Dehydroepiandrosterone treatment attenuates oestrogen-induced pituitary hyperplasia

C Suárez, I García Tornadú, W Khalil[1] and D Becu-Villalobos

Instituto de Biología y Medicina Experimental, CONICET V, Obligado 2490 (1428) Buenos Aires, Argentina

[1]The Lawson Research Institute, London, Ontario, Canada

(Requests for offprints should be addressed to D Becu-Villalobos; Email: dbecu@dna.uba.ar)

Abstract

The physiological importance of and therapeutic interest in dehydroepiandrosterone (DHEA) has been predominantly in relation to its action as an inhibitor of the promotion and progression of several kinds of tumours, including those of breast, prostate, lung, colon, liver and skin tissues. The aim of the present study was to determine the role of DHEA in diethylstilboestrol (DES)-induced pituitary hyperplasia. Female Sprague–Dawley rats were divided into four treatment groups: DES (implanted s.c. with a 20 mg DES pellet), DHEA (two 50 mg DHEA pellets), DHEA/DES (both DHEA and DES pellets), and controls (not implanted). Every week, all rats were weighed and cycled, and jugular blood samples were obtained. After 7 weeks, rats were killed. Hypophyses were removed and weighed, and serum prolactin, GH, IGF-I and leptin levels were assayed by RIA. DHEA cotreatment reduced pituitary enlargement by 39% in DES-treated rats. It also reduced the hyperprolactinaemia (280·4 ± 43·6 ng/ml for DHEA/DES vs 823·5 ± 127·1 ng/ml for DES) and partially reversed the loss of body weight induced by DES. DHEA treatment did not modify the effects of DES on serum GH, IGF-I and leptin levels. But DHEA *per se* also increased pituitary weight and induced hyperprolactinaemia, although to a lesser degree than DES. We conclude that DHEA administration has beneficial effects on oestrogen-induced pituitary hyperplasia and hyperprolactinaemia, but the fact that DHEA *per se* also induces diverse hormonal effects and a slight pituitary enlargement limits its use as a possible therapeutic drug.

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Introduction

Dehydroepiandrosterone (DHEA) and DHEA-sulphate (DHEA-S), synthesised by the adrenal gland, are the most abundant steroids circulating in the human blood. The normal range of concentrations between people is very wide, even in a homogeneous population with regard to sex and age. In rats, plasma DHEA concentrations are lower, but this animal model has been used to study the effects of DHEA treatment on various different disorders (for review see Svec & Porter 1998, Hinson & Raven 1999). Recently, there has been a strong resurgence of interest in DHEA because of its suggested antitumoural and anti-ageing effects, even though its biological action is still controversial and not clearly defined.

DHEA, synthesised from pregnenolone by the cytochrome P450-C17, can be converted to androstenedione by the 3β-hydroxysteroid dehydrogenase (3β-HSD), and later to testosterone by the 17β-HSD. Both androgens can be converted to oestrogens by an aromatase. In this way, DHEA is a precursor not only of androgens but also of oestrogens, and furthermore, it is supposed to act on both oestrogenic and androgenic receptors. DHEA can be metabolised in many tissues, mainly to 7α-hydroxy DHEA and to 5-α-androstene-3β,17β-diol (ADIOL) which can also be α-hydroxylated (Rose *et al.* 2001). Introduction of a 7α-hydroxyl group alters the property of both steroids to compete with oestradiol for the oestrogen receptor (Li *et al.* 1978).

When administered in pharmacological doses this adrenal steroid can inhibit experimental carcinogenesis in a variety of target tissues, including mammary gland, skin, prostate, lung, liver and colon (Schwartz & Tannen 1981, Nyce *et al.* 1984, Pashko *et al.* 1985, Schwartz *et al.* 1989, Gatto *et al.* 1998, Rao *et al.* 1999). It has also been described as antioxidant, protective against cardiovascular disorders (Jarrar *et al.* 2000), diabetes mellitus (Ladriere *et al.* 1997), obesity (Shepherd & Cleary 1984, Gansler *et al.* 1985), osteoporosis (Labrie *et al.* 1997) and stress (Hu *et al.* 2000). DHEA was even proposed as an inhibitor of the replication and reactivation of HIV-1 (Yang *et al.* 1994). In the central nervous system, it has been reported to have beneficial effects on processes like memory, learning (Frye & Lacey 1999, Wolf & Kirschbaum 1999)

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and sleep (Schiffelholz *et al.* 2000). DHEA would also be an antidepressive and an inducer of an inner feeling of general welfare (Arlt *et al.* 2000).

