Dehydroepiandrosterone-Induced Peroxisome Proliferation in the Rat: Evaluation of Sex Differences (43805)

M. S. Rao,[1] B. Reid, H. Ide, V. Subbarao, and J. K. Reddy
Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611

Abstract. **Dehydroepiandrosterone (DHEA) is a newly identified peroxisome proliferator that causes hepatomegaly, peroxisome proliferation, and induction of peroxisome-associated enzymes in rats and mice, and hepatocellular carcinomas in rats. In the present study, we have systematically analyzed sex differences and the effect of castration on DHEA-induced peroxisome proliferation in male and female rats, since no information is available on this subject. DHEA was fed in diet at a concentration of 0.45% for 2 weeks and livers were analyzed for hepatomegaly, peroxisome volume density, peroxisome proliferator associated Mr 80,000 polypeptide (PPA-80), and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (PBE) mRNA. Both intact and castrated rats showed similar response to DHEA characterized by increased peroxisome volume density, PBE mRNA, and PPA-80. Significant difference was observed in the liver weights between castrated and intact animals in both the sexes. Castrated rats that received DHEA had 20%–30% more liver weight than DHEA-administered intact rats. These results clearly indicate that peroxisome proliferative effect of DHEA is not influenced by sex hormones and it is equally potent in both males and females.** [P.S.E.B.M. 1994, Vol 207]

A variety of structurally diverse groups of compounds that include hypolipidemic drugs, phthalate ester plasticizers, industrial solvents, and herbicides are shown to induce peroxisomes and peroxisome-associated enzymes, and are designated as peroxisome proliferators (PP) (1–3). The mechanism of induction of peroxisomes by some of the structurally varied PP is through activation of peroxisome proliferator-activated receptor (PPAR), a member of the steroid receptor superfamily (4, 5). PP exhibit a marked species and tissue specific effects in inducing peroxisomes. Although peroxisome proliferation can be induced in rodents, nonrodents, and primates, maximum effects are observed only in rats and mice (1, 6, 7). Even in the most responsive species such as rats and mice, tissue specific effect of PP is striking. Peroxisomes and peroxisomal enzymes are induced markedly in hepatocytes, to a limited extent in the kidney tubular epithelial cells, heart muscle, and small intestine epithelium, and not at all in many other types of tissues (8).

In rats, the effect of hypolipidemic drugs and plasticizers appear to be sex dependent. With weak peroxisome proliferators such as clofibrate and di-(2-ethylhexyl)phthalate, the magnitude of induction of peroxisomes and some of the peroxisomal enzymes was much less in females when compared with males (9, 10). However, sex differences are not observed with potent PP (11, 12).

Dehydroepiandrosterone (DHEA), a steroid secreted by adrenal cortex in mammals including humans, is a new addition to the constantly expanding list of PP. DHEA is shown to induce peroxisomes, peroxisome-associated enzymes, and microsomal enzymes in the liver of rats and mice (13–15). Like other PP, DHEA is also shown to exhibit species specific effect (16). The mechanism by which this naturally

[1] To whom requests for reprints should be addressed at Department of Pathology, Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, IL 60611.

Received February 15, 1994. [P.S.E.B.M. 1994, Vol 207]
Accepted June 12, 1994.

0037-9727/94/0000-0186$10.50/0

occurring steroid induces peroxisome proliferation is not known. Unlike other PP, DHEA do not activate PPAR (4, 5, 17). At present, sex differences in the peroxisome proliferative effect of DHEA, which serves as a precursor for both androgens and estrogens, is not fully evaluated. In this study, we have systematically evaluated the peroxisome proliferative effect of DHEA in intact male and female rats and in castrated rats.

