

MOLECULAR CLONING OF STEROID HORMONE RECEPTORS OF THE AMERICAN ALLIGATOR

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In all species of crocodylians, sex is determined not by genetic mechanisms alone, but also by the temperature at which the egg is incubated. In the American alligator (*Alligator mississippiensis*) the thermosensitive period (TSP) for sex determination is a 7- to 10-day window within stages 21-24 of development. Treating embryos with estrogen during the TSP produces female offspring, even at male incubation temperatures. Therefore, it has been suggested that estrogens play a role in determining sex in the alligator. However, the mechanisms of estrogen action on sex determination in the alligator are still uncertain. Further, studies of contaminant-exposed alligators have shown alterations in steroid action. Whether these abnormalities are due, in part, to alterations in steroid receptor expression is unknown. To begin to understand the mechanism of steroid action in alligators, we isolated cDNA encoding the estrogen receptors (ER) and the progesterone receptor (PR). Degenerated primers specific to ER were designed according to a comparison of nucleotide sequences of ERs from other species. Partial DNA fragments were amplified by PCR using alligator ovary RNA. Two DNA fragments (ER_α and ER_β) were obtained, and the RACE technique was utilized to clone full-length alligator ER_α cDNA in the 5' and 3' directions. Comparison of the amino acid sequence from the alligator ER_α with that of human, chicken and zebrafish ER shows that alligator ER is very similar to chicken ER (91 %). We also isolated a DNA fragment encoding a partial progesterone receptor (PR) of the alligator. We examined the expression levels of three steroid receptors (ER_α, ER_β and PR) in the ovary of juvenile alligators. Thirty hours after a single E₂ (270 mg/kg) injection, total RNA was extracted from the ovary. cDNA was synthesized, and the expression levels of each of the above receptors were analyzed with quantitative RT-PCR. Intriguingly, ER_α transcript decreased significantly with E₂ treatment. ER_β and PR transcripts were not changed. These results suggest that the expression of ER_α is sensitive to estrogen in the ovary of the juvenile alligator. However, in this study the expressions of ER_β and PR were not affected by estrogen treatment. Further study are underway to examine the expression of these receptors during embryonic development and reproductive cyclicity.