Journal of Steroid Biochemistry, 1977, Vol. 8, pp. 985–987. Pergamon Press Printed in Great Britain

DOSE-RELATED EFFECTS OF THE SEX HORMONES AND CORTISOL ON THE GROWTH OF THE BURSA OF FABRICIUS IN CHICK EMBRYOS

JAMES M. NORTON and CHARLES R. WIRA*†
Department of Physiology, Dartmouth Medical School, Hanover, NH 03755, U.S.A.

(*Received* 21 *January* 1977)

SUMMARY

The administration of a single dose of either testosterone propionate progesterone, estradiol, or cortisol *in vivo* to developing chick embryos on the fifth day of incubation significantly alters the size of the bursa of Fabricius measured on day 19. Depending on dose, testosterone propionate or progesterone can have either stimulatory or inhibitory effects on bursal growth. When low doses were administered (0.02–2.0 μg), comparable to what the chick embryo might be exposed to during development, both hormones were stimulatory; at higher levels (20–2000 μg), both either partially or entirely inhibited bursal growth. In contrast, estradiol and cortisol were found to have no stimulatory effects at low levels, but markedly inhibited bursal growth at higher concentrations (20–2000 μg/egg). The results of this study suggest that the steroid hormones play an important role in the growth of the bursa of Fabricius in developing chick embryos. This phenomenon may have bearing on subsequent immunological responsiveness.

Numerous investigators have examined the role of steroids in the development of the bursa of Fabricius in chickens and observed inhibitory effects [1–4]. The most pronounced of these effects results from pretreatment with androgens such as testosterone and 19 nor-testosterone. Administration of these steroids to eggs during the fifth day of incubation produces chicks that at hatch have bursae that are either markedly reduced in size or completely absent. The steroid-induced involution of this organ in turn alters normal humoral immune development by interfering with lymphocyte differentiation and the synthesis of both antibodies and immunoglobulins [2–7]. Development of this hindgut lymphoepithelial organ is also inhibited by high levels of several estrogens, progestins and glucocorticoids [8, 9].

Some studies, however, suggest that steroids may have stimulating effects on the bursa. Glucocorticoids, for example, mobilize bursal lymphocytes and enhance their differentiation into mature, antibody-secreting plasma cells [10]. The data presented by Erickson and Pincus[8] are not statistically significant, but certain differences between groups of data suggest that progesterone at lower levels might be stimulatory. In view of the potential importance of steroid hormones in the humoral immune system and of the unclear status of information now available, we have undertaken a study to clarify the nature of the action of the individual steroid hormones when administered at doses comparable to what the developing chick might be exposed to during embryological development.

Eggs containing viable 5 day embryos were injected with either testosterone propionate, progesterone, estradiol or cortisol. In the initial experiments, steroids were dissolved in sesame oil but when it was realized that this vehicle had inhibitory effects on both bursa and embryo size, subsequent experiments were carried out with steroids dissolved in sterile saline. Data from experiments in which both vehicles were used are included in Fig. 1 and Table 1.

As shown in Fig. 1(a), the 20–200 ng dose of testosterone propionate increased bursal growth by about 25% relative to controls. This stimulatory effect occurred without a concomitant increase in embryo weight, as indicated in Table 1, suggesting that the effect of testosterone propionate was selective for bursal development. In contrast, the higher dose of testosterone propionate (200 μg) inhibited bursal weight by 60%. At 0.6–2.0 mg, not shown in the figure, total inhibition of bursal development was observed.

The molar concentration of steroid in each egg, calculated by assuming that the steroid is distributed evenly throughout the egg (approximate vol. 50 ml), is approximately 1.0 nM for a 20 ng injection. This concentration of testosterone is very close to the physiological range reported in the plasma of both male and female chick embryos between 5 and 17.5 days of incubation [11].

With progesterone, (Fig. 1(b)), a marked inhibition of bursal growth was observed at the 200 μg level.

* To whom correspondence should be sent.

† Supported in part by Research Grant AM 03535, CA 17323 and AI 13541 from the National Institutes of Health, U.S.P.H.S.

