Decrease of Core Body Temperature in Mice by Dehydroepiandrosterone

FERNANDO CATALINA,* LEON MILEWICH,† WILLIAM FRAWLEY,§ VINAY KUMAR,* AND MICHAEL BENNETT[1]*

*Departments of *Pathology, †Obstetrics and Gynecology, and §Academic Computing Services, University of Texas Southwestern Medical Center, Dallas, Texas 75390-9072*

Dietary dehydroepiandrosterone (DHEA) reduces food intake in mice, and this response is under genetic control. Moreover, both food restriction and DHEA can prevent or ameliorate certain diseases and mediate other biological effects. Mice fed DHEA (0.45% w/w of food) and mice pair-fed to these mice (food restricted) for 8 weeks were tested for changes in body temperature. DHEA was more efficient than food restriction alone in causing hypothermia. DHEA injected intraperitoneally also induced hypothermia that reached a nadir at 1 to 2 hr, and slowly recovered by 20 to 24 hr. This effect was dose dependent (0.5–50 mg). Each mouse strain tested (four) was susceptible to this effect, suggesting that the genetics differ for induction of hypophagia and induction of hypothermia. Because serotonin and dopamine can regulate (decrease) body temperature, we treated mice with haloperidol (dopamine receptor antagonist), 5,7-dihydroxytryptamine (serotonin production inhibitor), or ritanserin (serotonin receptor antagonist) prior to injection of DHEA. All of these agents increased rather than decreased the hypothermic effects of DHEA. DHEA metabolites that are proximate (5-androstene-3β, 17β-diol and androstenedione) or further downstream (estradiol-17β) were much less effective than DHEA in inducing hypothermia. However, the DHEA analog, 16α-chloroepiandrosterone, was as active as DHEA. Thus, DHEA administered parentally seems to act directly on temperature-regulating sites in the body. These results suggest that DHEA induces hypothermia independent of its ability to cause food restriction, to affect serotonin or dopamine functions, or to act *via* its downstream steroid metabolites.

[Exp Biol Med Vol. 227(6):382–388, 2002]

Key words: DHEA, 16α-chloroepiandrosterone, hypothermia, food restriction, neurotransmitters

Dietary dehydroepiandrosterone (DHEA) has the ability to induce hypophagia, a type of food restriction. Both DHEA and food restriction have similar biological effects, including the prevention of diseases such as cancer and arteriosclerosis (1–3). Such data raise the possibility that DHEA acts primarily via food restriction. Indeed, we recently observed that both DHEA and food restriction can induce apoptosis of lymphocytes with inhibition of lymphopoiesis and immune functions (4), as well as alterations in hypothalamic neurotransmitter levels (5). Food restriction can reduce body temperature in mice and in monkeys (6, 7). To determine if DHEA mediates hypothermia via food restriction and/or altered hypothalamic neurotransmitters, we treated mice either with dietary DHEA (0.45% w/w of food) or by food restriction (pair feeding). Both methods reduced core body temperature, but DHEA was more effective than food restriction alone. To detect other possible mechanisms of hypothermia, DHEA was injected intraperitoneally (i.p.). We analyzed three different strains of mice for sensitivity to the hypothermic effect of DHEA to test for genetic regulation. The requirements for the hypothalamic neurotransmitters serotonin and dopamine in mice injected i.p. with DHEA were also studied using reagents that affect dopamine or serotonin function. To determine if induction of hypothermia involves metabolites of DHEA, we compared DHEA with 5-androstene-3β, 17β-diol (A-diol), androstenedione (A-dione), and estradiol-17β (E_2). An analog of DHEA that shares biological effects with DHEA, 16α-chloroepiandrosterone (Cl-epi), was also tested. The results challenge the ideas that food restriction is the only cause of hypothermia, that dopamine and/or serotonin function are required, or that metabolites of DHEA are responsible/required for DHEA-induced hypothermia.

Materials and Methods

Animals. Male C57BL/6 (B6), (B6 × DBA/2)F1 (B6D2F1), BALB/c, and (CBA × B6)F1 mice were obtained from a conventional colony at this University, and were used at ages 10 to 16 weeks. The mice were housed at an environmental temperature of 22° ± 1°C and were maintained on a 12:12-hr light:dark cycle with lights out at 1900

This work was supported in part by the National Institutes of Health MSTP training (grant 5T32 GM08013 and research grants CA36822, CA47073, AI38939, AI20451, and CA43311).

[1] To whom requests for reprints should be addressed at Department of Pathology NB6.402, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390–9072. E-mail: michael.bennett@utsouthwestern.edu

Received January 25, 2001.
Accepted February 14, 2002.

1535-3702/02/2276-0382$15.00

hr. Groups of five mice were used except for experiments presented in Figure 3 ($n = 6$).

Diets. Mice were fed Purina Lab Chow until the start of experiments (Day 0). Groups of five mice were then fed pelleted AIN-76A diet (Dyets Inc., Bethlehem, PA) containing either no additive or DHEA (0.45% w/w) between 0900 and 1000 hr. Diets were stored at 4°C for no longer than six months to maintain optimal activity. Mice were given the diets *ad libitum,* except for mice that were pair fed to mice treated with DHEA. The amounts of AIN-76A diet the pair-fed mice received were determined by the weight of food consumed by the DHEA-fed mice on a daily basis. Body weights (grams) were measured at different time points starting at Day 1 and ending at Day 59. Daily food intakes (grams per day) were determined by weighing the food consumed per cage of five mice. The mean ± SEM values were calculated for weeks 1 to 8 ($n = 7$); week 9 had only 3 days.

