The Effect of DHEA Given Chronically to Zucker Rats (43883)

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Abstract. **Dehydroepiandrosterone (DHEA) has been reported to exert antiglucocorticoid activity. When administered to obese, hypercorticosteronemic Zucker rats, it causes a diminution of food intake and a reduction in their rate of weight gain. This experiment was conducted to evaluate whether this biologic effect could be ascribed to chronic adrenal insufficiency. Obese and lean Zucker rats were treated with DHEA as a food supplement for 28 days. Upon sacrifice, organ weights and serum chemistries were measured, along with neurotransmitter levels in regions of the hypothalamus. Results showed that although the obese animals gained weight more slowly, had lower insulin levels, and ate less, their serum glucose, corticosterone, and ACTH levels were not different from control. Hypothalamic neurotransmitters in the obese rat were unaffected by chronic DHEA treatment. We concluded that, although DHEA clearly affects Zucker weight gain, it does not induce chronic adrenal insufficiency.**
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Dehydroepiandrosterone (DHEA) and its sulfated derivative (DHEA-S) are the most abundant steroids of the adult human adrenal. As they can be converted in the periphery to potent androgens, they are frequently referred to as "adrenal androgens." However, many of their actions cannot be accounted for by conversion to testosterone.

In humans, DHEA has been investigated because of potential cardioprotective properties. Barrett-Connor *et al.* reported that in men the serum level of DHEA was inversely related to the likelihood of a myocardial event (1). This observation is supported by the finding that those with atherosclerotic disease have lower DHEA levels than matched controls (2–4).

Much of the experimental work conducted to delineate the actions of DHEA in animals has used rodent models of obesity (5–8). Cleary's group was among the first to demonstrate that feeding DHEA as a dietary supplement could lead to reduced weight gain and a reduction in hyperinsulinism in rats (5–6, 9–10). The rat model used most frequently to demonstrate this effect, the fatty Zucker rat, manifests hypercorticosteronemia in the obese state (11–14).

Other groups have studied the action of DHEA as an immunomodulator in mice. In these cases, DHEA improved immune function, particularly under stressful conditions (15–17). It was speculated that DHEA may act to modulate the immune suppressive effects of glucocorticoids. In a test of this possibility, Blauer *et al.* (18) showed that DHEA blocked dexamethasone-induced thymic involution and its suppressive effect on lymphocytes.

These observations led our group to hypothesize that DHEA's actions in obese rodents may be due to its action as an antagonist of glucocorticoids (19, 20). In one direct test of this hypothesis, we reported that DHEA blocked the effect of exogenous glucocorticoids on Zucker rat hepatic tyrosine aminotransferase, an enzyme involved in gluconeogenesis (19).

These favorable results led us to explore whether some of DHEA's favorable effects on more complicated physiologic pathways might be based upon its actions as an antiglucocorticoid. Recently, we studied the effects of DHEA on food intake in the hypercor-

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ticosteronemic, obese Zucker rat (21, 22). Hyperphagia and obesity in this animal are felt to be based, at least partially, on the hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis (23). We found that DHEA, given acutely, either as a food supplement, intraperitoneal injection or intracerebroventricular injection, diminished the caloric intake of the obese Zucker, with a particularly pronounced effect on fat food preference (21, 22, 24; unpublished observations). We have also shown that acutely, DHEA elevates serotonin levels in the lateral hypothalamus of this animal (25). Chaouloff has reviewed evidence that glucocorticoids affect central hypothalamic serotonin systems (26), and Castonguay's group has reported that dexamethasone increases fat food intake (27). These observations suggest another link among DHEA, glucocorticoids, and food intake.

