Effect of Dehydroepiandrosterone on Neurotransmitter Levels and Appetite Regulation of the Obese Zucker Rat

The Obesity Research Program

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The obese Zucker rat is a model of youth-onset obesity associated with hyperphagia. In this study, dehydroepiandrosterone's effect at decreasing food intake and body weight in the obese Zucker rat was investigated. Rats were treated with a dehydroepiandrosterone-supplemented diet (0.0, 0.06, 0.15, 0.3, or 0.6%) for 7 days. The 0.3 and 0.6% treatment groups showed a dramatic decrease in daily food intake, which was evident the 1st day. In addition to the reduction in food intake, body weight changes also were affected significantly in the high-dose treatment groups. The possibility that these dehydroepiandrosterone-induced changes were correlated to perturbations in central neurotransmitter levels associated with appetite control was investigated. The hypothalamus, frontal cortex, striatum, and hippocampus of dehydroepiandrosterone-treated animals were assayed for neurotransmitters known to have inhibitory or stimulatory effects on feeding behavior (serotonin, dopamine, norepinephrine, and epinephrine). Significant differences from steroid-free controls were noted only in the levels of hypothalamic serotonin in animals treated with dehydroepiandrosterone. Serotonin in the hypothalamus has been shown to decrease feeding behavior. The magnitude of dehydroepiandrosterone's effect on hypothalamic serotonin correlated with its effect on feeding behavior. Thus, dehydroepiandrosterone may reduce hyperphagia by altering hypothalamic levels of serotonin. ***Diabetes*** **42:662–69, 1993**

Several observations suggest that perturbations of the hypothalamic-pituitary-adrenal axis are involved in maintaining the hyperphagia of the obese Zucker rat. Adrenalectomy reduces hyperphagia, whereas corticosterone administration to these adrenalectomized animals restores hyperphagia (1). Administration of the synthetic antiglucocorticoid RU 486 to the intact obese animal diminishes food intake (2), as does administration of CRF (3). Recently, Langley and York (4) have reported that hypothalamic glucocorticoid receptor number is abnormal in the obese Zucker rat.

DHEA, the chief product of the human adrenal gland, like RU 486, reduces the obesity of the fa/fa Zucker rat (5). This salutary action of DHEA may be mediated by a variety of mechanisms. We recently reported that DHEA has antiglucocorticoid activity in rodent liver (6). Cleary and colleagues (5,7,8) have presented a large body of evidence that DHEA exerts a significant effect on energy metabolism in hepatic mitochondria.

We (9) and others (10) have found that DHEA reduces food intake in the young obese animal. Little is known of the mechanisms of this particular action of DHEA. Knowledge of the neural pathways controlling food intake in any experimental animal is rudimentary. Still, some potentially important CNS sites have been identified. The hypothalamus, for example, participates in food intake regulation (11). In this region, changes in catecholamines (NE and EPI) and the monoamines (serotonin and DPM) have been implicated as modulators of feeding behavior. Evidence exists that steroids may exert their influence on feeding behavior either by controlling the amount of neurotransmitter or the efficacy of these agents.

In this study, we investigated whether DHEA-induced changes in food intake can be correlated with changes in

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DHEA; dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone 3-sulfate; NE, norepinephrine; EPI, epinephrine; CRF, corticotrophin-releasing factor; RIA, radioimmunoassay; SOS, sodium octyl sulfate; DHBA, 3,4-dihydroxybenzylamine hydrobromide; HPLC, high-performance liquid chromatography; DPM, dopamine; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid; ANOVA, analysis of variance; GABA, γ-amino buytyric acid.

central neurotransmitters. It is proposed that these studies not only will serve as probes of how DHEA influences food intake but also may provide insights into the general mechanisms of abnormal appetite control.

RESEARCH DESIGN AND METHODS

Female Zucker rats (aged 13–18 wk) were obtained from the colony maintained in the Department of Physiology at LSU Dental School in New Orleans. Obese (fa/fa) animals, initially weighing 250–450 g, were used for the experiments. All animals were maintained on a 12-h light-dark cycle (lights on at 0600) in a room whose temperature was maintained at 22 ± 1°C. All animals were maintained on Purina rodent chow 5001 in pellet form before beginning the experiment. The manufacturer states that the physiological fuel value of the rodent chow is 3.30 cal/g, and the proportions of calories as carbohydrate, protein, and fat are 63.7, 30.4, and 5.9%, respectively. Food and water were provided ad libitum. Throughout the experiment animals were housed individually in wire mesh cages.

