.18 ± 0.64	0.80 ± 0.10
	T	5.70 ± 0.51* (374)	2.40 ± 0.40* (218)	2.91 ± 0.27 (92)	0.81 ± 0.09 (101)
Catalase (units/g liver)	C	64.5 ± 5.2	45.0 ± 3.0	88.2 ± 12.4	276.2 ± 41.9
	T	90.4 ± 5.7* (140)	67.4 ± 6.8* (150)	81.2 ± 13.7 (92)	247.4 ± 37.8 (90)
Carnitine acetyltransferase (μmol/min/g liver)	C	0.60 ± 0.16	0.74 ± 0.09	6.44 ± 1.04	5.98 ± 1.16
	T	13.58 ± 1.29* (2263)	1.65 ± 0.33* (224)	6.34 ± 1.26 (99)	6.24 ± 1.57 (104)
Carnitine palmitoyltransferase (μmol/min/g liver)	C	2.51 ± 0.48	3.02 ± 0.34	2.36 ± 0.19	1.90 ± 0.44
	T	6.49 ± 0.50* (258)	4.48 ± 0.55* (148)	2.18 ± 0.30 (92)	1.86 ± 0.14 (98)
Lauric acid ω-hydroxylation (nmol/min/mg protein)	C	1.22 ± 0.17	1.01 ± 0.21	2.30 ± 0.40	0.94 ± 0.18
	T	8.06 ± 0.53* (663)	3.02 ± 0.52* (299)	5.81 ± 0.55* (252)	0.93 ± 0.23 (100)
1-Acyl-GPC acyltransferase (nmol/min/mg protein)	C	76.4 ± 14.2	40.7 ± 8.6	38.0 ± 11.7	39.8 ± 5.2
	T	164.3 ± 10.4* (215)	49.0 ± 6.1 (120)	75.8 ± 14.9* (200)	46.6 ± 4.4 (117)
Malic enzyme (nmol/min/mg protein)	C	25.4 ± 4.0	70.0 ± 18.4	35.6 ± 5.9	6.6 ± 1.7
	T	189.2 ± 21.6* (745)	143.6 ± 28.0* (205)	35.3 ± 3.8 (99)	7.6 ± 0.6 (115)
Palmitoyl-CoA hydrolase (nmol/min/mg protein)	C	14.8 ± 2.2	20.3 ± 2.1	12.8 ± 2.4	11.8 ± 1.6
	T	43.0 ± 3.9* (291)	26.7 ± 5.3† (132)	13.2 ± 1.6 (103)	12.0 ± 4.0 (102)

Animals were treated with 300 mg of DHEA/kg body weight for 14 days. Values are means ± SD of five animals. Figures in parentheses refer to values expressed as percentage of corresponding control. Abbreviations: C = control; T = treated.

*,† Significantly different (Student's *t*-test) from control data: * $P < 0.01$, and † $P < 0.05$.

