J. steroid Biochem. Vol. 35, No. 5, pp. 607–609, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

ESTRADIOL SECRETION BY THE OVARY OF 19-DAY-OLD HYPOPHYSECTOMIZED AND SHAM-OPERATED CHICK EMBRYOS

J.-P. WENIGER,* J. CHOURAQUI and A. ZEIS
Laboratoire de Zoologie et d'Embryologie Expérimentale de l'Université Louis-Pasteur, 12, rue de l'Université, F-67000 Strasbourg, France

(*Received* 31 *July* 1989; *received for publication* 17 *November* 1989)

Summary—The amounts of estradiol released into culture media by ovaries from 19-day-old hypophysectomized (decapitated) and sham-operated chick embryos were determined by RIA. Per single ovary, ovaries from decapitated embryos secreted slightly less estradiol than ovaries from sham-operated ones during a 4-h culture period (837 ± 413 vs 935 ± 378 pg), but this difference was not significant. On an ovarian weight basis, ovaries from decapitated embryos secreted slightly more estradiol than ovaries from sham-operated ones (1.15 vs 0.91 ng), but this difference was not significant either. It is concluded from these results that the hypophysis does not control estradiol secretion by the ovary in the 19-day-old chick embryo.

INTRODUCTION

Whether or not the hypophysis controls ovarian estrogen secretion in the chick embryo has been much debated. Woods and Weeks[1] observed a reduction by one half in histochemically demonstrable Δ_5-3β-hydroxysteroid dehydrogenase activity in ovaries of chick embryos hypophysectomized by means of partial decapitation at 36 h of incubation. This reduction in Δ_5-3β-HSD activity was first observed on day 13.5 and then throughout the whole incubation interval of 14.5–19.5 days. These observations were interpreted as constituting evidence that the hypophysis exerts an effect on Δ_5-3β-HSD activity, and thus on steroid hormone synthesis, in the ovary of the chick embryo from 13.5 days of incubation. However, Akram *et al.*[2] and Akram and Weniger[3] did not notice any difference in the production of estrone and estradiol from ^{14}C-labelled sodium acetate, whether the cultured ovaries were from 17.5-day-old normal or hypophysectomized chick embryos. Woods and Erton[4] interpreted the initial appearance of immunocytochemically demonstrable estrone and estradiol in the cortical cords and cortical interstitial cells of the normal left ovary on day 13.5 of incubation as indicating that estrogen synthesis at these cellular sites comes under the control of hypophysial gonadotrophins at this time. Woods and Brazzill[5] suggested that the elevated plasma estradiol levels in the normal female chick embryo from day 13.5 on reflect an increase in ovarian estradiol secretion in response to an enhanced secretion of LH. However, Weniger and Zeis[6] expressed a different view. Since ovaries from intact and hypophysectomized 16-day-old chick embryos released identical amounts of estradiol during a 24-h culture period, they concluded that the hypophysis does not control ovarian estrogen secretion, at least not before 16 days of incubation. In the present study, we compare the production of estradiol by ovaries from hypophysectomized and sham-operated 19-day-old chick embryos in organ culture.

EXPERIMENTAL

In a typical experimental series, 60 fertilized white Leghorn eggs were incubated at 38°C for 42 h. After this time, embryos had reached the stage of 11–14 somites. Three fourths were hypophysectomized by the partial decapitation method of Fugo[7], the remainder being sham-operated. Sham-operated embryos had the tip of the prosencephalon severed, while decapitation consisted of a transverse cut through the neural tube at mid-mesencephalon, both presumptive anlagen of the hypophysis, i.e. the infundibulum and Rathke's pouch, being suppressed in this way. Partial decapitation is an acknowledged means of hypophysectomy [7, 8]. Absence of the upper beak and eyes warrants total hypophysectomy.

Embryos surviving after 17 more days were killed, the left ovary was taken from the females and cut into 6–7 slices which were cultured in a plastic Petri dish in 0.7 ml of Medium 199 at 37°C for 4 h. After this 4-h culture period, media were collected and kept at −20°C until the time of analysis.

The amounts of estradiol released into the culture media were determined by direct RIA. The radioactive antigen used was [2,4,6,7-^{3}H]estradiol (C.E.A.,

*To whom correspondence should be addressed.

Table 1. Dry weight of ovary from decapitated and sham-operated 19-day-old chick embryos and amounts of estradiol (E_2) released into culture media

	Decapitated ($n = 22$)	Sham-operated ($n = 34$)	Significance
Amount of E_2/20 μl	23.9 ± 11.8 pg	26.7 ± 10.8 pg	NS
Weight of ovary	0.518 ± 0.195 mg	0.735 ± 0.210 mg	$P < 0.001$
Correlation coefficient	0.17	0.44	
Amount of E_2/mg of ovary	46.1 pg	36.3 pg	NS

Values are means ± SD. Explanations in the text.

Gif-sur-Yvette; SA: 100 Ci/mmol). The antiserum, a gift from Roussel-Uclaf (Romainville), was toward 7-carboxymethyloxime estradiol-bovine serum albumin; it was used at a final working dilution of 1/250,000 (1 ml). Free estradiol was removed with a charcoal-dextran mixture. The standard curve was constructed with triplicate samples between 2 and 120 pg/tube, and 20 μl volumes of culture media were analyzed in duplicate. At this dilution, the culture medium did not displace the standard curve. Quantities of estradiol found were the same with and without extraction and known amounts of estradiol added to the culture medium were found in full. The sensitivity of the assay was 2 pg/tube. The coefficient of intra-assay variation was 9.2% and that of inter-assay variation 15.5%.

