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Induction of Hepatic Mitochondrial Glycerophosphate Dehydrogenase in Rats by Dehydroepiandrosterone[1]

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Feeding the thermogenic steroid, 5-androsten-3B-ol-17-one (dehydroepiandrosterone, DHEA) in the diet of rats induced the synthesis of liver mitochondrial sn-glycerol 3-phosphate dehydrogenase to levels three to five times that of control rats within 7 days. The previously reported enhancement of liver cytosolic malic enzyme was confirmed. The induction of both enzymes was detectable at 0.01% DHEA in the diet, reached plateau stimulation at 0.1 to 0.2%, and was completely blocked by simultaneous treatment with actinomycin D. Feeding DHEA caused smaller, but statistically significant increases of liver cytosolic lactate, sn-glycerol 3-phosphate, and isocitrate ($NADP^+$ - linked) dehydrogenases but not of malate or glucose 6-phosphate debydrogenases. The capability of DHEA to enhance mitochondrial glyceropbosphate dehydrogenase and malic enzyme was influenced by the thyroid status of the rats; was smallest in thyroidectomized rats and highest in rats treated with triiodothyronine. 5-Androsten-3β,17β-diol and 5-androsten-3β-ol-7,17-dione were as effective as DHEA in enhancing the liver mitochondrial glycero-phosphate dehydrogenase and malic enzyme. Administering compounds that induce the formation of cytochrome P_{450} enzymes enhanced liver malic enzyme activity but not that of mitochondrial glycerophosphate dehydrogenase. Arochlor 1254 and 3-methylcholanthrene also increased the response of malic enzyme to DHEA feeding.

The 17-keto steroid dehydroepiandrosterone (5-androsten 3B-ol-17-one) (DHEA). produced in the adrenals. testes and brain (1, 2) serves as an intermediate in the biosynthesis of both estrogens and androgens from 17a-hydroxy *pregnenolone*. The possibility that DHEA itself has biological activity was suggested from the work of Yen et *al. (3)* who found it to cause weight loss in obese mice without affecting food intake. In edition to its antiobesity effects, DHEA lessens the severity of diabetes in animals (4), enhances resistance to viral infections (5), and inhibits the formation of spontaneous (6) and carcinogen-induced (7) tumors in mice. Alterations of DHEA amounts and distribution of its metabolites are associated with some types of mental retardation (8, 9).

As other steroid hormones do. DHEA or its metabolic products would be expected to combine with a specific receptor protein and the receptor-hormone complex would activate transcription of specific genes. Searching for enzymes whose synthesis might be induced by DHEA disclosed large reposes by rat liver cytosolic malic enzyme (10-14), P IVA 1 (15), palmitoyl CoA oxidase (15, 16), 3-hydroxyacyl CoA dehydrogenase (17), acylCoA transferases (17, 19), and fatty acyl CoA hydrolase (11).

The proposal (20, 21) that mitochondrial w-glycerol 3-phosphate dehydrogenase (GPDH) might provide a pathway for electron transport from cytosol to mitochondria with for electron transport from cytosol to mitochondria with diminished phosphorylation efficiency and consequently increased thermogenesis prompted an investigation of the influence of thyroid hormone, a classic thermogenic agent, on that enzyme (22, 23). Feeding 0.2% desiccated thyroid powder in the diet of rats for 10 days enhanced liver mitochondrial GPDH 20-fold above the activity in livers of euthyroid rats (23) which is consistent with this enzyme participating in the thermogenic effect of thyroid hormone. Because DHEA is also a thermogenic agent we investigated the effect of this steroid on the activity of GPDH as influenced by diet composition and thyroid hormone status of rats. For comparison, assays for malic enzyme were also conducted in all experiments,

EXPERIMENTAL PROCEDURES

Materials Were from the Named Sources-S-Androsten-3B, 16B-diol-17-one, Steraloids, Wilton, NH; DHEA and other steroids, biodothymnine (sodium salt), and 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyl tetrazolium chloride (grade 1), Sigma Chemical, St. Louis; Bradford reagents, Bio-Rad Laboratories, Richmond, CA. A semipurified high-sucrose diet (containing casein, 20%; DL-methionine, *0.3%;* cornstarch, 15%; sucrose, 50%; corn oil, 5%; fiber-celufil, 5%; and AIN mineral mix, 3.5%), United States Biochemical, Cleveland, OH. Purina rat chow (22% protein; carbohydrate content is from corn meal and protein; carbohydrate content is from corn meal and soybean meal) was fed in most experiments A generous sample of 5-androsten-3β-ol-7, 17dione was provided by Professor Charles Sih, School of Pharmacy, University of Wisconsin-Madison. 16α-Bromoepiandrosterone was synthesized in this laboratory; its melting point (164°C) agreed with the reported value. Pregnenolone-16α-carbonitrile was a gift from Dr. P.O. O'Connell of the Upjohn, Kalamazoo.

