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Original article

17ß-estradiol secretion in normal and hypophysectomized chick embryos

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Summary — Ovaries from decapitated, sham-operated and intact 18-day-old chick embryos were cultured in Medium 199 for 6 h, and the amount of 17ß-estradiol released into the culture media was determined by radioimmunoassay. Ovaries from decapitated embryos secreted significantly lower amounts of 17ß-estradiol than those from intact embryos, but there was no difference when they were compared to ovaries from sham-operated embryos in this respect. On an ovarian weight basis, 17ß-œstradiol production was significantly different between the 3 groups of embryos, the ratio being highest in the decapitates. 17ß-Estradiol concentration was the same in serum from both decapitated and intact female embryos.

Considering these results, it is concluded that the hypophysis does not control 17ß-estradiol secretion by the chick embryo ovary even near hatching time.

17ß-estradiol — hypophysectomy — chick embryo ovary

Résumé — Sécrétion d'estradiol-17ß par l'embryon de poulet normal ou hypophysectomisé. *La présente étude concerne la sécrétion d'estradiol-17ß par l'ovaire de l'embryon de poulet de 18 jours, normal, hypophysectomisé ou simplement décalotté. Les ovaires ont été cultivés pendant 6 h sur le milieu 199, après quoi l'estradiol-17ß y a été dosé par radioimmunologie. La sécrétion d'estradiol-17ß des ovaires et des embryons décapités était plus faible que celle des ovaires des embryons normaux, mais ne différait pas de celle des ovaires des embryons décalottés. Rapportée au poids de l'ovaire, la production d'estradiol-17ß était significativement différente dans les 3 groupes d'embryons, le rapport étant le plus élevé chez les décapités. La concentration sérique d'estradiol-17ß était la même chez les embryons femelles décapités et normaux.*

Ces résultats conduisent à la conclusion que l'hypophyse n'intervient pas dans la sécrétion d'estradiol-17ß par l'ovaire de l'embryon de poulet, même à 3 jours de l'éclosion.

estradiol-17ß — hypophysectomie — ovaire embryonnaire de poulet

Introduction

Opposite views are held concerning a possible role of the hypophysis in estrogen secretion by the chick embryo ovary. According to Woods and Brazzill (1981) and Woods and Thommes (1984), 17ß-estradiol secretion is under pituitary

control from 13 days of incubation, whereas in the opinion of Weniger and Zeis (1987) this was not the case even at 17 days, when the amount of 17ß-estradiol released into the culture medium was not significantly different, whether the ovaries were from normal or decapitated embryos. Although mortality of decapitated embryos is high near hatching time, it was decided to try to gain one more day. The present study compares 17ß-estradiol production by ovaries from 18-day-old intact, decapitated and sham-operated embryos in organ culture. In addition, serum 17ß-estradiol levels were determined in the 3 sorts of embryos.

Materials and methods

Embryos of the white Leghorn strain were used. After 36 h of incubation (8—13 pairs of somites), 2 ml of albumin were withdrawn from all the eggs, and an opening was made in the shell. Some of the embryos were decapitated, others were sham-operated and still others were left intact. The opening was reclosed with a piece of scotch tape, and all the eggs were returned to the incubator for a further 17 days.

Partial decapitation is an acknowledged means of hypophysectomy (Fugo, 1940; Betz, 1975). It consists of removing the anterior part of the head by sectioning transversally the embryonic brain at the level of the constriction which separates the mesencephalon from the rhombencephalon. In this way, both anlagen of the hypophysis, *i.e.* the infundibulum and Rathke's pouch, are removed. Sham-operated embryos were those from which only the tip of the prosencephalon was taken. Embryos in eggs which were just windowed after albumin withdrawal were considered intact.

After 18 days of incubation, blood was collected from the surviving embryos through an incision made in superficial veins or arteries of the chorio-allantoic membrane. Blood was only taken from embryos whose chorio-allantoic membrane was dry, in order to avoid its being soiled with amniotic or allantoic fluid. No more than 0.05—0.35 ml of blood could be obtained per embryo, and so sera had to be pooled to obtain the volume of 0.5 ml necessary for radioimmunoassay.

