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Ergosteroids: Induction of thermogenic enzymes in liver of rats treated with steroids derived from dehydroepiandrosterone

HENRY LARDY, BRUCE PARTRIDGE, NANCY KNEER, AND YONG WEI

Institute for Enzyme Research, University of Wisconsin, 1710 University Avenue, Madison, WI 53705

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ABSTRACT **Dehydroepiandrosterone (DHEA), an intermediate in the biosynthesis of testosterone and estrogens, exerts several physiological effects not involving the sex hormones. When fed to rats it induces the thermogenic enzymes mitochondrial *sn*-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme in their livers. Animals and humans, and their excised tissues, are known to hydroxylate DHEA at several positions and to interconvert 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, 7-oxo-DHEA, and the corresponding derivatives of androst-5-enediol. We report here that these 7-oxygenated derivatives are active inducers of these thermogenic enzymes in rats and that the 7-oxo derivatives are more active than the parent steroids. We postulate that the 7α-hydroxy and 7-oxo derivatives are on a metabolic pathway from DHEA to more active steroid hormones. These 7-oxo steroids have potential as therapeutic agents because of their increased activity and because they are not convertible to either testosterone or estrogens.**

We wish to report the biological activity of some known and some newly discovered steroids structurally related to dehydroepiandrosterone (androst-5-ene-3β-ol-17-one; DHEA). DHEA is produced in the adrenals and brain and is the most abundant steroid in the blood of adult humans; it reaches a maximum concentration at 20–25 years of age and declines thereafter. It circulates mainly as the sulfate ester but the ester and free steroid are metabolically interconverted (1, 2). DHEA is an intermediate in the metabolic conversion of cholesterol to testosterone and estrogens and it also exerts several physiological effects independent of the sex hormones. In relatively large doses, it causes weight loss in genetically obese (3) and normal (4) animals without affecting food intake; it depresses blood cholesterol levels in men (5), rats (6), and dogs (7); it decreases blood sugar concentration in diabetic mice (8); it enhances resistance of mice to viral infections (9, 10); it reduces the incidence of spontaneous (11, 12) and carcinogen-induced (13) tumors in mice; and it improves memory in aged mice (14). Body weight responses in humans treated with DHEA are questionable (5, 15–19).

The diversity of the responses to DHEA and the large doses (≈0.5% of the diet for animals) required to elicit most of them led to the postulate that DHEA might be the precursor of steroid hormones other than the sex hormones and that these metabolites might be active in smaller doses and might display specificity for one, or only a few, of the effects described above.

We therefore synthesized known and possible metabolites of DHEA in the hope of finding compounds with greater biological activity. Another objective was to find metabolites of DHEA that retained the activities of the parent except for the ability to form androgens and estrogens. DHEA should not be used by women for extended periods because it increases their circulating testosterone and dihydrotestosterone manyfold above normal concentrations (16); they become hirsute and exhibit other signs of masculinization.

It was demonstrated by Tagliaferro *et al.* (20) that feeding DHEA to rats enhanced heat production and decreased efficiency of food utilization. We therefore measured its effect on the production of liver mitochondrial *sn*-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme, two enzymes that had been demonstrated (21–24) to be induced by a classic thermogenic agent, the thyroid hormone. Both enzymes were increased severalfold in livers of rats fed a diet containing 0.2–0.5% DHEA for 6 days (25, 26). A possible mechanism by which these enzymes function to decrease metabolic efficiency was proposed (25) and experimental results are supportive (27). We have used assays for these thermogenic enzymes as a guide to our synthesis program.