The ovarian slices were dried and weighed on a microbalance (sensitivity: 1 μg). The significance of the difference between means was tested by analysis of variance and covariance.

RESULTS

Mortality was highest on the first 2 days after the operation, when nearly one third of the decapitated embryos died. Out of 411 decapitated embryos, 41 survived after 19 days of incubation (19♂ and 22♀), and out of 148 sham-operated embryos, 69 survived (35♂ and 34♀). Decapitated embryos lacked both eyes and the upper beak. In a few cases, even the lower beak was missing. Sham-operated embryos had the top of the skull and the most anterior part of the forebrain missing. In a few cases, one eye had been resected, but on examination the hypophysial area proved intact. On an average, decapitated embryos had a slightly smaller size than sham-operated ones.

Table 1 gives uncorrected values, i.e. the quantities of estradiol found in 20 μl of culture medium. It is seen that ovaries from decapitated and sham-operated embryos did not produce significantly different amounts of estradiol. In contrast, the difference between mean ovarian weights was highly significant. Although the size of the ovary varies greatly even in normal chick embryos, when estradiol production was referred to ovarian dry weight, the calculated correlation coefficient (line 3) indicated that a correlation existed between the amount of estradiol secreted and the mass of the ovary in the case of the sham-operated embryos ($P = 0.01$), but not in that of the decapitated ones ($P > 0.10$). Analysis of covariance showed that the difference in estradiol secretion per unit weight of ovary between decapitated and sham-operated embryos was not significant.

DISCUSSION

The aim of the present study was to compare the secretion of estradiol by ovaries of 19-day-old hypophysectomized and sham-operated chick embryos. There was no significant difference in estradiol production between both kinds of ovaries, neither per single ovary nor on an ovarian weight basis. However, it seems worth mentioning that the amount of estradiol released per mg of ovarian tissue was greater in the decapitated than in the sham-operated embryos. It is known that cortical development is reduced in ovaries of decapitated embryos, while the medulla develops normally [7]. Since the medulla contains the estrogen-secreting cells [9–12], the relative importance of the steroidogenic tissue increases in the ovary of decapitated embryos. It is concluded from the present investigation that the hypophysis does not control estradiol secretion by the ovary even in the 19-day-old chick embryo, i.e. 2 days before hatching. This conclusion is in agreement with the conclusions of previous studies performed at 16–17 days of incubation [2, 3, 6], while it is at variance with the long expressed view of Woods and co-workers[1, 4, 5] that estrogen secretion is under pituitary control from 13.5 days of incubation.

REFERENCES

1. Woods J. E. and Weeks R. L.: Ontogenesis of the pituitary-gonadal axis in the chick embryo. *Gen. Comp. Endocr.* **13** (1969) 242–254.
2. Akram H., Zeis A. and Weniger J.-P.: Biosynthèse d'oestrogènes par l'ovaire de l'embryon de Poulet hypophysectomisé: aspect quantitatif. *C.R. Acad. Sci. Paris* **276** Série D (1973) 359–361.
3. Akram H. and Weniger J.-P.: L'hypophyse est sans influence sur la synthèse d'oestrogènes chez l'embryon de Poulet. *C.R. Acad. Sci. Paris* **278** Série D (1974) 2669–2670.
4. Woods J. E. and Erton L. H.: The synthesis of estrogens in the gonads of the chick embryo. *Gen. Comp. Endocr.* **36** (1978) 360–370.
5. Woods J. E. and Brazzill D. M.: Plasma 17β-estradiol levels in the chick embryo. *Gen. Comp. Endocr.* **44** (1981) 37–43.

6. Weniger J.-P. and Zeis A.: 17β-estradiol secretion by the ovary of the hypophysectomized chick embryo. *Gen. Comp. Endocr.* **65** (1987) 9–11.
7. Fugo N. W.: Effects of hypophysectomy in the chick embryo. *J. Exp. Zool.* **85** (1940) 271–297.
8. Betz T. W.: Partial decapitation of chicken embryos. *Acta Endocr. (Copenh.)* (Suppl. 198) (1975) 1–28.
9. Mintz B. and Wolff E.: The development of embryonic chick ovarian medulla and its feminizing action in intracoelomic grafts. *J. Exp. Zool.* **126** (1954) 511–536.
10. De Simone-Santoro I.: Morphologie ultrastructurale de l'ovaire de l'embryon de Poulet au cours de sa différenciation et de son organogenèse. *J. Microscopie* **8** (1969) 739–752.
11. Weniger J.-P.: Recherches sur la nature chimique des hormones sexuelles embryonnaires de Poulet. *Ann. Embr. Morph.* **2** (1969) 433-444.
12. Budras K. D. and Preuss F.: Elektronenmikroskopische Untersuchungen zur embryonalen und postembryonalen Genese der Eierstockzwischenzellen des Haushuhns. *Z. Zellforsch.* **136** (1973) 59–83.