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PEROXISOME PROLIFERATION AND INDUCTION OF PEROXISOMAL ENZYMES IN MOUSE AND RAT LIVER BY DEHYDROEPIANDROSTERONE FEEDING

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Summary—Dehydroepiandrosterone (DHEA) treatment is effective in the prevention of various genetic and induced disorders of mice and rats. In studies designed to define some of the basic mechanisms that underline the beneficial chemopreventive effects exerted by the action of this steroid, we found that the liver undergoes profound changes that result in: (i) hepatomegaly; (ii) color change from pink to mahogany; (iii) proliferation of peroxisomes; (iv) increased cross-sectional area and volume density of peroxisomes; (v) increased or decreased number of mitochondria per cell; (vi) decreased mitochondrial cross-sectional area; (vii) marked induction of the peroxisomal bifunctional protein enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase; (viii) increased activities of enoyl-CoA hydratase and other peroxisomal enzymes assayed in this study, viz. catalase, carnitine acetyl-CoA transferase, carnitine octanoyl-CoA transferase, and urate oxidase; and (ix) increased activity of mitochondrial carnitine palmitoyl-CoA transferase. In addition, feeding DHEA to mice resulted in increased plasma cholesterol levels in two strains of mice evaluated in this study, and either slightly decreased or markedly increased plasma triglyceride levels, depending on the strain. Whether liver peroxisome proliferation, induced by DHEA feeding to mice and rats, plays a role in the chemopreventive effects elicited by this steroid remains to be established.

INTRODUCTION

Dehydroepiandrosterone (DHEA) feeding (0.2–1.0%, w/w) of genetically susceptible mice and rats is effective in preventing or delaying the onset of obesity [1–3], diabetes [4], hemolytic anemia [5], and lupus [6]. DHEA treatment also is effective in the prevention of spontaneous, virus-induced breast cancer in mice [7] as well as of carcinogen-induced neoplasias, viz. lung tumors (induced by 7,12-dimethylbenz[a]anthracene and urethane) [8], colon tumors (induced by 1,2-dimethylhydrazine) [9], and skin papilomas (induced by topical application of 7,12-dimethylbenz[a]anthracene and phorbol ester) [10, 11]. Treatment with DHEA protects mice against acute lethal viral infections [12] and, also, is effective in reducing adipose tissue mass in men [13]. A dose–response effect of DHEA has been demonstrated in obese lethal yellow mice where it was found that, with increased levels of dietary DHEA, weight gains decreased in a dose-dependent manner [3]. In addition to the beneficial therapeutic effects described, the treatment with DHEA also has been shown to display androgenic and estrogenic effects as demonstrated by weight increases in the seminal vesicles of castrated male rats and in the uterus of prepubertal female rats [14]. Moreover, DHEA-treatment promotes the development of polycystic ovaries in mice [15], and of ovarian granulosa cell tumors in SWXJ-9 inbred mice [16].

The common features resulting from DHEA treatment to mice and rats are as folows: (i) decreased body weight gain (Refs [1] and [17], and this paper), (ii) hepatomegaly (Refs [18] and [19], and this paper), (iii) change in liver color from pink to mahogany (Ref. [18], and this paper) and (iv) marked induction of a liver protein of relative molecular mass of approximately 72,000 ($M_r \sim 72$ K) [18]. At the present time our understanding of the molecular mechanisms involved in the many manifestations of DHEA treatment is limited. Thus, to evaluate the impact of the liver changes elicited by DHEA administration, we conducted studies by use of liver tissue obtained from mice and rats that were treated with either a DHEA-containing diet (0.45% in food, w/w)

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or a control diet in an attempt to identify the protein of $M_r \sim 72$ K [18], and to determine whether morphometric changes in hepatocyte organelles occur in association with DHEA feeding.

EXPERIMENTAL

Animals

Mice used in the conduct of these studies were of the strains (NZB × NZW)F_1, BALB/c, C57BL/6, (C57BL/6 × DBA/2)F_1, female NZW, male BXSB and C3H/HeJ, which were purchased from Jackson Laboratory (Bar Harbor, Me); the mice were maintained in the Animal Resource Center of this institution and were fed pelleted AIN-76A, without and with DHEA (0.45% w/w), *ad libitum*. (NZW × BXSB)F_1 mice were bred at our institution. Food diets were compounded for us by ICN Biochemicals (Cleveland, Ohio).

Male Sprague–Dawley rats (60–80 g) were purchased from the Holtzman Company (Indianapolis, Ind.) and were maintained on AIN-76A and drinking water *ad libitum* until the body weights were approximately 100 g. Thereafter, the rats were fed either the AIN-76A diet alone or the same diet that contained DHEA (0.45%, w/w) for 7 days. Alternatively, rats were treated by intraperitoneal injection with DHEA (100 mg/kg body wt/day) in corn oil for 7 days while on the AIN-76A diet. Control rats were injected intraperitoneally with corn oil alone.