EXPERIMENTAL METHODS

Rats (body wt, 150 ± 10 g; Sprague–Dawley strain; Sasco, Omaha, NE) were fed pulverized Purina chow. Steroids to be assayed were dissolved in alcohol or ether, added to the chow, allowed to dry in air, and mixed well. After consuming the diet for 6 days the rats were sacrificed on day 7 and the left lobe of the liver was excised, weighed, and rinsed in cold 250 mM mannitol/70 mM sucrose/3 mM Hepes, pH 7.4. Preparation of mitochondrial and cytosolic fractions and assays for mitochondrial glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme were as described elsewhere (26). In each experiment, the enzyme activities in livers of rats fed the same diet without added steroid are recorded and activities in the treated rats' livers are reported as a percentage of the controls; a group receiving DHEA was also included to provide a standard response. Responses to any given compound are usually consistent between animals in an experiment, as are the controls, but there is considerable variation in the control values from one experiment to another. Control activities for glycerol-3-phosphate dehydrogenase have ranged from 3.2 to 10.2 nmol per min per mg of protein and activities for malic enzyme range from 16 to 36 (same units). Therefore, we consider compounds active only if they increase enzyme activity to >150% of the control value. To improve sensitivity, assays were conducted with less than maximally effective amounts of steroid in the diet. Food consumption was not altered by including the steroids in the diet. The limited number of rats in some groups mirrors our funding.

DHEA and androst-5-ene-3β,17β-diol were purchased from Steraloids (Wilton, NH). The 7-oxygenated steroids were synthesized by procedures described in the literature; some will be described elsewhere.

RESULTS AND DISCUSSION

Hydroxylations, oxidations, and reductions are major routes of steroid alteration in tissues (1, 2, 28). We have applied chemical reactions to prepare a wide variety of steroids related

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Abbreviation: DHEA, dehydroepiandrosterone.

Table 1. Induction of thermogenic enzymes by steroids oxygenated at position 7

Steroid	% of diet	No. of rats	% of control	
			GPDH	Malic enzyme
3β-ol-A-17-one (DHEA)	0.2	4	285	645
	0.1	61	276 ± 44	417 ± 64
	0.05	65	242 ± 41	311 ± 66
	0.01	5	121	96
3β,7α-diol-A-17-one	0.05	2	292	423
	0.033	2	308	374
3β-acetoxy-7β-ol-A-17-one	0.05	3	219	339
3β-ol-A-7,17-dione	0.05	3	366	521
	0.01	3	183	299
A-3β,17β-diol	0.2	6	254	473
	0.05	2	294	304
A-3β,7α,17β-triol	0.1	2	227	611
A-3β,17β-diol-7-one	0.05	5	378	418
	0.01	5	175	210

Control rats, whose enzyme activities were recorded as 100%, were from the same litters as those fed the steroids. A, androst-5-ene; GPDH, *sn*-glycerol-3-phosphate dehydrogenase.

to DHEA, and among these the 7-oxygenated ones are the most active (Table 1).

Whereas DHEA does not detectably enhance enzyme activity when fed at only 0.01% of the diet, 7-oxo-DHEA and 7-oxoandrost-5-enediol clearly do. They are also more effective at the other concentrations tested. The corresponding 7-oxygenated androstane derivatives do not induce these thermogenic enzymes. Acyl esters of most of the steroids listed in Table 1 are nearly as effective as the free steroids. The sulfate ester of DHEA is much less active than DHEA when administered either orally or intraperitoneally (26).

The presence of 7α-hydroxy- and 7-oxo-DHEA in tissues and urine was first reported by Fukushima, Gallagher, and coworkers (29), and hydroxylation at position 7 has been demonstrated in slices and extracts from several different tissues (30–36). The hydroxylase (37, 38) and 7α-hydroxysteroid dehydrogenase (39) involved in the formation of these compounds have been characterized. It seems likely that these 7-oxygenated steroids are on a metabolic pathway to a more active hormone; however, their biological activities have not been reported until recently (26, 40–43).

The 7-oxo steroids should prove to be more useful therapeutic agents than DHEA, for they are more active, are not aromatized (36), and cannot be converted to testosterone. We have prepared 7-hydroxytestosterone and it does not influence rat seminal vesicle weight nor does it induce thermogenic enzymes (data not shown).

Sunde *et al.* (44) have reported that 7α-hydroxytestosterone has no androgenic activity as measured by its lack of effect on maintenance of acid phosphatase in the prostate of castrated rats.

The 7-oxygenated compounds listed in Table 1 remain to be tested for activities other than weight loss and induction of thermogenic enzymes. Because of their influence on energy metabolism, they can appropriately be called ergosteroids.

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