Food Restriction-like Effects of Dietary Dehydroepiandrosterone. Hypothalamic Neurotransmitters and Metabolites in Male C57BL/6 and (C57BL/6 × DBA/2)F1 Mice

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Dehydroepiandrosterone (DHEA) is a precursor of sex hormones in mammals. Dietary DHEA serves to prevent or inhibit various diseases and also lengthens life spans of animals. Moreover, dietary DHEA inhibits food intake in certain strains of mice. We administered DHEA (0.45% w/w of food) to C57BL/6 (B6) and (B6 × DBA/2)F1 (BDF1) mice for 5 weeks. Food intake was inhibited in both strains of mice during the first week. Thereafter, B6, but not BDF1, mice consumed less food. Because hypothalamic serotonin and/or dopamine regulate appetite, satiety and other behaviors, the hypothesis tested was that hypothalamic concentration of serotonin, dopamine and/or their metabolites are affected differentially in B6 and BDF1 mice fed DHEA. In another study, mice were fed the AIN-76A diet with or without DHEA for 1 and 7 days or were pair-fed to DHEA-fed mice for 7 days. On Day 1 of DHEA feeding (acute effects) hypothalamic levels of serotonin, dopamine, and metabolites were unchanged in B6 mice, but levels of dopamine were increased and levels of dopamine metabolites were decreased in BDF1 mice. On Day 7 of DHEA feeding, levels of serotonin were increased in BDF1 but not B6 mice. On Day 7 of pair-feeding there were decreased levels of hypothalamic dopamine metabolites in BDF1 but not B6 mice. Paraventricular nuclei of BDF1 mice had decreased levels of serotonin but not of dopamine in all groups. Serum levels of DHEA and its metabolite, 5-androstene-3β,17β-diol, correlated significantly only with serotonin concentrations in BDF1 mice. The salient findings of these experiments are that DHEA inhibits food intake to a greater extent in B6 than in BDF1 mice. However, alterations of hypothalamic neurotransmitters were greater in BDF1 than in B6 mice. Because BDF1 and B6 mice share B6 genes, relevant gene(s) derived from DBA/2 mice might mediate the different responses detected. [Exp Biol Med Vol. 226(3):208–215, 2001]

Key words: DHEA; food-restriction; hypothalamus; serotonin; dopamine

Dehydroepiandrosterone (DHEA, 3β-hydroxy-5-androsten-17-one) given in food (0.2%–1.0% w/w) ameliorates a number of pathologies in animal models of human disease (1). Dietary DHEA also prolongs the life span of rodents (2). This treatment also decreases food intake in C57BL/6J and other, but not all, strains of mice (3). Reduction of food consumption is beneficial in many of the same models of disease in which dietary DHEA also is effective (reviewed in Ref. 3) and also prolongs life span and inhibits deleterious aging changes (4). Therefore, it is possible that one mechanism by which DHEA may exert its beneficial effects is by regulation of food intake (3). Because dietary DHEA treatment and food restriction have similar beneficial effects, it is important to understand the basis for these effects. How might DHEA decrease food intake in some, but not all strains of animals (3)?

Hypothalamic neurotransmitters such as serotonin (5-HT) and/or dopamine (DA) are involved in food intake regulation/satiety (5, 6), and responses to DHEA may be reflected in selective changes of neurotransmitter levels. DHEA and its sulfate derivative are neurosteroids, and appear to be neuromodulatory (7, 8). Dietary DHEA increases hypothalamic serotonin levels in obese Zucker rats in a manner that correlates with inhibition of food consumption (1).

In the present studies, we compared the effects of dietary DHEA (0.45%, w/w) on food intake and body weights of male C57BL/6 (B6) and (C57BL/6 × DBA/2)Fl (BDF1) mice over a 5-week period and the effects on brain neurotransmitters in mice fed DHEA for up to 7 days. The major findings were that "blunted" neurotransmitter responses of B6 mice to dietary DHEA correlated with a prolonged de-

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crease in food consumption, while "brisk" responses in BDF1 mice were associated with a very short-term effect on food intake.

