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# Alternative mechanism for anti-obesity effect of dehydroepiandrosterone: Possible contribution of 11β-hydroxysteroid dehydrogenase type 1 inhibition in rodent adipose tissue

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ABSTRACT

Dehydroepiandrosterone (DHEA) has been suggested to have an anti-obesity effect; however, the mechanism underlying this effect remains unclear. The effect of DHEA on adipocytes opposes that of glucocorticoids, which potentiate adipogenesis. The key to the intracellular activation of glucocorticoids in adipocytes is 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which catalyses the production of active glucocorticoids (cortisol in humans and corticosterone in rodents) from an inactive 11-keto form (cortisone in humans and 11-dehydrocorticosterone in rodents). In humans and rodents, intracellular glucocorticoid reactivation is exaggerated in obese adipose tissue. Using differentiated 3T3-L1 adipocytes, we demonstrated that DHEA inhibited about 15.6% of 11β-HSD1 activity at a concentration of 1 μM within 10 min. Inhibition was also observed in a cell-free system composed of microsomes prepared from rat adipose tissue and NADPH, a coenzyme of 11β-HSD1. A kinetic study revealed that DHEA acted as a non-competitive inhibitor of 11β-HSD1. Moreover, conversion from DHEA to estrogens was not observed by sensitive semi-micro HPLC equipped with electrochemical detector. These results indicate that the inhibition of 11β-HSD1 by DHEA depends on neither the transcriptional pathway nor the non-specific manner. This is the first demonstration that the anti-obesity effect of DHEA is exerted by non-transcriptional inhibition of 11β-HSD1 in rodent adipocytes.

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## 1. Introduction

Glucocorticoid influences a wide variety of physiologic functions, including glucose and lipid metabolism, immune, and anti-inflammatory responses. It also plays a pivotal role in the local regulation of adipose tissue function, development and distribution. It has been demonstrated that chronically elevated glucocorticoid levels, especially in adipose tissues, cause visceral obesity, diabetes, hyperlipidemia and hypertension, as seen in patients with Cushing's syndrome. Recently, evidence has accumulated that intracellular glucocorticoid amplification in adipocytes by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) contributes to central obesity and as a consequence promotes metabolic diseases [1–4]. 11β-HSD1, which is abundantly expressed in adipose tissue and the liver, reactivates cortisol from cortisone in humans (corticosterone from 11-dehydrocorticosterone in rodents) [5].

Dehydroepiandrosterone (DHEA) is a steroid hormone derived from cholesterol by the adrenal glands. In humans, DHEA is largely secreted as the sulfate ester, DHEAS, which is the most abundant steroid hormone circulating at micromolar levels. Serum concentrations of DHEA and its sulfate show peak levels between 20 and 30 years of age, followed by a steady, age-dependent decline [6]. Supplementation of DHEA has attracted widespread attention owing its beneficial effects on age-related diseases. Further, a variety of epidemiological studies and clinical trials in humans have demonstrated that the decline in serum levels of DHEA and DHEAS inversely correlated with the development of a series of metabolic diseases, such as obesity [7], diabetes [8], hyperlipidemia and atherosclerosis [9], and cardiovascular disease [10,11]. In rodents, a number of studies have also shown that DHEA reduced body weight [12], visceral fat [13], and improved glucose [14] and insulin levels [15]. Thus, DHEA has been suggested to have an anti-obesity effect; however, the mechanism underlying this effect remains unclear.

DHEA has also been reported to inhibit adipogenesis [16] and the proliferation of 3T3-L1 adipocytes [17], whereas glucocorticoids induce adipocyte differentiation. Apostolova et al. [18] demonstrated that DHEA inhibited the expression of 11β-HSD1 in adipocytes through CCAAT/enhancer-binding protein α (C/EBPα), which has been shown to be a potent transcriptional activator of 11β-HSD1 [19].

*Abbreviations:* DHEA, dehydoepiandrosterone; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; $K_m$, Michaelis constant; $K_i$, inhibition constant.

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In the present study, we demonstrated for the first time that DHEA acts as a non-competitive inhibitor of 11β-HSD1 and that inhibition occurred on the non-transcriptional pathway in rodent adipocytes.

## 2. Materials and methods

### 2.1. Animals

Male Wistar rats (7 weeks old) were obtained from SLC Co. Ltd. (Shizuoka, Japan). They were housed under a controlled 12-h/12-h light dark cycle (lights on from 7:00 a.m.) with room temperature of 23 ± 1 °C and humidity of 55 ± 5%, and fed ad libitum. After an acclimation period of 1 week, the animals were killed by bleeding from the abdominal aorta under light ether anesthesia. The experimental procedures were approved by the Kobe Pharmaceutical University Animal Care and Use Committee.

### 2.2. HPLC

To measure 11β-HSD1 activity (A) and to examine the conversion of DHEA to estradiol and other metabolites (B), steroids in the extracts of incubation medium were analyzed using HPLC (NANOSPACE SI-1; Shiseido Co. Ltd., Tokyo) equipped with a Capcellpak UG 120, 3 μm, 75 mm × 2.0 mm I.D. column (Shiseido), a UV detector and an electrochemical detector (ECD) according to the methods described elsewhere with minor modification [20,21]. The flow rate was 0.1 ml/min, and the column temperature was 40 °C. The extracts were dissolved in 200 or 400 μl mobile phase solvent and 10 μl of the sample was applied to HPLC.

