Rapid Publications

Therapeutic Effects of Dehydroepiandrosterone (DHEA) in Diabetic Mice

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SUMMARY

Dehydroepiandrosterone (DHEA), a major adrenal secretory steroid in humans, was therapeutic when fed in a concentration of 0.4% to C57BL/KsJ mice with either non-insulin-dependent or insulin-dependent diabetes. Genetically diabetic (*db/db*) mice of both sexes develop obesity and a glucose intolerance and hyperglycemia associated with insulin resistance by 2 mo of age, and exhibit beta-cell necrosis and islet atrophy by 4 mo. In contrast, DHEA feeding initiated between 1 and 4 mo of age, while only moderately effective in preventing obesity, did prevent the other pathogenic changes and effected a rapid remission of hyperglycemia, a preservation of beta-cell structure and function, and an increased insulin sensitivity as measured by glucose tolerance tests. DHEA feeding was also therapeutic to normal C57BL/KsJ male mice made diabetic by multiple low doses of streptozotocin (SZ). While DHEA treatments did not block either the direct cytotoxic action of SZ on beta-cells or the development of insulitis, the steroid significantly moderated the severity of the ensuing diabetes (reduced hyperglycemia and water consumption, and increased plasma insulin and numbers of residual, granulated beta-cells). DIABETES *31*:830–833, September 1982.

Dehydroepiandrosterone (DHEA, androst-5-ene-3-β-ol-17-one) and its sulfate derivative are major adrenal secretory products in humans, yet no biologic function of this steroid is known. Decreased secretion of DHEA is associated with advancing age in both sexes and with breast cancer in women. Biologic effects of DHEA in mice are related to its effects on obesity, tumor development, aging, and immune function.[1–3] The effects of DHEA were not associated with decreased food intake.[1] It has long been known that caloric restriction of otherwise adequate diets in rodents has an ameliorative effect on most age-associated renal, cardiovascular, and neoplastic lesions,[4,5] and can significantly extend lifespan.[6] These results suggest that chronic treatment with DHEA has many of the effects of caloric restriction without actually decreasing the amount of food eaten.

This report deals with the therapeutic effects of DHEA on both a genetic and chemically-induced model of diabetes in the mouse. The mutation, diabetes (*db*), produces obesity and a hyperinsulinemic, insulin-resistant state that progresses to a severe diabetes syndrome only when the mutation is placed on a susceptible inbred strain.[7] On the C57BL/KsJ (BL/Ks) background, diabetes is characterized by obesity and transient hyperinsulinemia, followed by beta-cell necrosis and atrophy. The sequence of events observed are predictable and culminate in premature death between 6–8 mo. The diabetes is more severe and develops more rapidly in males and can be improved or circumvented by combined estradiol and progesterone treatment.[8] Feeding severely restricted amounts of a complete diet, or ad libitum amounts of a carbohydrate-free diet has little effect on the development of obesity but inhibits the development of the severe diabetes.[8–11] Normal (+/+) BL/Ks males are susceptible to induction of severe, insulin-dependent diabetes following administration of multiple low doses of streptozotocin (SZ). These SZ diabetic mice provide an insulinopenic model to compare with the insulin-resistant genetic model.

MATERIALS AND METHODS

Diabetes (*db/db*) and normal control (+/+) mice of the BL/Ks strain were obtained from our research colony. Mice of both sexes were selected by age to obtain three groups of diabetes mice in different stages of development. Typically, mutants 1 mo of age had mild diabetes (with normal blood sugar concentrations 120–160 mg/dl and hyperinsulinemia), those 2 mo of age had moderate diabetes (blood sugar 200–300 mg/dl with marked hyperinsulinemia), and those mice 3–4 mo of age with severe, terminal diabetes (blood sugar > 350 mg/dl, with more moderate and decreasing concentrations of insulin, associated with almost

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total insulin resistance). The characteristic morphologic changes associated with the developing syndrome have been described.[7,8,10] Mice at each stage of the diabetes condition were divided into two groups, the control group fed powdered chow (Old Guilford 96), and the experimental group fed identical chow supplemented with 0.4% DHEA. The powdered diets were fed in food cups that were filled every 2 days. To more accurately determine daily food consumption, powdered chow with or without DHEA was repelleted in a press (5000 pounds/sq in.) and weighed amounts of these pellets were given. The amount of food eaten was determined by weighing the amount of food remaining each day. The ameliorative effects of DHEA were exhibited whether it was fed in powdered or pelleted chow.

