Studies on Sex-Organ Development

ONTOGENY OF CYTOPLASMIC OESTROGEN RECEPTOR IN CHICK MÜLLERIAN DUCT

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Oestradiol receptors were observed in the cytoplasm of the chick Müllerian duct at several embryonic stages. The sedimentation coefficients and the dissociation constants of the receptor protein remained unchanged throughout the various stages of development. Specific binding of cytoplasmic receptor to [³H]oestradiol assayed *in vitro* was shown to be saturable at a concentration of 10 nM or higher. The number of oestradiol-binding sites on a per-cell basis increased linearly from day 8 to day 12 of incubation and then levelled off from day 12 to the fourth day after hatching. These results indicate that in the developing embryonic sex organ, the same receptor protein is present throughout prenatal development. The concentration of the oestradiol receptor increases and reaches a constant value, but the capacity for the receptor to interact with the hormone does not change.

It has been observed that steroid hormones are involved in the normal development and function of various embryonic organs, e.g. glucocorticoids in the small intestine and lung of the mouse (Moog, 1971; Kotas & Avery, 1971), cortisol in the retina and pancreas of the chick (Moscona & Piddington, 1967; Cohen & Kulka, 1974), oestrogen in chick neural tissue (Vernadakis, 1971) and androgens in the normal function of the rat hypothalamus (Barraclough & Gorski, 1961). Previous experiments suggested that sex steroids influence the involution of the male Müllerian ducts and of the female right duct, as well as the development of the left Müllerian duct in the female chick (Wolff & Wolff, 1947, 1951; Hamilton, 1961; Groenendijk-Huijbers, 1962).

However, little is understood about the effect of the steroid hormones on the development of the embryonic sex organ. We reported the presence and described the principal characteristics of oestradiol receptors in the left Müllerian duct of the embryonic female chick in the preceding paper (Teng & Teng, 1975). In the present studies, the change of cytoplasmic oestradiol-binding sites in the left Müllerian duct and their interaction with oestradiol during the course of embryonic organ development have been investigated.

Materials and Methods

Almost all of the materials and methods used in this study are described in the preceding paper (Teng & Teng, 1975). The remaining details of experimentation and procedure are described in the relevant Figures.

Results and Discussion

Specific binding of [³H]oestradiol to cytosol receptor

Different concentrations of [³H]oestradiol were used to bind to the receptor in the cytosol of the Müllerian ducts from various developmental stages (Fig. 1*a*). Specific-binding curves obtained for the oestrogen-tissue interaction indicated that the bindings were saturable at an [³H]oestradiol concentration of 10 nM and that they levelled off when the added labelled hormone was increased further. The cytoplasmic oestradiol-receptor concentration in the developing Müllerian duct increased linearly from the 8th to the 12th day, and became saturated at the 12th, 15th and 18th day of development. The receptor concentration remained the same in the 4-day-old chick oviduct. The corresponding Scatchard plots (Scatchard, 1949), based on the specific-binding data (Fig. 1*b*), are straight lines, which indicated that within this range of steroid concentration and throughout the developmental stages investigated, a single class of binding sites in Müllerian ducts is measured. The values of dissociation constant (K_d), calculated from the slopes of the Scatchard plots, range from 3.0×10^{-9} to 3.2×10^{-9} M; these values indicate that the receptor affinity to oestrogen is basically unchanged during development. This observation is comparable with the study of oestrogen-receptor ontogeny in the newborn rat uterus, in which no distinguishable change of cytosol receptor-binding affinity to oestrogen was observed (Clark & Gorski, 1970).

Fig. 1. Specific binding of $[^3\text{H}]$ oestradiol to cytosol receptor

(a) Specific binding of $[^3\text{H}]$ oestradiol to the cytosol of foetal and newborn chick oviduct. Cytosols were prepared from the following sources: ■, 8-day, △, 10-day, ▲, 12-day, ●, 15-day and ○, 18-day left Müllerian ducts of embryonic female chicks, and □, the oviduct of 4-day-old female chick. All data presented are expressed as specific binding [after the subtraction of non-specific binding from total binding as described by Teng & Teng (1975)]. (b) Scatchard plot derived from the specific cytoplasmic $[^3\text{H}]$ oestradiol-binding data presented in (a). The symbols representing the stage of development are as in (a).

Glycerol-gradient analysis of the oestradiol receptor

Glycerol-gradient analyses (Fig. 2) showed that the binding of $[^3\text{H}]$ oestradiol to the Müllerian-duct cytosol of several developmental stages involves 8 S and 4.5 S receptors; these receptors were obtained from Müllerian duct of different stages and have the same sedimentation values. Their properties in the left Müllerian duct of the 15-day foetal female chick have been described previously (Teng & Teng, 1975).

Fig. 2. Glycerol-density-gradient sedimentation of Müllerian-duct cytosol receptors from 8-, 10-, 12- and 18-day female chick embryos

Samples (350 μl) of Müllerian-duct cytosols (1.5 mg of protein) prepared in buffer were incubated with 10 nM $[^3\text{H}]$ oestradiol (final concentration) at 0°C for 1 h, and free oestradiol was then removed by dextran-coated charcoal (with 0.25% charcoal and 0.025% dextran in the final volume). Incubation mixtures were layered on a linear 5–35% glycerol gradient (4.5 ml, prepared in buffer) and centrifuged at 150000g for 18 h at 0°C in the Beckman SW 50.1 rotor. Each gradient was fractionated with a Buchler piercer into 30 fractions (nine drops per fraction). Scintillation fluid (4 ml) was added to each fraction and radioactivity assayed in a mini counting vial with a Beckman model LS-250 scintillation spectrometer. Sedimentation markers (200 μg of aldolase, bovine serum albumin and γ -globulin) were run in a separate gradient. The symbols representing the stage of development are as in Fig. 1(a).

