Original Articles

Antihyperglycemic Effect of Dehydroepiandrosterone Analogue 16α-Fluoro-5-androsten-17-one in Diabetic Mice

LAURA L. PASHKO AND ARTHUR G. SCHWARTZ

The adrenocortical steroid, dehydroepiandrosterone, has been shown previously to produce an antidiabetic effect in C57BL/KsJ *db/db* mice. Preliminary clinical data suggest that this steroid may enhance insulin sensitivity in humans. The therapeutic use of dehydroepiandrosterone may be limited by its androgenic action. In a previous study, high-dose dehydroepiandrosterone therapy to postmenopausal women produced marked elevations in plasma testosterone (9-fold) and dihydrotestosterone (20-fold) levels. We previously developed the synthetic steroid, 16α-fluoro-5-androsten-17-one, which lacks the androgenic action of dehydroepiandrosterone yet has retained other biological activities of the native steroid. In this study, administration of 16α-fluoro-5-androsten-17-one in the diet (0.2 and 0.3%) to male C57BL/KsJ *db/db* mice markedly reduced plasma glucose levels. In contrast, treatment with dehydroepiandrosterone was effective in reducing plasma glucose levels at the 0.2% dose but had no effect at the 0.3% dose, possibly as a result of the androgenic state induced at the higher dose. Dehydroepiandrosterone treatment also produced a 25-fold elevation in plasma testosterone levels and a significant increase in seminal vesicle weights, whereas treatment with 16α-fluoro-5-androsten-17-one had no apparent effect on the weight of the seminal vesicle glands. *Diabetes* 42:1105–08, 1993

From the Fels Institute for Cancer Research and Molecular Biology, Temple University Medical School, Philadelphia, Pennsylvania.

Address correspondence and reprint requests to Dr. Arthur Schwartz, Fels Institute for Cancer Research and Molecular Biology, Temple University Medical School, 3420 North Broad Street, Philadelphia PA 19140.

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DHEA, dehydroepiandrosterone; HDL, high-density lipoprotein; 8354, 16α-fluoro-5-androsten-17-one; TLC, thin-layer chromatography, HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; MS, mass spectroscopy; IR, infrared; RIA, radioimmunoassay.

The steroid, DHEA, produces marked biological effects in laboratory animals, and evidence exists that this substance may be an important adrenocortical hormone (1–3). Administration of DHEA to experimental animals produces antiproliferative and cancer preventive (1), anti-obesity (4), antidiabetic (5), anti-atherosclerotic (6), and immunomodulating (7,8) effects. The therapeutic use of DHEA in humans, particularly in women, may be limited by the sex hormonal side effects of the steroid. DHEA is a biosynthetic precursor of testosterone and estrone and produces estrogenic and androgenic effects in laboratory rats (9,10). Administration of pharmacological doses of DHEA to 6 postmenopausal women for 28 days produced a marked elevation of plasma testosterone (9-fold) and dihydrotestosterone levels (20-fold), with only modest increases in plasma estrone and estradiol levels (2-fold; 11). DHEA treatment induced insulin resistance in these women and significantly lowered plasma HDL levels, very likely as a result of the androgenic state induced.

In addition to the sex hormonal side effects of DHEA, treatment of mice and rats with the steroid produces hepatomegaly with peroxisome proliferation (12,13), and long-term administration of DHEA to Fischer rats induces a high incidence of hepatocellular carcinoma (14).

We have developed the synthetic steroid, 8354, which does not demonstrate the androgenic, estrogenic, and liver-enlarging properties of DHEA, yet has retained the antiproliferative, cancer preventive, and anti-obesity actions of the native steroid (10,15,16). We report that 8354 is a very effective antidiabetic agent in insulin-resistant diabetic C57BL Ks/J *db/db* mice. Unlike DHEA, which significantly reduces hyperglycemia at low-dose administration but not at the high dose, possibly as a result of its dose-related androgenicity, 8354 is a very effective antihyperglycemic agent with marked efficacy at the highest dose.

RESEARCH DESIGN AND METHODS

Male C57BL/KsJ *db/db* mice were obtained from the Jackson Laboratory (Bar Harbor, ME) at 8 wk of age. The mice were housed 3 per cage in plastic shoe box cages on corncob bedding in the Fels Animal Facility at 72 ± 3°F with 12 h of alternating light and darkness. The mice had ad libitum access to Purina 5015 chow (Buckshire Feeds, Lansdale, PA) and acidified water.

Mice were weighed, earmarked, and after light anesthesia with methoxyflurane (Metofane), bled from the orbital sinus (between 1000 and 1200) for initial determination of plasma glucose levels. Glucose concentrations were determined using Sigma (St. Louis, MO) kit 510.

Five days later, mice were given Purina chow containing either 0.2% (wt/wt) DHEA, 0.3% DHEA, 0.2% 8354, or 0.3% 8354 or chow without drug. DHEA was obtained from Diosynth (Chicago, IL). Compound 8354 was synthesized as described previously (10). The purity of the steroid (mp 170–172°C) was confirmed by both TLC and HPLC. The identity of the compound was confirmed by elemental analysis, NMR, MS, and IR spectroscopy.