Materials and Methods

Animals, Diets, and Determination of Body Weight and Food Intake. Details have been described elsewhere (3). Male B6 and BDF1 mice 8–16 weeks old were housed at 22 ± 2°C and maintained on a 12-hr light/dark cycle in a conventional facility. The Institutional Animal Care and Use Committee approved the studies. Mice were fed Purina Lab Chow until the time of the experiments. Thereafter, mice were fed AIN-76A diet as pellets (Dyets Inc., Bethlehem, PA) containing either no additives (control) or DHEA (0.45%, w/w of food) (Sigma, St. Louis, MO). The calculated caloric content of the AIN-76A diet was 4.11 calories/gram. All animals except pair-fed mice were given food and water *ad libitum*. The amount of food administered to pair-fed mice was determined by the weight of the food consumed by the DHEA-treated mice on a daily basis. Groups of 10 male B6 and 12 BDF1 mice were split into groups of 5 and 6, respectively, and fed either the control AIN-76A diet containing no DHEA or the 0.45% DHEA diet for 5 weeks. Remaining food weights were determined daily between 9:00 and 10:00 AM. Any spilled food was weighed and added to the corresponding food weight. Food intake was determined as the means from the two cages in each group (grams consumed per animal) and the values were averaged for each week (mean ± SEM). Intake per gram of body weight was determined by dividing the mean food intake of mice in a cage by the mean body weight of mice in the same cage. Body weights were determined weekly.

Intraperitoneal (ip) Administration of DHEA. Four independent experiments were conducted. B6 and BDF1 mice were split into 4 groups, each containing 4–6 mice per group. Powdered DHEA was suspended in a 13% solution of low molecular weight polyethylene glycol (PEG, mol wt 3,350, Sigma) in distilled water. Weights of mice and food were determined just before and 24 hr after mice were injected with 0, 2.5, 5, or 10 mg of DHEA between 9:00 and 10:00 AM. Each mouse received 0.5 ml of one of the DHEA suspensions or 13% PEG–water (vehicle control) ip using 18-gauge needles.

Isolation of Whole Hypothalamus. Thirty-two male B6 mice and 32 male BDF1 mice were divided into 4 groups of 8 mice (2 cages of 4 mice each/group). For each strain, groups of mice received (i) control diet for 7 days, (ii) the DHEA diet for 7 days, (iii) the control diet for 6 days and DHEA diet for 1 day, or (iv) 1.1–1.5 g of control diet per animal on Day 1 (as determined from preliminary experiments) and then were pair-fed to mice fed DHEA from Days 2 to 7. Mice were killed by rapid decapitation between 9:00 and 10:00 AM, and blood was collected for steroid analysis. We chose this approach, rather than fasting overnight, to maintain the continuum of food intake. The hypothalamus was dissected by the technique of Glowinski and Iversen (9) modified for mice, and it was immediately frozen on powdered dry ice and stored at −70°C for later analysis.

Isolation of Paraventricular Nucleus (PVN). Thirty-two male BDF1 mice were divided into 4 groups of 8 mice each and treated as described above. Mice were killed by decapitation, and whole brains were frozen on powdered dry ice and stored at −70°C. PVN tissue was obtained by sectioning the whole brain in a cryostat (−15°C) in alternating 200- and 30-μm sections through the hypothalamus as previously described (10). The thick sections were maintained frozen, while the thin sections were stained with thionin (Nissl). The PVN was identified in the thin sections, and the area of the corresponding thick section was removed using a 0.5-mm diameter punch and placed into plastic tubes at −70°C until analysis.

Determination of Neurotransmitter and Metabolite Concentrations in Whole Hypothalamus and PVN. Each tissue sample was thawed and processed shortly before analysis. Prior to sonication, ascorbate oxidase (Sigma, 2 μl, 0.5 U) was added to each sample. Whole hypothalamus or PVN was sonicated by use of a Kontes micro-ultrasonic cell disruptor in mobile phase prepared in distilled water and containing, per liter, EDTA (20 mg), $NaPO_4 \cdot 12\ H_2O$ (7.1 g), sodium citrate (6.3 g), NaN_3 (20 mg), sodium octyl sulfate (25 mg), at pH 4. The homogenate was centrifuged for 5 min (Beckman Microfuge at 4°C), and aliquots of the supernatant were diluted in running buffer. The neurotransmitters, 5-HT and DA, as well as their metabolites, were separated by high-performance liquid chromatography (HPLC). The HPLC apparatus was equipped with a guard column, a reverse-phase C_{18}-analytical column (5-μm diameter particles), and an electrochemical detector (BioAnalytical Systems, West Lafayette, IN). The working electrode potential was set at +0.85 V against a silver/silver chloride reference electrode. Solutions containing the standards were injected after each 6–8 samples to ensure proper calibration, and their peak heights were used to compare with those of neurotransmitters and their metabolites in the experimental samples. The standards used were 5-hydroxytryptamine-creatinine sulfate for serotonin (5-HT), the 5-HT metabolite 5-hydroxyindole-3-acetic acid (5-HIAA), 3-hydroxytyramine hydrochloride (dopamine, DA), the DA metabolites 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), and the norepinephrine metabolite, 4-hydroxy-3-methoxyphenylglycol hemipiperazinium salt (MHPG). The total protein content in each hypothalamus or PVN used for assay was determined using a modified Lowry protein assay (11) and a microplate reader (Bio-Rad). Neurotransmitter and metabolite concentrations were expressed as ng/mg of total tissue protein. Metabolite/neurotransmitter ratios were calculated by dividing metabolite concentrations by parent neurotransmitter levels in each sample, i.e.,

5-HIAA/5-HT, HVA/DA, and DOPA/DA (12), as an indirect measure of turnover.