So far, most of the reports on the chronic effect of DHEA on the Zucker rat have dealt with quantitating its effect on body weight, organ weight, and food intake (5–10, 21, 22, 24). Little has been done to determine whether DHEA interferes with the physiologic actions of glucocorticoids. It is possible that some of the change in rate of weight gain and diminution of insulin found during chronic administration may be due to DHEA-induced adrenal insufficiency. Further, whether the effect of DHEA on hypothalamic neurotransmitters is maintained with chronic administration has not been addressed. In this work, we investigated these questions by studying the effect of DHEA administered to lean and obese Zucker rats for 28 days.

Materials and Methods

Lean and obese female Zucker rats (12–16 weeks old) were obtained from our colony, randomly assigned to control or treatment groups and placed in individual cages. They were maintained on a 12:12-hr light:dark cycle (lights on at 6:00 AM) and given water *ad libitum*. Initially, rats from all four groups were given an *ad libitum* diet of powdered Purina Rodent Chow 5001 (3.3 cal/g) (Ralston-Purina, St. Louis, MO). At the beginning of the experiment, the diets for the lean and obese treatment groups were changed to powdered chow containing 0.6% DHEA ($n = 7$ in each group). This diet was maintained for 28 days. Similar rats ($n = 6$ in each group) consuming an unadulterated powdered diet served as controls. Food jars were weighed daily. Spilled food was collected daily on paper towels and weighed. Body weights were measured three times per week on a triple beam animal balance.

On the 27th day, rats were placed individually in a Controlled Acoustical Environments Activity Chamber (Industrial Acoustics Co. Inc., Bronx, NY). Each rat was allowed 6 min to adjust to the environment, and then activity was monitored for 9 min. Motions were counted and expressed per minute of time. All animals were studied sequentially on the same day. Statistics were performed by one-way analysis of variance by Superanova (MacIntosh version). A P value of less than 0.05 denoted significance.

Animals were sacrificed after 28 days by rapid decapitation. Core blood was collected in glass tubes and centrifuged, and the serum analyzed by routine clinical automated techniques. DHEA-S, DHEA, corticosterone, and ACTH were measured by radioimmunoassay (RIA) using commercial kits from Diagnostic Products Corp. (Los Angeles, CA). Insulin was measured using a kit from Linco (St. Louis, MO). The detection limit of the assay was 0.02 pmol/ml and the cumulative intra- and interassay coefficient of variations were 6.3% ± 0.4% and 7.4% ± 0.5%, respectively (±SEM). C-peptide was measured by a kit from Linco. The detection limit was 0.025 pmol/ml, and the cumulative intra- and interassay coefficient of variations were 5.8% ± 0.3% and 6.3% ± 0.4% respectively (±SEM). Cyclo (His-Pro) was measured in our laboratory using a radioimmunoassay whose characteristics and performance have been described (28, 29).

Organs were excised and weighed on an analytical balance. The brain was removed, and the hypothalamus isolated. The hypothalamus was dissected into regions and analyzed for neurotransmitters using HPLC with an electrochemical detector. Our technique has been described (22). Recoveries of neurotransmitters from hypothalamic extracts range from 75% to 90% (22).

Statistical analysis was performed using a statistics package in PC! Info (Retriever Data Systems, Seattle, WA) or a software program SuperAnova (Abacus Concepts, Berkley, CA). Significance ($P < 0.05$) was measured using Fisher's LSD giving the exact P value. All mean values reported are group averages ± SEM.

Results

DHEA caused several changes in the serum chemistries of the treated Zucker rats (Table I). Glucose was higher in the obese animals, but there was not a treatment effect in either group. Neither lipid levels nor renal function were significantly altered. There were slight changes in the elements of mineral metabolism. Phosphate levels were increased by 9%, and magnesium levels increased in the DHEA-treated lean group. On the other hand, levels of these two parameters decreased in the treated obese rat. In general, the normalcy of most serum values suggests the animals were neither dehydrated nor ill.