Preparation of food with steroid. A weighed amount of steroid was ground in a glass mortar using a glass pestle. A small amount of chow was added to the mortar, and the drug and chow were ground together. The contents of the mortar were added to the rest of the chow and mixed with an electric mixer for 10 min. Water was added (683 ml of water to 831 g of chow plus steroid) with mixing continued for another 5 min. The chow was kneaded and weighed into 48-g balls (containing 26 g of dry food). The chow balls were wrapped in aluminum foil and frozen at −20°C until use. Each day mice received a fresh allotment of food. Mice were weighed weekly, and food consumption, with correction made for food falling into the bedding, was done weekly as described previously (15).

Once a week, between 1000 and 1200, the mice were lightly anesthetized with methoxyflurane and bled from the orbital sinus for plasma glucose determinations.

Plasma testosterone determination and seminal vesicle weights. At the conclusion of the experiment (after 39 days of steroid treatment), plasma samples were taken from mice 4.5 h after animals began consuming food containing 0.2% or 0.3% DHEA. This was done because the plasma concentration of testosterone may vary with the time of DHEA intake. Food was removed from the mice at 1530 and replaced at 0800 the next morning. After the overnight fast, mice commenced eating chow during the morning hours. Mice were lightly anesthetized with methoxyflurane 4.5 h after receiving chow and bled from the orbital sinus. Mice from all the treatment groups were then killed by an overdose of pentobarbital sodium (Nembutal), and the seminal vesicles were excised, stripped free of the coagulating glands, compressed to expel the fluid, and weighed. Plasma samples were centrifuged at 2000 *g* for 20 min and frozen at −20°C. Plasma samples were sent 1 wk later to Endocrine Sciences (Calabasas, CA) for testosterone determinations by RIA.

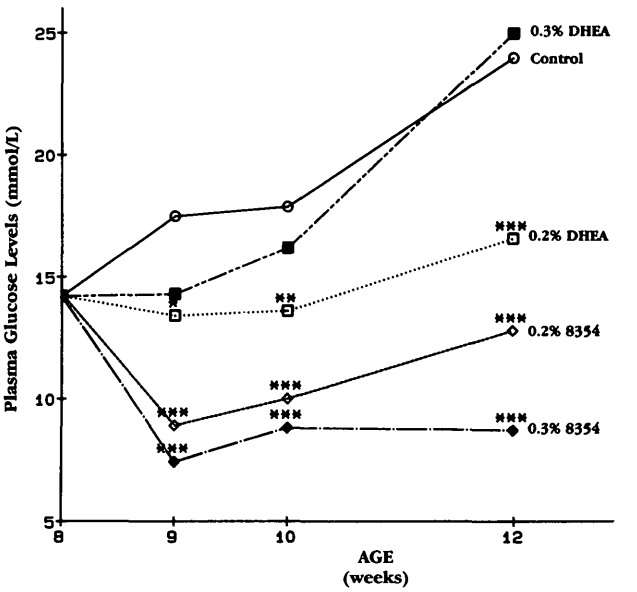

FIG. 1. Mean plasma glucose determinations (n = 6) of mice in different treatment groups. Significance was determined by Student's t test. *Significantly less than control, $P < 0.05$. **Significantly less than control, $P < 0.01$. ***Significantly less than control, $P < 0.001$.

RESULTS

As shown in Fig. 1, treatment of mice with 0.2% DHEA significantly reduced hyperglycemia, whereas treatment with 0.3% DHEA was without apparent effect. Treatment with 0.2% 8354 was significantly more effective than 0.2% DHEA at 9, 10, and 12 wk of age ($P < 0.05$).

No significant differences occurred in the mean body weights or mean food consumption of the various dosage groups over the 4-wk treatment period (Fig. 2).

Treatment with 0.2% or 0.3% DHEA for 39 days produced a dose-related increase in seminal weights of the mice, whereas treatment with 8354 had no apparent effect (Fig. 3). The mean plasma testosterone levels were

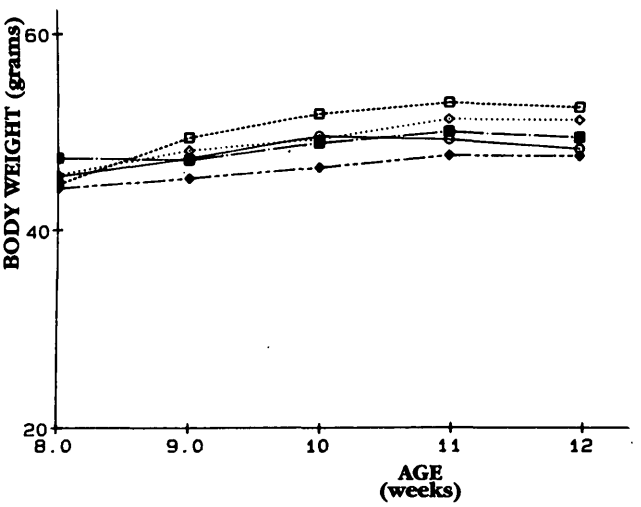

FIG. 2. Mean body weights of mice in different treatment groups. Data are means ± SD. Mean daily food consumption per mouse for mice (n = 3) in each group were: control (○), 5.1 ± 0.6; 0.2% DHEA (□), 5.5 ± 0.8; 0.3% DHEA (■), 5.7 ± 0.3; 0.2% 8354 (◇), 5.1 ± 0.6; and 0.3% 8354 (◆), 5.7 ± 0.8 g.