Determination of Serum DHEA and 5-Androstene-3β,17β-diol (A-diol) Levels. Blood was collected into 1.5-ml Eppendorf tubes, placed on ice, allowed to clot, and centrifuged (Brinkmann Microfuge). The supernatants were collected and stored at −20°C for later analysis. Samples were thawed, volume was determined, and 100-μl aliquots were transferred to glass tubes. Distilled water was added to obtain 1 ml total volumes. [1,2,6,7-^{3}H]DHEA (Amersham, Arlington Heights, IL) and [1,2,6,7-^{3}H]A-diol (synthesized by reduction of tritiated DHEA with $NaBH_4$ in isopropyl alcohol (13)) were added to each sample (approximately 2,000 dpm/sample) as internal recovery standards. Steroids were extracted with ethyl ether, the solvent was evaporated and the residue was dissolved in isooctane containing 5% ethyl acetate (0.5 ml). To avoid possible cross-reaction with other steroids, DHEA and A-diol were isolated using partition column chromatography. Briefly, samples were transferred to compacted minicolumns containing 1 g of Celite/ethylene glycol (20:15 w/v) that were preconditioned with isooctane (3 ml). Air pressure was used throughout to accelerate elution. The glass tubes were rinsed once more with 5% ethyl acetate in isooctane (0.5 ml), and the extracts were transferred to the corresponding minicolumns. Thereafter, consecutive elutions were conducted with isooctane (4 ml), 5% ethyl acetate in isooctane (6 ml), and 20% ethyl acetate in isooctane (9 ml). Partial peak fractions containing DHEA and A-diol, as determined from preliminary studies, were used for radioimmunoassay (RIA). Aliquots of these fractions were counted to determine recoveries. Mean percent ± SD recoveries were 69% ± 9.0% for DHEA and 80% ± 12% for A-diol. Duplicate aliquots of each peak fraction containing the steroids were dried and dissolved in borate buffer for RIA, which was conducted as previously described (14, 15). The antisera used were anti-DHEA (lot no. D7-421, Endocrine Sciences, Calabasas, CA) and anti-A-diol (antibody R 14-02 kindly provided by Dr. Rogerio A. Lobo, University of Southern California, Los Angeles, CA).

Statistics. For all studies, both parametric and nonparametric statistical analyses were conducted using the VAX 8000 computer statistical package (UTSTAT v88.6) at U.T. Southwestern Medical Center. Comparisons across groups were performed using the Newman–Keuls multiple group analysis procedure. Correlation coefficients were obtained using the Spearman analysis. Spearman's rho ranges from −1 (indicating that high ranks of one variable occur with low ranks of the other variable) through 0 (no correlation) to +1 (indicating that high ranks of one variable occur with high ranks of the other variable). Significant difference was based on $P < 0.05$.

Results

The weekly food intake by mice fed DHEA for 5 weeks, expressed as mean ± SEM, is based on the average food intake over the preceding 7 days (Fig. 1). For example, Week 1 represents Days 1–7, and Week 2 represents Days 8–14. In preliminary experiments we observed that housing mice singly or in groups of 3, 5, or 6 per cage had similar effects on food intake and body weight. Both B6 and BDF1 mice fed DHEA ate much less than control mice during the first week of treatment (Fig. 1). During Weeks 2, 3, and 5, B6 mice fed DHEA ate less than controls, $P < 0.05$ (Fig. 1A) while DHEA-fed BDF1 mice ate similar amounts as their controls, $P > 0.05$ (Fig. 1B). If intake was expressed as food consumed per body weight in grams, similar results were obtained (data not shown). The body weights of mice fed the control diet increased during the 5 weeks of study, whereas the weights of mice fed DHEA decreased sharply during the first week of treatment (Fig. 2) and remained significantly reduced at all time points, $P < 0.05$. Body weights gradually increased in DHEA-treated BDF1 but remained almost at the level achieved after the first week in B6 mice fed DHEA. Nevertheless, the weights of B6 and BDF1 mice fed DHEA did not differ significantly from each other.

