

# Dehydroepiandrosterone regulation of prolactin gene expression in the anterior pituitary does not depend on galanin induction

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## Abstract

**OBJECTIVES:** The effects of dehydroepiandrosterone (DHEA) on galanin (GAL) and prolactin (PRL) mRNA expression in the anterior pituitary of Fischer 344 rats were studied, taking in consideration that: (1) DHEA is an androgen with estrogenic activity on pituitary lactotrophs; (2) estrogens induce prolactinomas in Fischer 344 rats; and (3) GAL has been considered the main mediator of estrogen-induced lactotroph proliferation.

**DESIGN:** Female rats were ovariectomized and used as controls or treated during 2 weeks with DHEA (500 µg/kg/day or 5 mg/kg/day or 50 mg/kg/day) or estradiol (E2, 50 µg/kg/day), as a positive control for pituitary growth and GAL induction. GAL and PRL mRNA expression were studied by *in situ* hybridization.

**RESULTS:** Both DHEA and E2 induced PRL mRNA synthesis. However, DHEA neither produced pituitary enlargement nor GAL induction, in contrast to E2.

**CONCLUSIONS:** Our results shows that GAL is not involved in the estrogenic activity of DHEA on pituitary lactotrophs, and suggest that DHEA effects are exerted directly on the PRL gene or through another mechanism(s) not related to GAL.

## ABBREVIATIONS AND UNITS

DHEA	- dehydroepiandrosterone
PRL	- prolactin
GAL	- galanin
E2	- estradiol
OD	- optical density

**Introduction**

Estrogens increase prolactin (PRL) expression, synthesis and secretion in the rat [1]. When given chronically, estrogens also induce lactotroph proliferation and pituitary enlargement [2]. These effects are enhanced in the Fischer 344 (F344) rat [3–5], and seem to depend on the expression of the peptide galanin (GAL), which is positively regulated by estrogens [4, 6–8]. While GAL is present at very low levels only in somatotrophs in the ovariectomized rat [9], the increment observed in GAL content after estrogenization is due to its expression in lactotrophs [9, 10]. GAL released by some lactotrophs regulates the function of the same or other lactotrophs in an autocrine/paracrine fashion [6, 11].

The adrenal androgen dehydroepiandrosterone (DHEA) exerts estrogenic actions at the pituitary level. Studies with adenohipophyseal cells showed that DHEA increases PRL cell content and reduces lactotrophs' sensitivity to the inhibitory action of dopamine, through a direct interaction of DHEA with estrogen receptors [12]. *In vivo* studies showed that DHEA increases PRL mRNA content in pituitaries of intact or gonadectomized rats, and also diminishes tyrosine hydroxylase expression in the arcuate nucleus, thus decreasing the dopaminergic inhibitory input on PRL [13].

The present study was performed to test the hypothesis that DHEA modulates PRL expression through the regulation of the GAL gene, by virtue of its estrogenic activity. In addition, we evaluated the possible tumorigenic action of DHEA on the anterior pituitary of F344 rats.

**Materials and methods**

**Experimental animals:** Animal experimentation followed the National Institutes of Health Guide for the Use and Care of Laboratory Animals (NIH Guide; Instituto de Biología y Medicina Experimental Assurance Certificate No. A5072-01). Female F344 rats (180–200 g) were ovariectomized, allowed to recover from surgery for 7 days and divided into five groups, which received daily subcutaneous injections of different doses of steroids dissolved in sunflower oil for 14 days, as follows: (1) DHEA 500 µg/kg/day; (2) DHEA 5 mg/kg/day; (3) DHEA 50 mg/kg/day; and (4) estradiol (E2) 50 µg/kg/day. Group (5) was used as control (CONT), and received vehicle only. Rats were sacrificed between 9.00 and 10.00 h, and pituitaries were immediately removed, weighed, frozen on dry ice and stored at –70°C until use. Blood was collected, and serum was stored at –20°C for PRL determination, which was performed by RIA as previously described [4, 5].

**In situ hybridization for GAL and PRL mRNA:**

We followed a previously published method using isotopic-labeled oligonucleotide probes for the detection of GAL and PRL mRNAs [4]. Briefly, probes were 3' end labeled using (<sup>35</sup>S)-dATP and terminal deoxynucleotidyl transferase, and then purified by chromatography on Sephadex G-25 prepacked columns. Tissues were sliced every 16 µm in a cryostat and sections were then fixed for 30 min at 4°C in 2% paraformaldehyde, washed and stored at –70°C until used. On the day of the hybridization, sections were acetylated with 0.25% acetic anhydride and hybridized with 1x10<sup>6</sup> cpm of (<sup>35</sup>S)-labeled probe. After an overnight incubation in a moist atmosphere at 42°C, sections were washed, allowed to dry, and exposed to Kodak Biomax Films for 18 h. At least 10 images of sections of each pituitary were captured using a CCD-X77 videocamera coupled to a Mackintosh computer and the whole anterior pituitary present in each autoradiogram was analyzed using the NIH-Image software V1.5. The arbitrary optical density measured minus the background was used to calculate the relative abundance of each mRNA. Data were pooled for individual rats, and each experimental group was composed of 6 rats. Results were expressed as percent of the optical density corresponding to E2 50 µg/kg/day group, considering that this treatment was used as positive control for induction of PRL and GAL genes. Specificity of the hybridization was confirmed using (<sup>35</sup>S)-labeled sense probes, in which case the signal was less than 5% of that obtained with antisense probes.