Injection of Steroids. DHEA, A-diol, A-dione, and E_2 (Sigma, St. Louis, MO), and Cl-epi, the latter synthesized as described (8), were suspended in 13% polyethyleneglycol (PEG, MW 3350; Sigma) in distilled water. Groups of five mice (or six mice in Figure 3) received 0.5 ml of vehicle or suspension of steroid i.p. between 0900 and 1000 hr. In this and other similar studies, the amount of food eaten by mice during the 24-hr period (grams per day) was determined by weighing the food before and after the 24-h period. The data are presented in Figures 2 through 6 in parentheses after the group names.

Administration of Haloperidol (Haldol). Haldol (McNeil Pharmaceuticals, Spring House, PA), a dopamine receptor antagonist (9), was added to the drinking water at concentrations of 0.9, 9, or 90 μg/ml, starting 4 days prior to injection of DHEA. The temperature values at Time 0 h reflects any changes induced by Haldol alone.

Administration of 5,7-Dihydroxytryptamine (DHT). DHT (Sigma), a specific serotonergic neurotoxin (10), was dissolved in the vehicle, 0.9% NaCl containing 1 mg/ml ascorbic acid (Sigma). Mice were injected i.p. with 100 mg/kg body weight ketamine hydrochloride (Ketaset; Aveco Co. Inc., Fort Dodge, IA) and 5 mg/kg body weight Xylazine (Rugby Laboratories Inc., Rockville Center, NY) to induce anesthesia. A dose of 25 μg of DHT in 20 μl of vehicle was injected into a lateral cerebral ventricle (i.c.v.) as described (11). The site of injection was 2 mm from either side of the midline on a line drawn through the anterior base of the ears. The 27-gauge needle was inserted to a depth of 3 to 4 mm. DHEA suspensions or vehicle only were injected i.p. 7 days after the i.c.v. injections (groups of five mice). The temperature values at Time 0 reflect any changes induced by DHT alone (Figure 5A). To test the accuracy of injections, separate mice were injected with 30 μl of 10% India ink solution in the same location. The mice were sacrificed (CO_2 inhalation) and the brains were removed, rapidly frozen, and stored at −70°C. The frozen brains were sectioned at 50 μm intervals and were examined with a dissecting microscope.

Administration of Ritanserin. Ritanserin (RBI Biochemicals, Natick, MA), a serotonin receptor antagonist (12), was dissolved in methanol (2 mg in 1 ml), and thereafter was diluted in 79 ml of distilled water to obtain a concentration of 25 μg/ml. Mice received 0.5 μg/g body weight i.p. 30 min before injections of DHEA or vehicle (groups of five mice).

Measurement of Internal Body Temperature. Body temperature was determined by inserting a Tele-thermometer probe (Yellow Springs Instrument Co., Yellow Springs, OH) 25 mm into the recta of mice that were not anesthetized. The temperature value was registered 60 sec after probe insertion. Mice on diets were examined daily between 0900 and 1000 hr for 27 days, and then every 7 to 9 days in the study described in Figure 1. Mice injected i.p. with steroids and/or drugs were tested just before and 30 min to 24 hr after injection as denoted in the figures.

Statistics. The data were analyzed using a repeated measures model in which the covariance between any two observations on the same animal was modeled as the product of an overall variance and an autoregressive correlation coefficient raised to the power of absolute value of the time difference between the observations. Baseline observations (Time 0) were used as a covariate, and Time 0 values are not presented in the figures. The models were implemented in the MIXED procedure (13) using the SAS software. For the data derived from three strains of mice in Figure 3, contrasts were made within-strain, evaluating DHEA versus Vehicle. For the remainder of the data, all treatment groups were compared with the Vehicle groups. The baseline observations could not be used as a covariate in two studies because Haldol (Figure 4) and DHT (Figure 5A) were given some days before injection of DHEA. The SEM values were <10% of the means, and are not depicted in the figures, for clarity. However, the symbols for mean values at given times were filled in black when the mean experimental value was significantly different from the control diet or Vehicle control. In all testing, observed significances less than 0.05 are taken to be statistically significant. The ranges for the values of SEM are given in each figure legend. Differences in daily food intake between the control and the DHEA group of Figure 1 were analyzed by a paired t test.

Results

Dietary Studies. DHEA in the diet (0.45 % w/w) of male B6 mice (groups of five mice) treated for 8 weeks led to significant decreases in body temperature compared with mice fed the control AIN-76A diet (Fig. 1A). A similar comparison indicated that control and pair-fed mice were also significantly different (Fig. 1B). Animals fed DHEA had significantly lower temperatures than mice fed the control diet 26/29 times tested (Fig. 1A); mice pair fed to those on the DHEA diet were less affected, with 8/29 values significantly lower than in mice fed AIN-76A *ad libitum*