Animal Maintenance Sprague-Dawley rats, including immature young males (weighing 125-150 g at 40 days), adult males (weighing 225-250g at 55 to 60 days), and immature females (weighing 125-l 50g at 45 days), were used in the present study. They were allowed free

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Abbreviations: DHEA, dehydroepiandrosterone (5-androsten 3β-ol-17-one); GPDH, *sn*-glycerol 3-phosphate dehydrogenase; ME, malic enzyme; 7-ox&DHEA, 5-androsten 3β-ol-7, 17-dione; T_3, triiodothyronin; Thx, thyroidectomized.

access to water and either rat chow or the semi-purified high-sucrose diet during the experimental period. Immature young males were used for the experiments on the time course of induction and the dose-response relationship Of DHEA dependent GPDH and ME stimulation, as well as the effects of triiodothyronine (T3) and a cafeteria diet on the activities of these two enzymes, because these animals showed a greater enzyme response to the various treatments than did adult males. Because they were easier to thyroidectomize, the larger males were used in the studies of the relationship between thyroid hormone and DHEA, Afler operation, thyroidectomized animals were weighed daily for at least 2 weeks and only rats that maintained stable body weights were selected for experiments. Normal euthyroid rats in the control *groups* for these *experiments* were also maintarned for 2 weeks before use. In all other experiments body weight and food intake were measured every 2 to 3 days throughout the entire experimental period.

Diet Preparations and Hormone Administration--DHEA-containing diets were prepared by dissolving suitable amounts of the steroid in either, mixing evenly with either the pulverized Purina Chow or the semi-purified high sucrose diet, then drying in air. Most of the experiments were conducted with diets containing 0.2% DHEA for a 6 day period, unless mentioned otherwise. The cafeteria diet examined in this study contained chocolate chip cookies, cake, peanut butter, salami, sweetened condensed milk, and cheese. These palatable foods were changed every day and continuously offered for 2-3 weeks. Hormones to be injected were suspended in olive oil, the suspensions were prepared fresh daily.

Reagent Preparation and Application--L-Triiodothyronine (T,) solution was prepared by dissolving suitable amounts of the hormone in a minimum of 0.01 N NaOH, then diluting with saline to the desired concentration. The amounts of T_3 administered and the duration of treatments are described in the legends to figures and tables.

Preparation of Mitochondrial and Cytosolic Fractions-Mitochondria from various tissues were prepared as described (24), except that white adipose tissues were homogenized at room temperature. and skeletal muscle was ground with a polytron, then filtered through cheesecloth. Mitochondria-containing fractions were resuspended with 35% (w/w) glycerol and stored at -80°C. The supematant from the first 1,000 x *g* spin of mitochondrial preparation was centrifuged for another 30 min. at 85,000 x *g* to obtain the cytosolic fractions that were also stored at -80°C , until enzyme activities were assayed.

Protein Determination-Protein concentrations were measured by means of the dye binding method (25). Mitochondrial fractions were dissolved with Triton X-100, and compared to bovine serum albumin plus the detergent, whereas cytosolic fractions were assayed with bovine serum albumin alone as the standard.

Enzyme Assays-The activity of mitochondrial GPDH was determined by the method of Wernette *et al. (26) with the* concentration of DL-glycerophosphate at 100 mM and with an incubation time of 30 min. Its specific activity was designated as nmol reduced iodoformazan formed /(mg mitochondrial protein x min). The activity of cytosolic malic enzyme (ME) was measured according to the method of Hsu and Lardy (27). The specific activity of the enzyme was expressed as nmol NADPH formed (mg cytosolic protein x min). To determine the activities of lactate dehydrogenase, isocitrate dehydrogcnase. cytosolic glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenasc, and malate dehydro- genase, the methods of Bergmeyer (28) were used. Specific activities of these enzymes were expressed as the formation or oxidation of NADPH or NADH in nmol / (mg cytosolic protein x min).

