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JOURNAL ARTICLE

P450 aromatase messenger ribonucleic acid expression in male rat germ cells: detection by reverse transcription-polymerase chain reaction amplification

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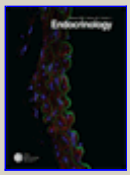
We have previously demonstrated that cytochrome P450 aromatase (P450arom) protein, an estrogen-synthesizing enzyme, is present and active in germ cells of the adult mouse testis. To establish that P450arom mRNA is expressed in germ cells of other species, we examined expression of P450arom in adult rat germ cells by employing reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from Staput separated germ cells and reverse transcribed. The resulting cDNA was amplified by nested PCR reactions using oligonucleotide primers selected from a highly conserved region of the P450arom gene. RT-PCR analysis yielded cDNA products of 334 bp in length that corresponded to the predicted size expected from the final nested amplification. The identity of the germ cell P450arom PCR products was confirmed by restriction enzyme analysis and direct nucleotide sequencing. Rat genomic DNA was subjected to PCR to verify that P450arom DNA products were not obtained from genomic DNA contamination. Rat genomic DNA yielded a nested PCR product for P450arom of approximately 2000 bp, suggesting that, as is the case with the human P450arom gene, the rat P450arom gene contains an intron in the amplified region. In addition, a semiquantitative technique was utilized to eliminate the possibility that the P450arom RT-PCR products were derived from Leydig cell contamination of Sta-put-separated germ cell preparations. RT-PCR for P450arom and 3-beta-hydroxysteroid dehydrogenase (3 beta-HSD), a Leydig cell-specific steroidogenic enzyme, was carried out on Sta-put-separated germ cells and interstitial cell preparations containing Leydig cells. P450arom and 3 beta-HSD RT-PCR reactions were stopped at three cycle intervals to detect and compare the earliest appearance of RT-PCR reaction products in various cell types. Results indicated that P450arom mRNA is detected in round spermatids before it is detected in interstitial cells, whereas 3 beta-HSD was detected only in interstitial cells, suggesting that the P450arom mRNA detected in germ cells is not due to interstitial cell contamination of germ cell preparations. Therefore, our results indicate that P450arom mRNA is expressed in adult rat germ cells and that testicular germ cells are a potential source of estrogen in the male reproductive tract.



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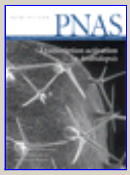

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