Constitutive Androstane Receptor Expression in the Rat Cervix During Gestation

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OBJECTIVE: *To investigate expression of the constitutive androstane receptor (CAR) in the pregnant rat cervix.*

METHODS: *Rat uterine tissue was obtained on gestational days 12, 16, 20, 21, and 22 (the day of parturition), and postpartum day 1. In addition, liver, lung, kidney, heart, and skeletal muscle tissue were obtained. Expression of the two known CAR isoforms was evaluated using reverse transcriptase-polymerase chain reaction.*

RESULTS: *These studies confirmed CAR expression in the liver; however, CAR was not demonstrated in the myometrium or cervical tissue.*

CONCLUSIONS: *The currently described CAR1 and CAR2 isoforms are not expressed in rat uterine tissue; therefore, they do not appear to participate in parturition in the pregnant rat. (J Soc Gynecol Investig 2000;7:355–7) Copyright © 2000 by the Society for Gynecologic Investigation.*

KEY WORDS: 5-α-Reductase, CAR receptor, parturition defects.

-α-Reductase, an enzyme responsible for the conversion of testosterone to dihydrotestosterone, is also responsible for the formation of many other 5-α-reduced steroids whose functions are yet to be defined. The discovery of two isoforms of 5-α-reductase has led investigators to begin to characterize their function in various cells. These two enzyme isoforms are coded by two different genes; each isoform appears to have unique physiologic properties and tissue-specific expression patterns. The gene for 5-α-reductase type I has been localized to chromosome 5, and for the type II isoform to chromosome 2 in humans.[1] Using Northern blots, the expression of the 5-α-reductase type I isoform has been demonstrated in multiple tissues including the liver, lung, adrenal, brain, colon, intestine, and kidney in rats; in contrast, the type II isoform has been observed in epididymis, prostate, and seminal vesicles.[1] Although the role of the type II isoform has been linked to normal male sexual development, the role of the type I enzyme is unclear. Unexpectedly, pregnant mice deficient in the 5-α-reductase type I enzyme have been found to be severely compromised in their ability to effect parturition; this defect is reversible with the administration of 5-α-androstan-3-α-17β-diol, a 5α-reduced androgen.[2] Recently, Mahendroo et al[3] proposed a mechanism explaining this parturition defect in 5-α-reductase type I–deficient mice. These investigators demonstrated that the problem with delivery resulted from improper cervical dilatation; however, the cellular signaling events underlying this defect in cervical ripening are yet to be elucidated.[3] Forman et al[4] recently described an orphan nuclear receptor that binds 5-α-reduced androgens such as 5-α-androsten-3α-ol and 5-α-androstan-3α-ol. This orphan nuclear receptor, which is highly expressed in the liver, has been named constitutive androstane receptor (also known as the constitutively active receptor [CAR]) because it is transcriptionally active in absence of ligand and is inhibited in the presence of 5-α-reduced androgens. To date, two isoforms of the CAR have been reported, the second isoform (CAR2) being a truncated variant of the receptor missing the C-terminal portion of the ligand binding/dimerization domain.[5] The studies described in this report sought to test the hypothesis that CAR is expressed in the pregnant rat cervix, and thereby might play a role in the apparent requirement for 5-α-reduced androgens for the normal dilatation of the cervix during parturition.

METHODS

For these studies, timed-pregnant Sprague-Dawley rats, gestational days 12, 16, 20, 21, and 22 (the day of parturition) and postpartum day 1 were used for the collection of uterine and cervical tissue with a protocol approved by the Animal Care and Utilization Committee at the University of Chicago. Liver, kidney, lung, heart, and skeletal muscle tissues were also isolated from pregnant rats on gestational days 16 and 22. These tissue samples were initially incubated in an RNase inhibitor solution (RNALater; Ambion Corp., Austin, TX) overnight at 4C, then stored at −70C until processed. Total RNA was isolated using the acidic guanidinium-thiocyanate-phenol-choloroform extraction technique (TRIzol reagent; Gibco Life Technologies, Grand Island, NY). Subsequently, the RNA samples were treated with DNAase (Stratagene Corp., LaJolla, CA) to remove any traces of genomic DNA.

