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Interference by atrazine and bisphenol A with progestin binding to the ovarian progestin membrane receptor and induction of oocyte maturation in Atlantic croaker

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Abstract

Recent studies have shown that several environmental chemicals which disrupt classical genomic steroid actions can also interfere with nongenomic steroid actions initiated at the cell surface. The effects of bisphenol-A and atrazine on the nongenomic actions of a progestin, 17,20 β ,21-trihydroxy-4-pregnen-3-one (20 β -S), on oocyte maturation (OM) were investigated an in vitro Atlantic croaker (*Micropogonias undulatus*) oocyte bioassay. Treatment of croaker follicle-enclosed oocytes with either bisphenol-A or atrazine blocked OM in response to 20 β -S in a concentration-dependent manner at 10-25 μ M (2.2-5.7 ppm) and higher concentrations. These compounds were also effective competitors at concentrations of 10^{-6} to 10^{-5} M of [3 H]-20 β -S binding to the ovarian progestin membrane receptor that mediates the OM response to 20 β -S. The results suggest that diverse classes of environmental chemicals can disrupt nongenomic progestin actions via receptor-mediated mechanisms.

Keywords: Atrazine; Bisphenol-A; Oocyte maturation; Nongenomic endocrine disruption; Progesterone membrane receptor; Atlantic croaker

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In addition to the classic genomic mechanism of steroid action via binding and activation of nuclear steroid receptors, steroids can also act at the cell surface of many target tissues including the brain, pituitary, bone, heart, gonads and gametes to initiate rapid, nongenomic responses mediated by binding to steroid membrane receptors. Recent studies have shown that nongenomic steroid actions, like genomic ones, are susceptible to interference by environmental estrogens and nonestrogenic endocrine disrupting chemicals (EDCs, Ghosh and Thomas, 1999; Nadal et al., 2000; Thomas and Doughty, 2005). In the present study the effects of an estrogenic EDC, bisphenol-A and a nonestrogenic one, atrazine, on progesterone induction of oocyte maturation (OM) and binding to the ovarian membrane progesterone receptor were investigated in Atlantic croaker. Currently, there is considerable interest and controversy over the mechanisms of action of these two EDCs. Interference with a nongenomic progesterone action was investigated in an *in vitro* oocyte maturation bioassay which determines the percentage of oocytes that undergo germinal vesicle breakdown (GVBD), an easily observable endpoint during oocyte maturation (Ghosh and Thomas, 1995). Fragments of Atlantic croaker ovaries containing

approximately 100 full grown follicle-enclosed oocytes (diameter > 450 μm) were primed for 8-11 h with human chorionic gonadotropin (15 IU/ml) prior to 12 h treatment with the maturation-inducing steroid, 17,20 β ,21-trihydroxy-4-pregnen-3-one (20 β -S, 87 nM) in the presence of the test chemicals (1 -50 μM). Atrazine (ChemService, Inc., Westchester, PA,) and bisphenol-A (City Chemical (Westhaven, CT) were added dissolved in methanol and ethanol, respectively (final conc. <1%). The carrier solvents were also added to the controls treated with 20 β -S alone. Atrazine significantly impaired 20 β -S-induced OM at concentrations of 10 μM and above in a dose-dependent manner, with fewer than 10% of the oocytes maturing at the highest concentration, 50 μM (16.4ppm, Fig.1A). The herbicide also significantly impaired maturation of spotted seatrout oocytes in a parallel in vitro bioassay at concentrations of 0.1 μM and 1 μM (results not shown). Bisphenol-A treatment also decreased OM in response to 20 β -S, although it appeared to be less potent than atrazine, since maturation of croaker oocytes (Fig.1B) and spotted seatrout oocytes (results not shown) was not significantly decreased by concentrations below 25 μM .