Because DHEA might cause food restriction by its possible effect on the sense of taste or by its effect on the gastrointestinal tract, DHEA was suspended in 13% poly-

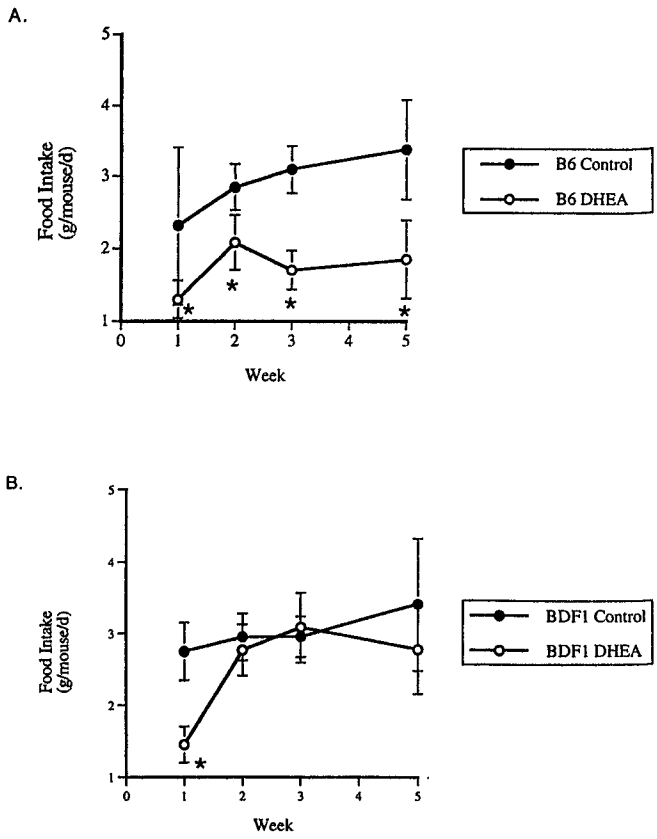

Figure 1. Food intake (mean ± SEM, g/mouse/d) by mice fed either the control diet or the diet containing 0.45% DHEA *ad libitum* for 5 weeks. Values were based on 7 days of measuring food intake by mice in two cages for each strain, e.g., Week 1 was the average of Days 1–7. (A) Male B6 mice. (B) Male BDF1 mice. *Mean value significantly different from the control group, $P < 0.05$ ($n = 7$ for each group).

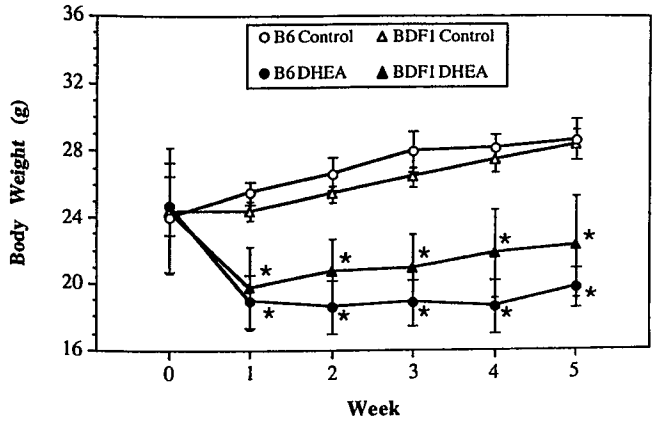

Figure 2. Body weights (g, mean ± SEM) of B6 (n = 10) or BDF1 (n = 12) mice fed the control diet or a diet containing 0.45% DHEA *ad libitum*. *See Fig. 1. The body weights are significantly different from those of controls but not from each other at each time point (P > 0.05).

ethylene glycol–water and was injected ip to bypass the oropharynx and gut. DHEA inhibited food intake in a dose-dependent manner during a 24-hr period in both B6 and BDF1 mice (Fig. 3). B6 mice were affected to a greater degree than BDF1 mice at the 5 mg dose, $P < 0.05$, but not at the 2.5 or 10 mg doses. It remains possible that injected DHEA affects taste, because DHEA acts at the central nervous system level (7, 8).

HPLC was used to determine 5-HT, DA, and metabolite concentrations in the hypothalamus of mice given the control or DHEA-containing diets for 1 or 7 days, or of mice pair-fed to the DHEA-treated mice for 7 days. In DHEA-treated B6 mice, no changes in neurotransmitters were observed on Day 1 (Table IA), and only 5-HIAA and MHPG levels were decreased on Day 7 compared to control and pair-fed mice ($P < 0.05$). Pair-fed B6 mice had increased

