In vivo effects of cadmium on the estrogen signaling pathway during spermatogenesis.

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The increasing incidence of male reproductive disorders in humans and wildlife has been ascribed to estrogen-like chemicals (xenoestrogens, XE) in the environment, which are believed to compete with hormonal estrogen (E) for estrogen receptor (ER) binding. Less attention has been given to chemicals that could impact E-mediated intratesticular signaling mechanisms by altering expressed levels of cytochrome P450 aromatase (P450arom, estrogen synthetase) or ER. Compared to conventional laboratory mammals, the dogfish shark (*Squalus acanthias*) has proven to be advantageous for stage-by-stage analysis of spermatogenesis, Sertoli cell-germ cell relations, and steroid and toxicant effects on testicular processes. A polymerase chain reaction (PCR) cloning strategy was used to isolate dogfish-specific P450arom and ER complementary DNAs (cDNAs). A 2118 base pair (bp) P450arom cDNA isolated from ovary encoded a protein of 527 amino acids (aa), which had high overall sequence identity when compared to P450arom species derived from the gonads of other vertebrates: e.g., 56 and 60% vs. goldfish ovarian and human placental P450arom, respectively. A much higher degree of conservation was found in functionally important aromatase-specific (74-100%) and heme-binding (70-90%) domains. An 1812 bp ER cDNA isolated from liver encoded a protein of 542 aa. Pairwise comparisons and phylogenetic analysis using available ER sequences indicated that the isolated dogfish ER was of the ER_-subtype. Northern and reverse transcription (RT)-PCR analysis with gene-specific probes and primer pairs showed that mRNA levels are differentially distributed by tissue-type: e.g., P450arom = ovary > testis > brain > liver; ER__ liver > kidney > brain > testis > ovary. Within the testis, mRNA distribution was gene-specific and stage-related. Both genes were expressed at highest levels in ZD regions (zone of degeneration), where spermatocysts contain Sertoli cells but germ cells are degenerate, implying that P450arom and ER are localized mainly in Sertoli cells. In regions other than ZD, P450arom mRNA was highest in M stages (meiotic cysts with spermatocytes), lower in PrM stages (premeiotic cysts with stem cells and spermatogonia), and lowest in PoM stages (postmeiotic cysts with spermatids). By contrast, ER_ mRNA was highest in PrM stages and decreased progressively through maturation. These patterns of mRNA expression during spermatogenesis are consistent with aromatase and ER binding activities as measured by radiolabeled tracer analysis, and reinforce the view that locally synthesized E serves as a paracrine signal from more mature to less mature stages. To examine effects of a known spermatotoxicant on components of the estrogen signaling pathway, animals were given a single cadmium injection ($CdCl_2$, 5 mg/kg ip) 3 d before sacrifice. This treatment regimen increases intratesticular Cd accumulation, induces synthesis of a metallothionein-like protein, compromises blood-testis barrier function in PoM stages, and increases the rate of apoptosis in spermatogonia, but does not alter DNA or overall protein synthesis. In the present study, Cd reduced P450arom mRNA in all stages without changing the developmental pattern, and decreased ER_ mRNA in M-stages preferentially. These results provide evidence that certain XE-like perturbations of E-dependent reproductive processes can be due to environmental chemicals that target P450arom or ER expression rather than ER binding *per se.* Supported by a grants from the EPA (R825434) and NIEHS (P42 ES07381).