Materials and Methods

F-344 rats (16 males, 16 females) weighing 90–100 g were purchased from Charles River Laboratories (Wilmington, MA). Rats were housed in groups of four as per sex in plastic cages on San-i-cel bedding, and after a week acclimatization they were divided into eight equal groups as shown in Table 1. Eight males and 8 females were castrated under metofane anesthesia using aseptic conditions. Experimental rats were fed a diet containing 0.45% DHEA acetate (Sigma Chemical Co., St. Louis, MO) for 2 weeks. Control rats were fed Purina rat chow. Castrated animals were fed control diet for 1 week before they were started on DHEA diet. Care and use of these animals were in accordance with the recommendations provided in the "Guide for Care and Use of Laboratory Animals" (DHEW Publication No. NIH 86–033). Animal weight and food consumption were measured daily. At the end of 2 weeks of DHEA administration, all animals were sacrificed under ether anesthesia, and body and liver weights were recorded. For quantitative morphometric analysis of peroxisomes, fragments of liver were processed for electron microscopy (18). To evaluate the induction of peroxisome proliferation associated Mr 80,000 polypeptide (PPA-80) 10 μg of solubilized postnuclear fractions were analyzed by SDS-PAGE (18). For assessing the level of PBE mRNA, total RNA was prepared and analyzed by Northern blot hybridization using [^{32}p]-labeled PBE cDNA (8). The levels of mRNA were measured by densitometric scanning of autoradiographs. Results in the table are the mean ± SE. Data were analyzed using a two-tailed Student's t test, and differences were considered significant when $P < 0.05$.

Table I. Experimental Design

Group[a]	Treatment
1. Intact males	Control diet
2. Orchiectomy	Control diet
3. Intact males	DHEA[b]
4. Orchiectomy	DHEA
5. Intact females	Control diet
6. Ovariectomy	Control diet
7. Intact females	DHEA
8. Ovariectomy	DHEA

[a] Each group consisted of four animals.
[b] DHEA is mixed in Purina Rat Chow at a concentration of 0.45% and fed for a period of 2 weeks.

Results

Body and liver weights, average daily food consumption, and the volume density of peroxisomes in liver cells from all eight groups are presented in Table II. In all the groups, body weight gain was 100% in males and 50% in females during the 2 weeks of experiment, although dietary consumption was comparable between males and females. In both males and females that were fed control diet, no differences in liver weights were observed between castrated and intact rats. Peroxisome volume density was also similar in these four groups. However, in intact and castrated rats, administration of DHEA resulted in a significant increase in the liver weight ($P < 0.001$), when compared with appropriate controls. Interestingly, the liver weight increase in castrated animals that received DHEA was 20%–30% more than intact animals given DHEA. Ultrastructural examination of liver cells of DHEA treated intact and castrated male and female rats showed a large increase in the number of peroxisomes. Quantitative morphometric analysis revealed 7.5- to 11-fold increase in peroxisome volume density in these groups over the corresponding controls (Table II).

SDS-PAGE analysis of postnuclear fractions prepared from livers of different groups are shown in Figures 1 and 2. In DHEA-treated intact and castrated males and females, a marked increase in the amount of PPA-80 was observed. Interestingly, in DHEA-treated orchiectomized rats the induction of PPA-80 was slightly more than in DHEA treated intact rats. However, no difference in PPA-80 levels was observed between castrated and intact females that received DHEA. In intact and castrated control rats the levels of PPA-80 were very low.

Northern blot analysis of total RNA from livers of DHEA-treated intact and castrated males and females showed a marked increase in PBE mRNA levels when compared with controls (Fig. 3 and 4). Castration alone did not cause any increase in the levels of PBE mRNA. Quantitative analysis of mRNA by densitometry revealed 6.5-fold increase in DHEA-treated males, and 5.5-fold increase in DHEA + orchiectomized group when compared with controls. Similarly, in intact and castrated females treated with DHEA, the increase in PBE mRNA was 5- and 6-fold respectively.