Tissue preparations

Mice were killed under ethyl ether anesthesia by cervical dislocation at various times after the initiation of DHEA treatment. Rats were killed by decapitation after 7 days of DHEA feeding or after the last intraperitoneal injection on day 7. Liver was removed, weighed, and homogenized (1:5 w/w) at 4°C in Tris buffer (50 mM, pH 7.4) that contained sucrose (0.25 M). Cytosolic fractions were prepared from liver homogenates by centrifugation at 105,000 *g* for 1 h. Homogenates and cytosolic fractions were stored at −70°C until the time of assay. Protein concentrations were determined by the method of Lowry *et al.*[20] with bovine serum albumin as the standard.

Enzyme assays

The enzymatic assays were conducted by spectrophotometric methods. Enoyl-CoA hydratase was assayed at 30°C with crotonyl-CoA as the substrate by the method described by Osumi and Hashimoto[21], using either 105,000 *g* supernatant fractions or liver homogenates as the enzyme sources to commence the reaction. Catalase activity was determined at 25°C by the method of Aebi[22]: hydrogen peroxide (45 mM) was used to initiate the reaction. Carnitine acetyl-CoA transferase activity was determined at 25°C as described by Bieber and Markwell[23]: homogenates and 105,000 *g* supernatant fractions were used as the enzyme source to initiate the reaction. Carnitine octanoyl-CoA transferase and carnitine palmitoyl-CoA transferase were assayed at 25°C as described by Halperin and Pande[24]: the reactions were initiated by addition of either homogenates or 105,000 *g* supernatant fractions as the enzyme source. Urate oxidase was assayed at 25°C by the method described by Schneider and Hogeboom[25] substituting borate buffer for phosphate buffer; the reactions were initiated by addition of sodium urate.

Gas-phase amino acid sequencing of the major rat liver protein induced by DHEA feeding

Cytosolic fractions prepared from liver homogenates of rats that were treated either with the DHEA-containing diet (0.45%, w/w) or the control diet for 7 days were used to identify the induced protein of $M_r \sim 72$ K [18]. Liver proteins were separated by SDS–PAGE on 7.5% polyacrylamide gels [26], transferred electrophoretically to nitrocellulose paper, and stained with Ponceau-S. The portion of the nitrocellulose paper that contained the induced protein was excised and treated by solid-phase tryptic digestion, as described by Aebersold *et al.*[27]. The tryptic fragments were separated by reverse phase high-pressure liquid chromatography (HPLC) on an Applied Biosystems Model 130A apparatus (Foster City, Calif.). Individual peptides were collected manually onto 1 cm diameter glass fiber discs. Selected peptides were reduced and alkylated as described by Andrews and Dixon[28] and treated by NH_2-terminal sequence analysis by use of an Applied Biosystems Model 470A Gas Phase Sequencer coupled to a Model 120A HPLC apparatus, using standard programming and chemicals provided by the manufacturer.

Ultrastructural studies

Sections of liver tissue (approximately 1 mm^3) were fixed for 1 h in phosphate buffer (0.1 M, pH 7.4) that contained glutaraldehyde (2.5%). The tissues were washed in buffer, then incubated in a solution that contained 3,3′-diaminobenzidine (to visualize catalase-containing peroxisomes), as described by Fahimi[29]. Subsequently, the tissues were embedded and sectioned, and areas of liver cells were photographed using a Phillips 301 electron microscope (Eindhoven, The Netherlands). Photographs of liver cells were taken at random and analyzed by morphometric techniques with the aid of ZIDAS image analyzer (Zeiss). Peroxisomes were identified as membrane-bound, electron dense organelles. The cross-sectional areas of peroxisomes and mitochondria were determined by planimetry. The volume density of peroxisomes and mitochondria were calculated as a function of the cytoplasmic area of the liver cell.

RESULTS

Effects of DHEA feeding on body weight gain and liver changes in mice

Mice fed DHEA (0.45% in food, w/w) had body weights that, in general, were smaller than those of corresponding animals treated with the control diet (Table 1). Mice of various strains treated with DHEA also developed livers that were significantly larger, both in actual weight (except for male C57BL/6 mice) and as a percentage of total body weight, than those of mice treated with the control diet (Table 1). These findings are in agreement with those reported by other investigators [1, 17]. In addition, we found that liver of mice treated with the DHEA-containing diet acquired a deep mahogany color compared with that of liver of control mice; this color change also was evident in liver mitochondria DHEA-treated mice and was not due to increased concentration of hemeproteins (data not shown). The cause(s) for the color change in liver have not been determined. The increase in liver size and change in color persisted for as long as the mice were fed DHEA, up to 7 months in this study. These liver changes were accompanied by the striking induction of a protein to which we assigned initially a $M_r \sim 72$ K, which was found to be localized to both mitochondrial–lysosomal (200 *g*–20,000 *g*) and cytosolic fractions; this protein was readily separated from others by SDS–PAGE [18].

