

cDNA sequencing of vitelline envelope protein and gene expression in *Cichlasoma dimerus* (Teleostei, Perciformes) induced by xenoestrogens

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A great variety of compounds released in wastewaters from industries and sewage effluents can act as endocrine disrupting chemicals, causing reproductive effects in human and wildlife populations. In fish, xenoestrogens (substances capable of mimicking/blocking the action of natural estrogens) produce feminization, abnormal induction of liver proteins, growth inhibition and abnormalities of testis and development of intersexes¹. Known xenoestrogens such as octylphenol (OP) and nonylphenol, come from degradation of alkylphenol polyethoxylates, a group of non-ionic surfactants widely used in detergents, paints, pesticides, cosmetics, textile, paper, and plastic industries⁵. Vitelline envelope proteins (VEP) are used as exposure biomarkers; they are involved in fertilization processes and contribute to the eggshell hardening, avoidance of polyspermy and protection of the embryo from physicochemical damage. Up to now, 3 types of VEP have been described in fish (α , β , γ)^{2,3,4}. In most fishes, VEP are under estrogenic control, synthesized by the liver and transported to ovaries through bloodstream. The aim of this work was to obtain partial sequences of VEP, site of production in females, and mRNA expression in a local (Argentina) freshwater fish exposed to OP.

C. dimerus females were ip injected with estradiol (E2) to induce VEP production 3 days before dissection; sham injected females were also used. Males were exposed to waterborne 4-(tert-octyl)phenol 97% (Aldrich) dissolved in ethanol or ethanol alone for 0-28 days. During dissection, organs were immersed in cold RNAlater and frozen at -20°C until departure from Buenos Aires to Maine. Once at MDIBL, RNA was purified and poly-A mRNA was reverse transcribed using oligo-dT and SuperScript III reverse transcriptase. Degenerate primers from VEP α, β, γ published in NCBI database for fish species were designed and previously obtained primers for a putative housekeeping gene, Na^+/K^+ -ATPase were used. Conventional PCR was performed at an annealing temperature of 55°C using RedTaq polymerase, and amplification products were isolated electrophoretically on 0.8% agarose gels. Following gel extraction, amplification products were sequenced, analyzed, trimmed and submitted to BLASTX analysis for tentative functional identification. Species-specific primers were designed for quantitative mRNA expression. cDNA was amplified in the presence of SYBRGreen dye using Qiagen Quantitect chemistry and the Stratagene MX4000 Multiplex Quantitative PCR System. A dilution series demonstrated a linear relationship between threshold cycle (Ct) and \log_{10} of template availability.

VEP β γ partial sequences of 400 and 230 bp were obtained from induced female and male livers. VEP gene expression was strongly induced by injection of E2 into females (Fig. 1). Ovary expressed both genes in the same level as brain and muscle (negative control organs). Exposure of male fish to OP (Fig. 2) caused a specific induction of liver VEP (β , γ), in the same order as the one obtained for estrogenized females. VEP expression was found neither in control liver, nor in muscle, intestine or brain from treated animals. The cyclic pattern of VEP expression seems to reflect a physiological response to OP since the results obtained for Na^+/K^+ -ATPase did not show the same (Fig. 3). Moreover, a clear inhibition of its expression due to OP was detected.

Fig. 1. Relative expression of vitelline envelope proteins (VEP) in different organs of *C. dimerus* females (E2= 10µg/g bw 17β-estradiol)

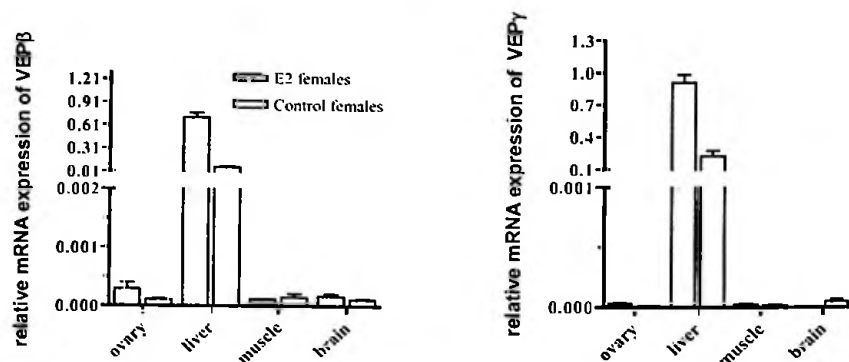


Fig. 2. Relative expression of VEP in liver of treated *C. dimerus* males. Fish were exposed to 150 µg/L octylphenol (OP).

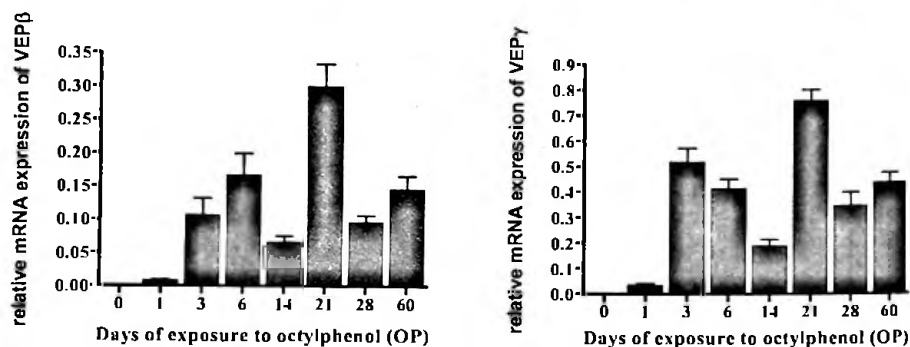
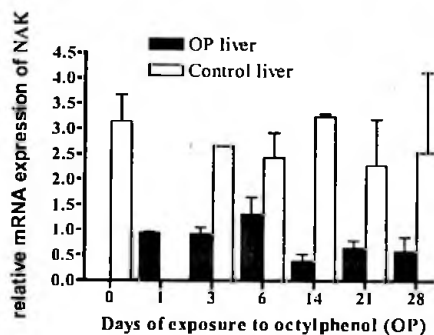


Fig. 3. Relative expression of Na⁺/K⁺-ATPase (NAK) in liver of *C. dimerus* exposed to 150 µg/L octylphenol (OP). Control fish samples from day 1 were lost.



In conclusion, VEP are normally expressed in the liver of *C. dimerus* females, as in several fish species. OP induces transcription and/or translation of VEP in liver from male fish on day 1, increases on day 3 and reaches a maximum on day 21 of exposition. Thus, gene expression constitutes a sensitive method to monitor endocrine disruptors in fish exposed for short period of time to OP.

This work was supported by NIA to Luquet and Genovese, CONICET traveling fellowship to Genovese, NSF DBI-0100394, NCRBRIN 1P20RR16463-01, PICT 14271 and UBACyT X118.

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