Discussion

The physiological role of DHEA, a steroid produced in large amounts in mammals, remains un-

Table II. Effects of DHEA on Body Weight, Liver Weight, and Peroxisome Volume Density in Male and Female Rats

Group	Treatment[a]	Body wt	Average daily food consumption (g/100 g body wt)	Liver wt/100 g	Peroxisome volume density (% of cytoplasmic volume)
1	Intact males + Control diet	167 ± 1[b]	11.7 ± 0.4	4.9 ± 0.1	0.6 ± 0.1
2	Orchiectomy + Control diet	170 ± 2	10.1 ± 0.2	4.6 ± 0.1	0.7 ± 0.1
3	Intact males + DHEA	162 ± 4	11.6 ± 0.4	6.6 ± 0.2[c]	5.0 ± 0.4[c]
4	Orchiectomy + DHEA	155 ± 3	11.1 ± 0.2	7.6 ± 0.1[c]	6.6 ± 0.5[c]
5	Intact females + Control diet	137 ± 1	10.6 ± 0.3	3.9 ± 0.1	0.5 ± 0.1
6	Ovariectomy + Control diet	139 ± 2	10.9 ± 0.3	4.0 ± 0.1	0.8 ± 0.1
7	Intact females + DHEA	130 ± 4	10.8 ± 0.2	6.7 ± 0.1[c]	5.5 ± 0.6[c]
8	Ovariectomy + DHEA	120 ± 2	10.7 ± 0.1	8.0 ± 0.3[c]	6.0 ± 0.7[c]

[a] DHEA was fed in diet at a concentration of 0.45% for 2 weeks.
[b] Mean ± SEM of four animals.
[c] $P < 0.001$ compared with controls (Student's t test).

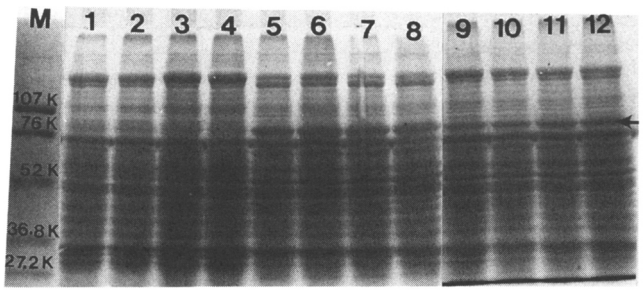

Figure 1. SDS-polyacrylamide slab gel electrophoresis of postnuclear fractions of liver from control (Lane 1–4), castration + DHEA-treated (Lane 5–8), and intact + DHEA-treated (Lane 9–12) male rats. M, molecular weight standards.

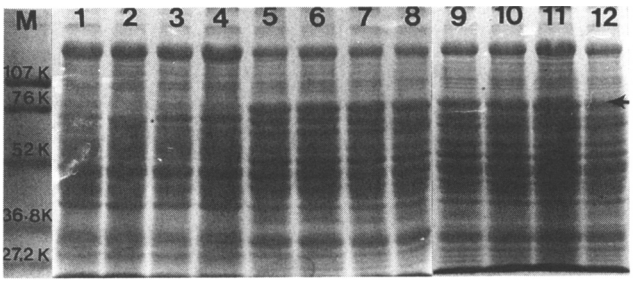

Figure 2. SDS-PAGE of postnuclear fractions of liver from control (Lane 1–4), castration + DHEA-treated (Lane 5–8), and intact + DHEA-treated (Lane 9–12) female rats. M, molecular weight standards.

known. Administration of large doses of DHEA has been shown to have a protective role against obesity, atherosclerosis, and carcinogenesis. DHEA is shown to be a PP in rats and mice (13–15, 19), and a hepatocarcinogen in rats (20–22). However, unlike other PP, DHEA is ineffective in inducing peroxisomes in isolated hepatocytes and in activating PPAR under *in vitro* conditions (4, 23). This indicates that a metabolite of DHEA rather than the parent compound is probably responsible for peroxisome proliferative effect. Several metabolites of DHEA tested so far are shown to have either weak or no peroxisome proliferative effect (24).