Identification of the major induced liver protein

Peptide fragments of the major protein induced in mice and rat liver by DHEA treatment (approximately 72 kDa by SDS–PAGE) [18] were obtained by solid-phase tryptic digestion of the transblotted protein and were isolated by reverse phase HPLC (Fig. 1). Selected peptide fragments were sequenced on a gas phase sequencer. Comparison of the sequences obtained for five different peptide fragments (Fig. 1) with those of known proteins enabled us to identify the protein as the bifunctional enzyme enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (EC 4.2.1.17), a component of the fatty acid β-oxidation system of liver peroxisomes [30, 31]. The bifunctional protein has an actual M_r of 77 K [32] rather than 72 K as estimated previously [18]. In liver of rodents fed the control diet, enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase could not be visualized on the gels obtained by SDS–PAGE by staining with Coomassie blue, as found in previous studies [18]; in lieu of the protein, there was a blank region in the corresponding region. The background level of this protein in control liver also was established by HPLC of possible fragment peptides obtained by tryptic digestion of the possible transblotted protein (Fig. 1).

Enoyl-CoA hydratase activity was determined in homogenates and cytosols prepared from liver tissue of rats and mice treated either with the control diet (AIN-76A) or the same food that contained DHEA (0.45%, w/w). Assays also were conducted with cytosols prepared from liver tissue of rats injected intraperitoneally with either DHEA (100 mg/kg body wt/day) in corn oil or the vehicle alone for seven consecutive days. The specific activity (sp. act) of enoyl-CoA hydratase in rat liver cytosol was approximately 40-times greater than that of controls (Fig. 2A). In the mouse, the sp. act of the liver enzyme was increased approximately 4-times in cytosols and 7-times in liver homogenates of animals treated with DHEA when compared with that of liver preparations of control mice (Fig. 2A).

Peroxisome- and mitochondrion-associated enzymes

On account of the finding of the induction of peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in mouse and rat liver by treatment with DHEA, we determined the specific activities of other peroxisomal enzymes in liver homogenates and cytosols of mice and rats that were treated either with the DHEA-containing diet or the control diet, viz. catalase, carnitine acetyl-CoA transferase, carnitine octanoyl-CoA transferase, and urate oxidase. In addition, we determined the specific activity of the mitochondrial enzyme carnitine palmitoyl-CoA transferase.

The sp.act of catalase (EC 1.11.1.6) was increased 2-times in liver homogenates and cytosols of rats and mice that were treated with DHEA (Fig. 2B). The sp.act. of carnitine acetyl-CoA transferase (EC 2.3.1.7) in liver cytosols of rats that were treated with DHEA was increased 16-times over control levels; similarly, approximately 3-times and 8-times increases were obtained with cytosols and homogenates prepared from liver of mice treated with the DHEA-containing diet (Fig. 2C). The sp.act of carnitine octanoyl-CoA transferase was increased approximately 11-times in rat liver cytosol, 3-times in mouse liver cytosol, and 2-times in mouse liver homogenate with DHEA treatment (Fig. 2D). The sp.act of urate oxidase (EC 1.7.3.3) was increased approximately 2-times in liver homogenates of mice fed DHEA (Fig. 2F); this enzymatic activity was not detected in either mouse or rat liver cytosol, an expected finding because this enzyme is associated exclusively with the crystalline core of peroxisomes and not with the peroxisomal matrix, as are the other enzymes evaluated in this study and which, upon homogenization, are found in the cytosolic fractions [33]. The sp.act of mitochondrial carnitine palmitoyl-CoA transferase (EC 2.3.1.21) was increased approximately 2-times by DHEA treatment both in rat and mouse liver cytosols and 4-fold in mouse liver homogenates (Fig. 2E).

Ultrastructural study of liver cells

The findings of induced peroxisome- and mitochondrion-associated enzymes prompted us to examine the ultrastructure of hepatocytes of mice treated either with the DHEA-containing diet or the control

Table 1. Body and liver weights of mice fed either a DHEA-containing diet of a control diet

Strain	*N*	Sex	Age at the time of killing (months)	Diet	Time on diet (months)	Mean ± SD Body weight (g)	Liver weight (g)	Liver weight as a percent of body weight
(NZB × NZW)F_1	5	Female	6	Control		40.9 ± 4.6	2.2 ± 0.4	5.4 ± 0.5
				DHEA	5	33.6 ± 1.2*	2.7 ± 0.1**	8.0 ± 0.4**
(NZB × NZW)F_1	5	Female	7	Control		38.9 ± 3.9	2.2 ± 0.3	5.7 ± 0.8
				DHEA	6	33.7 ± 1.0*	2.5 ± 0.09**	7.5 ± 0.2*
(NZW × BXSB)F_1	5	Female	5	Control		28.5 ± 0.6	1.6 ± 0.06	5.7 ± 0.2
				DHEA	3	24.9 ± 1.1**	1.8 ± 0.1	7.3 ± 0.2**
C57BL/6	4	Female	7	Control		23.4 ± 0.7	1.0 ± 0.1	4.2 ± 0.3
				DHEA	6	20.8 ± 0.6**	1.4 ± 0.2**	6.4 ± 0.5**
C57BL/6	4	Male	7	Control		32.7 ± 0.7	1.4 ± 0.2	4.4 ± 0.7
				DHEA	6	24.4 ± 0.9**	1.4 ± 0.02	5.7 ± 0.3*

*$P \leq 0.02$; **$P < 0.001$.

