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ANDROGEN TARGET CELLS IN THE PITUITARY OF THE CHICK EMBRYO

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SUMMARY

Androgen target cells are demonstrated in anterior pituitary and surrounding mesenchyme of the chick embryo using autoradiography. The proportion of [^{3}H]-dihydrotestosterone labeled cells increases from 30% at day 10 to 35–40% at day 15 of incubation. The results show for the first time a possible effect of androgens on the pituitary before birth which further points to a significant endocrine role during embryonic development.

INTRODUCTION

The results of recent studies show that in birds and mammals gonadal functions are influenced by the pituitary before birth. Identification of gonadotropin producing cells at day 16 of gestation in the rat fetus [1] and the presence of secretory granules in pituitary cells of the chick embryo before day 10 of incubation [2–4] suggest an early capacity of the pituitary to secrete gonadotropins. It has also been reported that the embryonic gonads secrete steroid hormones and their secretion is modulated by gonadotropin [5–10]. Whether or not gonadal steroids regulate by a feedback mechanism the secretion of the pituitary during embryonic development has not been investigated. The existence of "target cells" in the pituitary is essential to the eventual feedback of steroid hormones. Such target cells for estrogen and androgen are present in the adult pituitary [11, 12]. Only recently estrogen target cells have been demonstrated in fetal pituitary [13, 14]. The present article reports the existence of androgen target cells in the pituitary of the chick embryo, using thaw-mount autoradiography which has been applied successfully for the localization of steroid hormones in other embryonic tissues [15, 16]. Dihydrotestosterone (DHT) has been preferred to testosterone in order to preclude a possible aromatization to estradiol. Chick embryos at different stages of development were used in order to encompass the period of functional differentiation of the pituitary, including the time of appearance of differentiated secretory granules [3] and the onset of control of the gonadal steroidogenesis by the pituitary [5, 10].

MATERIALS AND METHODS

Eggs of White Rock chicken were incubated at 38°C in a humidified incubator. Embryos at day 10 (two males and two females), 12 (two males and two females) and 15 (two males and three females) were each injected intravenously with [1, 2, 4, 5, 6, 7, 16,17-^{3}H]-dihydrotestosterone (DHT), SA 190 Ci/mmol. Ten-day embryos received 0.03 μg, and 12- and 15-day embryos 0.04 μg each of [^{3}H]-DHT in 0.1 ml 5% ethanol-isotonic saline. In order to establish the specificity of the androgen localization, additional 15-day embryos were each treated with 4 μg non-radioactive hormone, 30 min prior to 0.04 μg [^{3}H]-DHT. The unlabeled hormone, either DHT (two males and one female), testosterone (one male and one female), or estradiol (one male and one female), was dissolved in ethanol–isotonic saline and applied to the chorioallantoic membrane. Embryos were decapitated 1 h after [^{3}H]-DHT injection, the pituitaries removed, then frozen in liquefied propane and stored in liquid nitrogen. Three μm sections were cut at −35°C in a Wide Range Cryostat (Harris Mfg. Co., North Billerica, MA) and thaw-mounted on photographic emulsion (NTB3) precoated slides. The slides were stored in the dark at −15°C for 6 months, photographically processed (D19 developer and rapid fixer, Kodak) and then stained with methyl green–pyronin. The autoradiographic technique has been described in detail by Stumpf and Sar [17]. For each embryo, cell countings were carried out on 1 or 2 sections (2,000–6,000 cells on each) including both the cephalic and caudal lobes.

RESULTS

All pituitary autoradiograms of both sexes of 10-, 12- and 15-day chick embryos show nuclear concentration of radioactivity in certain cells (Figs 1 and 2). Radioactively labeled cells are present in large

