IMMUNOCHARACTERISTICS OF OESTROGEN AND ANDROGEN TARGET CELLS IN THE ANTERIOR PITUITARY GLAND OF THE CHICK EMBRYO AS DEMONSTRATED BY A COMBINED METHOD OF AUTORADIOGRAPHY AND IMMUNOHISTOCHEMISTRY

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SUMMARY

The distribution of oestrogen and androgen target cells in the anterior pituitary gland of the chick embryo on days 10, 12 and 15 of incubation was studied 1 h after the injection of tritium-labelled steroid hormone using the thaw-mount autoradiographic technique. Oestradiol target cells were localized in the caudal zone that corresponds to the so-called 'caudal lobe', while androgen target cells were found throughout the rostral and caudal lobes of the anterior pituitary gland. With a combined autoradiography and immunohistochemistry technique, most of the oestrogen target cells showed immunoreactivity to turkey LH antiserum but not to adrenocorticotrophin (1-24) and β-thyrotrophin antisera. In contrast, androgen target cells did not show positive immunoreactivity to the three antisera used. The results suggested a direct and early involvement of oestrogens but not of androgens in the feedback regulation of pituitary gonadotrophin secretion in the chick embryo.

INTRODUCTION

In mammals and birds the anterior pituitary gland starts to regulate gonadal functions during embryonic life. Evidence for this early function of the pituitary gland has been provided by experiments with hypophysectomized or decapitated embryos (Fugo, 1940; Betz, 1967; Woods & Weeks, 1969; Akram & Weniger, 1973; Woods, Podczaski, Erton, Rutherford & McCarter, 1977) and fetuses (Jost, 1951; Weniger, Chouraqui & Zeis, 1977; Eguchi, Arishima, Nasu, Toda, Morikawa & Hashimoto, 1978) which have resulted in alterations in the development of the gonads and the production of steroid hormones. Association of embryonic gonads and pituitary tissue (Moszkowska, 1956; Pointis & Mahoudeau, 1977) in culture, as well as by addition of gonadotrophins to the culture medium (Cédard, Haffen & Guichard, 1968; Weniger & Zeis, 1974; Teng & Teng, 1977; Feldman & Bloch, 1978; George, Catt, Neaves & Wilson, 1978; Grassi-Milano & Pitini, 1978) have shown that the embryonic pituitary gland is active and that the embryonic gonads are sensitive to gonadotrophins.

The regulation of the steroid secretions by the anterior pituitary gland is believed to start after 16 days of gestation in the rat and mouse (Weniger et al. 1977; Eguchi et al. 1978; Pointis, Mahoudeau & Cédard, 1978) and at about 13 days of incubation in the chicken embryo (Woods & Weeks, 1969; Woods et al. 1977). A feedback effect of steroid hormones

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on the pituitary gland is known to exist in the adult but not in embryonic and fetal life, although it has been suggested that such an effect exists in the chicken embryo (Gasc, Stumpf & Sar, 1979) and the mouse (Stumpf & Sar, 1978) and rat fetus (Pointis et al. 1978).

Using an autoradiographic technique we have recently discovered androgen target cells in the anterior pituitary gland of the chicken embryo as early as day 10 of incubation after the injection of \(^{3}\text{H}\)dihydrotestosterone (DHT; Gasc et al. 1979). Under the conditions of this experiment approximately 30% of the anterior pituitary cells showed nuclear labelling with radioactivity. To understand the role of steroid hormones in the embryonic pituitary gland better, experiments were conducted to identify oestrogen target cells in addition to androgen target cells in both male and female embryos. We further investigated whether oestrogen and/or androgen target cells in the pituitary gland are gonadotrophin producing cells by using a combined technique of autoradiography and immunohistochemistry (Sar & Stumpf, 1979).

MATERIALS AND METHODS

Fertilized eggs of White Rock chickens, obtained from a local poultry farm, were incubated at 38 °C in a humidified incubator. After 4 days of incubation the eggs were opened and blood was collected from the extra-embryonic circulation for the determination of genetic sex (Omura, 1970; Gasc, 1973). The eggs were then resealed with adhesive tape and incubated until day 10, 12 or 15.