The 30 animals for experiment 1 were divided into 5 groups of 6 rats. The average weights and ages of the animal groups were within 1 SD of the group as a whole. Two days before the start of the measurements (day −4), all Zucker rats were introduced to a Purina diet that had been ground to produce a powder. No measurements of food intake were taken during the 2-day adjustment to the new food texture. Measurements of food intake began on day −2. On day 0, groups 2–5 received diets supplemented with DHEA (Sigma, St. Louis, MO) at levels of 0.06, 0.15, 0.3, and 0.6% (wt/wt). The steroid was mixed into the diet by manual and mechanical blending. Group 1, the control group, continued to receive a powdered DHEA-free diet. Each food dish was attached to the cage via a spring to reduce spillage. Food intake and body weights were measured daily between 0800 and 0900. At this time any spilled food from the collecting paper under the cage was gathered, air-dried if necessary, and weighed. Food dishes were weighed, and individual food intakes in grams were determined.

A second study was conducted to determine the effects of DHEA on neurotransmitters. Forty-eight obese female Zucker rats, aged 13–18 wk, were divided into 4 groups of 12 animals. Group 1 served as the control and received a DHEA-free diet. Groups 2–4 were fed a diet supplemented with 0.6, 0.3, or 0.06% DHEA, respectively. After consuming the diet for 24 h, 6 animals from each group were killed by rapid decapitation. The remaining animals were maintained on their respective diet for a total of 7 days and then killed by rapid decapitation.

The blood from each animal was collected in glass tubes (10 × 75 mm) and centrifuged for 15 min at −4°C. Four 500-μl aliquots of serum were taken, stored in a biological freezer (−80°C, and assayed later for levels of DHEA, DHEA-S, testosterone, and androstenedione. RIA kits used for these assays were purchased from Diagnostic (Los Angeles, CA).

The entire brain was removed immediately after death, placed in chilled buffer, and rapidly dissected into hypothalamus, frontal cortex, striatum, and hippocampus on a chilled glass surface. The regional dissection was accomplished by the method of Glowinski and Iverson (12). For monoamine analysis each brain part was homogenized in a buffer of 0.01 M citrate, 10% ethanol, and 250 mg/L SOS at pH 4. An internal standard of DHBA was added such that its final concentration was 1 ng/100 L of the HPLC injectate. The homogenate was centrifuged in a Sorvall RC2B centrifuge at 12,000 *g*, and the supernatant was frozen at −80°C until assayed. Recoveries ranged from 75 to 90% in our studies. The chromatographic system consisted of the following hardware and software. Dual Rainin Rabbit HP pumps equipped with a self-washing piston pump heads (capable of maximum flows of 10 ml/min) provided a flow rate of 1.5 ml/min.

Injection of samples was accomplished automatically using an Alcott model 728 autosampler. This feature allowed us to run as many as 40 samples overnight. The HPLC column consisted of a Rainin microsorb (5 M) C18 column (25 cm × 4.4 mm). A 1.5 cm × 4.6-mm guard column packed with microsorb C18 preceded the analytical column. Chromatographically separated monoamines were assayed by electrochemical detection using an ESA model 5100 Coulchem multielectrode array. This electrode array consisted of a model 5020 guard cell and a model 5010 dual analytical electrode cell. The guard cell voltage was 0.4 V. The two analytical cells were set at −0.04 V (detector 1) and 0.32 V (detector 2). NE, EPI, DPM, 5-HT, serotonin, and 5-HIAA were determined in unknown and standard samples by comparison with retention times and integrated areas of peaks. Cochromatography of spiked standards of each compound were initially run with brain parts to ensure that the peaks were authentic NE, EPI, DPM, 5-HT, and 5-HIAA.

Statistical analysis. Daily variations in food intake and body weight for each animal were examined by one-way ANOVA. Significance ($P < 0.05$) was measured using Fisher's least significant difference for the exact P value.

RESULTS

Thirty obese female Zucker rats were divided into 5 groups of animals. Four days (day −4) before the start of the treatment, all animals were introduced to a powdered form of standard rat chow. Before this, all rats had been maintained on the same diet in the pellet form since weaning. Starting on day −2, the animal's daily food consumption over the previous 24 h was measured and expressed as calories. At the same time, daily food intake was measured, and animal body weights were recorded. Starting on day 0, groups 2–5 received a 0.06, 0.15, 0.3, or 0.6% DHEA-supplemented diet, respectively. Group 1, the control group, continued to receive the DHEA-free diet.