DHEA concentrations were found to be significantly decreased in many pathological conditions, including various cancers, inflammatory diseases, type II diabetes mellitus and cardiovascular disorders (Kroboth *et al.* 1999). Therefore, there have been several studies focusing on the effects of DHEA administration both in animals and in humans in order to determine whether DHEA could be useful in the treatment of different disorders. But to date there is little consensus on the benefits of DHEA replacement therapy (Nippoldt & Nair 1998).

Even though DHEA appears to serve as an inhibitor of cell growth and proliferation *in vivo*, its hormonal activity and other side-effects may limit its clinical utilisation in chemoprevention. To this respect it has been described that a prolonged treatment with DHEA induces liver tumours (Rao *et al.* 1992), and that it stimulates proliferation of some oestrogen-sensitive mammary cancer cell lines (Maggiolini *et al.* 1999). Besides, DHEA can modify serum testosterone, oestrogen and prolactin levels.

On the basis of its broad range of suggested anticarcinogenic activity in animal model systems, and of data suggesting antiproliferative activity in transformed cells, DHEA is a candidate for evaluation as an inhibitor of pituitary hyperplasia. Chronic administration of oestrogens to rats induces enlargement of the anterior pituitary and increases the synthesis and secretion of prolactin (Diaz-Torga *et al.* 1998). Histologically tumours are composed of hyperplastic and hypertrophied lactotrophs, with involution of somatotrophs and gonadotrophs (De Nicola *et al.* 1978). Damage to hypothalamic dopaminergic neurons in response to oestrogen (Sarkar *et al.* 1982) and a direct action of oestrogen at the pituitary level have also been suggested. Both bromocriptine (Gonzalez Iglesias *et al.* 2000) and progesterone (Piroli *et al.* 1996) can attenuate pituitary tumour formation, but there is no evidence of the effect of DHEA. We therefore evaluated the effect of a chronic DHEA treatment on the development of pituitary hyperplasia induced by oestrogen. We also evaluated its hormonal effects by measuring serum prolactin, growth hormone (GH), insulin-like growth factor-I (IGF-I) and leptin levels during the treatment.

Materials and Methods

Animals

Female 60-day-old Sprague–Dawley rats were housed in an air-conditioned room with lights on at 0700 h and off at 1900 h. Rats were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Rats were divided into four treatment groups (each of six animals). Pituitary tumours were induced by s.c. implantation of a 20 mg pellet of diethylstilboestrol (DES) (Sigma) (DES group) for 7 weeks, in two groups. One of these groups was simultaneously implanted s.c. with two DHEA (Sigma) pellets (50 mg each) (DHEA+DES group). A third group of rats was implanted with two s.c. pellets of 50 mg DHEA (DHEA group). A fourth group was not implanted (control group: CON). Steroids were administered in a form that would provide a continuous and steady supply of each drug. Earlier studies demonstrated that large bolus treatments of DHEA cause cytotoxicity, androgenicity and insulin resistance, so the present slow-release implant is a more physiological approach (McIntosh *et al.* 1999). The experiment was repeated three times.

Rats were weighed before implantation of pellets and once a week until the seventh week. Vaginal smears were obtained for ten consecutive days on the first, third and sixth week. Jugular blood samples were taken under ether anaesthesia before (week 0) and 1–6 weeks after pellet implantation. On the seventh week rats were killed by decapitation and blood samples were collected. Anterior pituitaries were extracted and weighed.

RIAs

Prolactin and GH were assayed by RIA using kits provided by the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) (Bethesda, MD, USA). Results were expressed in terms of prolactin PRL RP3 and GH RP2. Intra- and inter-assay coefficients of variation were 7·2 and 12·8% respectively for prolactin, and 8·3 and 13·1% for GH.