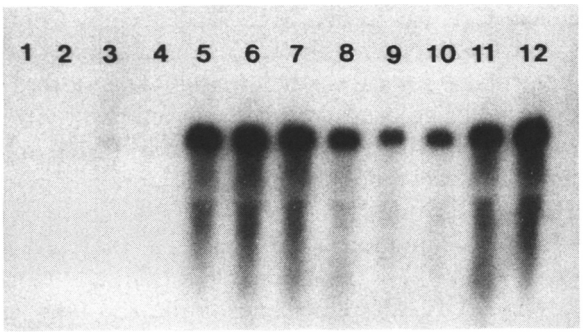

Figure 3. Northern blot analysis for PBE mRNA in livers from control (Lane 1–4), intact + DHEA-treated (Lane 5–8), and castrated + DHEA-treated (Lane 9–12) male rats. Total RNA derived from liver was tested for purity and integrity. Ten μg of RNA was denatured with glyoxal and electrophoresed through a 1% agarose gel, transferred to a nylon filter, and hybridized with [p^{32}]-labeled PBE mRNA. The autoradiograph demonstrates a 3.0-kb mRNA in all DHEA-treated animals.

The present study is designed to examine sex differences and effect of sex hormones on DHEA-induced peroxisome proliferation. The results as shown here clearly indicate that administration of DHEA in diet at 0.45% concentration resulted in hepatomegaly, increased peroxisome volume density, and increased levels of PPA-80 and PBE mRNA in both intact and castrated males and females. The level of increase in PPA-80, PBE mRNA, and peroxisome volume density between intact and castrated groups treated with DHEA was comparable. The only significant difference was an increase in the liver weights of

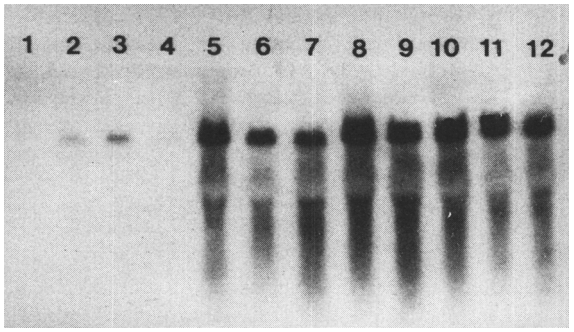

Figure 4. Northern blot analysis for PBE mRNA in livers from control (Lane 1–4), intact + DHEA-treated (Lane 5–8), and castrated + DHEA-treated (Lane 9–12) female rats. Northern blot analysis is performed as described in Figure 3.

castrated males and females when compared with the intact animals after DHEA treatment. The reason for such an increase in the liver weights in these groups is not clear. This disparity is not due to difference in the DHEA intake, since the dietary consumption in all the groups was comparable.

A comparable induction of fatty acyl CoA-oxidase was also observed by Leighton *et al.* (25) in male and female Wistar rats after DHEA administration. However, Yamada *et al.* (26) observed a significant sex difference in induction of peroxisomal β-oxidation enzymes by DHEA. In male rats, peroxisomal β-oxidation and acyl CoA oxidase levels increased by 7.9- and 6.4-fold respectively, whereas the increase in females was only 5.5- and 3.8-fold. The reason for the decreased induction in females in this study is not clear.

The absence of sex difference in peroxisomal inductive effect of DHEA in intact and castrated animals is interesting. Earlier studies by Svoboda *et al.* (9) have shown that clofibrate, at 0.25% dose level induced peroxisome proliferation and increased catalase activity only in male rats but not in females. In males the effect of clofibrate was markedly decreased after orchiectomy, indicating the role of testosterone in peroxisome proliferation. This was further corroborated in female rats where administration of clofibrate and testosterone to ovariectomized animals resulted in a marked increase in peroxisomes and catalase activity. Results of the present study clearly show castration had no effect on the peroxisome proliferative effect of DHEA in males and females indicating that sex hormones have no role in such an effect. However, it is possible that there are increased levels of circulating testosterone in these animals, since DHEA is actively metabolized to androgens and estrogens (27). In the present experiment, we did not measure the levels of testosterone in castrated animals receiving DHEA.

The mechanism by which DHEA induces peroxisome proliferation remains unknown. It is possible that not DHEA itself but a metabolic product yet untested may be responsible for peroxisome proliferation, or that DHEA itself is producing this effect through a receptor-mediated pathway different from PPAR.

The study was supported by Veterans Affairs Merit Review Grant and Public Health Service Grant GM23750.

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