Figure 1 presents total daily caloric intake of each treatment group throughout the experiment (day −2 through day 7). Points represent calories of food consumed over the prior 24 h. Before DHEA was introduced into the diet (days −2, −1, and 0), no animal group showed a significant difference in food intake. On intro-

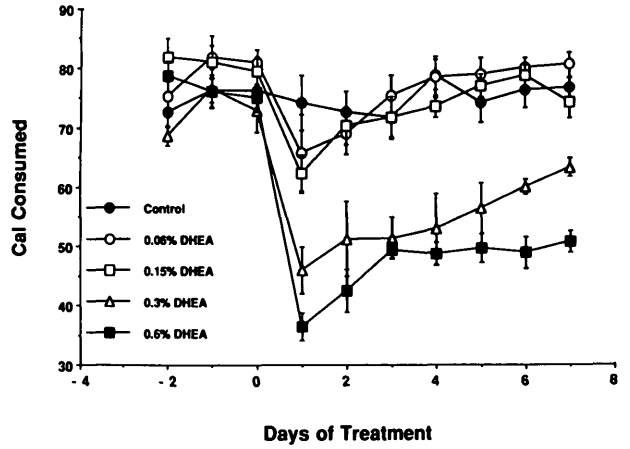

FIG. 1. Effect of DHEA on daily caloric consumption. For 3 days (days −2–0) all animals were maintained on a DHEA-free diet. After DHEA was added to diet, the number of calories consumed over next 24 h was plotted as day 1 value. Treatment was maintained for 7 days. Average caloric intake per day for each animal group was plotted against day of treatment. For more details see METHODS. Values are means ± SE for each animal group (n = 6).

duction of DHEA, however, statistically significant differences were evidenced after 1 day of treatment in both the 0.3 and 0.6% DHEA-treatment groups. These two doses versus all other doses were significantly different ($P < 0.0001$) on day 1 of treatment and remained significantly different ($P < 0.05$) during the subsequent 6 days of treatment.

After the 1st day of treatment, a continuous upward trend in food intake was evident in the 0.3 and 0.6% treatment groups. The lowest dose treatment group, which received a diet supplemented with 0.06% DHEA, showed a trend of decreasing total calories consumed on day 1. However, consumption clearly returned to control values on subsequent days of treatment. The 0.06 and 0.15% DHEA treatment groups never differed significantly from control values or from each other.

Table 1 compares the amount of DHEA calculated to be consumed on a per day basis. The amount of DHEA consumed on day 1 for each treatment group was lower than the average daily DHEA intake over the 7 days of treatment (Fig. 1).

TABLE 1
DHEA consumed during 7 days of treatment

Rat group	DHEA consumed		
	Day 1 (mg)	Over 7 days (mg)	Average daily (mg/initial kg body wt)
Control	0	0	0
0.06% DHEA	12	14	40
0.15% DHEA	28	33	89
0.3% DHEA	42	50	174
0.6% DHEA	67	85	232

Amounts of DHEA consumed daily by each animal group were calculated from food intake totals. Similar calculations were done to determine the average daily DHEA consumed over 7 days of treatment.

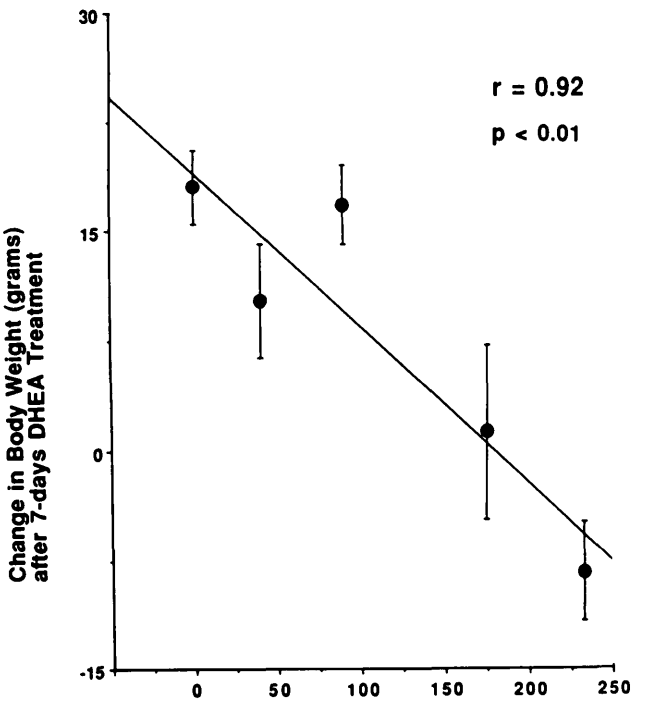

FIG. 2. Relationship between weight change and amount of DHEA consumed by obese Zucker rats. Change in rat weight during the week of DHEA treatment is plotted against the average daily consumption of DHEA. Exact values for DHEA intake are given in Table 1.