RESULTS

DHEA Induces Hepatic GPDH and ME Synthesis in Liver-Responses of hepatic GPDH and ME in immature male rats to various amounts of DHEA (00.01-0.5 %) in the diet, each of which was administered for a period of 6 days, arc shown in Fig. 1. Both enzymes exhibited a similar dose examined (0.01%) resulted in a small but detectable stimulation in activities of GPDH and ME. and maximum stimulation of these two enzymes required approximately 0.1-0.2% DHEA in the diet

The relationship between the doses of DHEA in the diet and the magnitude of hepatic enzyme stimulation detected in mature male rats (225-250g) was similar to that found in immature male rats, except that the d-response curve shifted slightly toward higher doses of DHEA in older animals.

Oral administration of DHEA at doses of 0.3-0.5% suppressed the food intake in both mature and immature male rats significantly; rats in these treated groups ate about 30% less than the controls. After 6 days on the above diets, the immature, treated rats had gained 40% less weight in comparison with their untreated counterparts and mature, treated rats exhibited a small reduction in body weight in contrast to a 5% increase by the controls. During the 6 day experimental period. diets containing no more than 02% DHEA did not suppress either food intake or weight gain significantly in male rats of both ages. However, after the long-term, 3-month treatment, the final body weights of rats fed 0.2% DHEA were 300400 g and those in the control group were significantly heavier at 450-500 g.

The time course of hepatic GPDH and ME induction by 0.2% DHEA in the diet of immature male rats is shown in Fig. 2. During the 20-day experimental period, the activity of GPDH in the liver of the control animals progressively decreased to about 60% of the initial level, and that of hepatic ME to approximately 40%. The effect of administration of DHEA on GPDH activity in this organ became discernible after the rats were fed the steroid-containing diet for 1-2 days, while that on the activity of hepatic ME required 2 days. A large difference in both enzyme activities between the treated and control groups was found after 4 days of treatment. The activity of liver ME reached maximal levels, 100-l 80 nmol / (mg cytosolic protein x min), at about 1 week after initiating the feeding of the steroid-containing diet, then gradually decreased throughout the rest of the experimental period, which seemed to parallel the intrinsic change in ME activity in control animals. The difference in GPDH activity between treated and control groups continued to increase during the second week of DHEA treatment. After 2 weeks on the steroid-containing diet, this enzyme activity reached 40-60 nmol / (mg protein x min).

Effect of DHEA Containing Diets on Activities of GPDH and ME. in Male and Female Rats at DifferentAges. -Administration of DHEA at 0.5 or 0.2% of the diet for 6 consecutive days remarkably enhanced the activities of GPDH and ME in the liver of the three types of Sprague-Dawley rats used(Table I). Compared with the activities in control animals fed the plain chow, hepatic ME activity was increased 5-to-8-fold and hepatic GPDH activity 2- to 5-fold in male rats of both ages. When fed for 75 days, 0.2% DHEA in the diet increased these respective activities 8-and 4-fold (Table IA). The DHEA-dependent increased in the activities of both enzymes in female rats was less than that found in male rats, but still substantial.

Characteristics of DHEA-Responsive Induction of Hepatic GPDH-Actinomycin D, an inhibitor of mRNA synthesis, blocked enzyme induction by DHEA. The inhibitor (2 ug/100 g body wt) was injected subcutaneously daily, 2 h before DHEA (28 mg/100g body wt) was injected intraperitoneally.

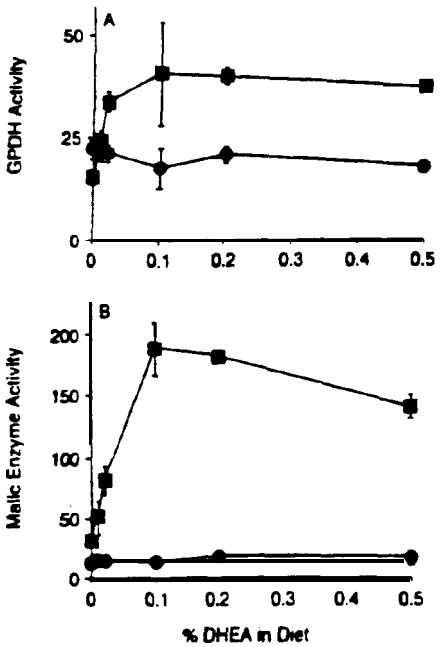

Fig. 1. **Dose-dependent effects of dietary DHEA on activities of CPDH and ME in liver and kidney.** Immature male rats were fed varying concentrations of DHEA in the diet for 6 days and were killed on day 7. Enzyme activities are recorded in nmol / (mg protein x min). Each data point represents the mean ± SD of 3 rats The enzyme assays were given in "EXPERIMENTAL PROCEDURES." GPDH activities are shown in A and those of ME in B. ■, assays of liver enzymes; ●, kidney.