Averaged over the entire course of the experiment, the DHEA treated rats consumed 124 or 115 mg DHEA/animal/day (lean or obese, respectively). When expressed in relationship to body weight, the lean animals consumed 658 mg DHEA/kg body wt/day and

Table I. Serum Chemistries of DHEA-Treated Zucker Rats

Substance	Lean		Obese	
	Control	Treatment	Control	Treatment
Glucose (mg/dl)	144.8 ± 3.2	139.0 ± 2.5	162.5 ± 5.0[b]	157.7 ± 2.9
Cholesterol (mg/dl)	121 ± 6.4	112 ± 6.0	131 ± 8.5	159 ± 14.2
Triglycerides (mg/dl)	93.3 ± 12.3	128 ± 16.9	681 ± 139[b]	465 ± 88.9
BUN (mg/dl)	22.0 ± 0.5	24.0 ± 1.5	28.0 ± 3.8	27.3 ± 1.0
Creatinine (mg/dl)	0.517 ± 0.017	0.500 ± 0.032	0.500 ± 0.126	0.457 ± 0.020
Uric acid (mg/dl)	2.00 ± 0.07	1.79 ± 0.07[a]	4.52 ± 1.00[b]	2.89 ± 0.17
Total protein (g/dl)	6.23 ± 0.12	6.81 ± 0.14[a]	7.43 ± 0.33[b]	7.49 ± 0.11
Calcium (mg/dl)	10.85 ± 0.17	11.02 ± 0.31	11.20 ± 0.09[c]	11.79 ± 0.19[a]
Phosphate (mg/dl)	7.15 ± 0.25	7.80 ± 0.27[a]	10.14 ± 0.61[b]	8.57 ± 0.45[a]
Magnesium (mg/dl)	2.00 ± 0.058	2.13 ± 0.042[a]	2.40 ± 0.11[b]	2.06 ± 0.05[a]
Bilirubin (mg/dl)	0.42 ± 0.017	0.42 ± 0.04	2.00 ± 0.44[b]	0.97 ± 0.08[a]
Lactic dehydrogenase (IU/liter)	603 ± 77	608 ± 73	540 ± 40	525 ± 40
ALT (IU/liter)	53 ± 1.1	55 ± 4.5	40 ± 12	57 ± 7.3
AST (IU/liter)	147 ± 12	147 ± 12	131 ± 11	147 ± 13
Alkaline phosphatase (IU/liter)	166 ± 11	180 ± 14	354 ± 83[b]	304 ± 76
GGT (IU/liter)	2.0 ± 0.3	3.0 ± 0.5[d]	5.8 ± 1.8[b]	3.8 ± 1.3
Amylase (units/liter)	1354 ± 88	1822 ± 107[a]	1752 ± 168[b]	1850 ± 87
Albumin (g/dl)	3.7 ± 0.12	4.9 ± 0.10[a]	4.1 ± 0.09[b]	5.1 ± 0.07[a]
ACTH (pg/ml)	45.2 ± 3.7	49.9 ± 6.7	80.0 ± 4.5	66.0 ± 6.4
Corticosterone (ng/ml)	119 ± 57	160 ± 65	92 ± 32	192 ± 73
DHEA-S (μg/dl)	1.90 ± 0.51	506 ± 131[a]	0.95 ± 0.43	1121 ± 168[a]
Insulin (p-mole/ml)	0.373 ± 0.037	0.319 ± 0.050	2.118 ± 0.277[b]	1.660 ± 0.204[a]
C-Peptide (p-mole/ml)	0.697 ± 0.050	0.778 ± 0.077	2.638 ± 0.244[b]	2.864 ± 0.171
HIC	1.900 ± 0.083	2.599 ± 0.232[a]	1.280 ± 0.067[b]	1.817 ± 0.141[a]

Note. Core blood was saved after decapitation, centrifuged, and analyzed for the indicated substances. Values reported reflect the mean ± SEM. ALT = alanine aminotransferase; AST = aspartate aminotransferase; GGT = gamma-glutamyl transpeptidase; HIC = hepatic insulin clearance = concentration of C-peptide/concentration of insulin.
[a] The value for the treatment group was significantly different from its phenotypic control ($P < 0.05$).
[b] The value from the obese control group was significantly different from that of the lean control $P < 0.05$.
[c] The value of the obese control group was different from that of the lean control group > 0.05; $P < 0.06$.
[d] The value from the treated group differs from that of its phenotypic control group $0.05 < P < 0.06$.

the obese animals consumed 399 mg DHEA/kg body wt/day. The serum level of DHEA-S was clearly increased in the treated animals (Table I); untreated animals had barely detectable levels of the hormone, while the treated animals had values that are three to seven times that found in adult humans.