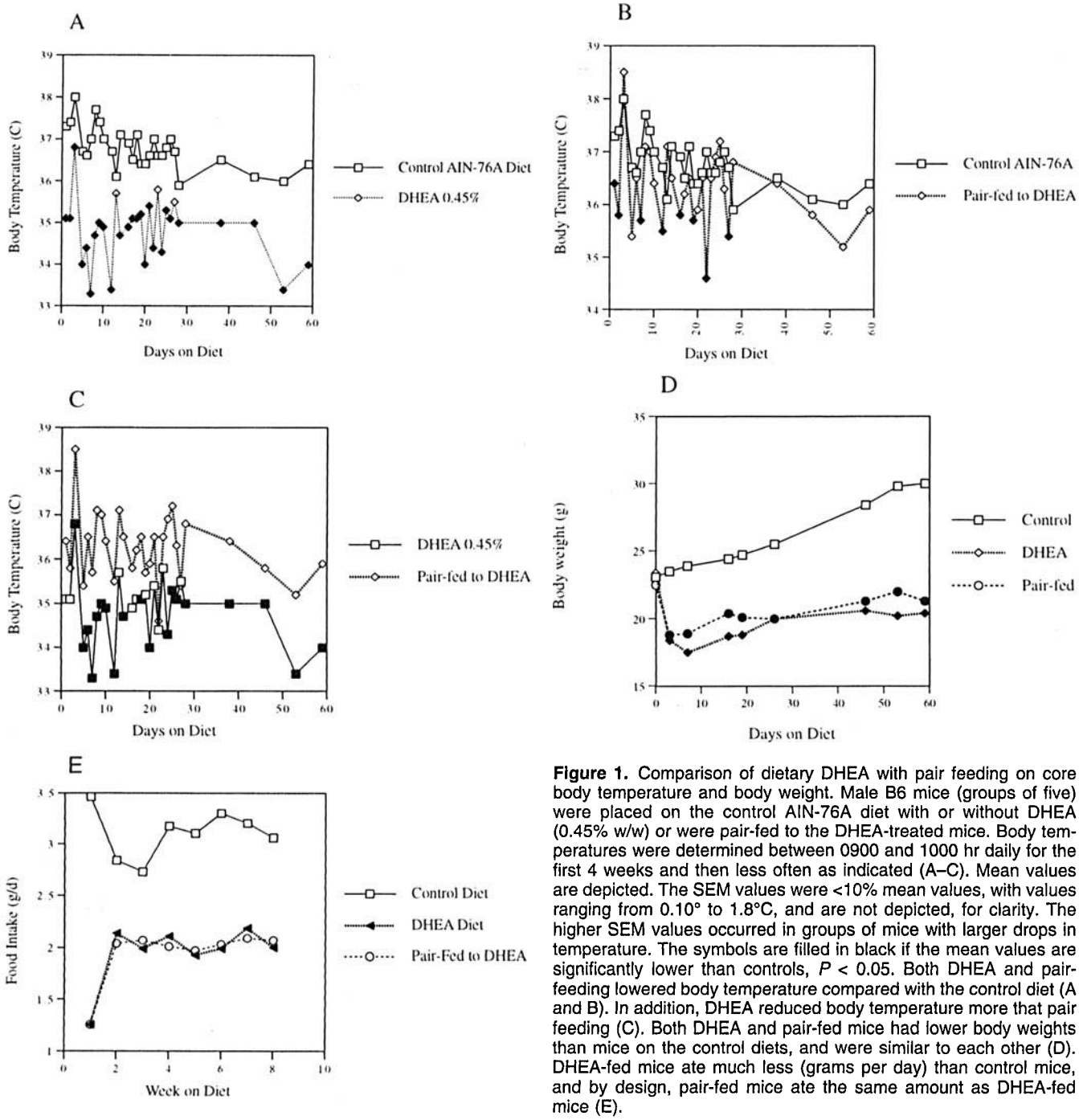

Figure 1. Comparison of dietary DHEA with pair feeding on core body temperature and body weight. Male B6 mice (groups of five) were placed on the control AIN-76A diet with or without DHEA (0.45% w/w) or were pair-fed to the DHEA-treated mice. Body temperatures were determined between 0900 and 1000 hr daily for the first 4 weeks and then less often as indicated (A–C). Mean values are depicted. The SEM values were <10% mean values, with values ranging from 0.10° to 1.8°C, and are not depicted, for clarity. The higher SEM values occurred in groups of mice with larger drops in temperature. The symbols are filled in black if the mean values are significantly lower than controls, $P < 0.05$. Both DHEA and pair-feeding lowered body temperature compared with the control diet (A and B). In addition, DHEA reduced body temperature more that pair feeding (C). Both DHEA and pair-fed mice had lower body weights than mice on the control diets, and were similar to each other (D). DHEA-fed mice ate much less (grams per day) than control mice, and by design, pair-fed mice ate the same amount as DHEA-fed mice (E).

(Fig. 1B). The temperatures of mice fed DHEA or pair fed to DHEA were significantly different 21/29 times tested (Fig. 1C). The values of body weights and food intake are depicted in Figure 1D, and E, respectively. Body weights were significantly greater in mice fed the control diet than in mice fed DHEA or pair fed to DHEA. Food intake (grams per day) from cages were averaged for each week ($n = 7$), except for Week 9 ($n = 3$). The amount of food intake was significantly decreased in mice fed DHEA. By design, mice pair fed to DHEA ate about the same amount. Thus, it appears that DHEA reduces body temperature by food restriction and by a separate mechanism.