**Statistical analysis:** Results were expressed as means ±SEM. Means were compared by one-way ANOVA, followed by post-hoc comparisons with the *Student-Newman-Keuls' test*, according to the GraphPad Prism V3.00 program. Values of serum prolactin were logarithmically transformed before comparison, due to proportional relation between means and variances [14]. Values of P < 0.05 were considered statistically significant.

**Results**

After two weeks of E2 treatment (50 µg/kg/day), F344 rats showed a 77% increment of the adenohipophyseal weight when compared to CONT rats (P < 0.001, Table I). This result validates treatment length and E2 choice as a positive control for pituitary enlargement, and is in accordance with previous reports in F344 rats [5, 15]. Animals receiving DHEA treatment for two weeks had no signs of anterior pituitary enlargement at any of the doses employed (Table I), even though the higher dose (50 mg/kg/day) was 10-fold that reported to affect PRL and tyrosine hydroxylase mRNA expression [13].

We also studied DHEA effects on serum PRL concentration. While the lower doses of DHEA was not effective to change serum PRL levels in comparison to CONT rats, DHEA 5 mg/kg/day produced a 29-fold increase in serum PRL values (P < 0.001, Table I), an effect also present with the highest dose of DHEA

**Table I:** Anterior pituitary weight, serum prolactin levels and prolactin mRNA optical density in control ovariectomized rats (CONT), rats treated for 14 days with 500 µg/kg/day, 5 mg/kg/day and 50 mg/kg/day of DHEA (DHEA 500 µg, 5 mg and 50 mg, respectively) and rats treated for 14 days with 50 µg/kg/day of E2 (E2 50 µg).

GROUP	Anterior pituitary weight (mg/100g BW)	Serum prolactin (ng/mL)	PRL mRNA (OD, % of E2 50 µg group)
CONT	7.33±0.51	0.93±0.31	4.05±0.61
DHEA 500 µg	7.53±0.46	1.85±0.43	1.60±0.62
DHEA 5 mg	7.62±0.40	27.40±7.20*	17.26±1.95*
DHEA 50 mg	7.48±0.20	38.30±9.77*	24.08±6.08*
E2 50 µg	12.98±1.01*#	351.0±69.0*#	100.00±7.56*#

Values are expressed as mean ± SEM of 6 animals/group.

\* P<0.05 or less vs. CONT, # P<0.001 vs. DHEA-treated groups, all the dosis (ANOVA and post-hoc comparisons with the Student-Newman-Keuls' test).

50 mg/kg/day (P<0.001, Table I). The effect of E2 50 µg/kg/day on serum PRL was higher than that of DHEA, a 378- and 8-fold increase being observed when compared to CONT rats or rats receiving DHEA 50 mg/kg/day, respectively (P<0.001, Table I).

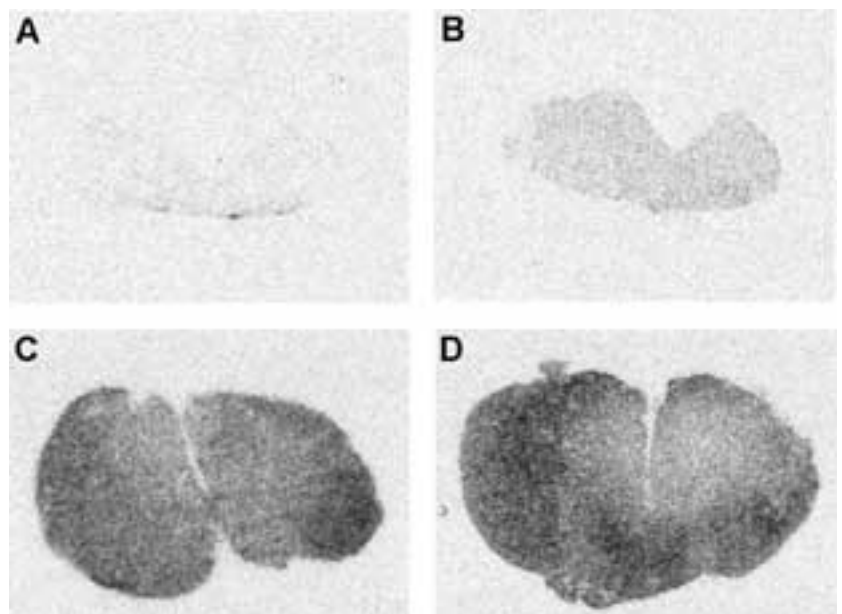
PRL mRNA content in pituitary glands was evaluated by *in situ* hybridization. Our study indicates that the expression was low in CONT rats (Table I and Fig 1A). While DHEA 500 µg/kg/day did not modify those values (Table I), DHEA 5 mg/kg/day or 50 mg/kg/day increased PRL mRNA expression 3.25- and 4.95-fold in comparison to CONT rats (P<0.05 for both comparisons, Table I and Fig 1B). E2 50 µg/kg/day exerted a marked stimulation of PRL mRNA expression, which was even larger than that following DHEA (p<0.001 vs. CONT and all DHEA doses, Table I and Fig 1C).

