Ontogenetic Aspect of Steroidogenesis by Gonads and Adrenals of Ducks and Its Role on Sex Differentiation

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Abstract Plasma testosterone, estradiol, corticosterone and cortisol levels together with gonadal testosterone and estradiol concentrations were determined by radioimmunoassay in the embryonic and post-embryonic Domestic Duck, *Anas platyrhynchos* var. *domestica*. The plasma testosterone and estradiol concentrations were higher in the female than in the male embryo, whereas no sex-difference was observed either in plasma corticosterone or cortisol. Both the ovarian estradiol and testosterone concentrations in the embryo were much higher in the testicular concentrations. The plasma corticosterone or cortisol concentration reached a peak one day after hatching, and decreased thereafter in both sexes. These results indicate that the ovary of embryonic duck is more active in secreting the sex steroid hormones than the embryonic testes, and suggest that the original sex of the duck (probably of all avian species) should be male (ZZ), and estrogenic hormones secreted from the embryonic ovary has an important role in sexual development (feminizing) of the female in the duck.

Introduction

Many works have been done on ontogenetic steroidogenesis in mammals (Rigaudière 1977, Geroge *et al.* 1978, Wilson 1978). Whereas in birds some works have been reported only in the chicken (Woods *et al.* 1975, Woods & Brazzill 1981, Guichard *et al.* 1977, 1979, Tanabe *et al.* 1979). Recently, it was found that H-Y antigen plays a pivotal role in the primary (gonadal) sex determination of mammals, and its regulatory gene exists on Y chromosome of XY male cells in mammals, but it exists in ZW female cells (possibly on W chromosome) in avian species (Wachtel *et al.* 1975 a b, Ohno 1979, Ohno & Matsunaga 1981, Müller *et al.* 1979, Zaborski *et al.* 1980).

The present work was undertaken to elucidate the relationship between ontogenetic steroidogenesis and sex differentiation in Domestic Ducks (*Anas platyrhynchos*).

Materials and Methods

The duck and ducklings were obtained from Osaka-Kairyō Breed, bred from a hybrid between Japanese native and Pekin duck. The eggs were incubated in an incubator at 38°C and 70% relative humidity, and hatched on the 27th day of incubation. Blood samples were collected with a heparinized syringe by heart puncture from 8 embryos of both sexes at 15, 19, 23 and 26 days of incubation, from 8 ducklings of both sexes at one and three days, and from 5 ducklings of both sexes at 7 and 14 days after hatching. The blood samples were centrifuged for separation of plasma.

One tenth ml plasma was used for the determination of testosterone, corticosterone

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and cortisol, and 0.2 ml plasma for estradiol. After killing the embryos or ducklings, left ovary or testis was removed. Ethyl ether extractions were repeated three times for the steroid hormones.

The radioimmunoassay without chromatography was carried out for estradiol, testosterone, corticosterone and cortisol (Shodono *et al.* 1975, Tanabe *et al.* 1979).

Fig. 1. Plasma testosterone (male $\circ$ — $\circ$, female $\bullet$ — $\bullet$) and estradiol (male $\square$ — $\square$, female $\blacksquare$ — $\blacksquare$) concentrations of embryonic and post-embryonic ducks. Each point and vertical line represent mean of 8 embryos or 5–8 ducklings and SEM, respectively.

Results

Plasma testosterone and estradiol concentrations of 15-, 19-, 23-, and 26-day-old embryos and 1-, 3-, 7-, and 14-day-old ducklings are illustrated in Fig. 1. Plasma testosterone levels were much higher in the female than the male throughout the embryonic and post-embryonic ages. Plasma estradiol levels were higher in the female than in the male embryo, whereas no significant amount of estradiol was detected in post-embryonic ducks of both sexes.