Possible interactions of these chemicals with the membrane progesterin receptor mediating progesterin-induced OM was investigated in competitive binding radioreceptor assays (Das and Thomas, 1999). Plasma membrane fractions of ovarian tissues containing full grown oocytes (>450 μm) and radiolabelled 20 β -S ($[^3\text{H}]$ -20 β -S, 41.9 Ci/mmol) were prepared as described previously (Patino and Thomas, 1990). Competition assays were conducted with 5 nM $[^3\text{H}]$ -20 β -S in the presence of different concentrations of the test chemicals (range 1 nM-10 μM , dissolved in 1-5 μl ethanol, which does not affect ligand binding in the receptor assays). After a 30-min incubation at 4°C with the membrane fractions, the reaction was stopped by filtration (Whatman GF/B filters, presoaked in assay buffer), washed, and bound radioactivity was measured by

scintillation counting. The displacement of the radiolabelled steroid binding by the steroid competitors was expressed as a percentage of the maximum specific binding of the steroid for its receptor. Both atrazine and bisphenol-A were effective competitors of [3 H]-20 β -S binding to the croaker ovarian membrane progestin receptor, causing significant displacement at a concentration of 10^{-6} M and approximately 50% displacement at 10^{-5} M (Fig.2). Scatchard binding analyses will be required to determine whether the binding of these compounds to the receptor is competitive (i.e. same binding site as 20 -S). Previous studies suggest Kepone binds competitively to the seatrout ovarian 20 -S receptor (Das and Thomas, 1999), whereas another estrogenic EDC, diethylstilbestrol, binds to a different site on the goldfish ovarian progestin receptor (Tokumoto et al., 2007).

The ovarian 20 β -S receptor mediating OM has been identified as a novel seven-transmembrane receptor, called membrane progestin receptor alpha (mPR α , Zhu et al., 2003). Recent studies suggest mPR α also mediates 20 β -S induction of sperm hypermotility in this species (Thomas et al., 2005). It is not surprising, therefore, that atrazine and bisphenol -A are also potent inhibitors of sperm hypermotility in response to 20 β -S in croaker (Thomas and Doughty, 2005). Overall, progestin stimulation of sperm motility appears to be much more susceptible to interference by chemicals than OM and is significantly impaired by less than one-tenth of the minimum effective concentrations observed in the present study (Thomas and Doughty, 2005). These differences may be due to lower effective concentrations of the test compounds at the oocyte surface than at the sperm membrane because the oocytes are surrounded by follicle cells. Bisphenol-A also binds to GPR30, the novel seven-transmembrane membrane estrogen receptor that has been characterized in breast cancer cells (Thomas et al., 2005), where it exerts an estrogen action (Thomas and Dong, 2006). GPR30 has also been

identified in croaker ovaries (Pang and Thomas, unpubl.), raising the possibility that this environmental estrogen could influence ovarian and oocyte functions by interfering with estrogen as well as progesterin nongenomic actions through interactions with both mPR α and GPR30.

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Figure captions

Fig. 1. Concentration –dependent inhibition by (A) atrazine, and (B) bisphenol-A of 20 β -S-induced maturation of Atlantic croaker oocytes in an in vitro bioassay. %GVBD: percent of oocytes that have completed germinal vesicle breakdown. Asterisks denote means significantly different from controls (0), Student's t test, N=4.

Fig. 2. Competition by atrazine and bisphenol-A for [³H]-20 β -S binding to Atlantic croaker ovarian membranes.

Figure 1

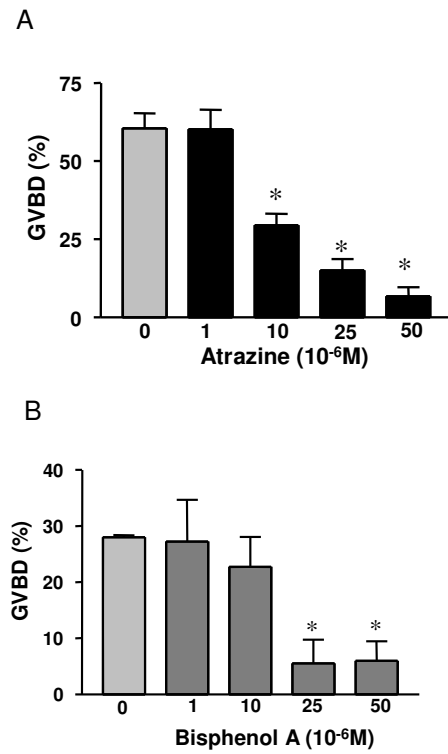


Figure 2