For IGF-I RIA serum samples (15 μl) and standards were subjected to the acid–ethanol cryoprecipitation method as previously described (Lacau-Mengido *et al.* 2000). IGF-I was determined using an antibody (UB2-495) provided by Drs L Underwood and J J Van Wyk, and distributed by the Hormone Distribution Program of the NIDDK. Intra- and inter-assay coefficients of variation were 8·2 and 14·1% respectively.

Leptin was determined using antimouse leptin (#AFP3011199) and recombinant mouse leptin (AFP 341C) for standard and iodinated hormone respectively (both reagents provided and distributed by the Hormone Distribution Program of the NIDDK and Dr A F Parlow). Increasing volumes of rat serum produced a parallel displacement of the mouse curve. The intra- and inter-assay coefficients of variation were 8·3 and 10·2% respectively.

Statistical analyses

Results are expressed as means ± S.E. Developmental patterns of body weight (BW), GH, prolactin and IGF-I were analysed by two-way ANOVA for the effects of week and drug treatment. If an F value of interaction was found significant, individual means were compared by Tukey's honest significant difference or Fisher's protected

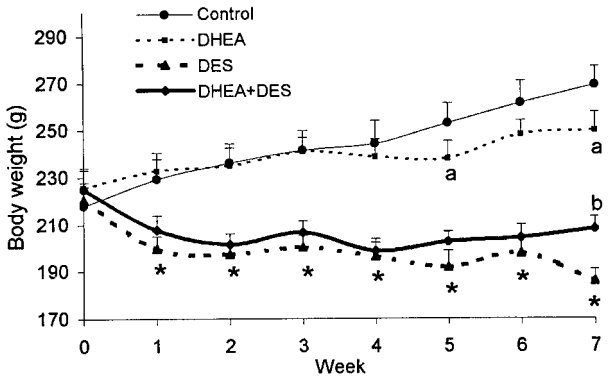

Figure 1 Body weight (BW) during treatment. *P<0·05, diethylstilboestrol (DES) and dehydroepiandrosterone (DHEA)/DES vs control (CON). [a]P<0·05, DHEA vs CON; [b]P<0·05, DHEA/DES vs DES. n=14–18 rats per group.

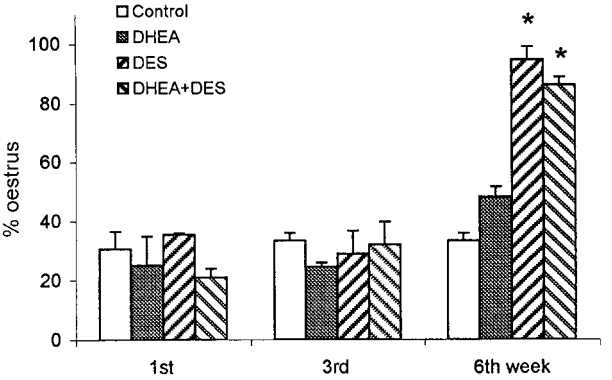

Figure 2 Percentage of oestrus occurrence during the first, third and sixth week of treatment. *P<0·05 vs week-matched CON. n=3 (each composed of six rats per group).

least significant difference (PLSD) tests. If it was not significant, groups of means were analysed by the same tests. Means of percentages of oestrus occurrence in the three independent experiments were compared using two-way ANOVA. Serum leptin and pituitary weights were analysed by one-way ANOVA. P<0·05 was considered significant.

Results

BW and vaginal smears

DES significantly decreased BW when compared with CON rats, from the first week of treatment onwards (F of interaction (21,336)=7·83; P<0·00028 for weeks 1–6, DES vs week-matched CON) (Fig. 1). DHEA by itself had also a minor negative effect on BW, and differences were significant at 5 and 7 weeks of treatment (P=0·023 and 0·048 respectively, DHEA vs week-matched CON). DHEA cotreatment was able to partially reverse the detrimental effect of DES on BW only at the last time point measured (P=0·0059, DHEA+DES vs DES).

A two-factor ANOVA indicated that there was a significant interaction between week and group in the percentage of oestrus occurrence (F(6,16)=8·19, P=0·00036). No differences were found among groups during the first and third weeks (Fig. 2). But 6 weeks after steroid treatment oestrus occurrence increased significantly in DES and DHEA+DES rats (P=0·00029 and 0·000870 vs CON respectively).

