The bursa of Fabricius of the chicken embryo: localization and ontogenic evolution of sex-steroid target cells

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SUMMARY

Androgenic hormones induce inhibition or regression of the bursa of Fabricius in the chick embryo. The high doses of hormones necessary to this involution raises the question of the processes involved and their putative role in the normal development of the bursa. If androgens play a role it is mediated by receptor sites in target cells. Using an autoradiographic technique, receptor sites for androgenic hormones were localized in mesenchymal cells of the bursa from the primordium (7-day embryo) up to the fully differentiated immune organ (15-day embryo). No target cells containing receptor sites in their nuclei were observed in the endodermic epithelium or the follicles. Oestrogen target cells in very small number are found in the mesenchyme of the bursa, in 15-day embryos. The early presence of receptor sites for steroid hormones in the bursa of Fabricius shows that the normal development may be influenced by androgens, but the actual effects are yet to be demonstrated.

INTRODUCTION

The effects of sex-steroid hormones in embryos have been primarily described in relation to reproductive organs and secondary sex characters. The regression of the bursa of Fabricius after testosterone treatment is a noticeable exception. First reported by Kirkpatrick & Andrews (1944) in adult animals and then by Glick (1956, 1957) in embryos, the effects of androgenic hormones on this organ of the immune system depend on the time the treatment is applied. Testosterone (1–5 mg per egg) administered before formation of the primordium inhibits bursal differentiation (Glick & Sadler, 1961; Aspinall, Meyer & Rao, 1961), while after bursal formation it induces the loss of lymphopoietic stem cells, and eventually the complete involution of the bursa (Glick, 1957; Rao, Aspinall & Meyer, 1962). In both cases the chicks born from testosterone-treated eggs are bursa-less and, consequently, display very low levels of humoral

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In contrast to high doses of testosterone, low doses, in the range of 100 nanograms per egg, have stimulatory effects on the growth of the embryonic bursa (Norton & Wira, 1977). In view of this dual effect of testosterone, and also some similarity between testosterone-induced involution in embryos and natural regression in adults, the actual role of androgens in the normal development of the bursa remains controversial.

If androgenic hormones are to play a physiological role in the normal development of the embryonic bursa, their target cells can be characterized by the presence in their nuclei of receptor sites for this type of steroid. In a preliminary note, using an autoradiographic technique we described the localization of receptor sites for testosterone in the bursa of Fabricius of the 15-day chicken embryo (Gasc, Sar & Stumpf, 1979). Only mesenchymal cells contain receptor sites, and not the cells of the endodermic epithelium. More recently, Le Douarin, Michel & Baulieu (1980) reported that receptors for androgens can be biochemically prepared from both the epithelium and the mesenchyme. The same authors also reported that after testosterone treatment the endodermic epithelium loses its differentiation capacities, not the mesenchyme.

In the present article, using the same autoradiographic technique, we provide new evidence which confirms and substantiates our earlier observations. The distribution of cells labelled with radioactivity in their nuclei is compared after injection of \(^{3}H\)dihydrotestosterone or \(^{3}H\)oestradiol to the embryo. Dihydrotestosterone was used to avoid the possible aromatization of testosterone and thereby overcome the uncertainty about the actual hormone bound to the receptor sites. Homologous and heterologous unlabelled steroid hormones were used in competition with tritiated hormones to test the specificity of the receptor sites. A wider sample of developmental stages was examined to cover the main steps of bursal differentiation from the primordium to the formation of follicles containing lymphocytes in the process of differentiation, that is, from day 7 to 15 of incubation.

Two questions were especially examined: (a) the exact location of the target tissue for androgen hormones, either the mesenchyme only, or the mesenchyme and the epithelium; and (b) the precocity of the receptor sites suggested by the early effects of testosterone on the primordium of the bursa.