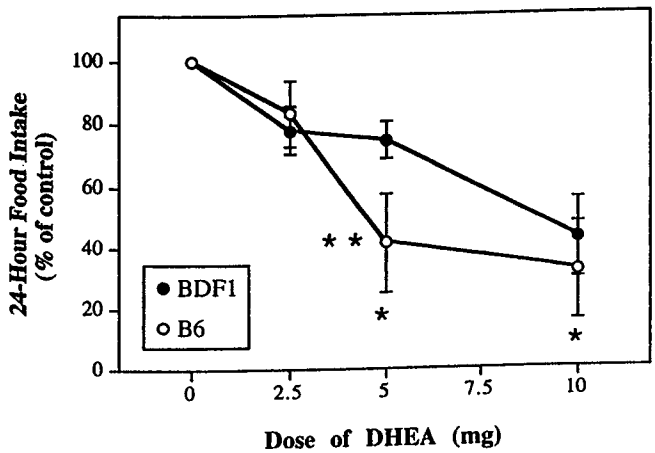

Figure 3. Food intake at 24 hr (mean ± SEM g/mouse/day) by groups of 4–6 male B6 and BDF1 mice injected ip with DHEA, expressed as % PEG vehicle controls. The mean ± SEM 24-hr food intake for control B6 and BDF1 mice were 2.9 ± 0.9 and 4.0 ± 0.3 g, respectively. *Mean values of B6 and BDF1 mice significantly lower than their control group, $P < 0.05$. **Mean value of B6 mice significantly lower than BDF1 mice at this dose, $P < 0.05$.

HVA levels (P = 0.06, Table IA) and increased HVA/DA ratios at 7 days ($P < 0.05$, Table II). Thus, no "acute" effects occurred on Day 1, and the changes at 7 days reflected either direct effects of DHEA or the indirect effect of food restriction on DA (or norepinephrine) metabolism. In contrast, in BDF1 mice undergoing the same food treatments several significant differences were found (Table IB). On Day 1, DA levels were increased and the metabolites DOPAC and HVA (and MHPG) were decreased ($P < 0.05$), which resulted in decreased metabolite/DA ratios (Table II). Hypothalamic 5-HT levels were increased by ~50% over controls and pair-fed mice after 7 days of DHEA, and DOPAC levels were decreased (Table IB). The DA levels had returned to control levels by Day 7. In pair-fed BDF1 mice, DOPAC, HVA, and MHPG levels were decreased relative to controls, $P < 0.05$, and HVA and MHPG levels were lower than in DHEA fed mice at Day 7 (Table IB). Thus, acute changes in DA and metabolites and later changes in 5-HT in BDF1 but not B6 mice may help mediate differences in food intake and body weight responses to dietary DHEA over time. Perhaps the "blunted" neurotransmitter responses in B6 mice contributed to the sustained decreases in both food intake and body weights over the 5-week study period.

In a similar study, the paraventricular nuclei of BDF1 mice were also evaluated for neurotransmitters and differed from those in the hypothalamus. For example, 5-HT was decreased in all treatment groups (Table III). There were no changes in DA on Day 1, although DOPAC and HVA levels were decreased, which led to lowered DOPAC/DA and HVA/DA ratios ($P < 0.05$, Table II). By Day 7, DHEA-fed and pair-fed mice had similar levels of neurotransmitters, $P > 0.05$.

When individual hypothalamic neurotransmitter and metabolite concentrations were plotted against corresponding serum DHEA and A-diol levels in BDF1 mice, only those of 5-HT showed a significant correlation (Fig. 4 and data not shown). Serum DHEA levels (mean ± SEM nmol/l) were 27 ± 11, 120 ± 28, 720 ± 180, and 220 ± 98 for control, DHEA (1 day), DHEA (7 days), and pair-fed mice, respectively. Serum A-diol concentrations were 8 ± 3, 16 ± 6, 280 ± 50, and 70 ± 33 for control, DHEA (1 day), DHEA (7 days), and pair-fed mice, respectively. Pair feeding alone raised levels of DHEA and A-diol ($P < 0.05$), suggesting that restriction of food intake independently affects production of these steroids.

Discussion

The major findings obtained in this study were that the differences in response to dietary DHEA by male B6 and BDF1 mice included a greater effect on food intake and body weight in B6 mice (Figs. 1 and 2) and a greater change in hypothalamic neurotransmitter levels in BDF1 mice (Table I). Effects of dietary DHEA, such as changes in lipid metabolism (16) or glucose-6-phosphate dehydrogenase ac-

Table I. Neurotransmitter and Metabolite Levels (Mean ± SEM, ng/mg Protein) in Hypothalami of Male Mice Fed AIN-76A (7 days) or AIN-76A Diet Containing DHEA (0.45% of Food) (1 or 7 days) or Pair-Fed to DHEA (7 days)[a,b]