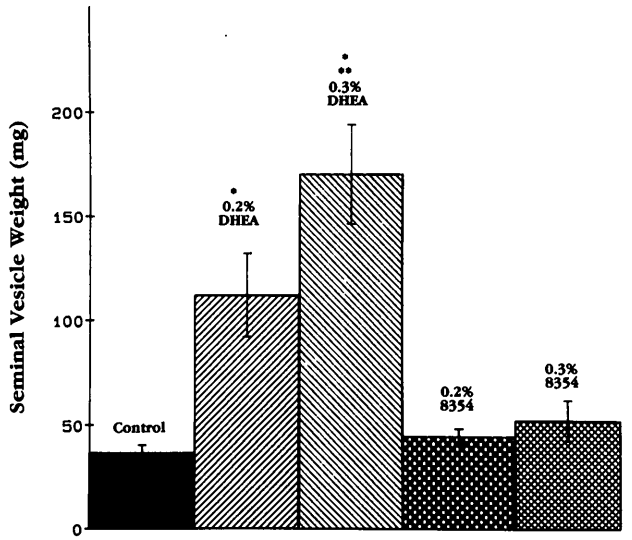

FIG. 3. Mean seminal vesicle weights of mice in various treatment groups. Data are means ± SD for 6 mice, except control (n = 2, average) and 0.3% DHEA (n = 5) groups. Significance was determined by Student's t test. *Significantly greater than control, $P < 0.01$. **Significantly greater than 0.2% DHEA, $P < 0.01$.

~25-fold higher in both DHEA treatment groups (Table 1).

DISCUSSION

The diabetes mutation produces obesity and a hyperinsulinemic insulin-resistant state that progresses to a severe diabetes syndrome when the mutation is placed on a susceptible inbred strain of mice (17). In C57BL/KsJ mice, the diabetes mutation produces obesity and transient hyperinsulinemia, followed by β-cell necrosis and atrophy. Treatment of these mice with oral hypoglycemic drugs was ineffective in controlling diabetes (17). Although insulin injections controlled the hyperglycemia in very young mice, once the plasma glucose levels exceeded 13.9 mM (250 mg/100 ml; 6–8 mo of age), injections of insulin ≤100 U/100 g were ineffective in reducing blood glucose levels to normal (17).

Coleman et al. (5) reported that DHEA administration (0.4% of diet) to C57BL/KsJ *db/db* mice produced a rapid remission of hyperglycemia, a preservation of β-cell structure and function, and an increased insulin sensitivity as measured by glucose tolerance tests. In these studies, we found that 0.2% DHEA administration significantly reduced hyperglycemia, whereas 0.3% DHEA was ineffective. DHEA treatment was highly androgenic, producing a 25-fold increase in plasma testosterone levels and a dose-related increase in seminal vesicle weights.

Based on their studies of patients with polycystic ovarian syndrome and hypertestosteronemia, as well as individuals with adrenal hyperplasia and high plasma DHEA levels, Buffington et al. (18) concluded that DHEA and testosterone have opposing actions on insulin sensitivity: testosterone levels correlated positively with various parameters of insulin resistance, whereas DHEA levels correlated positively with insulin sensitivity. Others (19,20) have also reported that androgens enhance insulin resistance. Thus it is possible that the androgenic state produced by the 0.3% DHEA dose overcame the antihyperglycemic effect of the DHEA. In contrast, 8354 produced no significant increase in seminal vesicle weight at either dose and at the higher dose was extremely effective in controlling the hyperglycemia.

Pourmotubbed et al. (21) reported that oral administration of DHEA (at a dose producing little change in plasma testosterone levels) to an obese, 17-yr-old female increased insulin sensitivity. High-dose DHEA therapy to 6 postmenopausal women, on the contrary, enhanced insulin resistance (11). The results reported herein with 8354 suggest that this steroid may have value in the control of hyperglycemia without the complicating side effects of DHEA.

TABLE 1
Plasma testosterone levels

Treatment group	n	Plasma testosterone (nM)
Control	4	0.4 ± 0.4
0.2% DHEA	6	10.7 ± 1.5*
0.3% DHEA	5	11.3 ± 3.5*

Data are means ± SD.
*Significantly greater than control, $P < 0.001$ by Student's t test.

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