Figure 2 shows the weight change over the treatment period and correlates it with the DHEA intake. Results from the two lower dose treatment groups (0.06 and 0.15%) were not different from those of the control group. The groups consuming the most DHEA (0.3 and 0.6%) showed either minimal weight gain or a net weight loss (1.16 ± 5.39 and −8.33 ± 3.49 g, respectively). The values for weight change show a positive correlation ($r = 0.92$) to DHEA intake per kilogram body weight, suggesting that DHEA intake influences weight.

The average body weights from day 1 to the end of the experiment and the calculated body weight change are presented in Table 2. There was a considerable variation

TABLE 2
Average body weights and body weight change

	Body weight (g)		Body weight change (g)
	Day 1	Day 7	
Rat group			
Control	296.0 ± 25.3	314 ± 24.8	18 ± 2.5*
0.6% DHEA	284.0 ± 38.7	275.7 ± 33	−8.3 ± 3.9*
0.3% DHEA	279.0 ± 34.5	280.2 ± 31	1.2 ± 5.9*
0.15% DHEA	289.8 ± 16	306.5 ± 18.7	16.7 ± 2.8
0.06% DHEA	282.7 ± 29	292.8 ± 25.7	10.1 ± 2.0

Data are means ± SE. Average body weight change: final body weight − initial body weight.
*$P < 0.05$, significant difference from control.

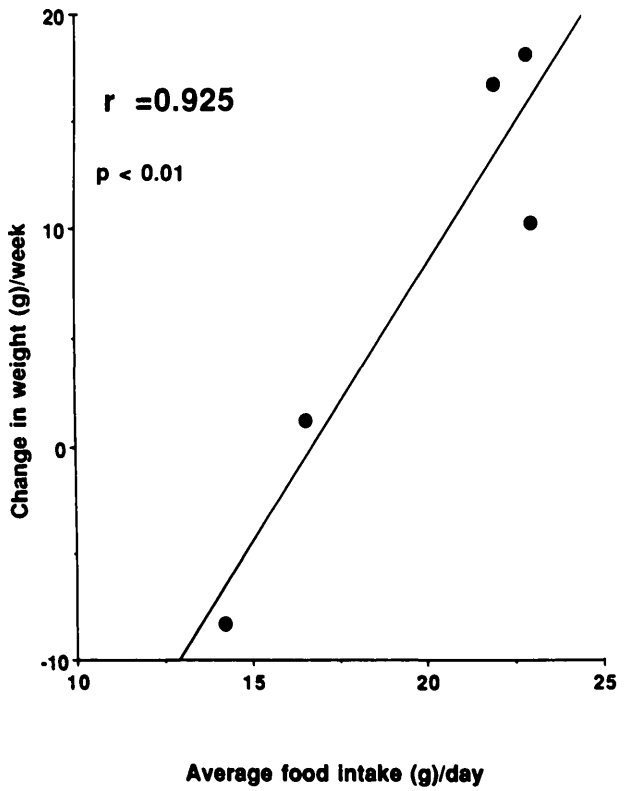

FIG. 3. Relationship of weight change to food intake in obese Zucker rats. Average weekly weight change of rats in Fig. 1 is plotted against the average daily food intake during 7 days of 0.0, 0.06, 0.15, 0.3, or 0.6% DHEA treatment.

in average body weight because of the fairly wide range of ages for adult obese animals. Even so, those in the control group gained 18 g, whereas the 0.6 and 0.3% groups either lost weight (−8.3 g) or gained only 1.2 g. When these data were expressed as body weight changes of each individual animal, significant differences were found between the control group and the 0.6% ($P < 0.0001$) and 0.3% ($P < 0.004$) groups but not the 0.15% ($P = 0.8$) or 0.06% $P = 0.2$) groups.

The average weight change over the 1 wk versus the average daily food intake during 7 day of DHEA treatment is presented in Fig. 3. Food intake shows a direct relationship with the change in weight ($r = 0.925$), suggesting again that changes in food intake are responsible for the changes in weight. In the high-dose treatment groups (0.3 or 0.6% DHEA-supplemented diet), food intake was 23 or 38% lower than control values, respectively. Because of this effect of DHEA on food intake, a second study was conducted to determine whether DHEA had an effect on neurotransmitters. It was postulated that this effect might provide insight into the biological mechanism of DHEA's action. Specifically, neurotransmitters known to have stimulatory or inhibitory effects on food intake were assayed by HPLC from brain parts.

In this study, after 1 or 7 days of DHEA treatment, the hypothalamus, frontal cortex, hippocampus, and striatum were assayed for levels of serotonin, 5-HT, 5-HIAA, NE, EPI, and DPM. Values are presented in Table 3. Of all brain parts assayed, the only significant difference between treatment groups and the control group was found in the hypothalamus. Specifically, DHEA caused a significant elevation in hypothalamic levels of 5-HT in the 0.3 and 0.6% treatment groups on day 1 and in the 0.6% treatment group on day 7.