**Materials and Methods**

All technical details are described in the preceding article (Gasc & Stumpf, 1981). Briefly, embryos at day 7, 10, 12 and 15 of incubation were injected intravenously with \(^{3}H\)dihydrotestosterone (DHT) or \(^{3}H\)oestradiol (E\(_2\)). For
Target cells for sex steroids in bursa of Fabricius

competition experiments a 100-fold excess DHT, testosterone, oestradiol or progesterone was administered 30 minutes before the injection of radio-labelled hormone. After 1 h the cloacal part of the embryo was dissected, frozen in liquid propane and cut at $-35\,^{\circ}\mathrm{C}$. Sections, $3\,\mu\text{m}$ thick, were thaw-mounted on glass slides coated with NTB3 photographic emulsion (Kodak), and then stored in the dark for 7 to 14 months. After photographic processing, slides were stained with methyl green–pyronine.

RESULTS

Androgen target cells. A nuclear concentration of radioactivity is observed in mesenchymal cells of the bursa of Fabricius after injection of $[^3\text{H}]\text{DHT}$ to 7-, 10-, 12- and 15-day embryos. In 7- (Fig. 1A) and 10-day embryos the labelled cells are exclusively located in the mesenchyme directly adjacent to the endodermic epithelium. This mesenchyme is in direct continuity with the cloacal mesenchyme which also contains labelled cells in great number (Gasc & Stumpf, 1981). In 12- and 15-day embryos the labelled cells are also located in the mesenchyme but do not appear so closely associated to the epithelium or the follicles (Fig. 1B). Though DHT-labelled cells are still found in higher density near the epithelium and the follicles, they are also present throughout the interepithelial and interfollicular spaces. The bursa stalk which forms a continuous channel of communication between the cloaca and the bursa contains a particularly high density of labelled mesenchymal cells which may belong to either the bursa or the cloaca.

The staining procedure used, methyl green-pyronine, does not allow one to distinguish between the two types of cells present in the mesenchyme, mesenchymal cells and lymphopoietic stem cells. The early presence of labelled cells at an age when no lymphoid cells have yet colonized the primordium of the bursa rules out the possibility that only lymphoid cells have receptors for DHT. Besides, at day 12 and 15 there are too many labelled cells for them to be only lymphoid cells. As no labelled cells are observed within follicles or the endodermic epithelium, it would seem that the lymphoid cells do not possess androgen receptors. A 100-fold excess of DHT or testosterone injected prior to $[^3\text{H}]\text{DHT}$ abolishes the labelling in mesenchymal cells (Fig. 1C), while an excess of unlabelled oestradiol (Fig. 1D) or progesterone only slightly reduces the nuclear concentration of radioactivity in the 15-day bursa.

Oestrogen target cells. In contrast to androgen, oestrogen target cells do not appear early in the development of the bursa, and when they appear, remain always scarce. Only in 15-day embryo are $[^3\text{H}]\text{E}_2$-labelled cells observed in the mesenchyme, usually as single cells in the vicinity of the follicles (Fig. 1E). Very few of these cells are found on each section; accordingly their density is drastically lower than the density of $[^3\text{H}]\text{DHT}$-labelled cells. Epithelial and follicular cells do not concentrate $[^3\text{H}]\text{E}_2$. Unlabelled oestradiol in excess
injected prior to $[^{3}H]$E$_2$ abolishes the nuclear labelling in the mesenchymal cells, but not an excess of unlabelled DHT (Fig. 1E).

DISCUSSION

The present results confirm our first demonstration of the presence of nuclear receptor sites for testosterone in mesenchymal cells of the bursa of Fabricius of the 15-day chicken embryo (Gasc et al. 1979). Receptors for DHT display the same distribution as those for testosterone, and are competed for by unlabelled testosterone. This similarity between the 2 androgenic steroids suggests there is only one type of receptor for androgen hormones as is generally admitted. Although the limited competition exerted by oestradiol or progesterone on $[^{3}H]$-DHT labelling in 12- and 15-day embryos was not accurately estimated, it appears far less pronounced than that exerted by DHT or testosterone (Fig. 1C), thus attesting to the specificity of the receptors for androgenic hormones. Our experimental conditions do not allow us to measure validly to what extent heterologous hormones such as oestradiol and progesterone compete with DHT for androgen receptors. Our observations, however, constantly show a weaker nuclear labelling in embryos of the ‘heterologous competition’ groups than the ‘control’ groups.