Group	*n*	5-HT	5-HIAA	DA	DOPAC	HVA	MHPG
(A) B6 Mice							
Control	8	4.24 ± 0.30	4.56 ± 0.20	1.79 ± 0.08	1.39 ± 0.15	1.49 ± 0.23	1.35 ± 0.08
DHEA (1 day)	8	4.42 ± 0.30	5.19 ± 0.44	1.86 ± 0.56	1.26 ± 0.10	1.82 ± 0.14	1.19 ± 0.06
DHEA (7 days)	8	3.46 ± 0.32	*3.64* ± 0.22	1.88 ± 0.19	1.08 ± 0.19	1.68 ± 0.16	*1.09* ± 0.09
Pair-fed	7	4.03 ± 0.36	5.36 ± 0.55	1.77 ± 0.20	1.48 ± 0.15	2.27 ± 0.30	1.39 ± 0.13
(B) BDF1 Mice							
Control	8	5.94 ± 0.57	4.76 ± 0.72	2.89 ± 0.43	2.12 ± 0.22	1.79 ± 0.16	2.01 ± 0.23
DHEA (1 day)	8	5.50 ± 0.51	4.13 ± 0.29	*5.18* ± 0.50	*1.15* ± 0.11	*1.11* ± 0.11	*1.37* ± 0.13
DHEA (7 days)	8	*8.92* ± 1.20	4.57 ± 0.50	2.32 ± 0.19	*1.48* ± 0.18	1.70 ± 0.18	2.17 ± 0.39
Pair fed	7	5.44 ± 0.35	4.94 ± 0.23	2.03 ± 0.24	*1.30* ± 0.13	*1.10* ± 0.07	*1.38* ± 0.08

[a] 5-HT, 5-hydroxy tryptamine (serotonin); 5-HIAA, 5-hydroxyindole-3-acetic acid (serotonin metabolite); DA, dopamine, 3-hydroxytyramine; DOPAC, 3,4-dihydroxyphenyl acetic acid; HVA, homovallinic acid, 4-hydroxy-3-methoxyphenyl acetic acid (DA metabolites); MHPG, 4-hydroxy-3-methoxyphenyl glycol (norepinephrine metabolite).
[b] Italicized means are significantly different from controls, $P < 0.05$.

Table II. Ratios of Metabolite/Neurotransmitter Levels in Brains of BDF1 Mice Fed DHEA (0.45% of Food)[a,b]

Tissue	Treatment	5-HIAA/5-HT	DOPAC/DA	HVA/DA
B6 hypothalamus	Control	1.11 ± 0.09	0.81 ± 0.12	0.82 ± 0.13
	DHEA (1 day)	1.21 ± 0.14	0.70 ± 0.04	1.03 ± 0.12
	DHEA (7 days)	1.07 ± 0.06	*0.59* ± 0.03	0.96 ± 0.15
	Pair-fed (7 days)	1.35 ± 0.11	0.86 ± 0.07	*1.30* ± *0.15*
BDF1 hypothalamus	Control	0.80 ± 0.09	0.81 ± 0.10	0.62 ± 0.09
	DHEA (1 day)	0.78 ± 0.05	*0.23* ± 0.02	*0.22* ± 0.02
	DHEA (7 days)	*0.58* ± 0.09	0.64 ± 0.06	0.73 ± 0.04
	Pair-fed (7 days)	0.93 ± 0.06	0.67 ± 0.06	0.58 ± 0.06
BDF1 paraventricular nuclei	Control	0.80 ± 0.08	1.45 ± 0.10	1.18 ± 0.12
	DHEA (1 day)	1.02 ± 0.15	*0.84* ± 0.12	*0.60* ± 0.14
	DHEA (7 days)	1.27 ± 0.26	1.22 ± 0.36	*0.64* ± 0.16
	Pair-fed (7 days)	*1.52* ± 0.18	1.34 ± 0.17	*0.73* ± 0.13

[a] See Table I; $n = 8$ for all groups except the pair-fed group ($n = 7$).
[b] See Table I.

Table III. Neurotransmitter and Metabolite Levels (Mean ± SEM, ng/mg Protein) in Paraventricular Nuclei of Male BDF1 Mice Fed AIN-76A (7 days) or AIN-76A Diet Containing DHEA (0.45% of Food) (1 or 7 days) or Pair-Fed to DHEA (7 days)[a,b]

Group	*n*	5-HT	5-HIAA	DA	DOPAC	HVA	MHPG
Control	8	2.40 ± 0.12	1.87 ± 0.12	0.55 ± 0.07	0.76 ± 0.08	0.62 ± 0.08	0.41 ± 0.04
DHEA (1 day)	8	*1.70* ± 0.26	*1.52* ± 0.10	0.54 ± 0.07	*0.41* ± 0.05	*0.28* ± 0.04	0.38 ± 0.04
DHEA (7 days)	8	*1.54* ± 0.22	1.71 ± 0.22	0.63 ± 0.13	0.61 ± 0.13	0.36 ± 0.10	0.34 ± 0.05
Pair-Fed	7	*1.31* ± 0.14	1.86 ± 0.12	0.44 ± 0.04	0.55 ± 0.05	*0.30* ± 0.04	0.46 ± 0.03

[a] See Table I.
[b] Italicized means differ from controls, $P < 0.05$.

tivity (17–19), cannot be excluded as possible mechanisms of weight loss. However, the decrease in food intake mediated by changes in hypothalamic neurotransmitters should be considered as a significant mechanism.