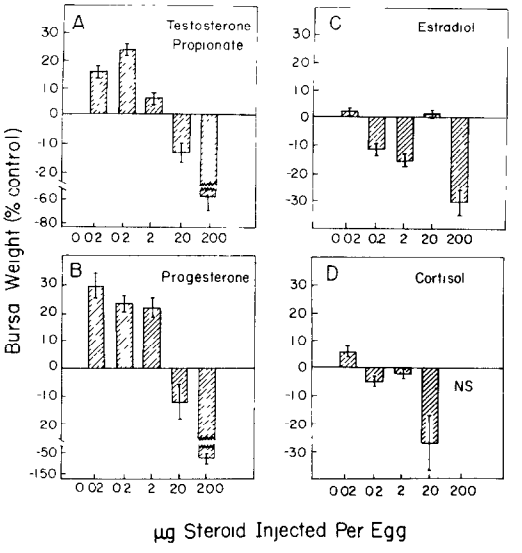

Fig. 1. Influence of steroids on the growth of the bursa of Fabricius in developing chick embryos. White Leghorn eggs purchased from SPAFAS, Inc., Norwich, CT, with viable 5 day embryos were pierced on the broad end with an egg punch and 0.1 ml of a sterile saline (0.02–2.0 μg/egg) or sesame oil 20–200 μg/egg) solution of either testosterone propionate (1(a)), progesterone (1(b)), estradiol (1(c)), or cortisol (1(d)) was injected with a sterile tuberculin syringe. Holes were sealed with two or three drops of liquid wax and eggs were placed in an incubator (100 F). Fourteen days later (day 19 embryo age) chicks were removed from their eggs, decapitated, and bursal weights were determined. Results are expressed as % of control ± SE (10–15 eggs/group) which received only sterile saline or sesame oil (NS = no survivors). Estimates of standard error were calculated as described in Meyer, S. L. *Data Analysis for Scientists and Engineers*. Wiley, New York (1975) pp. 40.

This inhibition was accompanied by a general inhibitory effect of progesterone on embryo development. As seen in Table 1, progesterone at 200 μg reduced body weight in surviving embryos to 61% of the control group. At levels of 200 μg and higher embryo survival was markedly reduced. With lower levels, as shown in Fig. 1(b), (2.0–0.02 μg) bursal weight increased 20–30%. As with testosterone, progesterone in low doses appears to affect the weight of the bursa and not of the embryo.

Estradiol and cortisol also had marked inhibitory effects at higher levels but did not stimulate bursal growth at 0.02–2.0 μg (Fig. 1(c) and (d)). Cortisol was the most embryotoxic of the steroids studied; at the 200 μg dose, no embryos survived, and upon examination all appeared to have stopped developing around the fifth day of incubation. Decreased survival rate of embryos, as well as a reduction in total body and bursal weight, was noted with the 2 and 20 μg range.

As a part of the bursal weight experiments, the histology of representative bursae was examined. No increase in the number of epithelial cell layers surrounding the lymphoid follicles was observed when bursae taken from birds exposed to low dose testosterone propionate or progesterone (0.2–2.0 μg) were compared to controls. This finding indicates that the increase in bursal weight following steroid exposure was due to an increase in the number of lymphocytes in the gland and not to an increase in epithelial cells.

The stimulation of bursal growth by testosterone propionate and progesterone is of particular interest in light of reports that the gonads in chick embryos from the 3rd to 18th day of development synthesize testosterone, estradiol and progesterone. These steroids were identified in ether extracts of both the culture media and the homogenized organs after 5–24 hr incubation [12, 13]. In a separate study that substantiates these findings, testosterone was identified in the circulation of both male and female embryos on day 5.5 of incubation [11]. These studies demonstrate that the developing bursa is exposed *in vivo* to steroid hormones at concentrations comparable to the low doses of exogenous testosterone propionate and progesterone that we observed to stimulate bursal growth. This would suggest that the endogenous steroids in the embryo play an important physiological role in bursal growth. While it is true that our findings do not constitute evidence that testosterone and progesterone modulate bursal development, they do nevertheless support such a view.

An influence of sex steroids on immunoglobulin production is implied by a recent study showing that chicks exposed to testosterone propionate *in ovo* on the third day of incubation, have higher serum IgM and IgG levels than controls at the time of hatch. These levels continued to rise during the 13 weeks after hatching [21]. The data suggest that early hormone exposure influences antibody production. Whether these changes in immunoglobulin production are the result of changes in bursal size that we have observed remains to be established.

There is evidence that bursal size is related to humoral immunity. Geneological lines of chickens with either large or small bursae at hatch exhibit different patterns of antibody production after antigen exposure [13]. When small-bursa chicks, bursectomized at the time of hatching, are exposed to red blood cells of sheep they fail to produce antibody for at least 5 weeks. In contrast, bursectomy of large-bursa chicks had only a limited and short-term effect on their immune response to sheep erythrocytes. In other studies bursal size has been related to immunoglobulin levels [5, 15]. The findings in this study suggest that sex hormone stimulation of bursal growth might be the mechanism whereby steroids enhance immunoglobulin and production.

There are several possible explanations for the steroid-stimulated increase in bursal weight. One is that both testosterone propionate and progesterone have direct effects on the epithelial cells of the bursa, as has been previously proposed in the case of testosterone [16]. Since these are the only cells present prior to lymphocyte infiltration at about day 13 of incubation, they might be stimulated to prepare a microenvironment more conducive to lymphocyte growth and differentiation. An alternative explanation of in-