Ovarian and testicular concentrations of estradiol and testosterone of ducks at the embryonic and post-embryonic ducks are illustrated in Fig. 2. Both estradiol and testosterone concentrations were much higher in the ovary than in the testis. The

Fig. 2. Concentrations of testosterone in testis ($\square$ — $\square$) or in ovary ($\blacksquare$ — $\blacksquare$) and estradiol in testis ($\circ$ — $\circ$) or in ovary ($\bullet$ — $\bullet$). Each spot and vertical line represent mean of 8 embryos or 5–8 ducklings and SEM, respectively.

ovarian concentrations of both hormones decreased after hatching. The levels of estradiol in the ovary were still higher than the testicular concentrations even 14-day-old ducklings. A similar sex-difference in the gonadal progesterone concentration was also observed (Tanabe *et al.* 1983).

Plasma corticosterone and cortisol levels in embryonic and post-embryonic ducks are illustrated in Fig. 3. Plasma corticosterone levels were low in 15-, 19- and 23-day-old embryos of both sexes, increased rapidly in 26-day-old embryos, reached a peak around the hatching time, and decreased rapidly thereafter. Similar changes in the plasma cortisol levels were observed although the cortisol levels were much less than the corticosterone concentration. No significant sex-difference was observed either in the plasma corticosterone or cortisol levels.

Fig. 3. Concentrations of plasma corticosterone (male $\circ$ — $\circ$, female $\bullet$ — $\bullet$) and plasma cortisol (male $\square$ — $\square$, female $\blacksquare$ — $\blacksquare$). Each spot and vertical line represent mean of 8 embryos or 5–8 ducklings and SEM, respectively.

Discussion

The present study indicates that duck embryonic and postembryonic ovaries are highly active in the production and secretion of testosterone and estradiol as compared with embryonic and postembryonic testes. Similar observations have been made in the embryonic chickens (Guichard *et al.* 1977, 1979, Tanabe *et al.* 1979). This is in contrast with the case of mammalian embryo, in which embryonic testes are much more active than embryonic ovaries (Rigaudière 1977, George *et al.* 1978, Wilson 1978).

In rabbits, gonadectomy in male or female embryo prior to the onset of phenotypic differentiation results in the development of the female phenotype (Jost 1972). On the contrary, in the avian species, the male secondaries such as the development of phallus and the laryngeal extension in ducks (Wolff & Wolff 1949) and the male type plumage in the chicken (Morgan 1920) is known to appear in castrated females.

It is to be noted that application of sex-steroid in the species with male heterozygosity (XX/XY) are apt to induce masculinization of the female (XX), whereas in the case of female heterozygosity (ZW/ZZ), similar treatments are tended to cause feminization of the male (ZZ), in amphibian, avian and mammalian species (Witschi 1967, Wolff & Wolff 1949). Furthermore, there exists abundant evidence indicative that in mammals XY male cells disseminate testis-organizing H-Y antigen, while in avian species ZW female cells disseminate ovary-organizing H-Y antigen (Wachtel *et al.* 1975a b, Ohno 1979, Ohno and Matsunaga 1981). Recently, Müller *et al.* (1978) reported the induction of H-Y antigen by estradiol benzoate in the gonads of male (ZZ) chicken embryo, thus ruling out a W chromosomal location of the structural gene of the antigen. This observation, along with the disappearance of H-Y antigen during the transformation of right gonad into testes in ovariectomized chicks (Zaborski *et al.* 1980), suggests that H-Y antigen is rather a differentiation factor than a determining factor in the organization of the ovary in the avian species, and that the expression of the antigen in avian species is regulated by estrogen. Taken all together, these observations on H-Y antigen indicate that the sex of the avian species is basically (inherently) male with homozygosity of sex chromosome (ZZ), and that the estrogens secreted from the embryonic ovary plays a very important role in sex differentiation and sexual development (feminizing) of the female. Furthermore, the major sex-determining (regulatory) gene is present on W chromosome, and the structural gene for H-Y antigen like-substance is present on autosomes; the avian female is the induced sex in contrast to mammals.

In the present study, plasma corticosterone levels reached a peak around the hatching time in both sexes of ducks. Although the plasma levels of cortisol were much less than those of corticosterone, they showed a similar change during embryonic and postembryonic (until 14 days of age) ducks as in the corticosterone concentrations. Both the corticosterone and cortisol levels decreased after hatching. It is likely that higher levels of corticosteroids are needed for newly hatched ducklings to adapt to a new environment after hatching.

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