Acute Effects of Injected DHEA. The i.p. injections of DHEA into groups of five B6D2F1 mice led to rapid decreases in body temperature, and after some time, values usually returned to normal by 24 hr (Fig. 2A and B). There were dose-dependent effects between 0.5 and 50 mg, i.e., doses of 0.5, 1, or 5 mg did not significantly lower body temperature, but doses of 10 mg or higher were highly effective in these two experiments. Some mice had seizures at the higher doses of 20 or 50 mg (one each) and some mice died (three and two at the next-to-last time points in Fig. 2A and B, respectively). The rapid onset of hypothermia (as early as 30 min) supports the idea that although DHEA

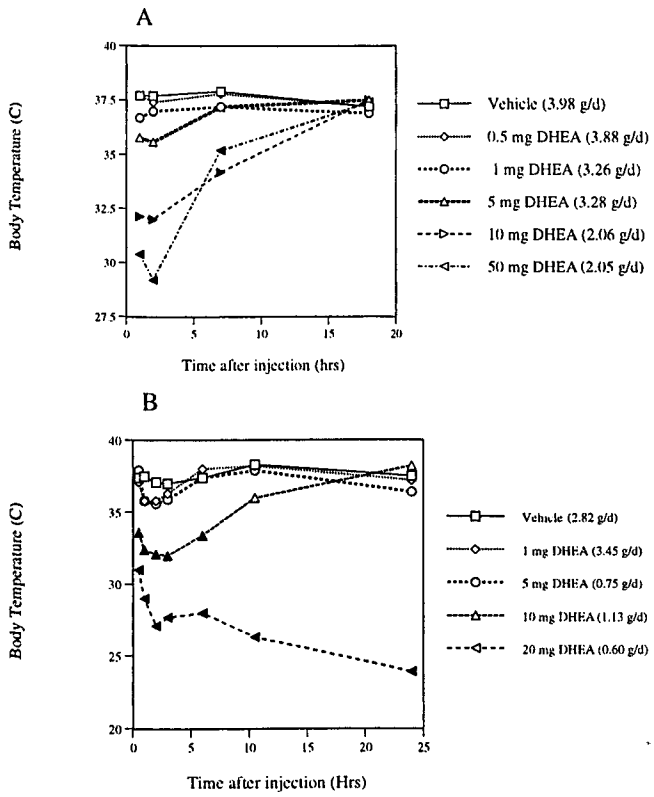

Figure 2. Effect of DHEA injected i.p. on core body temperature. Male B6D2F1 mice (groups of five) were injected with vehicle (13% PEG in water) or DHEA. Body temperature was measured before injection and 30 min to 24 hr later. Effects of 0.5, 1, 5, 10, and 50 mg of DHEA (A). Effects of 1, 5, 10, or 20 mg of DHEA (B). Mean values are depicted. The SEM values were <10% mean, with values ranging from 0.15° to 2.0°C, and are not depicted. The higher SEM values occurred in groups of mice with larger drops in temperature. The symbols are filled in black if the mean values are significantly lower than controls, $P < 0.05$ at a given time. Values in parentheses represent the amount of food eaten (grams per day per mouse) during the 24-h experimental period.

induces both hypothermia and hypophagia, hypothermia is not dependent solely on food restriction.

We previously observed that the hypophagia induced by dietary DHEA was under genetic control because B6 mice were much more sensitive than B6D2F1 mice (4, 5). This was reflected in different hypothalamic neurotransmitter levels of B6 versus B6D2F1 mice, i.e., they were "blunted" in B6 mice and significant in B6D2F1 mice (5). Preliminary studies suggested that this was not true for the effects of DHEA on body temperature. We compared B6, BALB/c, and (CBA X B6)F1 mice for their sensitivities to 10 mg of DHEA in the same experiment. All three types of mice had similar acute temperature responses to DHEA through 7 hr (Fig. 3). There were some variations in recovery, and BALB/c mice did not recover by 24 hr in this particular study. The data presented in Figures 3 and 2 (B6D2F1 mice were used) argue that the acute effects of DHEA are not under the same genetic control as those of the dietary effects of DHEA on food intake (4) and on hypothalamic levels of neurotransmitters (5).

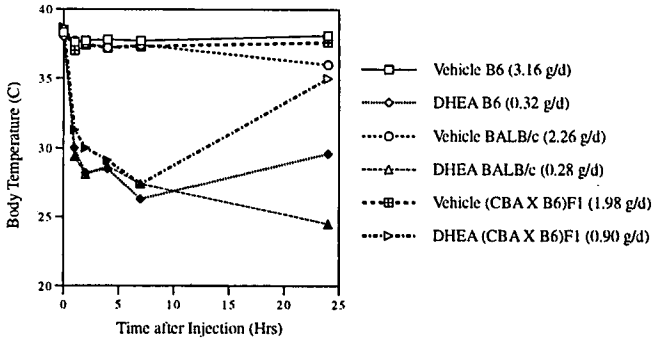

Figure 3. Effects of DHEA injected i.p. on body temperature in male B6, BALB/c, or (CBA X B6)F1 mice. Groups of six mice were injected with vehicle or 10 mg of DHEA. Body temperature was measured before and after injection at the indicated time points. Mean values are depicted. The SEM values were <10% mean values, ranged from 0.2° to 2.6°C, and are not depicted. The higher SEM values occurred in groups of mice with larger drops in temperature. The symbols are filled in black if the mean values are significantly lower than controls, $P < 0.05$. Values in parentheses represent the amount of food eaten (grams per day per mouse) during the 24-hr experimental period.