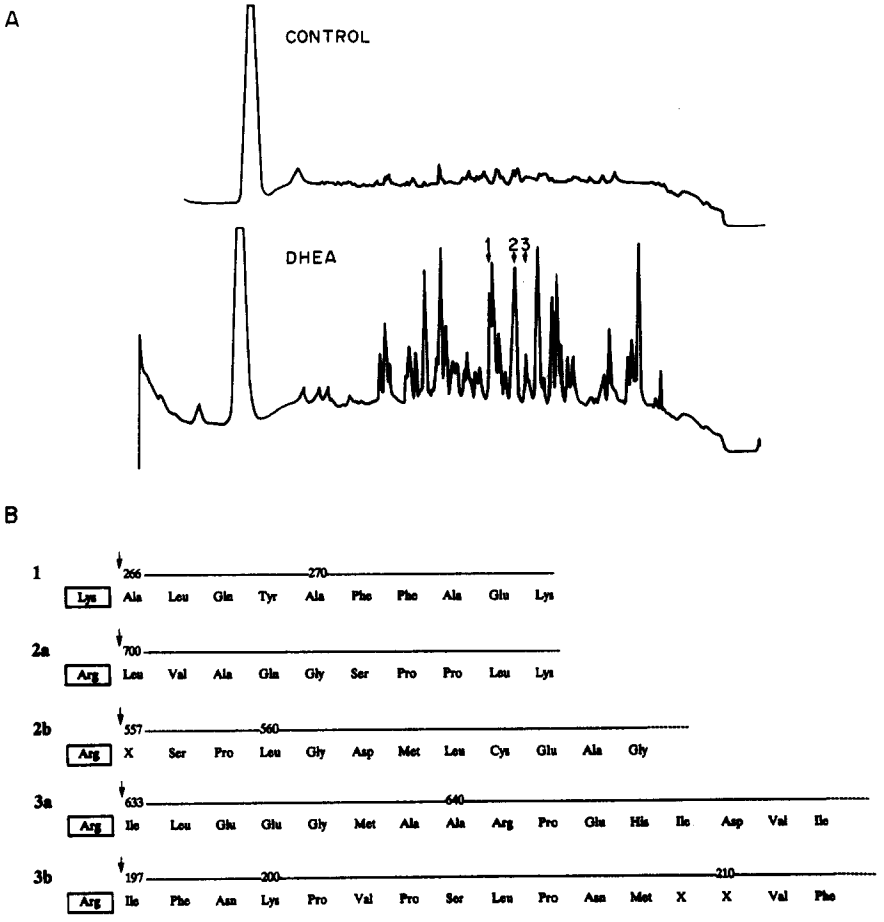

Fig. 1. Cytosolic fractions were prepared from liver tissue of Sprague–Dawley rats treated either with a DHEA-containing diet (0.45% in food, w/w) or a control diet for 7 days. Proteins were separated by SDS–PAGE on 7.5% polyacrylamide gels, transferred to nitrocellulose paper, and digested with trypsin as described in Materials and Methods. (A) Separation of tryptic peptides by reverse phase HPLC. Peaks labeled 1, 2, and 3 were reduced, alkylated, and sequenced (See Material and Methods). (B) The peaks labeled 2 and 3 were found to contain two polypeptides each (2a, 2b and 3a, 3b). Amino acid residues could be ascribed to the appropriate constituent peptides by measuring the relative abundance of the phenylthiohydantoin-amino acids at each position. The amino acid sequences obtained for each of the five tryptic fragment peptides analyzed are illustrated. These correspond to sequences found at the positions indicated in rat liver peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, as determined by molecular cloning and nucleotide sequence [31]. Phenylthiohydantoin-amino acids identified from a peptide mixture generated by *in situ* CNBr digestion of the intact protein were as predicted for peroxisomal enoyl-CoA hydratase/hydroxyacyl-CoA dehydrogenase. The positions of Lys and Arg in peroxisomal enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase at the appropriate sites of trypsin cleavage are boxed; the arrows indicate the positions of trypsin cleavage. The broken lines indicate that more amino acids may have been present in the trypsin fragments analysed, but were not sequenced. The X's indicate amino acids that were not identified.

