YOLK THYROID AND STEROID HORMONE METABOLISM AND THE CONSEQUENCES ON EMBRYONIC DEVELOPMENT: POTENTIAL SITES OF ACTION OF ENVIRONMENTAL ENDOCRINE DISRUPTORS

John Leatherland
Department of Biomendical Sciences, University of Guelph,
Guelph, ON N1G 2W1, Canada;
phone 519 824 4120, fax 519 767 1450; e-mail jleather@ovc.uoguelph.ca

Rakpong Petkam
Department of Biomedical Sciences, University of Guelph, Guelph, Canada

Jason Raine
Department of Biomedical Sciences, University of Guelph, Guelph, Canada

Colin Cameron
Department of Biomedical Sciences, University of Guelph, Guelph, Canada

EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

There is increasing evidence to show that some toxicological factors act as hormone mimics, or negatively impact hormone function in vertebrates (reviewed in Guillette and Crain, 2000), with these so-called environmental endocrine disrupting chemicals (EDCs) affecting various aspects of developmental and/or reproductive function.

Because of the chemical diversity of known EDCs, their mode of action is pluralistic, with some EDCs affecting aryl hydrocarbon receptors (AHR), some impairing hormone transport in the blood, and some interacting with hormone receptors. Although the molecular mechanisms of EDC action has been the focus of recent studies, there needs to be a continued balance between molecular events and the down-stream cellular and whole organism responses. Given the extremely complex cellular signaling events, and the remarkable information redundancy in cell systems, specific molecular changes brought about by a specific EDC may not necessarily translate into a biological response. The effects of EDCs on embyronic development is a case in point; there are well-characterized examples of a developmental delay between exposure of an embryo to a specific insult and the defined/diagnosed downstream biological response (Colborn et al., 1993; Crews et al., 2000; McLachlan, 2001).

Oocytes and Embryos of Oviparous Species in EDC Toxicology Research: the Roles of Yolk Hormones

Embryos of aquatic ectotherms are particularly appropriate for this "paradigm shift" in toxicology research. They are available in large numbers for experimental work, and the markers for their early developmental biology are beginning to be defined. Equally interesting, however, is the role played by the oocyte factors in the early developmental biology of oviparous species that produce very yolky eggs. The composition of the cytoplasmic milieu of the oocyte, and thus the factors affecting pronucleus, zygotic nucleus and early embryo nucleus activities, is determined by the physiological condition of the female. We know from studies of mammalian oocytes that early gene expression is significantly affected by cytoplasmic influences and epigenetic factors (Jenuwein and Allis, 2001). Thus, it must be assumed that a similar relationship exists between the zygotic and embryonic nuclei of oviparous ectotherms, such as fish. The functions of the many factors are still largely unknown, and the factors that influence oocyte viability are controversial. In addition to these naturally occurring molecules, the lipid-rich oocytes are a preferential partitioning tissue for lipophilic organochlorine compounds, some of which are suspect EDCs. If these compounds interfere with hormone metabolism, they may have profound effects on tissue- and gender-specific developmentally-programmed patterns of gene expression, giving rise to developmental abnormalities.

In this paper, we examine the influences of the yolk steroid and thyroid hormones of salmonid eggs on early developmental events. We are particularly interested in the capacity of the embryos to metabolize and excrete these hormones to maintain

its own steroid and thyroid hormone milieu. Figure 1 illustrates the potential sites of actions of toxicological agents on thyroid and steroid hormone economy of the ovarian follicle and early developmental stage embryo. We summarize several studies of ovarian and embryo endocrine physiology, that form the basis of ongoing toxicological studies.