Hypothalamic-pituitary-adrenal (HPA) function appears to remain unperturbed. ACTH and corticosterone levels between the treated and control groups were not statistically different, although there was a trend for higher values in the treated animals.

As expected, insulin levels were higher in the untreated obese animals compared with the untreated lean rats. DHEA treatment had no effect on the insulin levels of the lean animals but did significantly lower the insulin level of the obese. C-peptide was not altered by DHEA treatment in either group. Possibly, DHEA altered the hepatic clearance of insulin as reflected by the higher value for hepatic insulin clearance (HIC = C-peptide/insulin) in Table I. However, because these experiments were conducted to determine the effect of DHEA on the HPA axis, the animals had not been fasted before sacrifice for fear of altering adrenal function secondary to the stress of changing the animal's feeding schedule. Hence, the values of glucose, insulin, and C-peptide are nonfasting values.

Liver function appeared to remain stable or improve (Table I). In the lean rat, serum bilirubin remained low and hepatic enzymes remained at baseline levels except for a slight increase in gamma glutamyl transpeptidase. Albumin rose significantly. Since there was not a change in either BUN or creatinine, this increase in albumin probably reflects an anabolic effect of DHEA and not a change in the state of hydration. In a similar vein, serum liver enzyme levels remained stable in the obese treated rat, while albumin rose and bilirubin fell. Both changes may suggest an improvement in liver function.

Cyclo (His-Pro) is a cyclic dipeptide known to influence food intake (30). Levels of this peptide in the serum of the rats were changed by DHEA in both sets of animals (lean 2457 ± 174 vs 3138 ± 207 pg/ml, $P < 0.03$, control versus treated; and obese 2701 ± 157 vs 3135 ± 58 pg/ml, $P < 0.02$, control versus treated).

Levels of norepinephrine (NE), serotonin (5-HT), and 5-hydroxyindole acetic acid (5-HIAA) in three hypothalamic regions are shown in Table II. The only statistically significant treatment effects seen are in the lean animals where DHEA treatment is associated

Table II. Hypothalamic Neurotransmitter Levels in DHEA-Treated Zuckers

	NE	5-HT	5-HIAA
PVN			
Lean Control	2.830 ± 0.244	0.592 ± 0.207	0.505 ± 0.245
Lean Treated	1.840 ± 0.171[a]	0.558 ± 0.062	0.240 ± 0.020
Obese Control	1.248 ± 0.214	0.282 ± 0.045	0.148 ± 0.028
Obese Treated	1.620 ± 0.057	0.292 ± 0.043	0.148 ± 0.025
VMH			
Lean Control	1.088 ± 0.249	0.062 ± 0.036	0.205 ± 0.098
Lean Treated	0.800 ± 0.054	0.133 ± 0.041	0.187 ± 0.031
Obese Control	1.312 ± 0.158	0.177 ± 0.067	0.175 ± 0.041
Obese Treated	1.257 ± 0.152	0.208 ± 0.104	0.203 ± 0.042
LH			
Lean Control	0.873 ± 0.253	0.465 ± 0.026	0.327 ± 0.027
Lean Treated	1.335 ± 0.147[a]	0.555 ± 0.055	0.310 ± 0.009
Obese Control	0.905 ± 0.093	0.430 ± 0.053	0.288 ± 0.028
Obese Treated	1.318 ± 0.099	0.523 ± 0.048	0.327 ± 0.022

Note. Hypothalamic regions were isolated and analyzed for norepinephrine (NE), serotonin (5-HT), and 5-hydroxyindoleactic acid (5-HIAA). Values reported are the mean ± SEM. Values are expressed as ng/mg wet weight of tissue. PVN = paraventricular nucleus; LH = lateral hypothalamus; VMH = ventromedial hypothalamus.
[a] A significant difference ($P < 0.05$) treatment versus phenotypic control.

with a NE level that is slightly higher in the LH but slightly lower in the PVN.