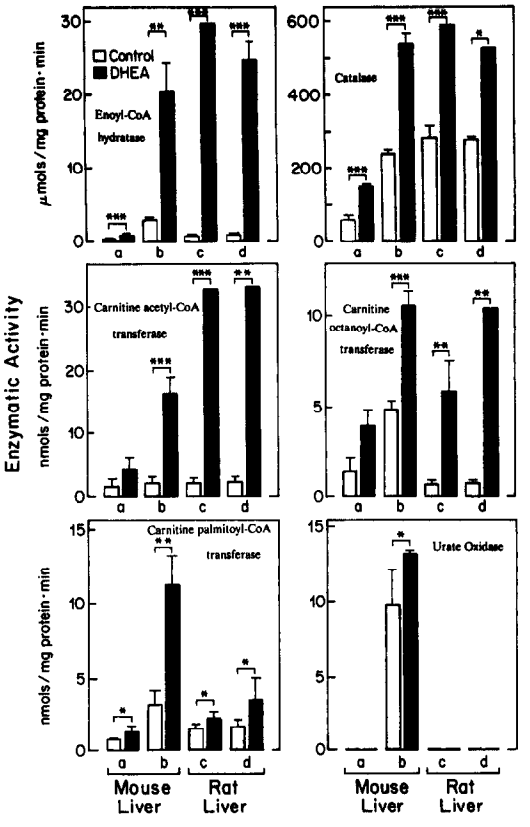

Fig. 2. The enzymatic assays described in this figure were conducted by spectrophotometric methods as described in Materials and Methods. Liver tissue of female (NZB × NZW)F_1 mice and male Sprague–Dawley rats were used in this study. The animals were fed *ad libitum* either the control diet (AIN 76A) or the same food that was supplemented with DHEA (0.45%, w/w). The mice were started on the DHEA-containing diet at 2 months of age and were maintained on it for 5 months. The homogenates were fractionated by centrifugation at 4°C, as described in Materials and Methods. Rats were fed either the DHEA-containing diet or the control diet for 7 days; alternatively the animals received DHEA (100 mg/kg body wt) in corn oil by intraperitoneal injection daily for 7 days. Control rats received corn oil only. 105,000 *g* supernatant fractions were prepared as described in Materials and Methods. (a) Mouse liver 105,000 *g* supernatant fraction; (b) mouse liver homogenate; (c) rat liver 105,000 *g* supernatant fraction (oral DHEA); (d) rat liver 105,000 *g* supernatant fraction (intraperitoneal DHEA). The bars represent the mean values ± SD obtained in enzymatic determinations conducted with individual liver preparations of 5 animals per group. $^*P < 0.05$, $^{**}P < 0.01$; $^{***}P < 0.005$.

diet. In these studies, we found significant morphometric differences in peroxisomes and mitochondria in hepatocytes of mice treated with DHEA compared with those in liver parenchymal cells of control mice. The cross-sectional area of hepatocyte peroxisomes, number of peroxisomes per unit of cytoplasmic area, and volume density of peroxisomes were studied in two mouse strains, viz., BALB/c and (C57BL/6 × DBA/2)F_1, and were found to increase significantly (Fig. 3 and Table 2). In addition, we found that the cross-sectional area of mitochondria in hepatocytes was decreased by DHEA treatment in three strains of mice evaluated in this study, viz. BALB/c, (C57BL/6 × DBA/2)F_1 and (NZB × NZW)F_1, and that the volume density of mitochondria in liver parenchymal cells was increased in (C57BL/6 × DBA/2)F_1 and (NZB × NZW)F_1 mice and decreased in BALB/c mice (Fig. 3 and Table 2).

Plasma triglycerides and total cholesterol

The action of other agents known to induce peroxisome proliferation in liver of mice and rats has been associated with hypolipidemia [34, 35]. To determine whether DHEA treatment of mice resulted in a similar effect, we assayed triglycerides [36] and total cholesterol [37] in plasma of mice that were fed either the control diet or the diet containing DHEA. We found that, depending on the strain of mice, plasma triglyceride levels were either markedly increased (C3H/HeJ and BALB/c mice, 47 and 92%, respectively) or slightly decreased [(NZW × BXSB)F_1 and (C57BL/6 × DBA/2)F_1 mice] (Table 3).

Cholesterol levels in plasma of mice treated with DHEA were increased significantly over those of animals that received the control diet in the two strains of mice evaluated, viz. BALB/c and (C57BL/6 × DBA/2)F_1; the increases in cholesterol levels in these mice by DHEA treatment were 87 and 57%, respectively (Table 3).

DISCUSSION

In this study, designed to identify some aspects of the involvement of the liver in the mechanism of action of DHEA, a steroid known to elicit therapeutic and chemopreventive effects in mice and rats of susceptible strains [1–12], we established that the major protein induced by action of this steroid in mouse and rat liver [18] was enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase. This bifunctional protein is associated with peroxisomes and is a component of the fatty acid β-oxidation system present in these organelles [21, 30]. The activity of enoyl-CoA hydratase was markedly increased (up to 40 times) in liver cytosols of rats that were either fed DHEA (0.45% in food, w/w) or injected intraperitoneally with DHEA (100 mg/kg body wt/day) for 7 days. The relative increase in the specific activity of enoyl-CoA hydratase in liver preparations of DHEA-treated mice (approximately 4 to 7 times) was relatively lower than that in rat liver cytosol. The striking increase in enoyl-CoA hydratase activity observed in the cytosolic fractions obtained from the liver of DHEA-treated rats is most probably a reflection of the large induction of peroxisomes and substantial leakage from these organelles during cytosol preparation. The changes in enoyl-CoA hydratase activity are correlated with the amount of peroxisomal protein of M_r ~72 K (by SDS–PAGE), which amounts to approximately 7% of the total protein found in the cytosolic fraction of liver of treated