Table 1. The effect of different steroid treatments on embryo weight

Dose (μg)		Progestesterone	Estradiol	Testosterone propionate	Cortisol
0.02	$\bar{x}$	26.227(13/15)	26.756(10/15)	29.251(10/15)	29.519(9/15)
(saline)	S.E.	±0.568	±0.614	±0.275	±0.733
	%C	101.226	97.456	102.976	101.301
	P	N.D.	N.D.	N.D.	N.D.
0.20	$\bar{x}$	26.723(8/15)	26.724(9/15)	28.259(14/15)	28.410(11/15)
(saline)	S.E.	±0.275	±0.972	±0.590	±0.909
	%C	103.14	97.340	99.483	97.498
	P	N.D.	N.D.	N.D.	N.D.
2.00	$\bar{x}$	26.55(11/15)	27.748(14/15)	27.026(12/15)	27.576(4/15)
(saline)	S.E.	±0.516	±0.609	±0.701	±1.384
	%C	102.478	101.072	95.143	94.634
	P	N.D.	N.D.	N.D.	N.D.
20.0	$\bar{x}$	18.171(7/10)	24.158(6/10)	22.798(7/10)	17.038(2/10)
(oil)	S.E.	±1.771	±0.896	±1.749	±2.433
	%C	105.706	90.969	110.247	79.912
	P	N.D.	N.D.	N.D.	—
200	$\bar{x}$	10.478(2/10)	23.620(5/10)	21.432(2/10)	0 (0/10)
(oil)	S.E.	±1.553	±1.517	±3.029	0
	%C	61.070	88.940	103.639	0
	P	—	N.D.	—	—

After *in vivo* exposure to testosterone propionate, progesterone, estradiol, and cortisol dissolved in either saline or sesame oil (see Fig. 1 for doses, injection route, etc.) and removal of yolk sacs, 19 day embryos were weighed (10–15 embryos/group). Results are presented as follows: the value of the day 19 mean embryo weights ± the standard error ($\bar{x}$ ± S.E.), the ratio of the number of surviving embryos in each group to the number present at the start of incubation (/), the ratio of the mean embryo weight of the hormone treated group to the mean embryo weight of the vehicle-injected control group expressed as percent control (%C), and the significance of the difference (*P*) between the mean embryo weights of the hormone-treated and control groups as based on the Student *t*-test analysis (N.D. = no significant difference).

creased bursal size is that testosterone and progesterone directly affect lymphoid cells. This possibility is suggested by studies indicating that lymphoid cells in mammalian systems remain under hormone influence after these cells leave the bursa and proliferate actively in the germinal centers of peripheral lymphoid organs [10, 17]. Enhanced immune responsiveness due to steroid treatment has been reported in some studies [18, 19] while in others chronically administered hormones appear to be deleterious [20]. Further studies must be undertaken to determine which of these possibilities is responsible for steroid hormone stimulation of bursal growth.

The authors wish to express appreciation to Ms. Patricia Bray for technical assistance during this study and to Mrs. Carola Phelan Sandoe for cooperation in the preparation of this manuscript.

REFERENCES

1. Ackerman G. A. and Knouff R. A.: *Anat. Rec.* **146** (1963) 23–27.
2. Cooper M. D., Peterson R. D., South M. A. and Good R. A.: *J. exp. Med.* **123** (1966) 75–102.
3. Dieter M. and Breitenbach R., *Proc. Soc. exp. Biol. Med.* **133** (1970) 357–364.
4. Erickson A. and Pincus G.: *J. Embryol. exp. Morph.* **16** (1966) 211–229.
5. Glick B.: *Poultry Sci.* **35** (1956) 843–851.
6. Glick B., Chang T. and Jaap R.: *Poultry Sci.* **35** (1956) 224–226.
7. Glick B. and Sadler C. R.: *Poultry Sci.* **40** (1961) 185–189.
8. Guichard A., Scheib D., Haffen K. and Cedard L.: *V. Intern. Cong. Endocrinol.* Hamburg, Fed. Rep. Germany (1976) pp. 279 ABS.
9. Isakovic K., Jankovic B. D., Popeskovic L., and Milosevic D.: *Nature* **200** (1963) 273–274.
10. Joshi U. M., Rao S. S., Kora S. J., Dikshit S. S. and Virkar K. D.: *Contraception* **3** (1971) 327–333.
11. Kenney J. F. and Gray J. A.: *Pediatr. Res.* **5** (1971) 246–255.
12. Kenney J. F., Pangburn P. C. and Trail G.: *Infect. Immunol.* **13** (1976) 448–456.
13. Landreth K. D. and Glick B.: *Proc. Soc. exp. Biol. Med.* **144** (1973) 501–505.
14. Meyer R. K., Rao M. A. and Aspinall R. L.: *Endocrinology* **64** (1959) 890–897.
15. Mueller A. P., Wolfe H. R. and Meyer R. K.: *J. Immunol.* **85** (1960) 172–179.
16. Nicol T., Bilbey D. L., Charles L. M., Cordingley J. L. and Vernon-Roberts B.: *J. Endocr.* **30** (1964) 277–291.
17. Rangneker K. N., Joshi U. M. and Rao S. S.: *Contraception* **5** (1972) 53–56.
18. Rusu V. and Cooper M.: *J. Immunol.* **115** (1975) 1370–1374.
19. Warner N. L., Szenberg A. and Burnet F. M.: *Aust. J. exp. Biol.* **40** (1962) 373–387.
20. Woods J. E., Simpson R. M. and Moore P. L.: *Gen. comp. Endocr.* **24** (1974) 413–423.
21. Glick B., Subba Rao D. S. V. and McDuffie F. C. *Fed. Am. Soc. exp. Biol.* **35** (1976) 278.