Treatment by radiolabelled hormones

\([2,4,6,7,16,17-^{3}\text{H}]\text{Oestradiol}\) (sp. act. 141 Ci/mmol, New England Nuclear, Boston, Massachusetts, U.S.A.) was injected into a vein of the extra-embryonic circulation on days 10, 12 and 15 of incubation. The doses of \([^{3}\text{H}]\text{Oestradiol}\) used increased with age: at day 10, 0.01 \(\mu\)g (three female and two male embryos), at day 12, either 0.01 \(\mu\)g (two female and two male embryos) or 0.02 \(\mu\)g (one female and one male embryo) and at day 15, 0.02 \(\mu\)g (two female and one male embryos). Competition experiments were carried out at days 12 and 15 by applying a 100-fold excess (2 \(\mu\)g in 0.1 ml 5% alcohol saline solution) of either unlabelled oestradiol-17\(\beta\) or unlabelled DHT on the chorio-allantoic membrane of the embryos 30 min before the injection of \([^{3}\text{H}]\text{Oestradiol}\). For the competition by oestradiol one female and two males at day 12 and two male embryos at day 15 were used, and for competition by DHT, one female and one male at day 12 and three female embryos at day 15 were used. For localization of \([^{3}\text{H}]\text{DHT}\), embryos received 0.03 \(\mu\)g at day 10 and 0.04 \(\mu\)g \([^{3}\text{H}]\text{DHT}\) at days 12 and 15, administered in the same way as \([^{3}\text{H}]\text{Oestradiol}\) (for details see Gasc et al. 1979). All embryos were killed by decapitation 1 h after the injection of radiolabelled hormone.

Preparation of tissues and autoradiographs

The pituitary gland was mounted on a brass holder either isolated completely or together with its surrounding cartilage. Minced rat liver was used as embedding material. The tissues were frozen in liquefied propane and stored in liquid nitrogen.

Blocks of frozen tissue were transferred to a cryostat at -35 °C (WR model, Harris Manufacturing Co., North Billerica, Massachusetts, U.S.A.). Frozen sections (3 \(\mu\)m) were cut and mounted directly on glass slides precoated with photographic emulsion (Kodak NTB 3). The technique of thaw-mount autoradiography has been described in detail (Stumpf & Sar, 1975).

After exposure for 6–12 months, the autoradiographs were developed, fixed (Kodak D19 and Rapid Fixer) and then stained with methyl green–pyronin. The sections used for immunohistochemistry were fixed in 3% paraformaldehyde for 30 s at 0 °C before photographic development. The unstained developed slides were then processed immunohistochemically.
Immunohistochemical technique

After photographic processing the autoradiographic slides were treated with $\text{H}_2\text{O}_2$ and egg albumin to eliminate non-specific staining in the immunoperoxidase staining method as described by Zehr (1978). After washing with phosphate buffered saline, the autoradiographs were incubated with antiserum to turkey luteinizing hormone (LH), $\beta$-subunit of bovine thyrotrophin (TSH) or adrenocorticotropic ($^1-24\text{ACTH}$) for 24 or 48 h at 4 °C in a humidified atmosphere and they were then stained by a modified immunoperoxidase method. The antisera were used at a dilution of 1 : 500 or 1 : 1000 (v/v). As a control, autoradiographs were incubated with serum from a normal rabbit before immunization. The turkey LH antiserum was provided by Dr B. Wentworth (University of Wisconsin, Madison, U.S.A.), bovine $\beta$-TSH antiserum by Dr J. Pierce (University of California, Los Angeles, California, U.S.A.) and $^1-24\text{ACTH}$ antiserum by Dr R. P. De Augustine (National Institute for Environment and Health Sciences, Research Triangle Park, North Carolina, U.S.A.). A detailed description of the combined thaw-mount autoradiography and immunoperoxidase staining method has been published (Sar & Stumpf, 1979).

RESULTS

One hour after the injection of $[^3\text{H}]\text{oestradiol}$, radioactivity was found to be concentrated in nuclei of certain cells of the anterior pituitary gland of all the embryos studied after incubation for 10, 12 and 15 days (Pl. 1). The labelled cells were more numerous after 15 than after 10 days but their topographical distribution appeared to be similar. There were no differences in the results between male and female embryos.

