EFFECTS OF *IN OVO* EXPOSURE TO DES AND TCDD ON GONADAL AROMATASE ACTIVITY AND SEX DIFFERENTIATION IN CHICKENS

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Introduction

The female chicken is the heterogametic sex (sex chromosome: ZW) and the male is homogametic (sex chromosome: ZZ). Chicken embryonic gonads are bipotential at an early stage. During development of the female, the left gonad differentiates to a single ovary / oviduct, and the right gonad regresses, developing a permanent female phenotype. This sexual differentiation occurs as a result of aromatase expression in the left gonad at day 6.5 and the production of estrogen from testosterone [1]. In the male genotype, both gonads develop into two testes. The *in ovo* treatment of a genetic female with an aromatase inhibitor before day 7 of incubation completely inhibits sexual differentiation to female, resulting in the development of a male phenotype. In contrast, the *in ovo* treatment of a genetic male with estradiol induces a sex-reversal to a female phenotype [2].

Autosexing chicks, a cross between Rhode Island Red males and Plymouth Rock females, indicate genetic sex by their specific color appearance at hatching, and these feature is useful for monitoring sex reversal. To examine the embryonic toxicity of chemicals, *in ovo* exposure has several advantages: the concentration of chemicals can be easily controlled, the applied chemicals are not eliminated until hatching, and the embryonic toxicity can be assessed independently of maternal influence. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is an anti-estrogen which binds to the Ah-receptor [3]. It is reported that TCDD have reproductive and teratogenic effects in several strains of mice, rats, rabbits, and chickens [4].

This study was undertaken to confirm sex reversal by *in ovo* treatment with an aromatase inhibitor, fadrozol (AI) and a synthetic estrogen, diethylstilbestrol (DES) using Rhode Island Red males and Plymouth Rock females, and to examine the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on sex differentiation and gonadal aromatase activity.

Materials and Methods

Materials

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was obtained from Cambridge Isotope Laboratories (Andover, MA). [1β-^{3}H]-androsto-4-ene-3, 17-dione ([^{3}H]-androstenedione) was obtained from NEN (Boston, MA). DES was obtained from Sigma (St Luis, MO). Fadrozol was supplied from Novartis (Tokyo, Japan).

Injection and incubation

Rhode Island Red males were crossed with Plymouth Rock females to obtain fertile eggs. DES, AI and TCDD were dissolved in 0.05 ml of propylene glycol and injected into the egg white on day 0 using a 1 ml syringe with a 23G needle. The eggs were incubated at 37.6°C at a relative humidity of 53% in a SHYOWA FURANKI incubator (model P03). Eggs were automatically turned once per hour.

Sex determination

At hatching, phenotypic sex was determined by an experienced vent sexer and genotype sex by the color of the head feathers, legs and mandible. Gonads were macroscopically examined and dissected under a microscope, frozen with liquid N_2 and kept at -80°C until the aromatase activity was assayed.

Assay for aromatase activity and mRNA expression

For the aromatase assay, gonads were homogenized with a glass homogenizer in 10 mM potassium phosphate buffer (pH 7.4) containing 100 mM KCl, 1 mM EDTA, 10 mM dithiothreitol and a protease inhibitor cocktail, and centrifuged for 10 min at 1800 g. Aromatase activity in the supernatant was assayed in terms of released ^3H_2O from ^{3}H-androstenedione as described by Lephart and Simpson [5] and Roselli and Resko [6]. Total RNA was purified using RNeasy (QIAGEN) and reverse transcribed to cDNA with MuLV reverse transcriptase and oligo d $(T)_{16}$ primer. Aromatase cDNA was amplified with specific primers by the PCR method and quantified using the LightCycler™ System (Roch Diagnostics, Mannheim, MA). Aromatase mRNA expression was estimated by the ratio to β-actin mRNA expression.

Results and discussion

At hatching, male chicks from Rhode Island Red males and Plymouth Rock females have white feathers on the top of their heads, yellow legs and mandibles while female chicks have black feathers, legs and mandibles. These genotypes were further confirmed by detection of W-chromosome-linked (female-specific) XhoI repeat sequence [7] using PCR amplification from liver genomic DNA. Hatchability of chicks was 70~80% on average. The genotype male was approximately 40% of all chickens used in this experiment. In a control group the genetic sex ratio coincided with the phenotypic sex ratio.

In the AI group, all genotypic female chicks vent sexed as males upon hatching whereas all genotypic male chicks vent sexed as females in the DES group. High aromatase activity was detected in the left ovary of normal females in the control group, but the activity in the testes of normal males was slightly detectable. AI treatment inhibited regression of the right ovary in genotype females while the aromatase activity increased approximately 4-fold in their bilateral gonads compared with the activity in the left ovary of normal females. However, the aromatase mRNA expression was not changed. The right remaining gonad showed testicular appearance regardless of the high aromatase activity.

Contrastingly, DES treatment induced the regression of the right gonad and ovarian shape in the left gonad of genotype males. This treatment also increased aromatase activity in both left and right gonads in sex-reversed males with the expression of aromatase mRNA. The effects of AI on genotype males and DES on genotype females were negligible.

The exposure of TCDD (2.5, 5.0 and 7.5 ng/egg) on day 0 did not influence hatchability whereas that of more than 10 ng/egg of TCDD completely inhibited hatching. The genetic sex ratio coincided with the phenotypic sex ratio in both control and TCDD treated groups. The gonadal aromatase did not change by TCDD exposure. These results suggest that *in ovo* TCDD exposure had no effect on sex differentiation in chicks. The sexual behavior of some chicks were to be examined when adults.

In conclusion: Aromatase primarily expressed in genotype females converts testosterone to estradiol which enhances expression of gonadal aromatase and induces permanent sex differentiation to phenotype females. An aromatase inhibitor, Fadrozole sex-reversed genotype females to phenotype males by inhibiting *in vivo* aromatase activity expressed in the ovary of genotype females. However, *in vitro* aromatase activity was highly up-regulated by Fadrozole, probably due to its aromatase stabilizing action. *In ovo* exposure of TCDD had no effect on sex differentiation in chicks.

References

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