Finally, we also studied the expression of GAL mRNA, which was undetectable in glands of CONT and DHEA-treated animals, for the range of doses employed, after 18 h of film exposure. On the other hand, E2 50 µg/kg/day induced a strong expression of GAL mRNA (Fig 1D). Longer exposure times of the films further magnified to saturation levels the signal for the E2-treated group, but failed to give evidences for GAL mRNA in the CONT and DHEA-treated rats.

## Discussion

The results of this study indicate that two weeks of DHEA treatment were effective to increase serum PRL and pituitary PRL mRNA in F344 rats, confirming previous reports in the Sprague-Dawley strain [12, 13, 16]. Our study was performed using ovariectomized rats as controls, in order to maximize DHEA effects on the PRL gene [13]. The use of higher doses of DHEA beyond those employed in this study were precluded by steroid insolubility into the vehicle. Most evidences indicate that DHEA effect on PRL gene is due to the direct interaction of the hormone with the estrogen receptor. *In vitro* studies showed lack of additive effect of maximal concentrations of E2 and DHEA on spontaneous PRL release and content in dispersed lactotrophs [12]. In addition, antiestrogenic treatment with LY156758 was able to abolish PRL accumulation due to DHEA in pituitary cells in culture [12]. Furthermore, DHEA is able to displace (<sup>3</sup>H)-E2 binding to cytosolic receptors in the pituitary, though the receptor affinity for DHEA is lower than for E2 [12, 17]. Although the present study did not include a dose-response curve for E2 to allow potency comparison, it is clear that the induction of the PRL gene exerted by DHEA 5mg/kg/day was about one-fourth of that provoked by E2 50 µg/kg/day, indicating that DHEA has a weak but still

**Figure 1:** Typical autoradiograms showing the hybridization signal for prolactin (A-C) and galanin (D) in the anterior pituitary gland. A: PRL mRNA expression in an ovariectomized-untreated animal (CONT). B: PRL mRNA in a rat treated during 14 days with DHEA 50 mg/kg/day. C: PRL mRNA in an animal receiving E2 50 µg/kg/day for 14 days. D: Galanin mRNA expression in an animal receiving E2 50 µg/kg/day for 14 days.



effective estrogenic action. We cannot rule out the possibility of DHEA transformation into estrogens *in vivo* [18]. If this was the case, however, DHEA could also be metabolized into androgens of higher biological potency, which are known to reduce PRL synthesis and release [19].

We studied the effects of DHEA on GAL, considering that this peptide has been proposed as the major mediator of estrogen action on lactotrophs [6, 11, 20]. Our results indicate that despite its effect on PRL mRNA, DHEA was not able to induce the expression of GAL mRNA, while E2 caused a strong induction of both genes. We could speculate that DHEA is able to stimulate PRL mRNA synthesis and PRL secretion through the release of preformed GAL. However, it has been demonstrated by us and others that GAL content in the anterior pituitary of ovariectomized rats is very low [4, 9–11, 21]. It has also been shown that mice carrying a loss-of-function mutation of the GAL gene have only a slight reduction in PRL mRNA expression and PRL content in the pituitary gland, and that their basal serum PRL levels are normal [20]. In addition, these animals are still responsive to E2 by increasing PRL mRNA expression and serum PRL levels, though to a lesser extent than wild type mice [20]. This is indicative that another factor(s) different from GAL could be involved in PRL synthesis/secretion under basal and DHEA-treatment conditions. Supporting this possibility, the expression of tyrosine hydroxylase mRNA in the arcuate nucleus of DHEA-treated rats is diminished when compared to control gonadectomized rats [13], an indication of a decreased inhibitory dopaminergic tone on PRL synthesis/secretion.

In contrast to E2, DHEA did not increase the anterior pituitary weight, a parameter highly sensitive to estrogens in the F344 rat [3–5, 15]. The lack of effects of DHEA on GAL mRNA expression and anterior pituitary growth are in agreement with the postulated role of GAL on lactotroph proliferation leading to tumor formation [6, 20]. In this context, we and others have shown that treatments that decrease pituitary growth in F344 rats under estrogenic treatment also decrease GAL mRNA expression in the gland [4, 21]. A tentative conclusion based on current evidence, is that DHEA acts as a weak estrogen increasing PRL mRNA and serum PRL levels through a mechanism independent of GAL, while E2 is able to increase GAL levels in the anterior pituitary, leading not only to increases in PRL but also to lactotroph proliferation. Further studies using adenohypophyseal cells in culture are planned to support this hypothesis.

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