Serum samples were assayed for DHEA, DHEA-S, androstenedione, and testosterone in these same animals (Table 4). Control values represent the lower limits of all assayed steroids. From these low control values, through to the 0.6% treatment group, serum levels of all hormones increased at both the 1- and 7-day treatment points. This suggests that DHEA was being converted to other steroids. After 1 day of treatment, the ratio of DHEA-S:DHEA ranged from 110:1 in the 0.06% treatment group to 150:1 in the 0.6% treatment group. The same ratio compared after 7 days of treatment was only about half as high (38:1 in the lowest and 83:1 in the highest treatment group).

The relationships between serum steroids and hypothalamic 5-HT are shown in Fig. 4. As serum levels of DHEA, DHEA-S, androstenedione, and testosterone increased, so did hypothalamic 5-HT content. This suggests that hypothalamic 5-HT content is elevated by DHEA or metabolites of DHEA. The values for these correlations are all significant ($P < 0.01$).

DISCUSSION

DHEA clearly decreased food intake in the obese, hyperphagic Zucker rat (Fig. 1). The changes seen here were rapid, occurring within the first 24 h, and dose dependent. At very low doses (0.06–0.15%), there was little change from baseline, whereas at higher doses (0.3 and 0.6%) there was an ~50% decrease in food consumption. At both of these higher doses the effect of DHEA waned by day 7.

Differences in weight gain were demonstrable during this 1-wk experiment (Table 2). This change in body weight was related to both the amount of DHEA consumed (Fig. 2) and the amount of food consumed (Fig. 3). These findings suggest that changes of food intake are important in influencing the weight of the DHEA-treated rat.

Numerous studies implicate the hypothalamus as an important region in controlling food intake. Leibowitz (11) has summarized evidence demonstrating that, in the rat, hypothalamic serotonin and DPM are the major feeding-inhibitory neurotransmitters, although both NE and EPI occasionally can act as feeding-inhibitory neurotransmitters.

To explore the possibility that DHEA caused a decrease in food intake in Zucker rats by altering neurotransmitters, a search was conducted for changes in monoamine levels in brains of DHEA-treated rats. The doses of DHEA that were evaluated were those that influenced food intake (Fig. 1). Specifically, rats were treated with either a low dose of DHEA (0.06%) that didn't affect appetite or higher doses (0.3 and 0.6%) that clearly diminished food intake. Animals were killed after either 1 or 7 days of treatment. The first time of death was chosen

TABLE 3
Neurotransmitter levels in Zucker rat brain parts after 1 or 7 days of DHEA treatment