To test the potential roles of dopamine in DHEA induced hypothermia, we administered the dopamine receptor antagonist, haloperidol (Haldol) in the drinking water, starting 4 days before the injection of 10 mg of DHEA. We measured the volume of drinking water daily to determine 24-hr intake of water per mouse; the value was approximately 5 ml. Thus, the doses of the drug were approximately 4.5, 45, and 450 μg/day. The concentrations of 0.9 or 9 μg/ml Haldol in drinking water did not affect temperature in the absence of DHEA (see Time 0 hr), nor did they affect body temperature in DHEA injected mice (Fig. 4). At 90 μg/ml, Haldol lowered core temperature by almost 2°C when given alone (Time 0, $P < 0.05$), and exacerbated the

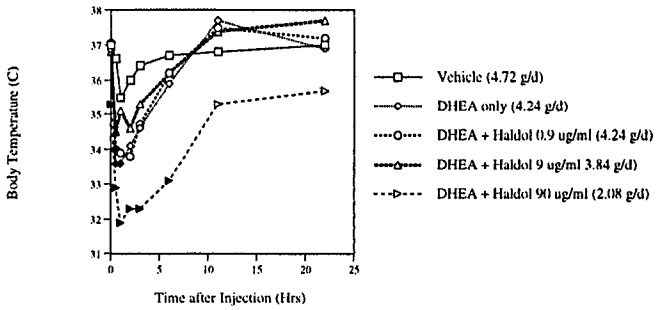

Figure 4. Test for the role of dopamine in DHEA induced hypothermia. Male B6D2F1 mice (groups of five) were placed on drinking water containing 0 (vehicle), 0.9, 9, or 90 μg/ml haloperidol (Haldol, a dopamine receptor antagonist). Four days later, the mice were injected i.p. with the vehicle, 13% PEG in water, with or without 10 mg of DHEA. Body temperature was measured before (time 0) and at the indicated time points after injection. Mean values are depicted. The SEM values were <10% mean values, ranged from 0.2° to 2.6°C, and are not depicted. The higher SEM values occurred in groups of mice with larger drops in temperature. The symbols are filled in black if the mean values are significantly lower than controls, $P < 0.05$. Values in parentheses represent the amount of food eaten (grams per day per mouse) during the 24-hr experimental period. The group receiving 90 μg/ml Haldol had significantly lower mean body temperature than controls at time 0 hr ($P < 0.05$).

effects of DHEA. Under these conditions, it would appear that dopamine may actually oppose the hypothermic effects of DHEA. Alternatively, Haldol and DHEA may act synergistically in an independent mechanism to lower body temperature.

To examine the role of serotonin in mediating the DHEA effects, we injected 25 μg of DHT, a serotonin production inhibitor, into a lateral brain ventricle of brains of B6D2F1 mice. One week later, we examined the ability of DHEA to induce hypothermia. DHT enhanced the hypothermic effect of DHEA (Fig. 5A). DHT alone did not lower body temperature (Time 0). Examination of the brains after i.c.v. India ink injection revealed that the ventricles were correctly targeted (data not shown). In a second approach, the serotonin receptor antagonist ritanserin was injected i.p. at a dose of 0.5 mg/kg body weight 30 min before DHEA was injected. Ritanserin, like Haldol and DHT, enhanced the hypothermic effect of DHEA (Fig. 5B). The latter two studies suggest that the DHEA-induced decrease in internal body temperature may be potentiated by the inhibition of serotonin action.

To determine if DHEA acts directly or via proximal or distal metabolites, we injected B6 mice with A-diol, A-dione, or E_2. Whereas DHEA (10 mg) led to significant decreases in body temperature, A-diol (10 mg) and A-dione (10 mg) produced no effects, and E_2 (2 mg) had a minor but not significant effect at 1 hr only (Fig. 6A). However, A-dione did lower body temperatures when doses of 20 or 50 mg were injected i.p. (data not shown). To determine if the analog of DHEA, Cl-epi, was effective in reducing body temperature, we injected B6D2F1 mice with various doses of this steroid i.p. Cl-epi significantly reduced body temperatures at doses of 10, 20, and 50 mg (Fig. 6B). Unlike DHEA, Cl-epi did not induce seizures or kill mice at the higher doses.