Ontogeny of cytoplasmic oestradiol-binding sites in Müllerian duct

From the 8th to the 12th day of development, DNA content in the left Müllerian duct was observed to increase slowly, but it increased rapidly from the 14th day to birth; the increase of cytosol protein concentration during Müllerian-duct development followed the same pattern. Oestradiol-binding sites in the Müllerian duct (in terms of fmol/200 μg of DNA) increased steadily from the 8th day to the 10th day and started to reach saturation at the 12th day of incubation; it then levelled off from the 12th day to the day of hatching (Fig. 3). On the basis of the observed DNA content of the female left Müllerian

Fig. 3. Correlation of the number of oestradiol-binding sites and the development of Müllerian ducts in the female chick embryos

Points on the graph representing the cytosol protein (●) or DNA (■) content per Müllerian duct were the average of three to four measurements. The measurement of oestradiol-binding sites (▲) was expressed as fmol of oestradiol bound/200 µg of DNA. For details see the preceding paper (Teng & Teng, 1975).

Table 1. Measurement of the left Müllerian ducts of the developing chick

Müllerian ducts excised from various developmental stages of female embryos were used immediately to determine length and weight. Approx. 50 Müllerian ducts per group were weighed in a Mettler model H-54 balance. The length of each freshly excised duct was measured individually by placing the duct on a piece of Whatman no. 1 paper that had been previously damped with Hanks solution (Hanks & Wallace, 1949).

Period of incubation (days)	Length* (mm/duct)	Wet weight† (mg/duct)
8	5.2±0.4	0.55±0.10
10	7.5±0.3	0.95±0.15
11	9.0±0.5	1.20±0.10
12	9.3±0.4	1.30±0.15
13	9.4±0.3	1.45±0.15
14	10.5±0.5	1.90±0.20
15	15.3±0.4	2.50±0.20
16	19.2±0.4	2.90±0.15
18	21.0±0.5	3.90±0.15
21 (hatch)	22.2±0.4	4.80±0.20
Newborn (4-day)	23.3±0.5	5.30±0.20

* Each value represents the mean±s.d. of 50 measurements.

† Each value represents the mean±s.d. of three measurements.

duct of different embryonic stages and of the knowledge that average DNA content is 2.5µg/10⁶ embryonic cells (Sober, 1968), the number of binding

sites per 10⁶ Müllerian-duct cells is 1.47, 3.55, 6.1, 5.4, 5.45 and 5.45fmol respectively for the 8-, 10-, 12-, 15- and 18-day embryonic Müllerian ducts and the 4-day-old chick oviduct.

The secretion of oestradiol from the ovary of the 6-day embryonic chick has been detected (Weniger, 1968). It could be argued that the lower number of oestradiol-binding sites observed in the cytosol of the 8-day and 10-day embryonic Müllerian ducts is due to a greater endogenous oestradiol secretion by the embryonic ovary; this greater secretion would decrease the measurable hormone-binding sites in the Müllerian-duct cytosol. This does not appear to be the case, however, since the observation by Guichard *et al.* (1973) indicated that the amount of oestradiol formed in the embryonic chick ovary increases progressively, corresponding to the age of the embryo. Although it is unlikely, the possibility that the measurable receptor sites in the Müllerian-duct cell of the 8- and 10-day embryo is lowered because of an accumulation of endogenous oestradiol should nevertheless not be ruled out.

What causes the increase of oestradiol-binding sites in the embryonic sex tract during development? Two major possibilities exist. (a) The increase could be caused by an autonomous mechanism endogenous to Müllerian-duct cells. The synthesis of the receptor in the embryonic stage may be equivalent to 'Period I' for synthesis of the oestrogen receptor in the rat uterus (Gorski *et al.*, 1971), and like that in the rat uterus, it is constitutive in nature. (b) The increase could be controlled by the endogenous oestrogen secreted from the ovary. This possibility has been

suggested by experiments reported previously (McGuire & Lisk, 1968; Feherty *et al.*, 1970; King *et al.*, 1971). We lack sufficient information to determine which possibility is valid.

Comparison of the morphological changes with the biochemical changes in the Müllerian duct

Two stages of morphological change in the left Müllerian duct (summarized in Table 1) were observed from the 8th day of incubation to the day of hatching. (a) A slow rate of change occurs from the eighth until the twelfth day of incubation. During this period of incubation, the wet weight and length of the left duct increased slightly. (b) A rapid rate of change begins on the fourteenth day of incubation and lasts to the fourth day after birth. During this period a rapid growth was observed in the left Müllerian duct. The increase of wet weight, length, cytoplasmic protein concentration and DNA content of the Müllerian duct throughout the course of development (Table 1 and Fig. 3) suggests that the left Müllerian duct grows from 50 to 80% faster between days 14 and 18 than between days 8 and 12. Yet the oestradiol-binding sites per unit of DNA or per cell in the day-12 Müllerian duct already have been shown to reach saturation, which occurs before the period of rapid growth of the Müllerian duct. It is possible that the 12th day of incubation is a critical period in the entire development of the embryonic organ; it may be that at this point, oestradiol-dependent metabolic activities related to the growth and future development of the organ are initiated.

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