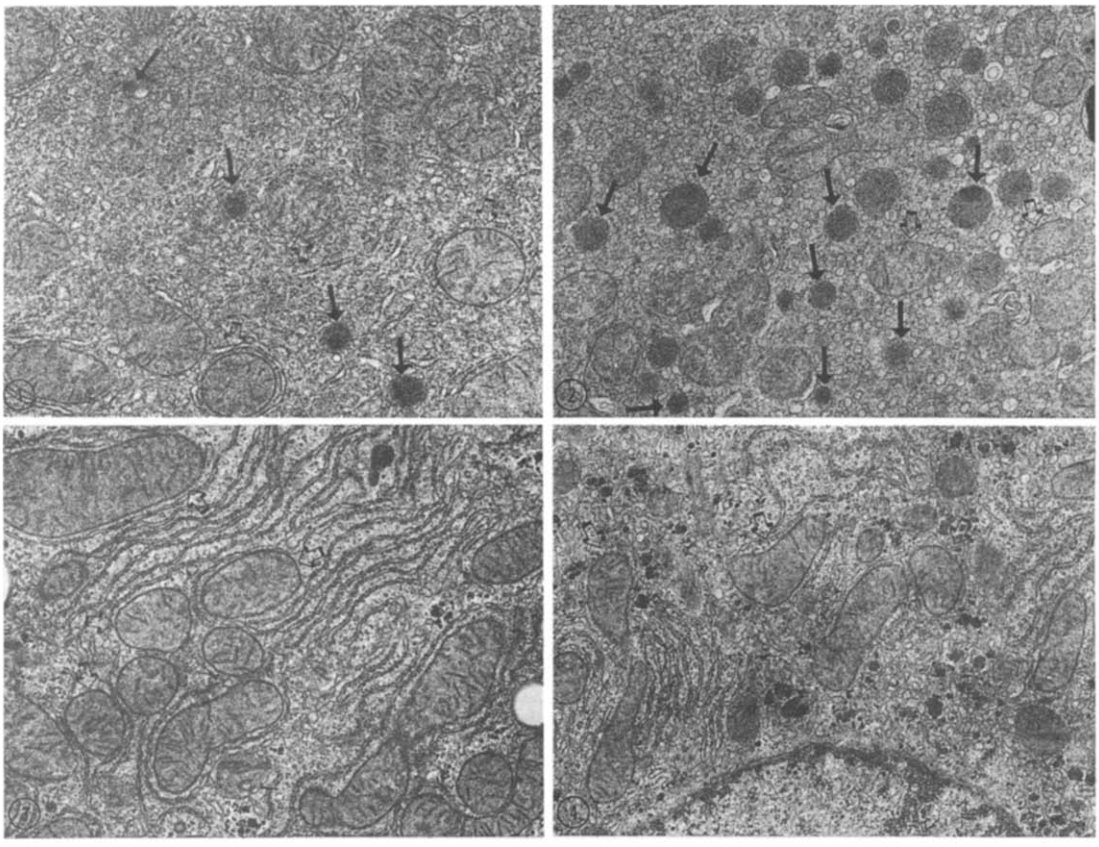

Fig. 3. Liver tissues were obtained from male BALB/c mice that were fed either the control or the DHEA-containing diet (0.45%, w/w) for 6 months. Tissue slices (~ 1 mm^3) were fixed for 1 h in phosphate buffer (0.1 M, pH 7.4) containing 2.5% glutaraldehyde. The tissues were washed in buffer and, thereafter, incubated in a solution that contained 3,3′-diaminobenzidine to visualize catalase-containing peroxisomes [29]. The tissues were subsequently embedded and sectioned. Comparable areas of liver cells were photographed using a Phillips 301 electron microscope. Photographs were taken at random at a constant magnification (×9100) and were analyzed by morphometric techniques with a ZIDAS (Zeiss) image analyzer. Peroxisomes were identified as membrane-bound, electron dense organelles. The cross-sectional area of peroxisomes and mitochondria was determined by planimetry. The number and volume density of peroxisomes and mitochondria were calculated as a function of the cytoplasmic area of the liver cell. The data were analyzed by Student's *t*-test (unpaired). Peroxisomes (black arrows) in hepatocytes of mice treated with DHEA appeared in clusters. Mitochondria (white arrowheads) were sought after fixing and embedding appropriate liver slices obtained from male BALB/c mice: micrographs of comparable areas of liver cells were obtained (×9100). (1) Control; (2) DHEA-treated; (3) Control; (4) DHEA-treated.

rats, and which hardly can be discerned in similar preparations obtained from liver of control rats (data not shown). This correlation makes it very unlikely that the measured enzyme changes are due to alterations in the levels of enoyl-CoA hydratase associated with the mitochondrial β-oxidation system.