An insulinopenic type of diabetes was produced in groups of normal BL/Ks mice by treatment with 5 subdiabetogenic doses of SZ (40 mg/kg body wt).[12] Two groups of 8 mice, one 8 wk of age and one 6 wk of age, were placed on the DHEA diet (0.4%) for 2 wk prior to streptozotocin treatment to assess any potential effects in preventing either the hyperglycemia or the insulitis associated with the development of SZ diabetes in this strain. The younger group was killed on days 10, 12, and 14 after initiation of SZ treatment to assess insulitis development, while the older group was studied for 10 wk to assess the rate of development of hyperglycemia. Another group of 18 male BL/Ks mice 8 wk of age was also treated with multiple doses of SZ. Twelve mice of this group were placed on the DHEA diet at the time of first injection. The other six mice remained on chow and the development of diabetes was compared over the next 10 wk.

Mice were weighed weekly at the time of blood sugar determination. Plasma insulin concentrations and glucose tolerance tests (1.5 g glucose/100 g body wt i.p.) undertaken on fed mice were carried out periodically during the course of the treatment immediately prior to the termination of each experiment. At killing, one-half of the pancreas was fixed in Bouin's solution for histologic examination and the other half was homogenized in acid-ethanol (1.5% concentration HCl in 70% ethanol) to determine pancreatic insulin content. Blood glucose and immunoreactive plasma insulin were determined as previously described.[7]

RESULTS

Diabetes (*db/db*) mice treated with DHEA gained weight in the early weeks of study at a rate somewhat less than mutants fed chow. At 3–4 mo, weight gain slowed in chow-fed mice while treated mice continued to gain weight. The final body weights (45–50 g) attained in treated and untreated mutants were the same, although the rate of weight gain in treated mutants was slower. Daily food intake measured over a period of 2 wk in a group of 5 treated and untreated mice was increased in DHEA-treated mutants (6.21 ± 0.19 versus 4.19 ± 0.23 g for chow-fed mutants). Figure 1 shows the changes in blood sugar concentration that occurred during a period of up to 18 wk in three groups of DHEA-treated mutants. When treatment was initiated in either the mild or the moderate stage of diabetes, blood sugar concentrations normalized within 1 wk of treatment. Older mutants (eight females, 4 mo of age), selected because of advanced diabetes, were unable to respond as well. Two of this group with blood sugar concentrations above 400 mg/dl continued to lose weight and died within 2 wk. The other six female mice with blood sugar concentrations less than 390 mg/dl survived and their blood sugar concentration returned to near normal after 2 wk of treatment (Figure 1). In contrast to the groups of male and female mice that were placed on a diet containing DHEA before severe diabetes developed, there was some tendency toward hyperglycemia in a few of these surviving mutants which caused the periodic fluctuations in blood sugar concentrations seen in this group of older female mice (Figure 1).

Glucose tolerance tests undertaken in *db/db* mice at various times after initiation of DHEA treatment showed that glucose intolerance was not nearly as severe and did not deteriorate with age in treated mutants compared to those fed control diet alone. Plasma insulin concentrations of untreated *db/db* mice typically rise rapidly to 5–10 times normal in the first 3 mo of life at which time a brief plateau is observed followed by decreases in insulin concentrations to

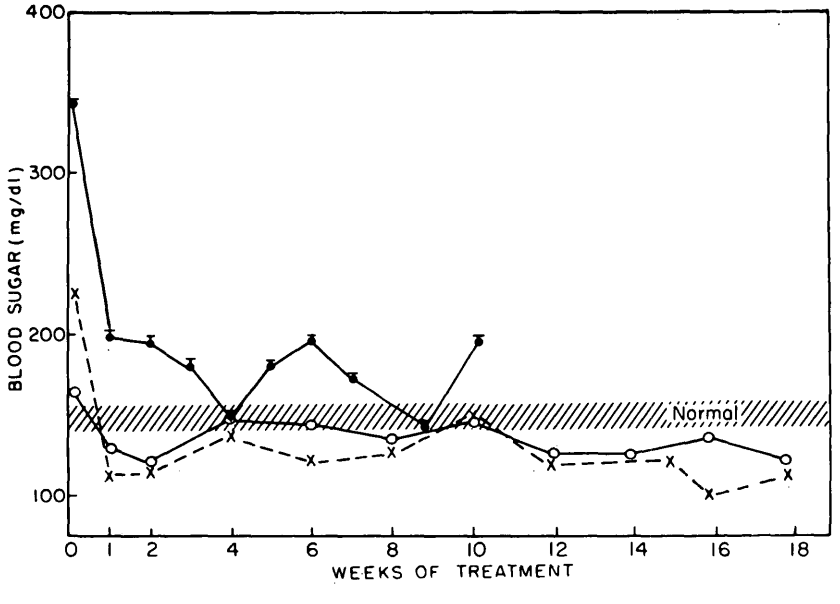

FIGURE 1. The effect of DHEA (0.4%) in the diet on blood sugar concentrations in BL/Ks (*db/db*) diabetes mice. Age at start; 1 mo for a group of 8 females (○—○), 2 mo for a group of 10 males (×---×), and 4 mo for a group of 8 females (●—●).

