

Z302 Effects of benomyl on animal cap explants and on whole-mount in Xenopus early embryo

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The teratogenic and cytotoxic effects of fungicide benomyl were investigated on whole-mount of *Xenopus* early embryos and on *Xenopus* animal cap explants. Late blastula embryos were exposed in 10 nM-100 uM benomyl in Steinberg's solution for 3 days. As a result, all embryos were dead in the concentration of 100 uM. For the finding of accurate lethal level, early embryos were exposed in 10-100 uM of benomyl, and all of them were dead in 80 uM. In 70 uM of benomyl, 60% of embryos were dead. In 40 uM or above concentrations, abnormal developments, distortion of axis and abnormal optic cup were occurred. The embryos were exposed in 50 um for 6 days, and most of them showed that the hypopigmentation, swelled abdomen, blisters and other internal abnormality except above malformations. Animal cap explants were cultured in 1 pM-100 uM benomyl in Steinberg's solution for 3days and all explants were destroyed in 100 uM concentration. For the finding of accurate lethal level, explants were exposed in 10-100 uM, and all of them were destroyed in 20 uM or above concentrations. These results may be due to the swelling of mitochondria, inhibition of tubulin multiplication, and the exhaustion of glutathione.

Z303 Three types of frog gonadotropin-releasing hormone receptors exhibit species- and type-specific ligand recognition and different signaling pathways

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Recently, we have identified three distinct types of the bullfrog gonadotropin- releasing hormone receptor (designated bfGnRHR-1, bfGnRHR-2 and bfGnRHR-3). In the present study, we also isolated three corresponding GnRHR clones (dyGnRHR-1, dyGnRHR-2 and dyGnRHR-3), in *Rana dybowskii*. Sequence analysis revealed high homology (~ 96% identity) of dyGnRHRs to their corresponding bfGnRHRs. Like bfGnRHRs, dyGnRHR-1 is mainly expressed in the pituitary, while dyGnRHR-2 and ?3 are expressed in the brain. Treatment of cells expressing the dyGnRHR and cyclic AMP response element (CRE)-luc reporter vector with various ligands (cGnRH-II, mGnRH, [Trp8]GnRH) stimulated luciferase activity with a pharmacological profile resembling that of bfGnRHR. However, the dyGnRHR-1 and ?2 showed a 10-fold less sensitivity to each ligand than bfGnRHR-1 and ?2, respectively, while dyGnRHR-3 exhibited a similar ligand sensitivity with bfGnRHR-3. It is quite interesting that dyGnRHR-1 and ?2 did not stimulate inositol phosphate accumulation after ligand treatment, while dyGnRHR-3, indicating that dyGnRHR-1 and ?2 are solely coupled with Gs protein but not Gq/11 protein. The data obtained here reinforce the presence of three types of GnRHR in amphibians, and suggest species- and type-specific ligand recognition and different signaling pathways of frog GnRHRs.