17β-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 17β-estradiol released into the culture media was determined by radioimmunoassay. Ovaries from decapitated embryos secreted significantly lower amounts of 17β-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, 17β-estradiol production was significantly different between the 3 groups of embryos, the ratio being highest in the decapitates. 17β-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 17β-estradiol secretion by the chick embryo ovary even near hatching time.

Introduction

Opposite views are held concerning a possible role of the hypophysis in estro-
control from 13 days of incubation, where-
as in the opinion of Weniger and Zeis
(1987) this was not the case even at 17
days, when the amount of 17β-estradiol
released into the culture medium was not
significantly different, whether the ovaries
were from normal or decapitated embryos. Although mortality of decapita-
ted embryos is high near hatching time, it
was decided to try to gain one more day.
The present study compares 17β-estra-
diol production by ovaries from 18-day-old
intact, decapitated and sham-operated
embryos in organ culture. In addition,
serum 17β-estradiol levels were deter-
mimed 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somites), 2 ml of albumin were withdrawn from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 Rath-
ke's pouch, are removed. Sham-operated
embryos were those from which only the tip of
the prosencephalon was taken. Eggs in
eggs which were just windowed after albumin
withdrawal were considered intact.

After 18 days of incubation, blood was col-
clected 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 O2 and CO2.

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 radioim-
munoassay of 17β-estradiol. [2,4,6,7-3H] Estra-
diol was supplied by C.E.A. (Gif-sur-Yvette) and
its radiochemical purity was ≥ 99%. The antise-
rum was a gift from Roussel-Uclaf (Romain-
ville); it was directed towards 7-carboxy-me-
thyloxime 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 sensitivi-
ty 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 17β-estra-
diol 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 fro-
zened, 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 dry-
ness. The residue was dissolved in 0.6 ml of
phosphate buffer, and 2―0.3-ml samples were
taken for radioimmunoassay of 17β-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 17β-estradiol secreted and the
weight of the ovary in each group of embryos
(Schwartz, 1963). Analysis of covariance was
used to compare 17β-estradiol secretion be-
tween the 3 groups of embryos on an ovarian
weight basis (Leilouch 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.

17β-Estradiol in culture media

Since pilot experiments had shown that after a 5-fold dilution of the culture medium the amount of 17β-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 17β-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 17β-estradiol released and the weight of the ovary were not correlated. However, between the groups, the production of 17β-estradiol

Table I. Amount of 17β-estradiol (E₂) released into culture media by ovaries from decapitated (A), sham-operated (B) and intact (C) 18-day-old chick embryos, ovarian dry weight, E₂/weight ratio and serum 17β-estradiol concentration.

<table>
<thead>
<tr>
<th></th>
<th>Decapitated (A)</th>
<th>Sham-operated (B)</th>
<th>Intact (C)</th>
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<tbody>
<tr>
<td>17β-estradiol</td>
<td></td>
<td></td>
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<tr>
<td>in culture medium (pg)</td>
<td>27 ± 8 (n = 28)</td>
<td>27 ± 9 (n = 26)</td>
<td>33 ± 12 (n = 33)</td>
</tr>
<tr>
<td>A/B : NS</td>
<td></td>
<td></td>
<td>A/C : P = 0.03</td>
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<tr>
<td>Weight of ovary (mg)</td>
<td>0.55 ± 0.13 (n = 23)</td>
<td>0.74 ± 0.26 (n = 19)</td>
<td>0.84 ± 0.18 (n = 31)</td>
</tr>
<tr>
<td>A/B : P &lt; 0.001</td>
<td></td>
<td></td>
<td>A/C : P &lt; 0.001</td>
</tr>
<tr>
<td>E₂/weight (pg/mg)</td>
<td>49</td>
<td>37</td>
<td>39</td>
</tr>
<tr>
<td>Serum 17β-estradiol</td>
<td>9.4 ± 2.6 (n = 7)</td>
<td>6.6 ± 3.2 (n = 3)</td>
<td>10.5 ± 3.8 (n = 8)</td>
</tr>
<tr>
<td>concentration (pg/250 μl)</td>
<td></td>
<td></td>
<td>A/C : NS</td>
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Values are mean ± standard deviation. NS : difference statistically not significant.
per unit weight of ovary was significantly different, as shown by analysis of covariance.

17β-Estradiol in serum

Pilot experiments showed that under the conditions used 96.7 ± 2.8% (n = 4) of a tracer amount of 3H 17β-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 17β-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 17β-estradiol concentration between female decapitated and intact embryos.

Discussion

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

17β-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 17β-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 17β-estradiol secretion by the 18-day-old chick embryo ovary.

The same conclusion was reached when the serum 17β-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 17β-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 17β-estradiol in crude ether extracts, whereas we introduced a purification step meant to eliminate the bulk of lipids.

In conclusion, 17β-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 et al., 1974).

References


Kinyon N. & Watterson R.L. (1958) Reduced endocrine activity of ovaries of hypophysectomized duck embryos as indicated by modified development of genital tubercle and syrinx. Physiol. Zool. 31, 60-72