TABLE 1
Effect of DHEA on plasma and pancreatic insulin concentrations in C57BL/Ks normal and diabetes (*db/db*) mice

Diet	Genotype and sex	Plasma insulin (μU/ml) at 2 mo	Plasma insulin (μU/ml) at 5 mo	Pancreatic insulin (U/g wet weight) at death (5 mo)
Chow	+/+ M	66.5 ± 2.0 (6)*		0.625 ± 0.25 (7)
	db/db M	291 ± 4.5 (7)	98.0 ± 1.9 (6)	0.116 ± 0.18 (3)
	db/db F	456 ± 7.0 (6)	92.0 ± 3.0 (5)	0.687 ± 0.18 (5)†
DHEA	+/+ M	18.5 ± 1.6 (3)		0.695 ± 0.16 (5)
	db/db M	542 ± 9.7 (5)	420 ± 5.0 (13)	0.655 ± 0.29 (6)
	db/db F	330 ± 6.1 (6)	1039 ± 8.5 (11)	2.07 ± 0.038 (7)

* Numbers of animals per group studied are in parentheses.
† Typical values observed in mutant females can range from 0.1 to 1.0 U per g wet weight depending on the severity of the diabetes.

values only slightly higher than that in normal mice.[7] This decrease in plasma insulin occurs in association with characteristic degenerative changes of the beta-cells. Islet atrophy is a consistent feature of untreated BL/Ks mutants by 4 mo of age. Plasma insulin concentrations in DHEA-treated diabetes mice remained high during the entire treatment period of 5 mo (Table 1) and were always associated with normal blood sugar concentrations whereas similar concentrations of insulin in untreated mutants are typically associated with severe hyperglycemia.[7,11] Hyperglycemia in spite of hyperinsulinemia is a consistent feature of older mutants and is attributed to the extreme insulin resistance associated with the obesity.

Histologic examination of the pancreases of treated mice revealed no signs of islet cell atrophy. Instead, islets were increased in number and size and showed signs of the hyperactivity usually associated with younger mutants. Some degranulation of beta-cells was evident, but this was not severe. Pancreatic insulin content remained much higher than in untreated mice of this age (Table 1) and although not quantified, increased numbers of mitotic figures were observed.

Mice treated with SZ and fed DHEA, regardless of whether DHEA treatment preceded or was simultaneous with SZ treatment, exhibited the characteristic development of diabetes in the immediate post-treatment period (2–3 wk). The group of young mice, pretreated with DHEA and killed at days 10–14, all had severe insulitis. The developmental pattern of diabetes seen in the group of 18 male mice treated with DHEA simultaneous with the first SZ injection is shown in Figure 2. After 2–3 wk on a DHEA diet, the blood sugar concentrations of treated SZ-diabetic mice diverged from those fed chow alone. Although DHEA-treated mice remained hyperglycemic, their blood sugar concentrations were significantly less than those of untreated mice and remained so during the entire treatment period. Associated with the lower blood sugar concentration was a significant decrease in water consumption in those diabetic mice treated with DHEA. All diabetic mice were hyperphagic and food consumption was increased still further in mice treated with DHEA (8.41 ± 0.37 vs 6.33 ± 0.32 g in chow fed mice). After 6 wk of treatment, 6 of 12 SZ-diabetic mice were taken off the diet and fed chow alone. Within 2 wk the blood sugar concentrations of these mice became significantly elevated over the values observed in mice that remained on treatment. Plasma insulin concentrations deter-