Tissue	5-HT	5-HIAA	NE	EPI	DPM
Day 1					
Control group					
Hypothalamus	0.907 ± 0.06*	0.770 ± 0.06	1.51 ± 0.34		0.307 ± 0.04
Frontal cortex	0.540 ± 0.03	0.135 ± 0.02	—	—	—
Hippocampus	0.387 ± 0.04	0.458 ± 0.06	0.403 ± 0.04	—	—
Striatum	0.542 ± 0.07	0.613 ± 0.08	—	0.713 ± 0.12	8.808 ± 0.78
0.6% DHEA group					
Hypothalamus	1.147 ± 0.12*	0.988 ± 0.07	1.828 ± 0.41		0.357 ± 0.04
Frontal cortex	0.517 ± 0.03	0.340 ± 0.03	—	—	—
Hippocampus	0.353 ± 0.02	0.428 ± 0.02	0.367 ± 0.03	—	—
Striatum	0.560 ± 0.02	0.757 ± 0.07	—	0.497 ± 0.11	8.705 ± 0.49
0.3% DHEA group					
Hypothalamus	1.267 ± 0.06*	0.828 ± 0.08	1.708 ± 0.34	—	0.400 ± 0.05
Frontal cortex	0.602 ± 0.07	0.272 ± 0.03	—	—	—
Hippocampus	0.373 ± 0.02	0.478 ± 0.04	0.412 ± 0.03	—	—
Striatum	0.607 ± 0.05	0.693 ± 0.07	—	0.668 ± 0.05	10.060 ± 0.31
0.06% DHEA group					
Hypothalamus	0.967 ± 0.02	0.610 ± 0.10	1.502 ± 0.27	—	0.335 ± 0.04
Frontal cortex	0.555 ± 0.05	0.287 ± 0.03	—	—	—
Hippocampus	0.400 ± 0.03	0.408 ± 0.03	0.362 ± 0.03	—	—
Striatum	0.627 ± 0.03	0.587 ± 0.07	—	0.710 ± 0.10	9.870 ± 0.34
Day 7					
Control group					
Hypothalamus	0.973 ± 0.09*	0.795 ± 0.06	1.32 ± 0.26		0.438 ± 0.05
Frontal cortex	0.685 ± 0.07	0.377 ± 0.05	—	—	—
Hippocampus	0.400 ± 0.02	0.480 ± 0.06	0.383 ± 0.06	—	—
Striatum	0.688 ± 0.05	0.755 ± 0.10	—	0.877 ± 0.13	10.125 ± 0.89
0.6% DHEA group					
Hypothalamus	1.185 ± 0.06*	0.890 ± 0.10	1.860 ± 0.32	—	0.407 ± 0.03
Frontal cortex	0.728 ± 0.04	0.448 ± 0.05	—	—	—
Hippocampus	0.397 ± 0.03	0.462 ± 0.04	0.435 ± 0.02	—	—
Striatum	0.668 ± 0.07	0.737 ± 0.11	—	0.732 ± 0.07	8.950 ± 0.46
0.3% DHEA group					
Hypothalamus	1.063 ± 0.07	0.677 ± 0.07	1.537 ± 0.25	—	0.358 ± 0.07
Frontal cortex	0.682 ± 0.03	0.303 ± 0.03	—	—	—
Hippocampus	0.412 ± 0.03	0.393 ± 0.03	0.413 ± 0.03	—	—
Striatum	0.607 ± 0.05	0.693 ± 0.07	—	0.668 ± 0.05	10.060 ± 0.31
0.06% DHEA group					
Hypothalamus	1.107 ± 0.08	0.887 ± 0.09	1.895 ± 0.34	—	0.428 ± 0.06
Frontal cortex	0.694 ± 0.05	0.364 ± 0.01	—	—	—
Hippocampus	0.415 ± 0.01	0.390 ± 0.02	0.432 ± 0.02	—	—
Striatum	0.730 ± 0.07	0.740 ± 0.06	—	0.700 ± 0.06	10.157 ± 0.65

Data are means ± SE of neurotransmitters assayed by HPLC and electrochemical detection. See METHODS for more details. —, neurotransmitter too low to be consistently measured.
*Significant difference. $P < 0.05$.

TABLE 4
Serum hormone levels after 1 or 7 days of DHEA treatment in the obese Zucker rat

	DHEA (μM)	DHEA-S (μM)	Androstenedione (μM)	Testosterone (nM)
Day 1				
Control group	0.019 ± 0.018	0.19 ± 0.05	0.004 ± 0.0003	0.28 ± 0.66
0.06% DHEA	0.188 ± 0.025	20.7 ± 6.1	0.112 ± 0.035	2.04 ± 0.42
0.3% DHEA	0.535 ± 0.125	71.7 ± 35.0	0.53 ± 0.19	6.98 ± 2.57
0.6% DHEA	1.19 ± 0.43	180 ± 157	0.99 ± 0.29	13.6 ± 5.5
Day 7				
Control group	0.050 ± 0.065	0.245 ± 0.109	0.003 ± 0.003	0
0.06% DHEA	0.265 ± 0.223	9.97 ± 4.93	0.085 ± 0.034	2.81 ± 1.25
0.3% DHEA	0.778 ± 0.225	28.7 ± 18.3	0.44 ± 0.10	5.49 ± 1.15
0.6% DHEA	2.52 ± 0.74	210 ± 130	1.58 ± 0.42	12.3 ± 1.7

Data are means ± SD for each animal group ($n = 6$).