Pituitary weight

As BW was significantly different between treatments on the day of pituitary extraction (see Fig. 1), we analysed pituitary weight in relation to BW. DES treatment significantly increased anterior pituitary weight relative to BW (P=0·00017, DES vs CON) (Fig. 3) and DHEA partially reversed this increment (DHEA/DES group vs DES group, P=0·045). DHEA *per se* also increased this parameter (P=0·0010, DHEA vs CON). Similar results were obtained when comparing absolute pituitary weights (not shown).

Serum levels of prolactin, GH, IGF-I and leptin

As previously described, DES-implanted rats had higher serum prolactin levels from the second week of treatment onwards (Fig. 4, left panel). DHEA treatment itself induced a significant increase in prolactin levels at the third and fourth weeks (P=0·00048 and 0·045, DHEA vs week-matched CON respectively), even though levels achieved were lower than in DES-treated week-matched rats. Cotreatment of DES rats with DHEA reduced high

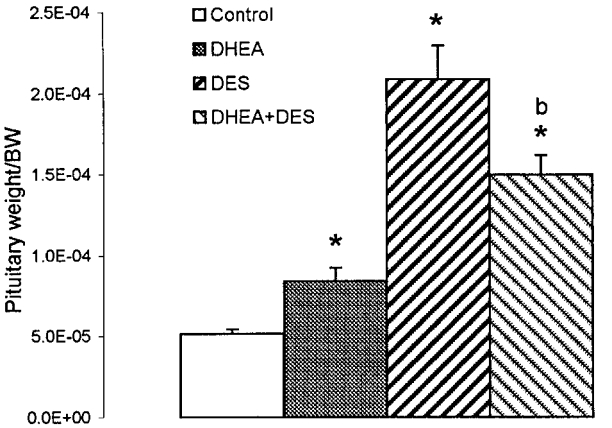

Figure 3 Pituitary weight (g)/BW (g) at the end of the treatment. *P<0·05 vs CON. [b]P<0·05, DHEA+DES vs DES. n=15–18 rats per group.

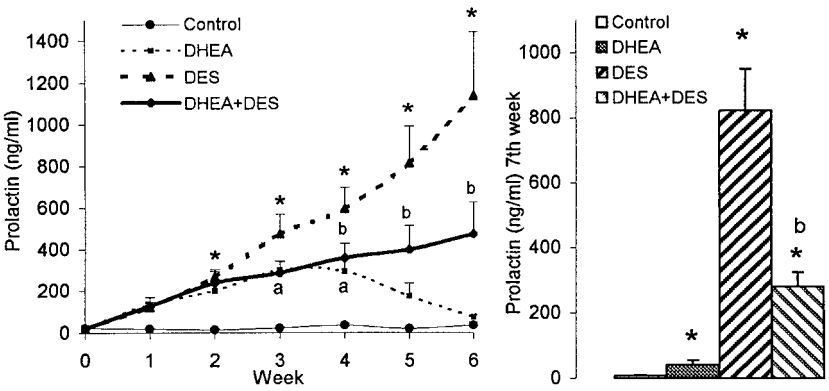

Figure 4 Left panel: serum prolactin levels during treatment. Jugular blood samples were obtained weekly under ether anaesthesia. *P<0·05, DES and DHEA/DES vs CON. [a]P<0·05, DHEA vs CON. [b]P<0·05, DHEA/DES vs DES. Right panel: serum prolactin levels at the end of the treatment. Blood samples were obtained after decapitation. *P<0·05 vs CON. [b]P<0·05 vs DES. n=15–18 rats per group.

prolactin levels from the fourth week onwards (P=0046, 0·000037 and 0·000029 for weeks 4, 5 and 6 respectively, DES vs DES+DHEA).

Serum prolactin at the seventh week is depicted separately, as samples were obtained from trunk collection, and not under ether anaesthesia as for the rest (Fig. 4, right panel). Even though absolute values were lower, DES rats still had significantly higher levels than CON or DHEA+DES rats. DHEA *per se* also significantly increased prolactin levels at this week (P=0·038, DHEA vs CON).