No untoward activity (self-mutilation, etc.) was noted in either of the DHEA-treated groups. In order to test one aspect of activity, animals were placed in an activity chamber on the 27th day of the experiment. This apparatus measures spontaneous movement in a darkened, sound-proofed environment. The activity of obese untreated rats was significantly lower than that of the lean untreated rats (Fig. 1). DHEA treatment resulted in a decline in the activity of the lean animals to that of the obese controls. This decline was significant ($P < 0.02$). However, DHEA treatment did not alter the activity of the obese animals.

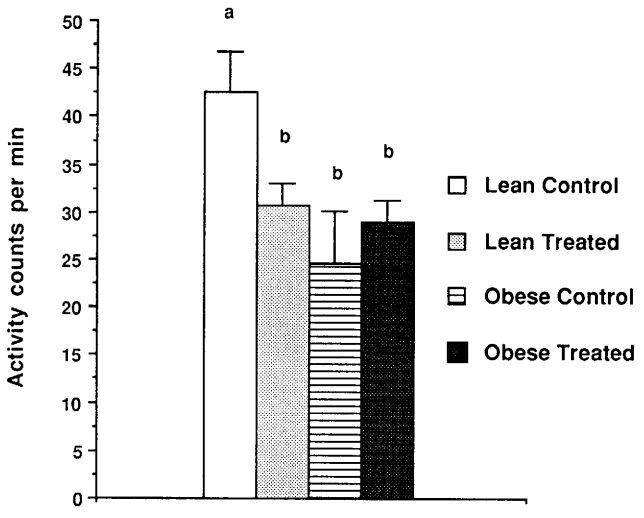

Figure 1. Effect of DHEA on the spontaneous activity of Zucker rats. On the 27th day of DHEA treatment, all rats were placed individually in a darkened, sound-proofed activity chamber. The values reported are the means + SEM of the activity counts per minute over a 9-min period.

Organ weights are reported in Table III. DHEA treatment caused a clear change in the size of the lean animals' livers and spleens (the former increased by 58% and the latter decreased by 13%). The obese animals showed similar trends: liver size increased by 28%, and the spleen decreased by 14%. DHEA also caused a significant decrease in the weights of obese rat hearts and parametrial fat pads. In the case of the heart, the final weight of the obese DHEA-treated group was similar to that of the lean animals; the heart weight of the obese untreated rat was significantly greater than those from either lean group. These findings confirm those already reported by Cleary's group (9).

Discussion

Many studies have documented that obese animals either lose weight or gain weight more slowly with DHEA treatment (5–10, 21, 22, 24). The mechanism(s) of this action is far from understood. Cleary's group suggested that the majority of the effect was through a change in mitochondrial lipid metabolism (5). Our studies suggest that DHEA-induced changes in total caloric intake are important in modulating the weight of the obese animal (21).

Additional possibilities must also be considered. For example, since DHEA has been shown by us (19, 20) and others (18, 31–33) to be an antagonist of glucocorticoid action, it is possible that chronic adrenal insufficiency is a cause of the change in weight. Searching for this potentially dangerous effect is important, because exogenous DHEA is being used experimentally in humans (34, 35).