As no competition experiments were carried out with 7- and 10-day embryos, we cannot extend the conclusion on the specificity of the receptors to the younger stages. However, the difference in distribution of the cells labelled with $[^{3}H]$E$_2$ or $[^{3}H]$DHT, in the bursa of Fabricius and also in the cloacal region (Gasc & Stumpf, 1981), strongly suggests that the receptors for either oestrogens or androgens appear early in the development with the same specificity properties they will display a few days later.

The presence of target cells for androgens in the bursa of Fabricius before

Fig. 1. Autoradiograms of the bursa of Fabricius after injection of either $[^{3}H]$oestradiol or $[^{3}H]$dihydrotestosterone.

In 7-day embryos (A) the epithelial primordium of the bursa is surrounded by mesenchymal cells that concentrate $[^{3}H]$DHT in their nuclei. Comparatively, epithelial cells display only a background labelling. In 15-day embryos (B) cells showing nuclear concentration of $[^{3}H]$DHT are exclusively located in the mesenchyme and not in the endodermic epithelium or the follicles. The specificity of the nuclear receptor sites for androgen hormones is attested to by the competition exerted by an excess of unlabelled DHT (not shown) or testosterone (C) while an excess of oestradiol only slightly diminishes the average labelling in bursal mesenchyme (D). In 15-day embryos that received $[^{3}H]$E$_2$ and a 100-time excess of unlabelled DHT only scarce cells (arrows) concentrate the oestrogen hormone (E), thus showing the presence of receptor sites for oestrogen hormones in a few rare mesenchymal cells.

Unfixed frozen sections stained with methyl green–pyronine. Exposure time: A, 11 months; B, C and D, 7 months; E, 14 months. Magnification: A, ×1700; B, C, D and E, ×1100. Abbreviations: e, endodermal epithelium; f, follicle; m, mesenchyme.
7 days of incubation and throughout embryonic development is consistent with the finding that androgen receptors can be biochemically prepared from the bursa during the same period (Le Douarin et al. 1980). However, Le Douarin et al. found receptors also in the epithelium after removal of the mesenchyme, whereas we observe a nuclear concentration of DHT only in the mesenchyme. As no particular concentration of radioactivity is observed in the cytoplasm of the epithelium in the autoradiograms, the discrepancy between the two techniques cannot be accounted for by a difference in location of the receptors inside the cells. Also intriguing is the fact that the epithelium, but not the mesenchyme, loses its differentiating capacities after treatment with high doses of testosterone (Le Douarin et al. 1980). A possible interpretation is that androgen hormones have two distinct effects on the embryonic bursa. At physiological concentration they exert an effect after binding to the receptor sites in the mesenchyme. The actual effect at this level has been little studied and remains unclear but might be an enhancement of growth as proposed by Norton & Wira (1977). The results of administering larger doses, which have received more attention, are irreversible damage to the developmental capabilities of the epithelium which then becomes incapable of housing the lymphoid cells and degenerates. This scheme may account for what occurs both in normal development and under experimental conditions. It does not, however, offer any explanation for the effects of other steroids, specially oestradiol, on the differentiation of the bursa (Erickson & Pincus, 1966; Norton & Wira, 1977). Concerning these weak inhibitory effects one may think that they are mediated by the androgen receptors which show a low affinity for oestradiol in our competition experiment as well as in biochemical studies (Le Douarin et al. 1980). The very few oestrogen target cells observed in the bursa at day 15 may also be involved, although their small number and late appearance does not lend support for a critical role during embryonic life. In our earlier studies no cells labelled with $[^3H]E_2$ were observed. The present use of $[^3H]E_2$ at a lower dose and higher specific activity probably accounts for the difference.

Our observations suggest that androgen hormones have a role in the normal development of the embryonic bursa at physiological levels, and this effect is mediated through androgen receptors in the mesenchyme. Since very high doses of testosterone are necessary to induce regression of the bursa, we submit that mechanisms other than physiological interactions are involved in these processes. This distinction between the effects of low and high levels of hormones, and the possible cross-reaction between different steroid hormones and receptors are requisite considerations for further studies of the relationship between steroids and the development of the bursa of Fabricius.

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