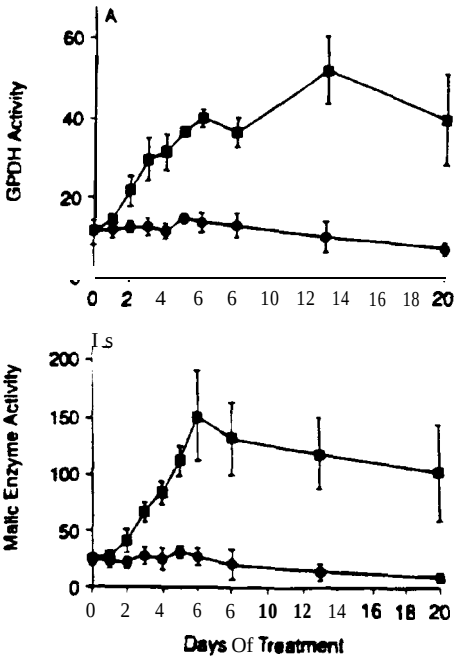

Fig. 2. **The time course of hepatic CPDH and ME induction by DHEA.** Immature male rats fed the diet containing 0.2% DHEA were killed at the indicated days after the initiation of steroid feeding. A describes the time course for hepatic GPDH induction and B for hepatic malic enzyme in nmol / (mg protein x min). Each data point represents the mean ± SD of 3 - 5 rats. ● , treated; ●, controls.

TABLE I. **Effects of DHEA-containing diet on activities of mitochondrial glycerophosphate dehydrogenase (GPDH) and cytosolic malic enzyme.** in sets A and B control tats were fed pulverized Purina Rat Chow. DHEA was added to this diet at the concentration designated. The T, was injected at the following daily doses per 100g body wt: a, 10 μg. C. 1 μg. In experiment C the high sucrose diet was fed for 12 days: DHEA was included in thal diet during the last 6 days. Enzyme assays are described in "EXPERIMENTAL PROCEDURES." Data are reported as mean ± SD from 3 to 5 animals. * indicates significant differences between control and treated groups at $p < 0.05$ and ** at $p < 0.01$

Type of rats and treatment	GPDH [nmol/(mg protein x min)]		Malic enzyme [nmol/(mg protein x min)]	
	Control	Fed DHEA	Control	Fed DHEA
A Immature males 0.5% DHEA, 6 days	14±2.2	37±1.7**	27±9	139±10**
Immature males + T_3, 6 days	41±0.9		66±10	
Mature males 0.5% DHEA 6 days	5.3±1.0	19±0.9**	lai1.3	79±11**
Immature females 0.2% DHEA 6 days	8.8±1.2	17±0.8**	13±8.1	44±11**
Immature males 0.2% DHEA 75 days	6.0±2.0	23±6.0**	8±2.0	65±43**
B. Mature males 0.5% DHEA 13 days	5.0±1.0	19±1.0**	loil.3	79±11**
Mature Thx males 0.5% DHEA, 13 days	1.2±0.3	611.1"	1.3±1.0	42±17*
Mature Thx males + T, 0.5% DHEA 13 days	38±8.3	79i2.8"	63±34	338±1 11"
C. Mature males. chow	5.6i1.4		I li2.6	
Mature males, Hi Suc. 12 d. 0.2% DHEA 6 days	14±4.0	29±4.3**	46±32	116±20*
Mature Thx males. Hi Suc, 12 d. 0.2% DHEA, 6 days	0.4±0.2	4il.7	4.tio.9	18±15
As shown above + T,. 10 days 0.2% DHEA 6 days	47i4.9	77±5.4**	213±77	390±144*
D. Immature males chow diet, 2 1 days	7.8il.3		11±3.0	
Immature males cafeteria diet 2 1 days	20±0.6		35±8.2	

TABLE II. **Effects of dietary DHEA on activities of liver cytosolic NAD- or NADP-linked dehydrogenases.** Activities of malate (MDH), lactate (LDH), glycerophosphate (cytosol GPDH), isocitrate (ICDH), and glucose-6-phosphate (G6PDH) dehydrogenases m immature male rats fed a diet containing 0.2% DHEA for 2 weeks are reported as means ± SD from 5 rats. * and ** as in Table 1.