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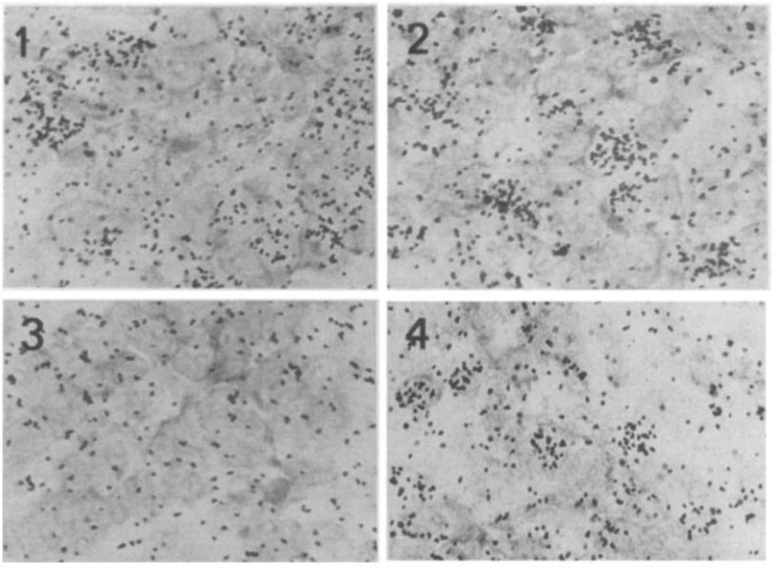

Figs. 1–4. Autoradiograms prepared from pituitary of 10- (Fig. 1) and 15-day (Fig. 2) chick embryos after [^{3}H]-dihydrotestosterone ([^{3}H]-DHT) injection show nuclear concentration of radioactivity in a large number of cells. When a 100-time excess of DHT is injected prior to [^{3}H]-DHT injection, nuclear concentration is abolished (Fig. 3), but with the identical dose of estradiol, nuclear concentration is only moderately reduced (Fig. 4). Three μm sections; 180-day exposure; stained with methyl green–pyronin; ×900.

numbers in both the cephalic and the caudal lobes of the anterior pituitary. The pattern of labeling is not uniform throughout the pituitary, but no area appears devoid of labeled cells. However, some differences in labeling intensity and density of labeled cells cannot be excluded between the two lobes. The proportion of labeled versus the total number of cells averages 30–33% at 10 and 12 days and 35–40% at 15 days. In addition to pituitary cells, mesenchymal-type cells surrounding the gland, especially in the infundibular area, show nuclear concentration of radioactivity.

In pituitaries of embryos pretreated with an excess of either unlabeled DHT (Fig. 3) or testosterone, no nuclear concentration of labeled hormone is seen. Pretreatment with an excess of unlabeled estradiol does not prevent the nuclear concentration of radioactivity but reduces its intensity (Fig. 4) and the density of labeled cells. The proportion of labeled cells was reduced to a mean percentage value of 23%.

DISCUSSION

The nuclear concentration of radioactivity in pituitary cells and the abolition of the nuclear labeling after androgen treatment, with both DHT or testosterone, indicates that [^{3}H]-DHT binds to the nuclei with high affinity and limited capacity. When unlabeled estradiol 100-fold in excess was applied, nuclear concentration of radioactivity was moderately reduced indicating a limited cross-reactivity at the level of either transport of hormones or receptor sites.

This is the first demonstration of androgen target cells in the pituitary of embryos. The results in chick embryos at days 10–15 of incubation point to a direct effect of this hormone on the pituitary at this early time of development. Among the regulatory mechanisms suggested by our observations, the existence of a feedback regulation between gonads and pituitary before birth appears obviously, although the exact time when this regulating mechanism is functional remains to be established. Other regulations, such as cellular proliferation, cannot, however, be excluded. Despite this uncertainty the present results reveal novel aspects of sexual physiology in the embryo.

In our study target cells for DHT are observed in both the cephalic and caudal lobes of the anterior pituitary. Since LH-producing cells appear to be located in the caudal lobe and FSH- and TSH-cells in the cepahlic lobe [18, 19], a histochemical clarification would be required to identify which cell types are target for androgens. This can be achieved using a combined technique of autoradiography and immunohistochemistry, which has previously been utilized to characterize androgen target cells in the adult rat pituitary [20].

The fact that specific target sites for androgen exist in the pituitary points to a role of these hormones in embryonic development. In the literature, this role appears to be underestimated in birds where androgen hormones are considered of secondary, if of any, importance before hatching. In mammals, direct effects of androgen hormones on pituitary and brain have been demonstrated in adults [21] but not yet in the fetus. The results of the present autoradiographic study strongly suggest the involvement of androgen hormones on neuroendocrine regulation during embryonic development.

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