After blood withdrawal, the embryos were killed. Males were discarded, and the left ovary was removed from the females. It was cut into 5—6 pieces, which were cultured in 0.7 ml of Medium 199 (Eurobio, Paris) at 38°C for 6 h in an atmosphere enriched with O_2 and CO_2.

At the end of the 6-h culture period, each medium was collected separately, and after 5-fold dilution with phosphate buffer (pH 7.4), 2 100-µl samples were taken for direct radioimmunoassay of 17ß-estradiol. [2,4,6,7-^{3}H] Estradiol was supplied by C.E.A. (Gif-sur-Yvette) and its radiochemical purity was ≥ 99%. The antiserum was a gift from Roussel-Uclaf (Romainville); it was directed towards 7-carboxy-methyloxime estradiol—bovine serum albumin, and was used at a final working dilution of 1/250,000 (1 ml). Free estradiol was removed with a charcoal—dextran mixture. The sensitivity of the assay was 2 pg/tube. The within and between assay percent coefficients of variation were 9.2% and 15.5%, respectively. The ovarian pieces were dried and weighed on an ultramicrobalance.

For the determination of serum 17ß-estradiol concentrations, 0.5 ml of pooled sera (from 2 to 9 embryos) were diluted by half with water and extracted with 2 ml of isooctane—ethyl acetate 7.3 (v/v). The aqueous phase was frozen, and the organic phase was decanted and evaporated. The residue was dissolved in petroleum ether, which was extracted with 70% methanol. The petroleum ether phase, which retained most of the lipids, was discarded by aspiration, while the methanolic phase, which contained the steroids, was evaporated to dryness. The residue was dissolved in 0.6 ml of phosphate buffer, and 2—0.3-ml samples were taken for radioimmunoassay of 17ß-estradiol.

Statistical analysis

Means were compared using analysis of variance. The test of independence between 2 quantitative variables was applied to determine whether a correlation existed between the amount of 17ß-estradiol secreted and the weight of the ovary in each group of embryos (Schwartz, 1963). Analysis of covariance was used to compare 17ß-estradiol secretion between the 3 groups of embryos on an ovarian weight basis (Lellouch and Lazar, 1974).

Results

Four hundred and seventy-four embryos were decapitated. Mortality was highest on the first 2 days after the operation, when about half of the embryos died. Fifty-eight embryos survived after 18.5 days of incubation : they were then killed. They had only a lower beak, or a stump of it, or no beak at all. In all cases, the absence of upper beak and eyes warranted total hypophysectomy. There were 30 males and 28 females. Out of 113 intact embryos, sixty-four survived after 18.5 days, and out of 111 sham-operated embryos, 58 survived. There were 26 and 33 females, respectively.

As a general rule, the decapitated embryos were smaller than the non-operated and sham-operated ones, the size of which was intermediate between those of the decapitated and the non-operated embryos. Coelosomic embryos or those bearing other malformations were rare. They were discarded.

17ß-Estradiol in culture media

Since pilot experiments had shown that after a 5-fold dilution of the culture medium the amount of 17ß-estradiol found did not differ whether extraction was performed or not, the assay was carried out on unextracted media. Values shown in Table I are uncorrected and represent the amount of 17ß-estradiol in 20 µl of culture medium. This amount was significantly lower in media of ovaries from decapitated embryos in comparison with the amount found in media of ovaries from intact embryos, but did not differ from that found in media of ovaries from sham-operated embryos.

As already mentioned, intact embryos were best developed, and, consequently, the dry weight of their ovary was highest (Table I). In one and the same group of embryos, the amount of 17ß-estradiol released and the weight of the ovary were not correlated. However, between the groups, the production of 17ß-estradiol

Table I. Amount of 17ß-estradiol (E_2) released into culture media by ovaries from decapitated (A), sham-operated (B) and intact (C) 18-day-old chick embryos, ovarian dry weight, E_2/weight ratio and serum 17ß-estradiol concentration.