Dietary treatment	NAD-linked			NADP-linked	
	MDH	LDH	GPDH [μmol/(mg protein x min)]	ICDH	G6PDH
0% DHEA	4.9±0.6	5.5±1.1	1.2±0.5	0.25±0.04	0.014±0.003
0.2% DHEA	4.9±1.3	11.3±0.7*	2.3±0.8	0.36±0.05*	0.014a0.004

TABLE III. Enzyme induction by steroids related to DHEA. The hormones were administered to immature male rats for a period of 6 days; the animals were killed on day 7. The intraperitoneal dose of 28 mg/100g body weight corresponds approximately to the amount of hormone consumed by the rats fed 0.2% in the diet There were 3 to 7 rats in each of the hormone-fed groups and 5 in each of the injected groups. * and ** as in Table 1

Treatment (g)	Liver wt	GPDH [nmol/(mg protein x min)]	ME
Control	8.9ztl.l	12.8±2.7	25.4±7.3
Steroid fed		(% of control activity)	
0.1 % DHEA	10.4±1.0	286±103	515±97
0.2% DHEA	11.0±1.0	287±39	613±173
0.2% DHEA sulfate	9.2i0.4	208±3 1	270128
0.2% 5-androsten-3β, 1 7β-diol	N.D	275±11	4941175
0.0 1% 7-oxo-DHEA	10.2±0.4	167±8	182±41
0.1% 16α Br-epiandro-sterone	8.4i0.7	96±14	83±9
		[nmol/(mg protein x min)]	
Control	8.2il.0	13.3±2.6	21.3±7.0
Steroid injected daily		(% of control activity)	
DHEA 14 mg/100g	N.D. 200±10	245±48	
DHEA 28 mg/100g	10.9±0.5	252±17	372±48
DHEA sulfate 28mg/100g	9.4±0.5	152±31	179113
5-Androsten-3β.17β-diol 28 mg/100g	9.6i0.6	337±27	444±108
7-Oxo-DHEA 7 mg/100g	10.Ji0.3	183±26	251±85
16α-Br-epiandrosterone 14 mg/100g	8.7zt0.6	126±19	128±9

Rats injected with DHEA and killed at daily interviews showed a linear increase of GPDH activity which in four days reached 3 times the activity of the control group. Liver GPDH rats given actinomycin alone, or followed by DHEA was not significantly different from that of control rats (29).

The effect of DHEA treatment on protein content in liver was also investigated. After an 8-day treatment, cytosolic fractions from treated and control groups contained 52±2 and 49±6 mg protein/g of liver, respectively; mitochondrial fractions, after 3 washings, retained 10 ± 2 and 21 ± 2.5 mg protein/g of liver ($p<0.01$). In addition, the relative weight of the liver (liver wt/body wt) in the treated animals was found to increase by 20-50%, in agreement with observations by Cleary *et al.* (11) and Kritchevsky *et al.* (30). Apparently, mitochondrials and peroxisomal protein content as well as liver size were significantly affected by DHEA administration, while cytosolic protein content was not These effects of the steroid were also discernible in rats after the 20-day treatment.

Effects of DHEA on Activities of Cytosolic Dehydrogenases–Because physiological functions of both ME and GPDH are related to NADPH generation and NADH oxidation, respectively, in the cytosol, activities of other hcpatic enzymes that interact with these coenzymes were assayed to determine whether they were affected by DHEA. Cytosolic NAD-linked lactate dehydrogenase, malate dehydrogenase, and glycerophosphate dehydrogenase, as well as NADP-requiring isocitrate dehydrogenase and glucose&phosphate dehydrogenase activities arc summarized in Table II. Feeding 0.2% DHEA in the diet for 2 weeks resulted in a statistically significant increase in activities of both lactate dehydrogenase (100%) and isocitrate dehydrogenase (50%), as well as a variable induction in the activity of cytosolic NAD-linked glycerophosphate dehydrogenase (an increase ranging from 0 to 100%). However. neither malate dehydrogenase nor glucose-6-phosphate dehydrogenase responded to the steroid.