(A) The column was eluted with mobile phase solvent ($H_2O$:methanol = 45:55 v/v%) for 12 min, and 11-dehydrocorticosterone and corticosterone were monitored by absorbance at 240 nm. Under these conditions, 11-dehydrocorticosterone and corticosterone appeared at 5.45 ± 0.01 min (mean ± SD, $n = 5$) and 8.47 ± 0.01 min ($n = 5$), respectively.

(B) The column was eluted with mobile phase solvent (33 mM phosphate buffer (pH 7.4):methanol:acetonitrile = 40:55:5 v/v%). DHEA and 3β-hydroxy-$\Delta^5$ steroids were determined by absorbance at 210 nm, and 3-keto-$\Delta^4$ steroids were measured by absorbance at 240 nm. Estradiol and its analogues with a phenolic hydroxyl group were detected by ECD, the electrode potential of which was held at 650 mV vs. Ag/AgCl reference electrode. Under these conditions, DHEA and estradiol appeared at 15.60 ± 0.04 min ($n = 5$) and 14.40 ± 0.08 min ($n = 4$), respectively.

### 2.3. Culture and differentiation of 3T3-L1 cells and microsome preparation

Differentiated 3T3-L1 adipocytes and microsomes from mesenteric fat pads and the liver of 8-week-old male Wistar rats were prepared according to the method described elsewhere [20,22].

### 2.4. Measurement of 11β-HSD1 activity

11β-HSD1 activity (11-oxoreductase) was measured using adipocytes from 3T3-L1 (a) and microsomes from rat mesenteric fat depots and liver (b) according to the method described previously [20].

(a) 3T3-L1 adipocytes were carefully washed in PBS (−) buffer and then preincubated in DMEM at 37 °C for 5 min. Immediately before starting the reaction, DMEM was changed to medium containing 1 μM 11-dehydrocorticosterone (Sigma) with or without DHEA (Sigma). Incubation was carried out in six-well plates at 37 °C for 10 min in a humidified atmosphere in the presence of 10% $CO_2$. The final concentration of dimethylsulfoxide (DMSO) solution, which dissolved the steroids, was below 0.05–0.1% in the incubation medium. The medium (2 ml) was extracted with dichloromethane (2.2 ml, twice). The organic solvent was collected and evaporated *in vacuo*. Then, the residue was reconstituted with 400 μl HPLC mobile phase solution ($H_2O$:methanol = 45:55 v/v%). The concentration of 11-dehydrocorticosterone and corticosterone in the sample was measured using HPLC under the conditions (A) described above. To validate the above assay, it was necessary to establish that steroid formation was proportional to both the incubation time and protein concentrations. The appearance of corticosterone was linear for a period of 10 min in the six-well plate and was proportional to the protein concentration (data not shown).

(b) 11β-HSD1 activity in microsomes from rat mesenteric fat depots or liver was determined in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (100 mM KCl, 20 mM NaCl and 20 mM MOPS, pH 7.4) containing 1 mM NADPH (Oriental Yeast, Tokyo), and 11-dehydrocorticosterone (1 μM) with and without DHEA. The reaction was started by the addition of 10 μl microsomes (protein concentration was diluted to 0.84 mg/ml (mesenteric fat) and 0.24 mg/ml (liver) with MOPS buffer) and the reactants were incubated at 37 °C for 40 min (mesenteric fat) or 20 min (liver). Steroid concentrations were measured by HPLC under condition (A). Under these conditions, the appearance of corticosterone was linear for 1 h (data not shown).

### 2.5. Kinetic assay for 11β-HSD1

Enzyme reactions were carried out in 1 ml MOPS buffer containing 1 mM NADPH and 0.25–4 μM 11-dehydrocorticosterone at 37 °C for 40 min (mesenteric fat) or 20 min (liver). If necessary, 1–25 μM DHEA was added to the buffer. The reaction was initiated by the addition of microsomes from rat mesenteric fat depots or liver. Steroid concentrations were measured by HPLC as above. Linearity of enzyme activity vs. time and protein concentration was confirmed. Michaelis constant ($K_m$) and inhibition constant ($K_i$) value estimations were averaged from Lineweaver–Burk plots and Dixon plots, respectively with triplicate samples.

### 2.6. Statistical analysis

Data are expressed as the mean ± SEM. Statistical analysis was performed using Dunnett's test for multiple comparisons. Differences between two groups were statistically compared by the two sample *t*-test. *P* values below 0.05 were considered significant.

## 3. Results

### 3.1. Effect of DHEA on 11β-HSD1 activity in 3T3-L1 adipocytes

To investigate the effect of DHEA on 11β-HSD1 activity, differentiated 3T3-L1 adipocytes were incubated in DMEM containing 11-dehydrocorticosterone with and without DHEA at 37 °C for 10 min. The suppression of 11β-HSD1 activity by DHEA (1–50 μM) occurred in a dose-dependent manner (Fig. 1A). In these 3T3-L1 adipocytes, we were not able to measure any conversion of corticosterone to 11-dehydrocorticosterone even after extended (8 h) incubation with the substrate, indicating that 11β-HSD1

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