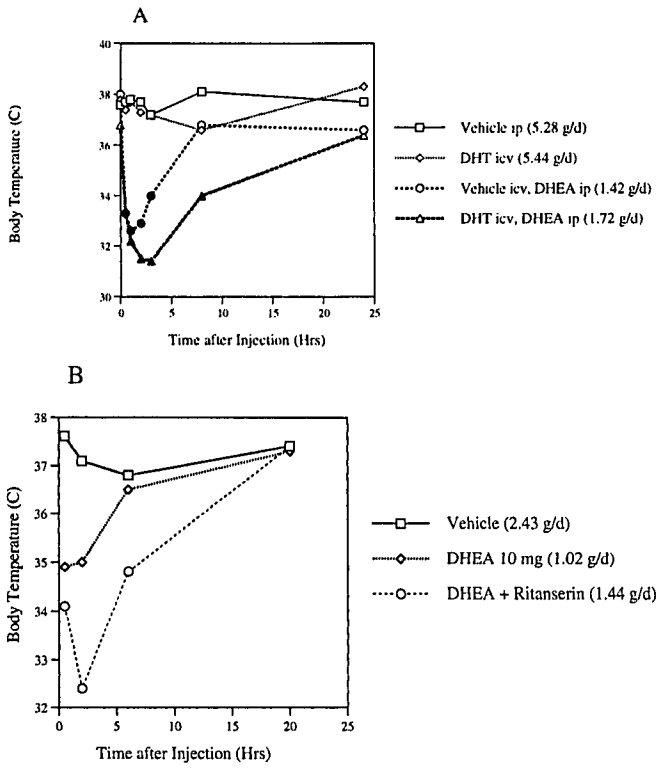

Figure 5. Tests for the role of serotonin in DHEA induced hypothermia. Male B6D2F1 mice (groups of five) were injected with vehicle (0.9% NaCl with 1 mg/ml ascorbic acid) with or without 25 μg of DHT (a serotonergic neurotoxin) into a lateral cerebral ventricle (A). Seven days later, the mice were injected i.p. with vehicle or 10 mg of DHEA. Male B6D2F1 mice (groups of five) were injected i.p. with vehicle or 0.5 mg/kg body weight ritanserin (a serotonin receptor antagonist) (B). After 30 min, mice were injected i.p. with 5 mg of DHEA. Body temperature was measured before (0 hours) and after injection at the indicated time points. Mean values are depicted. The SEM values were <10% mean values, ranged from 0.2° to 2.6°C, and are not depicted. The higher SEM values occurred in groups of mice with larger drops in temperature. The symbols are filled in black if the mean values are significantly lower than controls, $P < 0.05$. Values in parentheses represent the amount of food eaten (grams per day per mouse) during the 24-hr experimental period. The group receiving only DHT had mean body temperatures not significantly different from controls at time 0.

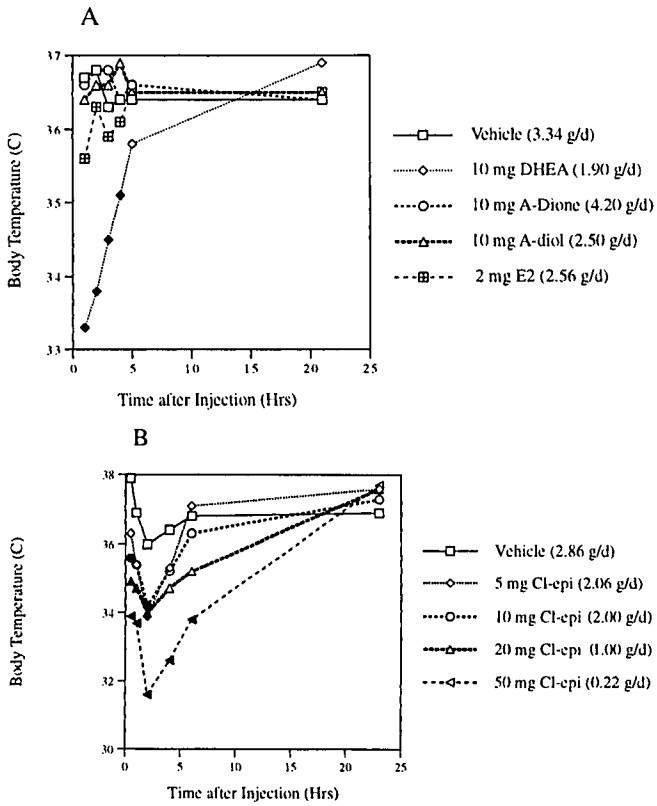

Figure 6. Comparison of the effects on body temperature of three metabolites and one analog of DHEA injected i.p. Metabolites: B6 mice (groups of five) were injected i.p. with vehicle only, A-diol (10 mg), A-dione (10 mg), E_2 (2 mg), or DHEA (10 mg) (A). Analog: B6D2F1 male mice (groups of five) were injected i.p. with vehicle containing 0, 5, 10, 20, or 50 mg of Cl-epi (B). Rectal body temperature was measured before and after injection at the indicated time points. Mean values are depicted. The SEM values were <10% mean values, ranged from 0.2° to 2.2°C, and are not depicted. The higher SEM values occurred in groups of mice with larger drops in temperature. The symbols are filled in black if the mean values are significantly lower than controls, $P < 0.05$. Values in parentheses represent the amount of food eaten (grams per day per mouse) during the 24-hr experimental period.

The amounts of food eaten during the 24-hr experiments were calculated and are presented in parentheses in the figures after the indicated treatment. In most cases, food intake (grams per day) reflected the degree of temperature reduction induced by a dose of an active agent. Perhaps the rapid drop in temperature caused a temporary loss of appetite and/or the agents, especially DHEA, independently induced a loss of appetite (5).

Discussion

We report here that DHEA induces hypothermia and food restriction when fed to mice, and hypothermia results from both food restriction and some other, undefined mechanism (Fig. 1). Hypothermia occurs rapidly when DHEA is injected i.p., and this effect does not involve food restriction, even though mice so injected often ate less during the 24-hr experiments (Fig. 2–6). This conclusion is based on the rapid onset of DHEA action, often by 30 to 60 min. This result differs from the similar effects of DHEA and food restriction on body weights, lymphocyte apoptosis, and inhibition of lymphopoiesis (4). Even though body temperature is largely regulated by thermogenic metabolism (1, 14), DHEA induces rapid hypothermia after i.p. injection. Dopamine and serotonin can induce hypothermia (15–17), and DHEA can affect serotonin and dopamine levels in the hypothalamus of mice and rats (4, 18). However, interference with dopamine or serotonin effects enhanced rather than inhibited the hypothermic effects of DHEA (Figs. 4 and 5). Thus, DHEA and the neurotransmitters, serotonin and dopamine, regulate hypothermia by different mechanisms.