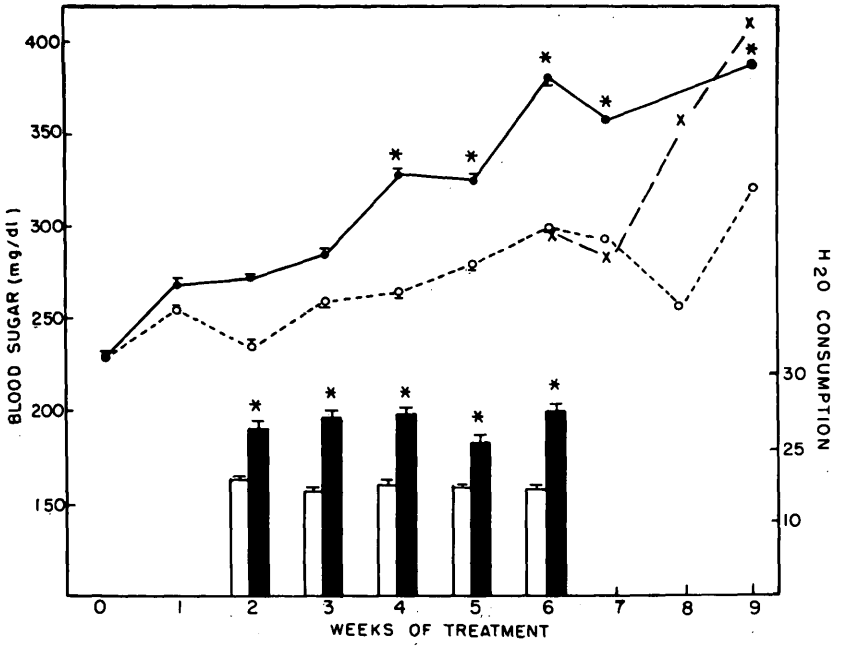

FIGURE 2. Effect of treatment with DHEA on blood sugar concentrations and water consumption (bar graph) in 18 mice made diabetic with streptozotocin. *, values significantly different from treated mice ($P < 0.01$, Student's t test). Circles and open bars represent data from 12 mice treated with DHEA. ×, mice (N = 6) previously fed diet containing DHEA for 6 wk and then refed chow.

mined after 4 and 8 wk on diabetes mice fed DHEA diets were higher than those of mice fed chow alone (17.3 ± 0.6 vs 11.5 ± 0.5 μU/ml). Histologic examination of pancreases of chow-fed diabetics revealed very few recognizable islets and even fewer granulated beta-cells. In contrast, sections of pancreas from DHEA-fed mice had some small islets with a few granulated beta-cells. The pancreatic insulin content in the SZ-treated diabetes mice (32.6 ± 1.1 and 24.9 ± 0.8 mU/g wet weight for DHEA-fed vs chow-fed animals, respectively) was greatly reduced from that of normal mice (Table 1) and is consistent with observed plasma insulin values.

DISCUSSION

Treatment of genetic diabetes with a standard mouse chow supplemented with 0.4% DHEA was very effective in lowering blood sugar concentrations to normal. This effect was observed if treatment was initiated at any time before the terminal stage of diabetes (blood sugar > 400 mg/dl). No sex differences were noted in the degree of improvement of the diabetes.

Histologic examination of the pancreases of DHEA-treated diabetes mice revealed no signs of the atrophy typical of older untreated mutants. The islets exhibited enlarged sinusoids and had other indications of hyperactivity. This observation suggests that DHEA feeding produces some beneficial effect that protects the islets from a stress that normally leads to beta-cell atrophy and degeneration. The association of high plasma insulin concentrations in DHEA-treated diabetics with normal blood sugar concentrations suggests that DHEA may be increasing sensitivity to insulin. An increase in insulin sensitivity would lower the demand for insulin and reduce or eliminate the chronically hyperglycemic state, thus allowing the hyperactive beta-cells to regranulate and otherwise avoid degenerative changes. It is also possible that DHEA encourages islet proliferation of regenerating or newly developing islets.

The studies with DHEA-treated SZ-diabetes mice show that dietary DHEA is not capable of blocking the insulitis associated with SZ treatment, although DHEA treatment produced some immunosuppression in normal mice as measured by the Jerne plaque assay (E. H. Leiter, unpublished observation). The increased plasma insulin concentration in DHEA-treated diabetics (although less than normal) coupled with a moderate decrease in blood sugar concentration suggests some potentiating effect of DHEA on the residual insulin left after SZ treatment. The increased insulin content and histologic appearance of pancreases from DHEA-fed mice suggest that DHEA might enhance survival and possibly replication of residual beta-cells. On the other hand, those mice removed from treatment redeveloped the severe diabetes, suggesting that any stimulation of residual islets or increased insulin sensitivity is dependent on the continued presence of DHEA.

DHEA, a precursor to both testosterone and estrogens, could establish a hormonal imbalance that might be responsible for its beneficial effects. Female BL/Ks mice have less severe diabetes whether it is induced by genetic means or chemically by SZ.[8,13] Our studies showing that estrogen treatment in some strains of diabetes mice produces a less severe response similar to that seen with DHEA treatment suggest that DHEA may have its effect via estrogens.[8] The previously observed beneficial effects of androgens fed in the diet to diabetic mutants were associated with decreased food consumption.[14] Decreased food consumption was not seen in these or in other studies feeding DHEA. This again suggests that estrogens, rather than androgens, may be responsible for the therapeutic effect of DHEA in diabetes. Regardless of the mechanism of action, more study is warranted of the potential therapeutic value of DHEA in treatment of various types of diabetes.

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