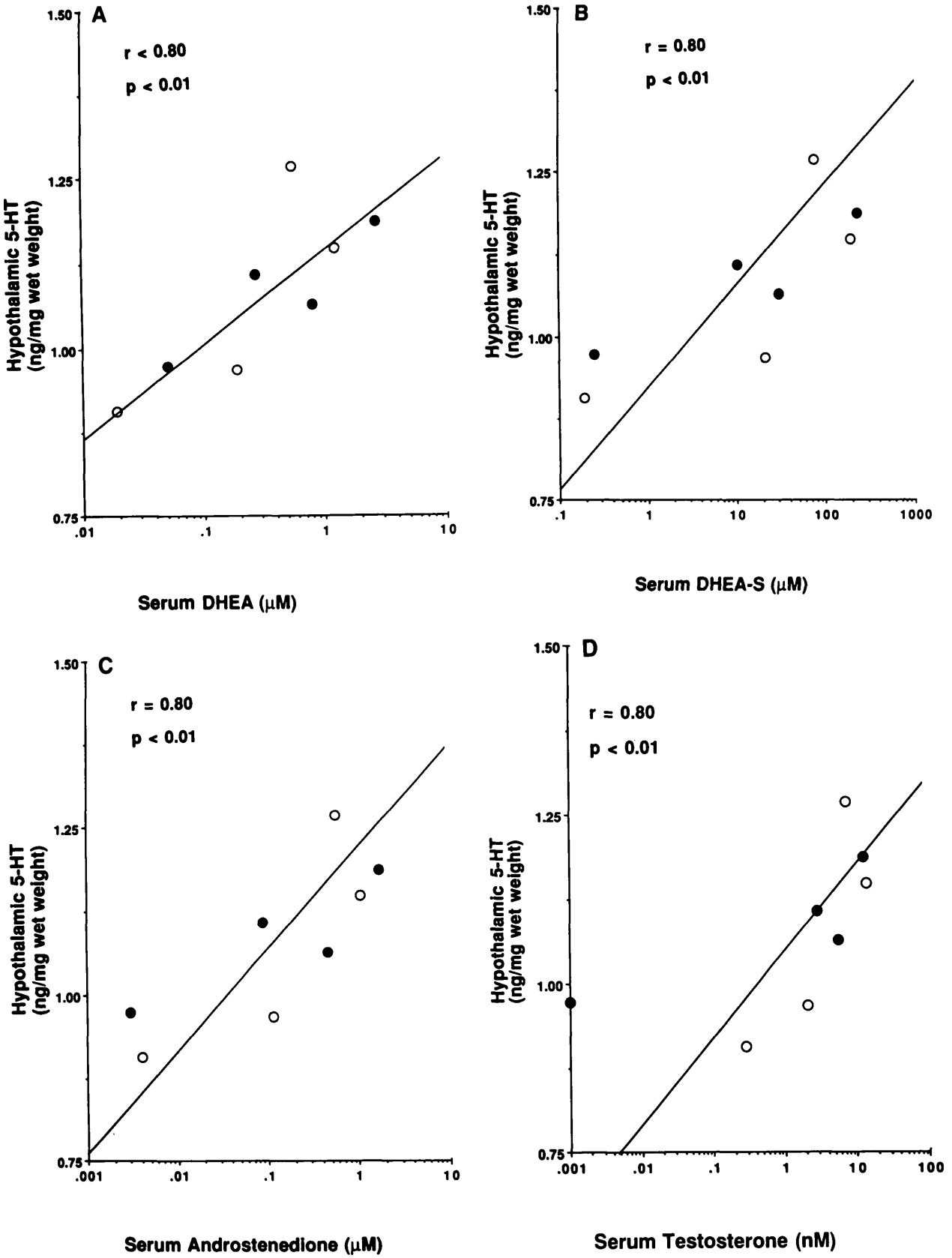

FIG. 4. Relationship between hypothalamic content of serotonin (5-HT) and serum steroid levels. Hypothalamic 5-HT levels (ng/mg wet weight) are plotted against serum steroid values. (○), Animals killed on day 1; (●), animals killed on day 7. *A-D*: values of DHEA, DHEA-S, androstenedione, and testosterone, respectively.

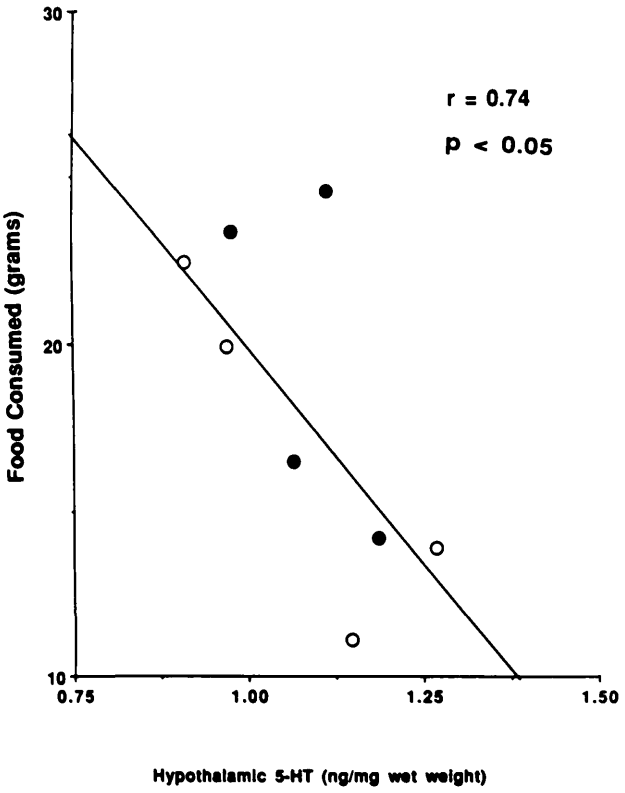

FIG. 5. Relationship between the amount of food consumed and hypothalamic levels of serotonin. Amount of food consumed by rats in Fig. 1 on days 1 and 7 is plotted against levels of serotonin measured in the hypothalamus of rats killed in Table 3. ○, Animals treated for 1 day; ●, animals treated for 7 days.