The finding of the induction of this peroxisomal protein by DHEA treatment led us to evaluate the activities of other peroxisome-associated enzymes in mouse and rat liver, viz. catalase, carnitine acetyl-CoA transferase, carnitine octanoyl-CoA transferase, and urate oxidase, which also were found to be increased significantly with DHEA feeding. In addition, the specific activity of carnitine palmitoyl-CoA transferase, a mitochondrion-associated enzyme, was increased in liver preparations of DHEA-treated animals compared with that in liver of control mice and rats. We have reported that the specific activities of other peroxisome-associated enzymes of rat liver, viz. catalase and KCN-insensitive palmitoyl-CoA oxidase, are increased by DHEA treatment compared with controls [38]. Similar increases in the specific activities of catalase and fatty acyl-CoA oxidase in rat liver produced by dehydroepiandrosterone acetate treatment have been reported previously by Leighton *et al.*[39].

The action of orally-administered DHEA does not appear to serve a significant role in decreasing lipogenesis in liver of lean mice and rats because the specific activity of glucose 6-phosphate dehydrogenase and other enzymes involved in *de novo* lipogenesis are either unchanged or elevated rather than decreased as a consequence of DHEA administration, e.g. malic enzyme [40, 41]. Significant decreases in the

Table 2. Morphometric analysis of liver cells from control and DHEA-treated mice

Organelle	Mouse strain	Treatment (weeks)	N	Cross-sectional area (mm^2)	P	Volume density (%)	P
Peroxisomes	BALB/c	Control	8	0.078 ± 0.004		0.107 ± 0.15	
	(male)	DHEA (25)	8	0.107 ± 0.005	<0.001	2.77 ± 0.57	<0.05
	(C57BL/6 × DBA/2)F_1	Control	8	0.057 ± 0.005		0.60 ± 0.21	
	(male)	DHEA (17)	8	0.091 ± 0.003	<0.001	1.79 ± 0.20	<0.001
Mitochondria	BALB/c	Control	8	0.513 ± 0.021		21.1 ± 1.40	
	(male)	DHEA (25)	8	0.269 ± 0.096	<0.001	10.8 ± 0.88	<0.001
	(C57BL/6 × DBA/2)F_1	Control	8	0.258 ± 0.014		5.59 ± 0.30	
	(male)	DHEA (17)	8	0.092 ± 0.006	<0.001	12.0 ± 0.84	<0.001
	(NZB × NZW)F_1	Control	7	0.649 ± 0.090		11.3 ± 0.03	
	female)	DHEA (1)	5	0.472 ± 0.058	0.1	9.98 ± 1-05	NS
		Control	5	0.778 ± 0.071		10.6 ± 0.36	
		DHEA (2)	9	0.396 ± 0.048	<0.01	11.4 ± 0.03	<0.05
		Control	22	0.958 ± 0.059		9.76 ± 0.23	
		DHEA (4)	13	0.572 ± 0.065	<0.001	11.6 ± 0.02	<0.001

Micrographs, at an enlargement of ×9100 were used to compute the parameters described for liver cells of BALB/c and (C57BL/6 × DBA/2)F_1 mice. The magnifications used in the micrographic studies for liver cells of (NZB × NZW)F_1 mice were in the range of × 5700– × 45,000. Values are given as mean ± SD. *N* represents the number of micrographs. The data were analyzed by Student's *t*-test (unpaired). NS, not significant. Mice were started on the DHEA-containing diet at 2 months of age.

levels of glucose 6-phosphate dehydrogenase and fatty acid synthetase in liver of obese Zucker rats and lethal yellow mice by DHEA action, however, have been reported [2, 3]; thus, it is possible that decreased lipogenesis may indeed serve a role in lowering triglyceride levels in obese animals.

Ultrastructural studies of mouse hepatocytes were conducted to establish whether the morphology of peroxisomes and mitochondria in these cells are altered by DHEA treatment. We found that peroxisomes appeared to cluster together and that their cross-sectional area, number per unit of cytoplasmic area, and volume density were increased significantly in liver parenchymal cells of DHEA-treated mice compared with those of control mice. In these studies, we also found that the cross-sectional area of mitochondria in mouse hepatocytes was decreased significantly by DHEA treatment. These findings are supportive of the view that DHEA administration to mice and rats causes proliferation of peroxisomes in hepatocytes, at least when administered in the large doses used in this and other studies [1–12].