To evaluate the issue of palatability of DHEA-containing food, we challenged mice with DHEA injected ip. The significant decreased food intake during a 24-hr period was readily apparent (Fig. 3). Because we observed that this treatment also lowers body temperature (data not shown), it is likely that DHEA acts directly on the central nervous system, involving the hypothalamus and possibly other brain structures.

The hypothalamus is an important site in the regulation of appetite, as 5-HT appears to be involved in regulation of food intake (20). Changes in hypothalamic neurotransmitter levels are associated with altered eating behavior (5, 6). Thus, obese Zucker rats treated with dietary DHEA ate less than rats fed the control diet, and this effect was associated with increased 5-HT levels in whole hypothalamus (21). In addition to 5-HT, DA also appears to be involved in regu-

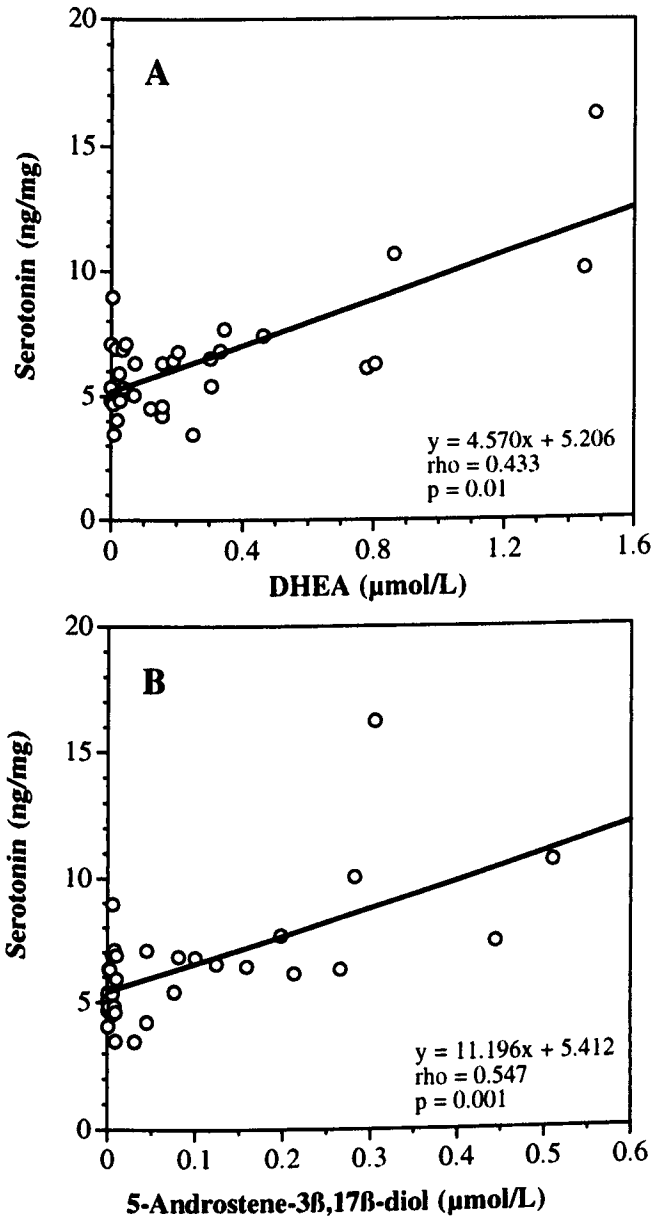

Figure 4. Relationship between hypothalamic serotonin levels (ng/mg tissue) and serum DHEA (A) or A-diol (B) levels (µmol/l) in BDF1 mice on the four treatment regimes (control diet, DHEA diet for 1 or 7 days, and pair-fed to DHEA-fed mice for 7 days).

lation of food intake (5). In this study, there was a striking difference in the "acute" 1-day effects of DHEA on B6 and BDF1 mice. Neurotransmitter and metabolite levels in B6 mice were not affected, whereas in BDF1 mice there were increased DA and decreased DA metabolite concentrations. The second marked difference between B6 and BDF1 mice was the increase in hypothalamic 5-HT in BDF1 but not in B6 mice after 7 days of DHEA feeding (Table I). These differences may or may not explain the differences in food intake and body weight changes in B6 and BDF1 mice, but they are consistent with other studies implicating a role for hypothalamic 5-HT in food intake. Decreased 5-HT concentrations have been reported in the brains of fasted or pair-fed mice (22), suggesting an association with the "hungry" state.