One idea is that DHEA blocks hypothalamic thermoreceptors, and there is evidence that this might be so in rats treated with DHEA at different ambient temperatures. At high temperatures (34° or 37°C), rats died within 5 days, and this could be prevented by DHEA injected at 50 mg/kg body weight/day (19).

A second possibility is that DHEA and other neurosteroids, e.g., DHEA sulfate (DHEAS), can affect the GABA-A receptor system to affect temperature, hypnosis, memory, and anxiety (20–22). To examine this possibility, it will be important to compare the hypothermic effects of DHEA with DHEAS. Because large amounts of DHEA (10–50 mg) were injected, a third possibility is that DHEA entered the brain by overwhelming transport restriction regulation to affect cell membranes in the brain nonspecifically. One study indicated that DHEA and DHEAS can interact with phospholipid membranes, albeit less efficiently than cholesterol (23). It would be important to know how well DHEA crosses the blood brain barrier (BBB) to enter the brain. Levels of unconjugated lipophilic steroids in human cerebrospinal fluid (CSF) and free steroids (unbound to proteins) in serum are very low, even in patients with disturbed BBB or brain-CSF barrier. DHEAS does not easily pass into the brain (24). The BBB appears to restrict passage of DHEAS and other similar molecules via the organic anion transporting polypeptide 2 (oatp2). For example, a substrate of oatp2, digoxin, interferes with uptake of DHEAS into a brain capillary endothelial cell line that expresses oatp2 (25). The authors noted that transport of neurosteroids into the brain is 10 times less efficient than transport from brain to serum.

A fourth possibility is that the induction of cytochrome P450/epoxygenase by DHEA might regulate temperature by regulating arachidonate metabolism (26). The fever induced by LPS in rats was inhibited by treatment with two different P450 inducers, DHEA and bezafibrate. Therefore, DHEA could cause hyothermia, in part, by affecting epoxyeicosanoid levels in the brain (26).

There are a number of other potential mechanisms by which DHEA may cause hypothermia thus far not investigated. For example, inhibition of orexin-A, which causes hyperthermia if injected i.c.v. in rats (27), is a possible target. Orexin-A increases sympathetic nerve firing rates in interscapular brown adipose tissue (IBAT), and this can be inhibited by lysine acetylsalicylate, an inhibitor of prostaglandin synthesis. IBAT is a potential target for DHEA activity because it is important for adrenergic non-shivering thermogenesis mediated by the uncoupling protein, UCP1 (28). Finally, in recent years, evidence for vagal nerve regulation of fever has been studied. Subdiaphragmatic vagotomy appears to regulate fever by interfering with both efferent and afferent innervation of liver and other organs (29, 30).

We fed mice a high dose of DHEA and injected high doses of DHEA in these and other experiments. DHEA and compounds like Cl-epi could be useful in reducing pathological fever in patients if they were active at lower doses. Beneficial effects of DHEA given parentally have been achieved at much lower doses than those used in the present study, e.g., 20 to 50 mg/kg body weight, or 0.6 to 1.5 mg per 30 g mouse (31, 32).

In conclusion, DHEA induces hypothermia when given in the diet or injected i.p, and food restriction cannot explain most of the effects observed. Serotonin and dopamine regulate temperature and DHEA can modify hypothalamic levels of these neurotransmitters. Nevertheless, these transmitters do not appear to promote hypothermia induced by the injected DHEA. We conclude that hypophagia and hypothermia caused by DHEA administration in the diet or by injection occur, at least in part, by separate mechanisms.

1. Svec F, Porter JR. The actions of exogenous dehydroepiandrosterone in experimental animals and humans. Proc Soc Exp Biol Med **218**:174–191, 1992.
2. Sabbiah MT, Siekert RG Jr. Dietary restriction and the development of atherosclerosis. Br J Nutr **41**:1–6, 1979.
3. Grasl-Kraupp B, Bursch W, Ruttkay-Nedecky B, Wagner A, Lauer B, Schulte-Herman R. Food restriction eliminates preneoplastic cells through apoptosis and antagonizes carcinogenesis in rat liver. Proc Natl Acad Sci U S A **91**:9995–9999, 1994.
4. Catalina F, Kumar V, Milewich L, Bennett M. Food restriction-like effects of dehydroepiandrosterone: decreased lymphocyte numbers