The distribution of the labelled cells was not uniform. Two zones may be recognized: caudally, labelled cells were consistently seen in all cords (Pl. 1, fig. 1), while rostrally labelled cells were sparse and encountered in only a few cords (Pl. 1, fig. 2). The limit between the two zones was characterized by an abrupt change in density of labelled cells. The caudal zone corresponded approximately to the caudal lobe and the rostral zone to the cephalic lobe described by Rahn (1939). However, it was not possible to ascribe the labelled cells strictly to the ‘caudal lobe’ since there was no clear delineation between the two lobes of the anterior pituitary gland.

In the caudal zone the labelled cells appeared either as single cells or in groups of cells not exceeding two at 10 days or four at 15 days of incubation. The ratio of labelled to unlabelled cells in the caudal zone was approximately 10% at day 10, 15% at day 12 and 20% at day 15. Administration of unlabelled oestradiol before the injection of $[^3\text{H}]\text{oestradiol}$ abolished the labelling (Pl. 1, fig. 4) whereas a similar dose of DHT affected neither the labelling nor the pattern of distribution of the labelled cells (Pl. 1, fig. 5).

After injection of $[^3\text{H}]\text{DHT}$ at 10, 12 and 15 days of incubation, the nuclear concentration of radioactivity was observed in cells distributed throughout the two lobes. The labelled cells were similarly distributed in both lobes, although there were differences in the intensity of labelling. The ratio of labelled to unlabelled cells in the anterior pituitary gland was approximately 30–40% and increased with age similarily in embryos of both sexes.

Autoradiographs of the pituitary gland prepared after administration of $[^3\text{H}]\text{oestradiol}$ were incubated with antiserum to turkey LH, $^1-24\text{ACTH}$ or the $\beta$-subunit of bovine TSH. Immunoreactive LH cells were found in both male and female embryos at days 12 and 15 of incubation. The distribution of LH-positive cells appeared to be similar at 12 and 15 days. These cells were located in the caudal zone and extended rostrally to the periphery of the rostral zone. The most anterior portion of the rostral zone did not contain LH-positive cells. The majority of the $[^3\text{H}]\text{oestradiol}$-labelled cells were stained for LH (Pl. 2, fig. 7) but not all LH-positive cells showed nuclear labelling with $[^3\text{H}]\text{oestradiol}$. The density of labelled cells positive for LH was higher in the caudal than in the rostral zone. Autoradiographs at 10, 12
and 15 days of incubation when stained with antiserum to $^{1-24}$ACTH showed that immunoreactive cells were confined to the cephalic lobe and were devoid of $[^3$H]oestradiol nuclear labelling. Thyrotrophin immunoreactive cells were observed at day 15 of incubation but not earlier. They were located mainly at the periphery of the rostral zone and did not appear to be labelled with $[^3$H]oestradiol.

Pituitary autoradiographs prepared after injection of $[^3$H]DHT and stained with antisera to turkey LH, $^{1-24}$ACTH and bovine $\beta$-TSH, showed immunoreactive cells in a manner similar to that shown by experiments with $[^3$H]oestradiol. However, none of these cells appeared to be labelled with $[^3$H]DHT (Pl. 2, figs 8, 9).

DISCUSSION

The concentration of $[^3$H]oestradiol in the nucleus of cells of the anterior pituitary gland indicated the existence of oestrogen target cells as early as day 10 of incubation in male and female embryos. These target cells contained binding sites saturable by and specific for oestrogenic hormones, as shown by the competition experiments with unlabelled oestradiol or DHT. Previously, the earliest target cells were demonstrated at day 17 of incubation in the chick embryo (Kim, Stumpf, Sar & Martinez-Vargas, 1978) and at day 16 of gestation in the mouse fetus (Stumpf & Sar, 1978).