because DHEA's effect was maximum at that point (Fig. 1), whereas the second time of death was selected because the effect of DHEA, although still present, was waning. In each case, rats maintained on a steroid-free diet served as control animals.

Neurotransmitter levels in the brains of animals treated for 1 day with DHEA are reported in Table 3 and reveal that the only statistically significant change, compared with control animals, was in the level of hypothalamic serotonin. Serotonin was elevated significantly in the animals treated with the higher doses of DHEA. A trend toward a higher value also was observed in the animals treated with 0.06% DHEA, although the change was not significant.

After 7 days of DHEA treatment, the only statistically significant difference between treated and control animals was again in the level of hypothalamic serotonin. In this case however, only the value from the 0.6% DHEA-treated rat was significantly different from the control value. The other two dosage groups showed trends for higher values but were not significantly different. These perturbations, in general, paralleled the changes seen in food intake (Fig. 1). Figure 5 demonstrates the correlation between hypothalamic serotonin levels and food intake at both time points. In Fig. 5, the food intakes are from the rats in Fig. 1 and are plotted against the levels of hypothalamic serotonin from the comparable rats presented in Table 3. There was a positive correlation between these two parameters ($r = 0.74$).

Figure 4 relates the peripheral levels of steroid hormones to the levels of hypothalamic serotonin. In each case, a relationship exists between the hormones and the level of serotonin. As testosterone, androstenedione, and DHEA-S are all derived from DHEA, it is not surprising that all four will show a similar relationship to the level of serotonin. Hence, although one may certainly conclude that DHEA influences hypothalamic serotonin, it is not certain whether it acts directly or through a steroid metabolite.

How DHEA elevates serotonin is not demonstrable from these data. Diminished catabolism, increased synthesis, or diminished release could all be involved. At the highest DHEA dose, an increase occurs in the level of 5-HIAA, a metabolic of 5-HT. This might imply that overall serotonin synthesis is increased. However, direct turnover studies are needed to answer this definitively. Likewise, the absence of a change in the levels of other transmitters does not exclude an effect of DHEA on these agents. Turnover could be modified and not recognized if the equilibrium values are not altered.

The identity of the molecular species with which DHEA (or its metabolite) binds to exert these effects is not known. DHEA and DHEA-S have been found to be synthesized in brain tissues and have been termed neurosteroids (12). It has been proposed that they exert their effects at membrane binding sites and not through classic, cytosolic steroid receptors. These sites may have a much lower affinity for steroids than the classic steroid receptors. Majewska (13), for example, has presented evidence that neuronal synaptosomes possess two sites of DHEA-S interaction. The first one has a K_d in the high micromolar range, whereas the second is on the $GABA_A$ receptor and has a K_d of 3 μM. These values are three orders of magnitude greater than those found with classic steroid receptors. Note that in this study serum DHEA-S is in the 30–200 μM range when it affects hypothalamic serotonin levels in the Zucker rat. This suggests that DHEA-S is acting on membrane sites and not with a cytosolic receptor.

DHEA has had inconsistent effects in trials on human weight and insulin resistance. Few doses have been evaluated, however. Nestler et al. (14) used 1600 mg/day in men of normal weight and found an effect on body fat. The same group of researchers (15), however, found no effect when the 1600-mg dose was used in men who weighed an average of 97 kg. These two trials used doses that can be calculated to be 21.3 and 16.5 $mg \cdot kg^{-1} \cdot day^{-1}$ and resulted in serum DHEA-S levels of 40 μM. If the data presented here on the effect of DHEA on the rat can be used as a guide, it can be hypothesized that these doses may be at the lower range of the biopotency curve (Table 1 and Fig. 1). Zucker rats that receive 40 mg $DHEA \cdot kg^{-1} \cdot day^{-1}$ have DHEA-S levels on the order of 10–20 μM. At these levels, DHEA's effect in Zucker rats is equivocal and not statistically significant. However, at doses of 200 $mg \cdot kg^{-1} \cdot day^{-1}$, which resulted in serum levels of DHEA-S of 100–200 μM, the effect is clear. Possibly evaluation of a wider range of DHEA doses in humans would document this hormone's beneficial action.

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