Tissue Specificity of Responses to DHEA-In contrast to the thyroid hormone, which induces GPDH in liver, kidney, heart, skeletal muscle, and adipose tissue (23), DHEA elicits a response only in liver. The failure of kidney to respond to DHEA is shown in Fig. 1. Feeding 0.4% DHEA in the diet for 3 weeks decreased the specific activity of GDPH 20% and malic enzyme 60% in brown fat (29).

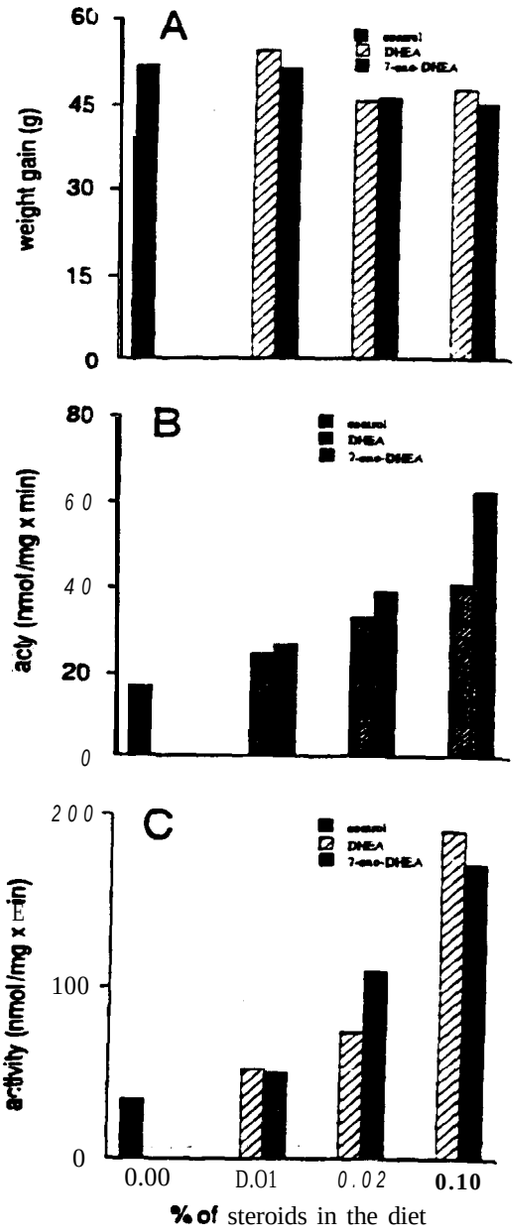

Fig. 3. Comparison of DHEA and 7-oxo-DHEA. The steroids were fed to immature male rats for 6 days. Data for GPDH are shown in B and for malic enzyme in C. Mean values for 3 animals are recorded.

DHEA-Responsive Induction of GPDH ond ME in Rats of Various Thyroid States--The relationship between DHEA and thyroid hormone in their effects on GPDH and ME induction was examined with oral administration of these steroid to animals of three different thyroid states: hypothyroid (thyroidectomized), euthyroid, and hyperthyroid (T,-injected thyroidectomized tats) (Exp. B in Table I). The experiment was initiated when the body weight of the thyroidectomized animals stabilized-about 2 weeks after surgery. Thyroidectomized rats ate very little of the diet containing 0.5% DHEA for about 1 week. Therefore the entire period of the experiment was extended to 13 days which allowed 6 days of food consumption in normal amounts. Activities of both GPDH (22, 23) and ME are extremely low in livers of thyroidectomized rats, but DHEA was effective in enhancing their activity, especially that of malic enzyme. T_3 administered at a relatively high dose (4 μg/100g body wt, daily) induced the formation of both enzymes and greatly augmented enzyme synthesis induced by DHEA.

TABLE IV. Inducers of cytochrome P_{450} enhance malic enzyme formation. Immature male rats, fed the chow diet with or without 0.2% DHEA for a total of 2 weeks, were injected ip with the inducers for the last 4 days. Arochlor 1254 was injected only once, 4 days before terminating the experiment. Data are the means ± SD from 5 to 10 rats for each treatment except for the higher dose of RU 38486 where only 2 animals were treated. • indicates significant difference between inducer-mated and control chow-fed rats at $p<0.05$ and • * at $p<0.01$.* indicates significant difference between inducer-treated and control groups of rats fed chow containmg DHEA at $p<0.05$ and ** at $p<0.01$.

