CLONING AND INITIAL CHARACTERIZATION OF NUCLEAR AND FOUR MEMBRANE PROGESTERONE RECEPTORS IN THE FATHEAD MINNOW, *PIMEPHALES PROMELAS*

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Both native progestagens and synthetic progestins have important effects on reproduction that are mediated through progesterone receptors (PRs). Progestagens regulate gamete maturation in vertebrates, are critical regulators of placental mammal pregnancy, and act as reproductive pheromones in some fishes. Progestins are components of human contraceptives and growth supplements in beef cattle. There are at least four PRs known in teleost fishes, including the nuclear progesterone receptor (nPR) and four membrane PRs (mPRα, mPRβ, and splice variants mPRγ-1 and mPRγ-2). Recently mPRδ and mPRε binding affinity and expression in the human brain were described. In this study, we have cloned and begun to characterize nPR, mPRα, mPRβ, and mPRγ in the fathead minnow (FHM). The FHM is a teleost fish species widely distributed in the United States, an important aquatic toxicology model, and an aquacultured baitfish. Full-length coding sequences were obtained through traditional cloning strategies using total RNA extracted from ovary (nPR, mPRα, and mPRβ) or gill (mPRγ-1 and mPRγ-2). Phylogenetic analyses indicate that, in general, predicted amino acid sequences of FHM PRs have the highest identities with other teleosts among vertebrates. Tissue specific expression of each of the PRs was evaluated by RT- and Q-PCR in male and female adult FHM brain, head kidney, heart, gill, liver, muscle, ovary, pituitary, spleen, and testis. In both sexes, nPR transcript was detected in brain, gonad, pituitary, and spleen. Surprisingly, nPR mRNA was detected in the male, but not the female, kidney. Of the four mPRs, mPRα transcript was the most widely expressed and was detected in all tissues examined in both sexes. In both male and female FHM, mPRβ mRNA was detected only in the brain, gonad, and gill. Expression of mPRγ mRNA was detected in male and female gill and kidney and in female intestine. We also examined the functional activity of FHM nPR as measured by activation of an MMTV promoter-driven firefly luciferase reporter construct. Cos-7 cells were transiently transfected with an empty expression vector or an expression vector for FHM nPR in combination with the reporter construct, and treated with progesterone (P4), or the fish progestagens: 17,20β,21-trihydroxy-4-pregnen-3-one (20β-S) or 17,20β-dihydroxy-4-pregnen-3-one (DHP). FHM nPR responded to each of the steroids, with the response of DHP > 20β-S > P4. In conclusion, we have successfully identified the coding sequences of four of the known PRs in FHM, investigated tissue-specific expression of each in adults of both sexes, and shown that nPR is activated by native progestagens and at least one potential environmental gestagen, P4. In ongoing research, we are examining ontogenetic expression of all mPRs from embryogenesis through adult stages and are comparing functional activity of all mPRs treated with P4, DHP, and 20β-S.

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