In these experiments there were no indications that DHEA induced adrenal insufficiency. If DHEA

Table III. Organ Weight of DHEA-Treated Zucker Rats

Tissue	Lean		Obese	
	Control	Treatment	Control	Treatment
Liver	8.25 ± 0.59	13.02 ± 0.019[a]	12.68 ± 0.87[b]	16.26 ± 1.54[a]
Kidney	1.51 ± 0.13	1.56 ± 0.08	1.82 ± 0.16[b]	1.94 ± 0.17
Heart	0.682 ± 0.033	0.699 ± 0.056	0.800 ± 0.102[b]	0.678 ± 0.069[a]
Spleen	0.410 ± 0.030	0.356 ± 0.039[a]	0.385 ± 0.070	0.332 ± 0.038[a]
Soleus	0.091 ± 0.008	0.089 ± 0.015	0.079 ± 0.005	0.083 ± 0.011
Parametrial fat pad	0.809 ± 0.237	0.694 ± 0.159	8.725 ± 2.102[b]	7.249 ± 1.128[a]
Adrenals	0.077 ± 0.030	0.063 ± 0.010	0.076 ± 0.030	0.061 ± 0.013

Note. At the end of the 28 days, experimental rats were sacrificed by rapid decapitation. Organs were removed, blotted, and weighed. The values recorded reflect the mean ± SEM of each group. The final body weights of the animals were: lean control = 215 g; lean treated = 204 g; obese control = 352 g; obese treated = 302 g.

[a] The value of the treatment group is different from that of the untreated control group of the same phenotype ($P < 0.05$).

[b] The obese control group had a different value than that of the lean control group ($P < 0.05$).

were a classic glucocorticoid antagonist, one would expect elevations of ACTH and corticosterone. Although serum concentrations of corticosterone in the DHEA-treated animals tended to be greater than those of the control, there were no statistically significant differences in the levels of either corticosterone or ACTH between groups. Levels of DHEA-S were very high at the time of sacrifice, so it was certainly present at the time corticosterone and ACTH were measured. It is recognized, however, that obtaining blood samples for corticosterone that are reflective of basal conditions is difficult. We have published that rat corticosterone measurements are volatile and easily elevated by handling (36). In these experiments, attempts were made to minimize this problem, but subtle differences between groups may have been obscured by stress caused by the experimental design.

DHEA diminished the spontaneous activity of the lean animals but did not affect that of the obese (Fig. 1). Insulin levels did fall but, adrenal weights were the same between treated and control groups. Spleen size did not increase as one might expect with diminished glucocorticoid activity, and there were no signs of dehydration or hypoglycemia. Thus, although DHEA clearly acts to antagonize exogenous glucocorticoids in some experimental paradigms, it does not appear to diminish the effects of the HPA axis under these chronic conditions.

DHEA seemed to have no effect on the level of hypothalamic neurotransmitters in the obese animal under these conditions. Previously, we have shown that DHEA administered for 1–7 days leads to a clear elevation in hypothalamic serotonin in the obese rat, which was most notable in the lateral hypothalamus (25). In the experiments in this paper, there was a strong tendency toward elevation of serotonin in the obese treated animals; however, the difference was not significant ($0.05 < P < 0.10$). These results suggest that the effects on the levels of hypothalamic neurotransmitters waned in obese animals. This is consistent with our previous reports on the effect of DHEA given as a 0.3% food supplement to obese animals (25). At that dose, DHEA clearly affected food intake for over 7 days, but its effect on hypothalamic serotonin, while seen clearly after 1 day of treatment, was no longer demonstrable after 7 days. It may be that DHEA affects serotonin levels at critical synapses, but total content returns to normal.

In conclusion, DHEA, an adrenal steroid that has been shown to antagonize the effects of glucocorticoids, does not seem to induce adrenal insufficiency when given for prolonged periods. Further, although it alters the levels of hypothalamic neurotransmitters when given acutely (25), during chronic administration this effect is no longer seen, suggesting that either these changes are not important in mediating DHEA's effects or that the effect involves more complex mechanisms (i.e., changes in receptor number or receptor sensitivity).

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