Inducer	0.0% DHEA		0.2% DHEA	
	GPDH	ME	GPDH	ME
	(% of control activities)			
Not treated	100	100'	454±73	915±234
Aroclor 1254 30 mg/100g	76±13**	219±55**	493±102	1814±184**
3-Methylcholanthrene 2.5mg/100g	72±13**	194±56**	491±61	1487±283**
Phenobarbital 7.5mg/100g	95±23	176±28**	371±35*	1171±255*
Isosafrole 15mg/100g	90±11	155±40**	445±64	1087±175
β-Naphthoflavone 4mg/100g	80±14*	230±44**	416±53	1014±257
Pregnenolone-16α carbonitrile 2.5mg/100g	90119	116±41	388±60	1223±387*
Dexamethasone 8mg/ 1 00g	7915"	92±21	318±50**	617±18**
RU 38486 0.3mg/100g	104±8	95±14	455±142	1169±183
RU 38486 3.0 mg/100g	88-92	123-125	440-466	1196-1215

The GPDH and ME activities in livers of the control rats were 10.2±3.0 and 12.1±3.5 nmol/(mg protein x min), respectively.

Dietary Studies--Feeding low-protein diets to animals enhances thermogenesis, decreases metabolic efficiency (31) and induces the formation of liver mitochondrial GPDH (32, 33, 14). As shown in Table IC, merely including 50% sucrose in the diet without altering protein or fat content, in comparison with the chow diet, causes both GPDH and ME to be induced 3- to 4-fold above control values,

Previous investigators (33-35) have demonstrated enhanced conversion of T_4 to T_3 in animals fed a low protein diet and it has been assumed (33) that the elevated level of T_3 is responsible for the induction of GPDH synthesis. That seems also to be the cause of enhanced GPDH and ME formation in rats fed the high sucrose diet because thyroidectomized rats showed no enzyme response to sucrose feeding (Table IC).

The high-calorie "cafeteria" diet which causes overeating and inefficient weight gain (36, 37) is known to induce liver GPDH (38). Table ID demonstrates that malic enzyme is induced as well.

Structural Specificity--The relatively high doses of DHEA required to obtain weight loss in obese animals (3.4) and the wide variety of metabolic processes influenced by this steroid indicate the possibility that it may be converted to active derivatives other than androgens and estrogens (39). The C_{19} steroids are subject to eruymic alteration by hydroxyiation, oxidation, reduction, and conjugation (1, 40, 41). To determine whether DHEA may be converted to more active metabolites a variety of steroids derived physiologically from, or related in structure to. DHEA have been tested for their influence on GPDH and ME activities in rats. DHEA sulfate was approximately half as effective as DHEA when fed and less effective when injected intraperitoneally in amounts even equal to those consumed by rats fed the steroid at 0.2% of the diet (Table III). Androstene diol, which is readily introconverted with DHEA (1) was as effective as equal doses of DHEA (13). 7-Oxo DHEA was as effective as DHEA in inducing ME (Fig. 3C) and somewhat more effective in enhancing GPDH, especially at low doses (0.01-0.1% of the diet) (Fig. 3B). These concentrations of DHEA and of 7-oxo DHEA did not alter food intake as compared with the control group. The slight depression of body weight gain by DHEA and its 7-oxo derivative in the 6day experiment is not statistically significant (Fig. 3A). It does however rule out any acute toxic effect of the 7-oxo derivative which has not previously been administered to animals.

The following compounds did not enhance the activity of GPDH or ME when included in the diet of rats for a period of 6 days; the amount fed was 0.2% of the diet except where lesser doses are designated: 1 7α-hydroxy pregnenolone, testosterone, dihydrotestosterone, estrone (0.005), estriol (0.005), equilin (0.01), 5-androsten-3β, 16β-diol-17-one (0.007), 5-androsten-3β, 17β-diol-16-one. 5-androsten-3.18-dione, epiandrosterone (5α-androstan-3α-ol-17-one), etiocholan-3α-ol-17-one, etiocholan-3β-ol-17-one, etiocholan-3, 17-dione, 5α-androstan-3α, 17β-diol, 16α-bromoepiandrosterone, and 5-androsten 3β, 16α, 17β-triol (0.0025). Each of the estrogens tested at the designated doses depressed food intake and suppressed ME activity in both liver and kidney to two thirds to one half of that found in untreated control rats.