Both DHEA- and DES-treated rats presented higher GH levels from the first to the fifth week when compared with week-matched CONs (P<0·006 and P<0·02 for DHEA and DES vs CON respectively) (Fig. 5, left panel). On the other hand DES+DHEA rats had similar GH levels to DES rats throughout the experiment (P>0·99, DES vs DHEA+DES). At the seventh week, when rats were killed, no differences were found among groups (P=0·47) (Fig. 5, right panel). In the CON group GH levels were significantly higher in the samples obtained by killing than those obtained under ether anaesthesia (compare left and right panels).

A two-factor ANOVA indicated that serum IGF-I levels were significantly decreased in DES and DHEA+DES rats when compared with week-matched CON rats at the first, second, fourth and fifth weeks (Fig. 6, left panel). These differences were recapitulated on the seventh week (Fig. 6, right panel). DHEA *per se* did not significantly modify serum IGF-I levels.

On the seventh week DES significantly enhanced serum leptin levels (Fig. 7) (P=0·022, DES vs CON), and cotreatment with DHEA did not modify this effect (P=0·027, DHEA+DES vs CON). On the other hand

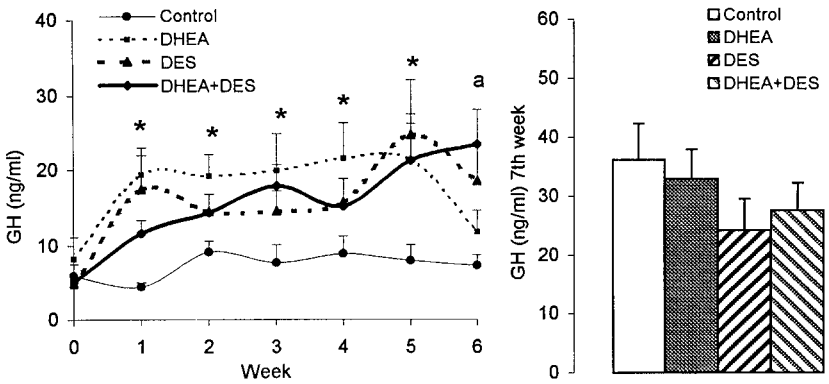

Figure 5 Left panel: serum GH levels during treatment. Jugular blood samples were obtained weekly under ether anaesthesia. *P<0·05, DHEA, DES and DHEA/DES vs CON. [a]P<0·05, DES and DHEA/DES vs CON. Right panel: serum GH levels at the end of the treatment. Blood samples were obtained after decapitation. n=15–18 rats per group.

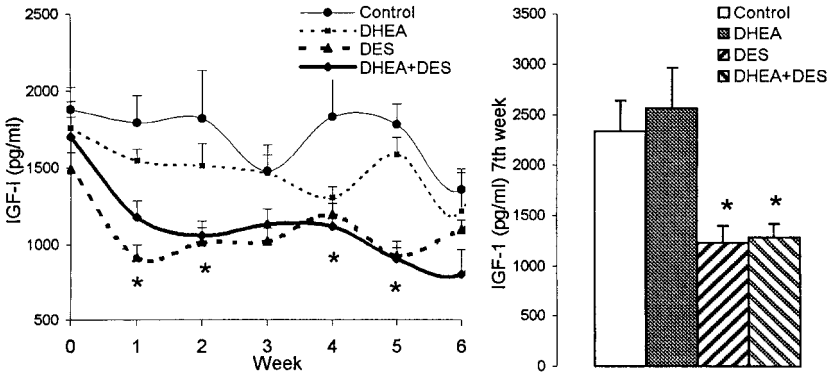

Figure 6 Left panel: serum IGF-I serum levels during treatment. Jugular blood samples were weekly obtained under ether anaesthesia. Right panel: serum IGF-I levels at the end of the treatment. Blood samples were obtained after decapitation. *P<0·05, DES and DHEA/DES vs CON. n=15–18 rats per group.

DHEA treatment *per se* did not alter leptin levels at this time point.