The findings of this study are an indication that there is a close similarity between the hepatic effects produced by DHEA treatment of mice and rats and those produced by the action of unrelated compounds known by the generic name of peroxisome proliferators. Namely, the oral administration of peroxisomal proliferators, e.g. clofibrate, to rats and mice has been shown to result in (i) development of hepatomegaly [42–45], (ii) marked induction of a "~80 kDa" protein [46–48], and (iii) induction of peroxisomal enoyl-Coa hydratase/3-hydroxyacyl-CoA hydratase [30, 49], viz. catalase, carnitine acetyl-CoA transferase, carnitine octanoyl-CoA transferase other effects produced in mouse and rat liver by DHEA treatment were similar to those produced by treatment with clofibrate and other peroxisome proliferators. Thus, the specific activities of various peroxisome-associated enzymes, in addition to enoyl-CoA hydratase [30, 49], *viz.* catalase, carnitine acetyl-CoA transferase, carnitine octanoyl-CoA transferase and urate oxidase [50–57], are increased by the action of peroxisome proliferators as found with DHEA treatment. In addition to the induction of peroxisomal enzymes, the specific activities of mitochondrial carnitine palmitoyl-CoA transferase [58], microsomal ω-hydroxylase [59–61], and cytosolic malic enzyme [62] also are increased significantly by clofibrate treatment, as demonstrated with DHEA [38, 40, 41].

Additional correlates between the effects of DHEA treatment and those obtained by administration of classical peroxisome proliferators such as clofibrate to mice and rats are reflected in morphologic changes in hepatocyte peroxisomes and mitochondria. Thus, peroxisomes proliferate and cluster together; the cross-sectional area, the number per unit of cytoplasmic area, and the volume density of peroxisomes are increased by clofibrate feeding [43–45, 63–66]. The changes on hepatocyte mitochondrial morphology

Table 3. Serum triglyceride and cholesterol levels in mice fed either a DHEA-containing diet or a control diet

Strain	Sex	Age at the time of killing (months)	Time on DHEA (weeks)	Triglycerides (mg/dl) Controls	Triglycerides (mg/dl) DHEA-treated	P	Cholesterol (mg/dl) Controls	Cholesterol (mg/dl) DHEA-treated	P
(NZW × BXSB)F_1	Female	14	50	105 ± 23 (5)	93 ± 10 (6)	NS	—	—	—
(NZW × BSXB)F_1	Female	15	56	117 ± 37 (5)	108 ± 12 (5)	NS	—	—	—
(C57BL/6 × DBA/2)F_1	Male	4	8	115 ± 12 (6)	99 ± 11 (6)	NS	—	—	—
(C57BL/6 × DBA/2)F_1	Female	3	4	103 ± 25 (10)	93 ± 15 (10)	NS	59 ± 6 (10)	93 ± 15 (10)	<0.001
C3H/HeJ	Female	3.5	5	76 ± 11 (5)	112 ± 27 (8)	0.01	—	—	—
BALB/c	Female	4.5	7	93 ± 15 (8)	179 ± 24 (9)	<0.001	52 ± 8 (8)	97 ± 11 (9)	<0.001

Triglycerides were determined as described by Spayd *et al.*[36] and cholesterol by the method of Allain *et al.*[37]. Mean ± SD are reported; the number of animals used per group are indicated in parenthesis. NS, not significant.

induced by clofibrate treatment also are similar to those produced by DHEA, viz. these organelles have a decreased cross-sectional area and there is an increase in the number of mitochondria in hepatocytes [66–68].

There is a widely held belief that peroxisomal proliferators produce hypotriglyceridemia; nonetheless, there is a great paucity of findings published to support this presumption. For example, it was reported that clofibric acid treatment lowers plasma triglyceride levels in the rat by approximately 13–38% [34]. In the same study it was found that gemfibrozil, a peroxisomal proliferator that is widely used as an hypolipidemic agent in clinical medicine, increases plasma triglyceride levels in the rat by approximately 18–27% [34]. Another widely accepted characteristic feature of peroxisomal proliferators is that these compounds exert a cholesterol-lowering effect. For example, cholesterol levels were reduced 15–50% in plasma of rats treated with clofibrate [35] and, in a study conducted with young men, it was found that DHEA administration (1.6 g/day) for 28 days resulted in a reduction of total serum cholesterol (~4%) and low-density lipoprotein levels (~4%) [13].

On the basis of these and similar reports, we determined triglyceride and cholesterol levels in plasma of mice that were treated either with a control diet or a diet containing DHEA to establish whether the action of the steroid also resulted in hypolipidemia in these animals. In the limited number of mouse strains evaluated in this study, we found that plasma triglyceride levels were either lowered slightly, but not significantly [(NZW × BXSB)F_1 and (C57BL/6 × DBA/2)F_1] or, otherwise, were increased significantly (C3H/HeJ and BALB/c, 47 and 92%, respectively).

Cholesterol levels in plasma of mice treated with DHEA were increased significantly (57–87%) in the two strains of mice evaluated in this study compared with those of controls. It has been reported that DHEA treatment of mice results in marked increases in plasma levels of testosterone and 5α-dihydrotestosterone, produced by extraglandular conversion [15, 16, 69], and thus it is possible that the elevation in plasma cholesterol levels is due to the action of these hormones.

The conclusions of this study are that DHEA treatment of mice and rats results in hepatomegaly that is temporally associated with peroxisome proliferation and induction of peroxisomal enzymes. Whether the beneficial chemopreventive and therapeutic effects that accrue with DHEA administration to susceptible rodents [1–12] are associated with the increased number of peroxisomes per liver cell and increased peroxisome-associated enzyme activities remains to be established.

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