The active compound 7-oxo DHEA could arise by oxidation of 7α-hydroxy DHEA produced by a cytochrome P_{450} hydroxylase system specific for the 7 position of steroids (4 1, 42). Introduction of oxygen at other positions on the steroid molecule may also depend on P_{450} type hydroxylases; therefore inducers of cytochrome P_{450} enzymes (43) were tested to determine whether such treatment influenced responses to DHEA Injecting Arochlor, 3-methylcholanthrene, and phenobarbital increased the response of malic enzyme to DHEA but did not enhance the response of GPDH (Table IV). The enhancement of ME activity by each of the P_{450} inducers in animals not given DHEA could have resulted from enhanad hydroxylation of endogenous DHEA. None of the P_{450} inducers affected food intake or weight gain during the 4 day period of their use; all caused liver enlargement.

The steroid derivative RU 38486 competes with glucocorticoids and progesterone for their respective receptors (44). It had no effect on GPDH or ME activities when injected into rats at 0.3 on 3.0 mg per 100 gm body weight daily. The lower dose is sufficient to block the action of progesterone and the higher block glucocorticoid function (45).

DISCUSSION

The ability of DHEA to cause weight loss in obese animals without altering food intake is based on its thermogenic activity (46). Introduction of mitochondrial GPDH synthesis by thyroid hormone (22, 23) or DHEA may provide a means to enhance thermogenesis because the pathway of ekctron transport through that enzyme (20, 2 1) is a third less efficiently coupled to phosphorylation than oxidation of NADH through the complete mitochondrial electron transport system. It must therefore generate more heat. Mitochondrial GPDH is usually considered to be one of two main, possible pathways for the transfer of reducing equivalents from cytosolic NADH to the mitochondrial electron transport chain. The NADH generated in cytosol during the conversion of carbohydrate to pyruvate represents only 1/6th of the reducing equivalents generated in the conversion of carbohydrate to CO_2. Decreasing the efficiency of that fraction by 1/3 could diminish the efficiency of energy capture during carbohydrate metabolism by only 5.6% which is far less than the effect elicited by thermogenic hormones and low-protein diets (47).

A postulated role for liver rnalic enzyme in the transport of reducing equivalent from mitochondrially generated NADH to the cytosolic pool

is described elsewhere (14). By that process a much larger fraction of reducing equivalent could be transported through the less efficient, more thermogenic, GPDH pathway.

DHEA enhances GPDH some fourfold and malic enzyme five to eightfold in liver but does not alter the activities in kidney, spleen, muscle, or white adipose tissue. Feeding DHEA for 3 weeks depressed GPDH in brown fat by 20% and malic enzyme by 60%. It seems likely that heat generated under the influence of DHEA may lessen the demand for thermogenesis by brown fat.

Administering DHEA elevates plasma concentrations of this steroid as well as of its metabolites including both estrogens and androgens (39, 48). In our study androgens and estrogens did not increase the activity of liver GPDH of malic enzyme. Androstenediol and DHEA sulfate, which are interconvertible with DHEA (1) induced both enzymes. However, 17α-hydroxypregnenolone, which generates DHEA by carbon bond cleavage at C17-C20, had no effect. Presumably the cleavage is limited by the amount of 17, 20 lyase, or indigenous DHEA may exert a controlling influence on this enzyme.

The great variety of physiological effects exerted by DHEA invites speculation that it may be converted to a number of more active compounds with differing functions. The synthetic analog–16α-bromoepiandrosterone is more active than DHEA in suppressing thymidine incorporation into epidermal cells (49) and in blocking the differentiation of 3T3-L1 preadipocytes (SO), yet it was found to have no effect on the activities of GPDH or malic enzyme (Table III). Etiocholanoloncs (both 3a and 3β) are antiobesity agents for diabetic, obese, and normal mice (51) but had no effect on GPDH or malic enzyme. Thus, species differences as well as functional differences may be involved in these discrepancies.

C_{19} steroids are hydroxylated at position 6, 7, 11, 15, and 16 in intact animals and by isolated microsomal fractions (1, 41). Hydroxylation at position 7 and formation of 7-oxo-DHEA by liver fractions have been reported (52-56) but such compounds have only recently been found to have biological activity (13, 57). An advantage of 7-oxo-DHEA for therapy is the fact that it is not convertible to either androgens or estrogens. The synthesis of several additional new compounds related to DHEA will be described elsewhere.

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