and functions with increased apopotosis. Proc Soc Exp Biol Med **221**:326–335, 1999.
5. Catalina F, Speciale SG, Kumar V, Milewich L, Bennett M. Food restriction-like effects of dehydroepiandrosterone: hypothalamic neurotransmitters and metabolites in mice. Proc Soc Exp Biol Med **226**:208–215, 2001.
6. Cheney KE, Liu RK, Smith GS, Meredith PJ, Mickey MR, Walford RL. The effect of dietary restriction of varying duration on survival, tumor patterns, immune functions and body temperature in B10C3F1 female mice. J Gerontol **38**:420–430, 1983.
7. Lane MA, Baer DJ, Rumpler WV, Weindruch R, Ingram DK, Tilmont EM, Cutler RG, Roth GS. Caloric restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. Proc Natl Acad Sci U S A **93**:4159–4164, 1996.
8. Abou-Gharbia M, Pashko L, Schwartz A, Swern D. Synthesis of dehydroepiandrosterone sulfate and 16α-halogenated steroids. J Pharmacol Sci **70**:1154–1157, 1981.
9. Froeming JS, Lam YW, Jann MW, Davis CM. Pharmacokinetics of haloperidol. Clin Pharmacokinetics **17**:396–423, 1989.
10. Finnegan KT, DeLanney LE, Ricaurteand GA, Langston JW. The amine-depleting effects of 5,7-dihydroxytryptamine (5,7-DHT) in C57BL/6 mice do not increase with age. Brain Res **496**:251–256, 1989.
11. Haley TJ, McCormick WG. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br J Pharmacol **12**:12–15, 1957.
12. Gao B, Cutler MG. Effects of acute and subchronic administration of ritanserin on the social behaviour of mice. Neuropharmacology **32**:265–272, 1993.
13. Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS System for Mixed Models. Cary, NC: SAS Institute, 1996.
14. Bobyleva V, Kneer N, Bellei M, Battelli, Lardy HA. Concerning the mechanism of increased thermogenesis in rats treated with dehydroepiandrosterone. J Bioenerg Biochem **25**:313–321, 1993.
15. Cox B, Kerwin RW, Lee TF, Pycock CJ. A dopamine-hydroxytryptamine link in the hypothalamic pathways which mediate heat loss in the rat. J Physiol **303**:9–21, 1980.
16. Matsuda T, Nakano Y, Kanda T, Iwata H, Baba A. Gonadal hormones affect the hypothermia induced by serotonin 1A (5-HT1A) receptor activation. Life Sci **48**:1627–1632, 1991.
17. Sugimoto Y, Yanada J, Horisaka K. Activation of peripheral serotonin-2 receptors induces hypothermia in mice. Life Sci **48**:419–423, 1991.
18. Porter JR, Abadie JM, Wright BE, Browne ES, Svec F. The effect of discontinuing dehydroepiandrosterone supplementation on Zucker rat food intake and hypothalamic neurotransmitters. Int J Obes Relat Metab Disord **19**:480–488, 1995.
19. Tal E, Sulman FG. Dehydroepiandrosterone-induced thyrotrophin release during heat stress in rats. J Endocrinol **67**:99–103, 1975.
20. Melchior CL, Ritzmann RF. Dehydroepiandrosterone enhances the hypnotic and hypothermic effects of ethanol and pentobarbital. Pharmacol Biochem Behav **43**:223–227, 1992.
21. Melchior CL, Ritzmann RF. Neurosteroids block the memory-impairing effects of ethanol in mice. Pharmacol Biochem Behav **53**:51–56, 1996.
22. Melchior CL, Ritzmann RF. Dehydroepiandrosterone is an anxiolytic in mice on the plus maze. Pharmacol Biochem Behav **47**:437–441, 1994.
23. Dicko A, Di Paolo T, Pezilet M. Interaction of dehydroepiandrosterone with phospholipid membranes: an infrared spectroscopy investigation. Biochem Biophys Acta **1368**:321–328, 1998.
24. Schwarz S, Pohl P. Steroid hormones and steroid hormone binding globulins in cerebrospinal fluid studies in individuals with intact and with disturbed blood-cerebrospinal fluid barrier. Neuroendocrinology **55**:174–182, 1992.
25. Asaba H, Hosoya K, Takanaga J, Ohtsuki S, Tamua E, Takizawa T, Terasaki T. Blood-brain barrier is involved in the efflux of neuroactive steroid, dehydroepiandrosterone sulfate, via organic anion transporting polypeptide 2. J Neurochem **75**:1907–1916, 2000.
26. Kozak W, Kluger MJ, Wachulec M, Dokladny K. Role of cytochrome P-450 in endogenous antipyresis. Am J Physiol (Integr Comp Physiol) **279**:R455–R460, 2000.
27. Monda M, Viggiano A, Mondola P, De Luca V. Inhibition of prostaglandin synthesis reduces hyperthermic reactions induced by hypocretin/orexin A. Brain Res **909**:68–74, 2001.
28. Ricquier D, Bouillaud F. The uncoupling protein homologues; UCP1, UCP2, UCP3, StUCP and AtUCP. Biochem J **345**:161–179, 1999.
29. Szekely M, Balasko M, Kulchitsky VA, Simons CT, Ivanov AI, Romanovsky AA. Multiple neural mechanisms of fever. Auton Neurosci **85**:78–82, 2000.
30. Gordon FJ. Effect of nucleus tractus solitarius lesions on fever produced by interleukin-1β. Auton Neurosci **85**:102–110, 2000.
31. Cheng GF, Tseng J. Regulation of murine interleukin-10 production by dehydroepiandrosterone. J Interferon Res **20**:471–478, 2000.
32. Oberbeck R, Dahlweid M, Kock R, van Griensven M, Emmendorfer A, Tscherne H, Pape HC. Dehydroepiandrosterone decreases mortality rate and improves cellular immune function during polymicrobial sepsis. J Crit Care Med **29**:380–384, 2001.