Discussion

DHEA, unconjugated or as its sulphate, is the major secretory steroidal product of the human adrenal gland. Its serum concentration is 20 times higher than that of any other steroid hormone in humans (Ebeling & Koivisto 1994). But, despite its abundance its physiological role is not completely known. In rats, DHEA is metabolised *in situ* in many tissues mainly to 7α-hydroxy DHEA by the α-hydroxylase CYP7B, or to ADIOL, which can also be α-hydroxylated, presumably by the same enzyme (Rose *et al.* 2001). *In vitro* and *in vivo* data suggest oestrogen- or androgen-like effects of DHEA and its metabolites, depending on sex hormone homeostasis (Poortman *et al.* 1975). The present results showed that in pituitary hyperplasia induced by oestrogen, DHEA had several endocrine and metabolic effects, which depended mainly on the endocrine environment.

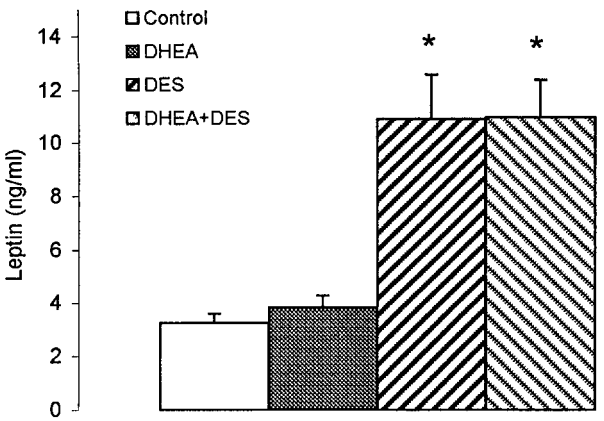

Figure 7 Serum leptin levels at the end of the treatment. Blood samples were obtained after decapitation. *P<0·05 vs CON group. n=9–15 rats per group.

It is well documented that oestrogen can reduce and testosterone can increase BW (Shulman *et al.* 1987, Borski *et al.* 1996). In the present experiments DES-treated rats had a progressive BW gain delay, which reached a 31% decrease in BW compared with CONs at the seventh week. DHEA treatment slightly decreased BW gain (only 7% at the seventh week), presenting an oestrogen-like action. This is in agreement with results showing that DHEA treatment produces an antiweight effect in genetically obese rats and, to a lesser degree, in non-obese rodents (Richards *et al.* 2000). It has been proposed that this action involves inhibition of hepatic lipogenesis as a result of a reduction in NADPH production (Parente & Berdanier 1993). Besides, DHEA or its 7-oxygenated derivatives can enhance heat production by inducing the liver thermogenic enzymes glycerol-3-phosphate dehydrogenase and the malic enzyme (Shepherd & Cleary 1984, Lardy *et al.* 1995). The effect of DHEA on food intake is not clear (Cleary 1991, Richards *et al.* 2000), and in our present experiments we did not find changes in serum leptin levels in DHEA-treated rats. When DES and DHEA were combined, we found that BW loss induced by the oestrogen was slightly reversed. This indicates that DHEA by itself may be acting as a weak oestrogen but in combination with DES it may antagonise its effect by partially displacing DES from oestrogen receptors, directly or via ADIOL (Poortman *et al.* 1975), or it may act as an androgenic compound. In this respect it has also been shown that DHEA produced a significant reduction of BW in unstressed rats, but that it reversed the inhibition of BW gain in stressed rats (Hu *et al.* 2000). These results would indicate that DHEA might be acting as a homeostatic factor in order to maintain BW in the normal range.

DHEA did not significantly increase oestrus occurrence. In other experimental models it has been described that the administration of DHEA induces precocious ovulation, acyclicity and anovulation in immature female rats (Knudsen *et al.* 1975) and ovarian cysts in adult cycling rats (Ward *et al.* 1978).