Oestrogen and androgen target cells were present on day 10 of incubation with a pattern of distribution different for the two types of hormones. Furthermore target cells to oestrogen constituted less than 20% of the cells in the cephalic lobe whilst those for androgen constituted at least 30% of all pituitary cells at day 10 of incubation. The differential distribution as well as the specificity of the binding sites for DHT and oestradiol provide strong evidence for the existence of two different types of target cells in the anterior pituitary gland of the chicken embryo.

Using the immunohistochemistry technique (Sar & Stumpf, 1979) the majority of oestrogen target cells were identified as gonadotrophin-producing cells, both in the caudal and cephalic lobes of the anterior pituitary gland of chicken embryos at day 12 and 15 of incubation. However, at day 10 of incubation oestrogen target cells could not be stained by this procedure. This was probably because these cells contained only small amounts of gonadotrophic hormone or gonadotrophin had been leached out during the preparation of the tissue. This assumption was supported by the immunohistochemical finding that LH cells can be detected in paraffin-wax-embedded pituitary tissues of 7-day-old embryos using the same turkey LH antiserum (J.-M. Gasc & M. Sar, unpublished results). The presence of LH-producing cells in the anterior pituitary gland (before day 12 of incubation), reported here for the first time, is in agreement with the description of Mikami, Hashikawa & Farner (1973) of different secretory granules being present at 9 days and the demonstration by Jozsa, Scanes, Vigh & Mess (1979) of cells immunoreactive to prolactin and adrenocorticotrophin antisera in 7-day-old chick embryos. This suggests an early functional differentiation of the pituitary gland during embryonic life.

Androgen target cells do not appear to react with antisera to LH, TSH or ACTH as do the oestrogen target cells. The results reported here do not, therefore, provide evidence that androgen target cells are involved in any endocrine function of the anterior pituitary gland of the chicken embryo, at least until day 15 of incubation. In addition, since the immunoreactivity of androgen target cells towards other pituitary hormones has not been explored, involvement of these cells in other functions cannot be excluded.

A feedback regulation from the gonads directly on the secretion of gonadotrophin requires the presence of receptors for steroid hormones in the pituitary cells. The present results show that most of the oestrogen target cells, but not the DHT target cells, are cells producing LH. This suggests that oestrogen but not androgen is involved in the direct feedback regulation of secretion of gonadotrophin during embryonic life.
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REFERENCES


DESCRIPTION OF PLATES

PLATE 1
Oestrogen target cells in the pituitary gland of the 10- and 12-day-old chick embryo. Three micrometre sections stained with methyl green–pyronin; exposure time 8 months (× 1000).

Fig. 1. At day 10 of incubation, the caudal zone of the pituitary gland displays approximately 10% of cells that concentrate radioactive hormone in their nuclei after injection of [3H]oestradiol into the embryo.

Fig. 2. Conversely, in the cephalic zone, on the same section as Fig. 1, labelled cells are very sparse.

Fig. 3. Caudal zone of the pituitary gland of a 12-day-old embryo after injection of [3H]oestradiol alone (compare with Figs 4 and 5).

Fig. 4. After injection of [3H]oestradiol and a 100-fold excess of unlabelled oestradiol into a 12-day-old embryo, the concentration of labelled hormone in the nuclei is abolished in cells of the caudal zone.

Fig. 5. Conversely, after injection of [3H]oestradiol and a 100-fold excess of unlabelled dihydrotestosterone (DHT), the concentration of labelled hormone in the nuclei is similar to that in Fig. 3.

Fig. 6. Remnant of Rathke’s pouch displaying only rare cells labelled with [3H]oestradiol.

PLATE 2
Immunohocharacteristics of oestrogen and androgen target cells in the pituitary gland of the 12- and 15-day-old chick embryo. Three micrometre sections, stained by immunoperoxidase technique (see text) and counterstained by light green (× 1000).

Fig. 7. Oestrogen target cells, revealed on autoradiograph after injection of [3H]oestradiol into 12-day-old embryo, are immunoreactive to an antiserum to turkey LH.

Fig. 8. Androgen target cells, labelled with [3H]DHT are not immunoreactive to an antiserum to turkey LH.

Fig. 9. Androgen target cells, labelled with [3H]DHT are not immunoreactive to an antiserum to adreno-corticotrophin (1–24).