Effect of (xeno)-estrogens on zebrafish P450c17 (C17, 17 alpha-hydroxylase/17, 20-lyase) mRNA and protein expression in gonadal tissue

by
François BRION (1), Nathalie HINFRAY (1), Olivier PALLUEL (1), Julien MAILLAND (1), Isabelle ANGLADE (2), Sélim AÏT-AISSA (1), Olivier KAH (2) & Jean-Marc PORCHER (1)

ABSTRACT. - The objectives of this study were to determine the expression and the cellular localization of P450c17 in gonadal tissues in the zebrafish and to investigate the effect of (xeno)-hormones on its expression at both mRNA and protein levels. We showed that gonads are sites for cyp17 gene expression and we documented for the first time the cellular localization of the cyp17 protein expression within ovarian and testicular tissues in a cyprinid fish. Moreover, we demonstrated the down-regulating effect of (xeno)-estrogens on testicular cyp17 expression.

Key words. - Steroidogenesis - P450c17 - Gene expression - Gonads - Zebrafish

Introduction
Cytochrome P450c17 is a critical enzyme for the biosynthesis of gonadal steroids in vertebrates. Recent progress has been made with respect to the cloning and sequencing of this gene in zebrafish (Wang and Ge, 2004). However, little is known about the expression and localization of this enzyme in the gonads as well as its regulation by (xeno)-hormones. The objectives of this study were to determine the expression and the cellular localization of P450c17 in gonadal tissues in adult zebrafish and to investigate the effect of (xeno)-hormones on its expression at both mRNA and protein levels.

Methods
Adult zebrafish were exposed to either 17α-estradiol (E2; 10 nM), 11β-hydroxyandrostenedione (11-OHA, 10 nM), ethynylestradiol (EE2; 10 nM) or nonylphenol (NP; 20, 100, 500 µg/L) during 7 days. Gonadal cyp17 mRNA levels, normalised to beta-actin, were measured by a specific bDNA assay (n = 6 fish/sex/group). Cyp17 protein expression was characterised by immunohistochemistry and western-blotting experiment using a specific zf-cyp17 polyclonal antibody developed in this study.

Results and discussion
We confirmed that P450c17 gene is expressed in gonads of non-treated adult fish with a dominant expression within the testis. In the ovary, P450c17 protein is mainly expressed in follicle cells surrounding oocytes. In the testis, positive immunoreactive-cells were detected in interstitial cells, presumably Leydig cells. We showed that exposure to 11-OHA had no effect on cyp17 mRNA level in both ovary and testis while exposure to E2 led to a dramatic decrease of the testicular expression of cyp17 at both mRNA (Fig. 1) and protein level. Interestingly, exposure of male fish to the xeno-estrogens EE2 and NP, to a lesser extent than estrogens, also decreased the testicular cyp17 gene expression.

Conclusions
For the first time, immunolocalization of the steroidogenic P450c17 enzyme is documented within the ovarian and testicular tissue in a cyprinid fish species. Additionally, we demonstrated that (xeno)-estrogens negatively affected testicular steroidogenesis by inhibiting P450cyp17 expression. This study provides new information on the mode of action of (xeno)-estrogens on the steroidogenic pathways allowing a more fully understanding of effects of endocrine disrupting chemicals on the biosynthesis of steroids hormones in the zebrafish (Hinfray et al., 2006, Cheshenko et al., 2007).

Figure 1. - Down-regulating effect of E2 on testicular cyp17 mRNA level (n = 6 fish /group).

(1) INERIS, Unité d’évaluation des risques écotoxicologiques, France. [francois.brion@ineris.fr]
(2) UMR CNRS 6026, Rennes, France.
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References