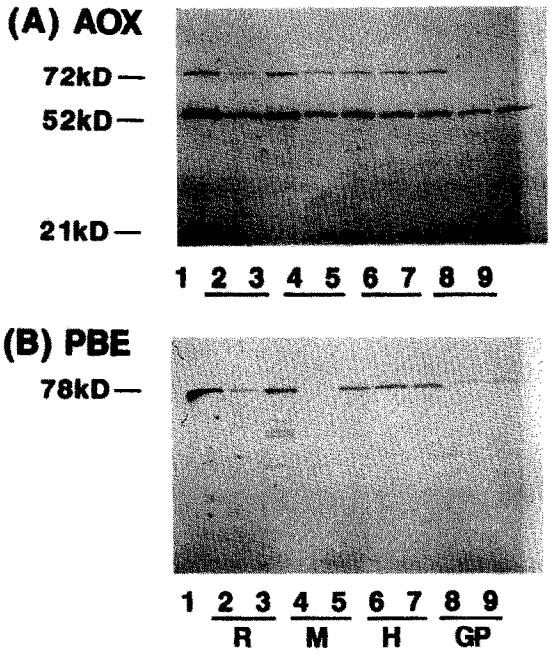

Fig. 1. Immunoblotting of acyl-CoA oxidase (AOX) and peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme (PBE). Lane 1, purified enzyme; lanes 2 and 3, rat; lanes 4 and 5, mouse; lanes 6 and 7, hamster; lanes 8 and 9, guinea pig; lanes 2, 4, 6 and 8, control; lanes 3, 5, 7 and 9, DHEA-treated. The positions of AOX subunits (72, 52, 21 kDa) and PBE (78 kDa) are indicated.

1, lanes 3 and 5). In hamsters and guinea pigs, DHEA did not produce appreciable induction of either of the enzymes (Fig. 1, lanes 7 and 9).

When the effects of DHEA on several other enzymes that were shown previously to be stimulated by DHEA [4] were examined, an increase in microsomal lauric acid (ω-1)-hydroxylation activity was observed in all the treated animals: 2.5-fold in rats, 1.5-fold in mice and hamsters, and 1.2-fold in guinea pigs (data not shown). The activity of microsomal carboxylesterase toward butanilicaine was also increased in rats (2.0-fold) and mice (1.6-fold) (data not shown).

DISCUSSION

The data presented here indicate that there are species differences in the inducing effect of DHEA on hepatic peroxisome proliferation-associated enzymes. Of the animals examined, rats were the most responsive species and mice were relatively less responsive to the enzyme induction by DHEA. Under the conditions of the DHEA treatment employed (300 mg/kg body wt, *per os*, 14 days), hamsters and guinea pigs did not respond to the induction. These differences in the responsiveness of the rodent species correlate well with those observed with other peroxisome proliferators [6–12]. Therefore, the present study supports our previous conclusion that DHEA is a typical peroxisome proliferator having properties common to many other proliferators [4].

Hamsters and guinea pigs are known to be less responsive species to peroxisome proliferators. In these animals, the marked induction comparable to those seen in rats and/or mice is not observed even when extremely high doses of the drugs are used [6–12]. However, they are not necessarily unresponsive species [10–12]. Therefore, a possibility still remains that DHEA treatment at a higher dose than that used here could lead to hepatic enzyme induction in hamsters and guinea pigs. The non-responsive nature of the animals toward DHEA observed here should be examined further. It should be noted that the activities of microsomal lauric acid ω-hydroxylation and 1-acyl-GPC acyltransferase were increased in hamsters (Table 2).

Frenkel *et al.* [3] have reported recently induction of enoyl-CoA hydratase, carnitine acyltransferases and catalase in the liver of mice treated with DHEA. When compared with their data, the induction of the mouse enzymes observed here was modest (Table 2). This may be due to difference in the conditions of DHEA treatment employed; they performed a high dose and long-term treatment by feeding DHEA-containing diet (0.45%, w/w) for 5 months [3].

Peroxisome proliferators are a diverse group of chemicals which include hypolipidemic drugs, phthalate ester plasticizers and agricultural chemicals [6–8]. Although the structural requirements for peroxisome proliferation are not fully understood, it is now proposed that a structure of hydrophobic anion may be required [10, 26–28]. However, DHEA has no anionic functional groups itself, and its steroidal structure is also unique among peroxisome proliferators known thus far. Therefore, as a structurally new type of peroxisome proliferator, DHEA would provide significant information in understanding the mechanisms involved in peroxisome induction by drugs. Recently, Issemann and Green [29] have cloned a putative peroxisome proliferator-activated receptor which belongs to the steroid hormone receptor superfamily. According to their report [29], DHEA seems not to stimulate the transcriptional activation mediated by the receptor. It is of interest to know how many kinds of, if any, receptor molecules exist for structurally diverse peroxisome proliferators and what structures of the proliferators are recognized by the receptor(s).

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