Metabolite/neurotransmitter ratios can be cautiously used as indicators of neurotransmitter turnover (12). After 1 day of DHEA treatment, both B6 and BDF1 mice ate less, but only BDF1 hypothalami had increases in DA and decreases in DA metabolites DOPAC and HVA, metabolite/DA ratios, and MHPG (Tables IB and II). There were no alterations in these values in the hypothalamus of B6 mice, suggesting that the DA changes or responses to the acute effects of DHEA help determine whether or not food restriction is temporary or long lasting. There were no acute effects of DHEA on hypothalamic 5-HT in B6 or BDF1 mice, but by 7 days 5-HT levels were increased in the hypothalamus of BDF1 but not B6 mice. This increase in 5-HT levels may be related to the feeding "recovery" in BDF1 mice.

Hypothalamic DA and 5-HT are involved in feeding behavior (5, 23), and the paraventricular nucleus may be an important site within the hypothalamus for 5-HT regulation of appetite (6). However, the PVN represents a small fraction of the total hypothalamic mass. Thus, any effect of DHEA on neurotransmitter concentrations at the PVN may not be detectable when evaluating the whole hypothalamus. The finding of lower 5-HT levels in PVN of BDF1 mice fed DHEA for 1 or 7 days or pair-fed for 7 days suggest that this transmitter was affected by food restriction as early as Day 1. The contrasting increase in 5-HT concentration in whole hypothalamus at 7 days of DHEA treatment of BDF1 mice indicates that if DHEA induces a decrease in food intake by increasing 5-HT concentrations, this occurs at a hypothalamic site other than the PVN. The lateral hypothalamus is an important site in obese Zucker rats (24), and it would be of interest to examine this site in DHEA-fed mice. Neuromodulatory peptides that are involved in feeding behavior are localized to the lateral hypothalamus (25).

There were significant relationships between serum levels of DHEA or A-diol and corresponding hypothalamic 5-HT concentrations in BDF1 mice subjected to the four different feeding regimes (Fig. 4). These results suggest that 5-HT may be critical component for the recovery from the reduced food intake in BDF1 mice induced by DHEA feeding. A-diol is a DHEA metabolite produced mainly in the liver (16) and which has estrogenic activity (26). Because hypothalamic 17β-estradiol implants in rodents regulate appetite (27, 28) in a manner approaching that of dietary DHEA, further studies are necessary to establish whether DHEA exerts its effects directly or via metabolite-mediated estrogen receptor binding.

In some instances significant differences in neurotransmitter or metabolite levels were present in pair-fed mice, e.g., decreased HVA levels in hypothalami and of 5-HT and HVA in PVN of BDF1 mice. The fact that these alterations also were present in pair-fed mice suggests that this may be a result of, and not the cause of, decreased food intake.

These observations highlight the importance of including pair-fed groups whenever conducting studies of this type. Without a pair-fed group, it is unclear whether observed neurotransmitter changes in treated animals are the cause of decreased food intake or rather the result of food restriction.

Food intake is also regulated by various polypeptides including neuropeptide Y, melanocyte-stimulating hormone, leptin, cocaine- and amphetamine-regulated transcript, urocortin, corticotropin-releasing factor, cholecystokinin-8, melanin-concentrating hormone, and glucagon-like peptide-1 (29–34). The inhibitory action of estrogen on food intake may be mediated through regulation of hypothalamic neuropeptide Y (35). Mutants of the *agouti* locus are associated with hyperphagia and obesity, and *mahogany* and *mahoganoid* mutant gene products can suppress both the *agouti* coat color changes and the obesity of mice bearing mutations of *agouti* (36). The complicated roles of gene products of these loci and the interaction between melanocortin receptors and the leptin regulated "adipostat" (37, 38) are under intensive investigation. How DHEA may interact with such a system is a subject of future investigation. The genetic data confirm the previous finding that C57BL/6 mice are more sensitive than closely related C57BL/Ks mice to DHEA induced food restriction (39). A future direction to clarify the mechanism by which B6 mice are especially sensitive to DHEA would be the identification of the gene(s) involved. This could be accomplished by evaluating mice that result from genetic crosses between B6 and C57BL/Ks (39) and/or B6 and DBA/2 (this paper) mice to compare genotypes with response to dietary DHEA.

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