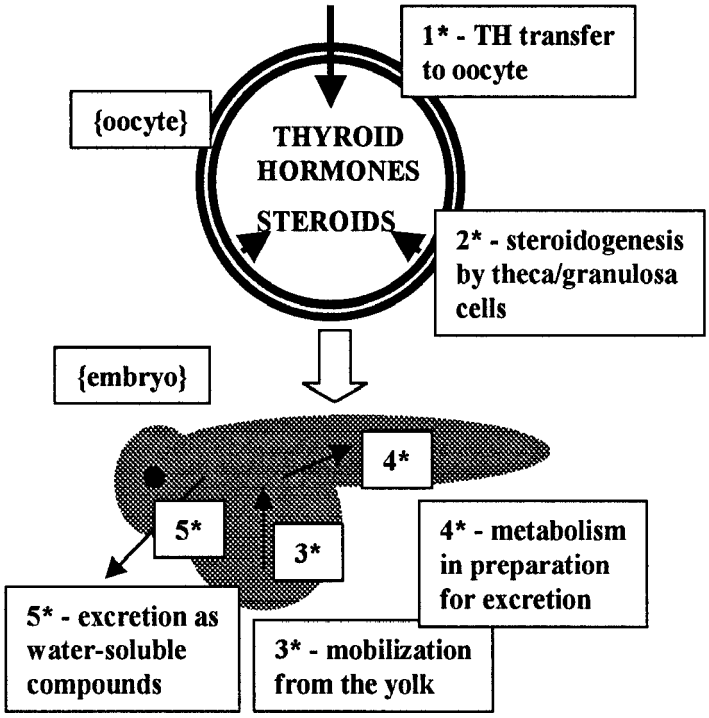

Figure 1. Diagrammatic representation of potential sites of action of xenobiotic interactions with the thyroid and steroid hormone economy and homeostasis of the maturing oocyte and early developmental stage embryo of a salmonid fish. These include: - 1* the transfer of thyroid hormone (TH) to the ooplasm, 2* steroid production by the theca and granulosa cells of the ovarian follicle, 3* mobilization of hormone products from the yolk, 4* the metabolism of thyroid and steroid hormones, and 5* the production of water-soluble excretory products for excretion

via the gills (and possibly the kidney).

Rationale for the Studies

The research focuses on the roles of yolk steroid and thyroid hormones, and to a lesser extent of GH, on early developmental events in salmonid fishes to address the questions: i) are these hormones essential for early development, or do they pose a potential hazard for the early embryo?, and ii) is normal embryo development influenced if hormone metabolism and clearance is compromised, as might be brought about by the presence of environmental contaminants in the yolk?

Experimental Work

The paper will describe studies at that we have made of several stages of hormone incorporation into yolk and hormone clearance from yolk, and preliminary reports on the actions of some suspect EDCs on these events.

Ovarian follicles

We have focused on factors that influence the trafficking of thyroid hormones between the plasma of the female, or the ovarian fluid in the body cavity of the female and the ooplasm, and the thecal/granulosal cells and the oocyte. Using an in vitro ovarian follicle model, and HPLC separation of steroid products, we have also examined the influence of many factors that impact on the steroidogenic pathways of theca and granulosa cells. In addition, we report on the actions of putative EDCs, and of AHR modulators, such as β-naphthoflavone, on steroidogenic pathways of the theca/granulosa cells.

Developing embryos

We report on the clearance of thyroid hormones (TH) from the developing embryos, and on physiological responses when TH levels are manipulated experimentally. We will also report on the actions of DDT and its metabolites on the ability of embryos to metabolize and clear TH. In additional studies, we are exploring the effects of experimentally-altered TH content of the yolk of oocytes and gene expression in embryos (with a particular interest in genes coding for the production of TH receptors).

We also report on a) the ability of developing salmonid embryos to metabolize, and conjugate a range of steroids, by specific (e.g., pregnenolone) or more general

steroidogenic pathways, and b) the influence of DDT and metabolites on the the ability of the embryos to metabolize, conjugate and produce water-soluble steroid forms that can be excreted.

References

Colborn, T., F.S. vom Saal and A.M. Soto. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Env. Health Perspect. 101: 378-384.

Crews, D., E. Willingham and J.K. Skipper. 2000. Endocrine disruptors: present issues, future developments. Quart. Rev. Biol. 75: 243-260.

Guillette, L. and D.A. Crain. 2000. Environmental Endocrine Disruptors: An Evolutionary Perspective. New York: Taylor & Francis. 355p.

Jenuwein, T. and C.D. Allis. 2001. Translating the hisone code. Science 293: 1074-1079.

McLachlan, J.A. 2001. Environmental signalling: what embryos and evolution teach us about endocrine disrupting chemicals. Endocrine Rev. 22: 319-341.