With regard to hormone secretion, we found that DHEA enhanced serum prolactin in CON rats but that it partially reversed DES-induced hyperprolactinaemia. Consequently, DES-induced pituitary enlargement was reduced by cotreatment with DHEA, but DHEA by itself slightly increased pituitary weight. In this regard, it has been described that DHEA can stimulate prolactin secretion both *in vitro* and *in vivo*, and that the effect is blocked using an anti-oestrogen, supporting a potential oestrogenic role of DHEA on lactotrophs (Simard *et al.* 1988). The fact that in the presence of high oestrogen levels the action of DHEA could be reversed may indicate that the endocrine environment conditions the effect of the adrenal steroid. As mentioned, there is evidence that DHEA can interact with several different classes of receptors, and there appears to be a considerable degree of tissue and species variation in the receptor type mediating the response to DHEA. In the rat mammary gland DHEA interacts with androgen receptors (Gatto *et al.* 1998, Sourla *et al.* 1998), whereas in some human breast cancer cell lines DHEA has been reported to act through either the oestrogen (Maggiolini *et al.* 1999) or the androgen (Boccuzzi *et al.* 1993) receptor. Furthermore, it has been suggested that the hormonal environment may influence the receptor type with which DHEA interacts (Boccuzzi *et al.* 1992, Ebeling & Koivisto 1994). Our results show that in a low-oestrogen milieu DHEA has an oestrogen-like effect, stimulating serum prolactin, anterior pituitary tumour growth and inhibiting BW gain, whereas in oestrogen abundance DHEA antagonises the tumour growth and the prolactin release induced by oestrogens, and enhances BW.

Serum GH levels in unstressed rats (seventh week) were not modified by oestrogen or DHEA treatment. Even though there is suggestive evidence that there is a stimulatory effect of oestrogens on GH secretion in humans, contradictory data have been obtained in rats (Müller *et al.* 1999). On the other hand, we observed that both DHEA and DES reversed the decrease in GH levels induced by ether anaesthesia. It is well documented that acute ether stress increases serum prolactin and decreases GH secretion (Diaz-Torga *et al.* 2002). This last effect is proposed to occur via stress-induced hypothalamic corticotrophin-releasing hormone stimulation of somatostatin release into portal blood (Arimura *et al.* 1976). Oestrogen, on the other hand, can antagonise the effect of somatostatin on pituitary GH *in vitro* (Hertz *et al.* 1989) and *in vivo* (Bray *et al.* 2001), thus preventing GH decrease. From our results it can be inferred that DHEA can also interfere with ether stress-induced GH decrement, even though we cannot be precise about the mechanisms of action involved.

We found that oestrogen lowered serum IGF-I levels. It has been reported that this decline may reflect in part an effect on the GH secretory response to GH-releasing hormone, and it may be also related to a direct effect of oestrogens on IGF-I gene expression in the liver and peripheral tissues (Shulman *et al.* 1987, Borski *et al.* 1996). On the other hand, it has been reported that DHEA treatment increases serum IGF-I levels in humans and rats (Morales *et al.* 1994, McIntosh *et al.* 1999, Arlt *et al.* 2000). In our experimental protocol (chronic treatment), IGF-I levels did not increase with DHEA treatment, and DHEA could not prevent the IGF-I decrease induced by oestrogen.

With regard to leptin regulation, it has been described that circulating leptin levels are higher in women than in men, even after correction for body fat (Paolisso *et al.* 2001). In humans, leptin concentration correlates positively with oestradiol, and negatively with testosterone and DHEA-S in males, and positively with oestradiol and negatively with DHEA in females (Paolisso *et al.* 2001). Furthermore it has been shown that acute oestradiol administration significantly increased leptin mRNA levels in adipose tissue, and serum leptin levels in rats (Brann *et al.* 1999). In accord, we found that DES rats had significantly higher serum leptin levels. It has been described that in females both DHEA-S and dihydrotestosterone induced a significant decrease in leptin secretion from omental adipose tissue *in vitro* (Pineiro *et al.* 1999). Besides, in obese rats DHEA treatment reduced body and fat pad weights, and slightly lowered leptin levels (Richards *et al.* 2000). We did not observe any effect of DHEA on leptin levels, and it is plausible that this effect may be better evidenced in obese rats.

The present results show that DHEA attenuates oestrogen-induced pituitary hyperplasia and lowers the resulting hyperprolactinaemia. Nevertheless the fact that DHEA *per se* produced diverse hormonal effects, such as prolactin and GH increase, and a slight pituitary enlargement should be considered in the applications of this drug.

Acknowledgements

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