	Decapitated (A)	*Sham-operated (B)*	*Intact (C)*
17ß-estradiol in culture medium (pg)	27 ± 8 (n = 28)	27 ± 9 (n = 26) A/B : NS	33 ± 12 (n = 33) A/C : P = 0.03
Weight of ovary (mg)	0.55 ± 0.13 (n = 23)	0.74 ± 0.26 (n = 19) A/B : P < 0.001	0.84 ± 0.18 (n = 31) A/C : P < 0.001
E_2/weight (pg/mg)	49	37	39
Serum 17ß-estradiol concentration (pg/250 µl)	9.4 ± 2.6 (n = 7)	6.6 ± 3.2 (n = 3)	10.5 ± 3.8 (n = 8) A/C : NS

Values are mean ± standard deviation.
NS : difference statistically not significant.

per unit weight of ovary was significantly different, as shown by analysis of covariance.

17ß-Estradiol in serum

Pilot experiments showed that under the conditions used 96.7 ± 2.8% (n = 4) of a tracer amount of ^{3}H 17ß-estradiol added to 0.5 ml of serum was extracted in the isooctane—ethyl acetate phase and that the overall recovery of 15 pg of radioinert 17ß-estradiol was 87.8 ± 14.8% (n = 6). Losses of ≈ 12% were considered acceptable, and no corrections were made. There was no difference in serum 17ß-estradiol concentration between female decapitated and intact embryos.

Discussion

In order to investigate the role of the hypophysis in 17ß-estradiol secretion by the late chick embryo ovary, 2 parameters were studied in 18-day-old decapitated, sham-operated and intact embryos, namely 17ß-estradiol production by cultured ovaries and serum 17ß-estradiol concentration. The response of the chick embryo ovary to exogenous LH has already been demonstrated (Guichard *et al.*, 1979; Gonzalez *et al.*, 1987).

17ß-Estradiol secretion by ovaries from decapitated embryos was the same as that by ovaries from sham-operated embryos and was 83% of that by ovaries from intact embryos. However, on a weight basis, ovaries from decapitated embryos secreted rather greater amounts of 17ß-estradiol than ovaries from intact and sham-operated ones. This can be explained if one recalls that hypophysectomy primarily affects the ovarian cortex (Fugo, 1940), and not the medulla, which contains the estrogen-secreting cells (Mintz and Wolff, 1954; De Simone-Santoro, 1969; Weniger, 1969; Budras and Preuss, 1973). Thus, it is concluded that the hypophysis does not influence 17ß-estradiol secretion by the 18-day-old chick embryo ovary.

The same conclusion was reached when the serum 17ß-estradiol concentration was measured : for serum from both decapitated and intact embryos, this concentration was about 40 pg/ml. However, it must be emphasized that this concentration is much smaller than that found in previous studies. Woods and Brazzill (1981) report a plasma 17ß-estradiol concentration of 1360 pg/ml in 17.5-day-old female chick embryos, and Tanabe *et al.* (1986) a concentration of 160 pg/ml in 18-day-old female embryos. The origin of this discrepancy is not obvious. It cannot lie in the manner of collecting blood. Although Tanabe *et al.* (1986) drew the blood from the heart or veins, both Woods and Brazzill (1981) and we ourselves drew it from extraembryonic vessels. What differs between the method of Woods and Brazzill (1981) and Tanabe *et al.* (1986) and our method is that the former authors assayed 17ß-estradiol in crude ether extracts, whereas we introduced a purification step meant to eliminate the bulk of lipids.

In conclusion, 17ß-estradiol secretion by the ovary is not under pituitary control in the normal female chick embryo, even as late as 18 days of incubation, although all the conditions exist which would make such a control possible : the ovary responds to exogenous LH from as early as 7 days of incubation (Weniger and Chouraqui, 1988), immunoreactive LH cells are already detectable in the primordium of the anterior hypophysis of the 4-day-old embryo (Gasc and Sar, 1981) and LH is measurable in the plasma from 10 days of incubation (Woods and Thommes, 1984; Tanabe *et al.*, 1986), and LH recep-

tors are present in the ovary from 8.5 days of incubation (Woods and Thommes, 1984). This issue requires further investigation. However, the situation is different in the duck, where the dependence of estrogen secretion on the hypophysis, first detected on morphological grounds (Kinyon and Watterson, 1958), has been confirmed by radiochemical methods (Akram and Weniger, 1973; Weniger *e t al.*, 1974).

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