Sense and antisense primers for a homologous region of the

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Table 1. Constitutive Androstane Receptor RT-PCR Information

Gene	Primer sequences	Accession no.	Amplicon identity
CAR1	Sense: ACC AGT TTG TGC AGT TCA GG Antisense: CTT GAG AAG GGA GAT CTG GT	AF009327 Z30425	Mouse = 91% Human = 83%
CAR2	Sense: As above Antisense: As above	AF009328	Mouse = 91%

two CAR isoforms were designed based on the conserved sequences of the human and mouse CAR1 and CAR2 gene sequences[5,6] using Gene Runner (v 3.02) DNA analysis software (Hastings Software Inc., Hastings, NY). Subsequently, reverse transcriptase-polymerase chain reaction (RT-PCR) assays were performed using these primers and the GeneAmp RNA PCR Kit (Perkin Elmer [Roche], Branchburg, NJ) on RNA samples from each tissue preparation. Three micrograms of total RNA was used for the RT-PCRs as follows: reverse-transcriptase step performed at 42C for 15 minutes, followed by 36 PCR cycles with melting at 94C, annealing at 54C, and extension at 72C. The 268-base pair (bp) amplicon product was resolved on 1.8% agarose-tromethamine (Tris)-borate-ethylenediaminetetraacetic acid (EDTA) gels and visualized after ethidium bromide staining of the gel and ultraviolet transillumination. To confirm the identity of the RT-PCR product, the amplicon was isolated from the agarose gel using the GeneClean Silica Matrix kit (Bio101 Inc., LaJolla, CA) and then sequenced in the sense direction using the automated fluorescent dideoxynucleotide DNA sequencing technique performed by the University of Chicago Core Sequencing Facility. The BLAST (basic local alignment search tool) homology search algorithm and the on-line Genebank database (National Center for Biotechnology Information at www.ncbi.nlm.nih.gov) were used to confirm the identity of the sequenced amplicon and compare it with the published human and mouse CAR sequences. Positive control RT-PCR studies were performed using total RNA from cervical and myometrial tissue along with primers for the β-isoform of actin (Clontech, Palo Alto, CA).

RESULTS

Sense and antisense primers, designed from the conserved regions of the human and mouse CAR1 and CAR2 sequences,[5,6] resulted in the generation of the anticipated 268-bp amplicon product (Table 1). Isolation and sequencing of this amplicon demonstrated 83–91% identity with the published CAR gene sequences. (Table 1). This sequence information is the first for CAR in the Sprague-Dawley rat; this partial sequence for rat CAR has been submitted to the Genebank database (accession number AF233431).

Consistent with reports using mouse tissues, CAR expression was observed in liver of the pregnant rat; in contrast, this

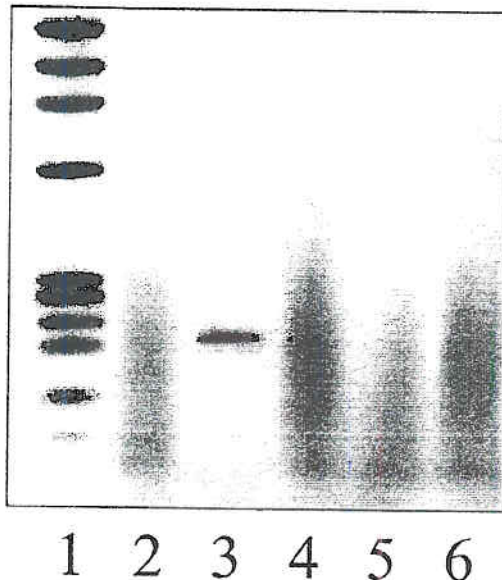

Figure 1. Ethidium bromide–stained gel (image color reversed) demonstrating the results of the RT-PCRs performed with CAR primers and total RNA from various tissues at day 16 of gestation (*lane 2 = kidney; lane 3 = liver; lane 4 = heart; lane 5 = skeletal muscle; lane 6 = lung*) along with *HaeIII* cut ϕX 174 DNA molecular size marker (*lane 1*). Tissue samples obtained from animals at day 22 of gestation produced identical results (data not shown).

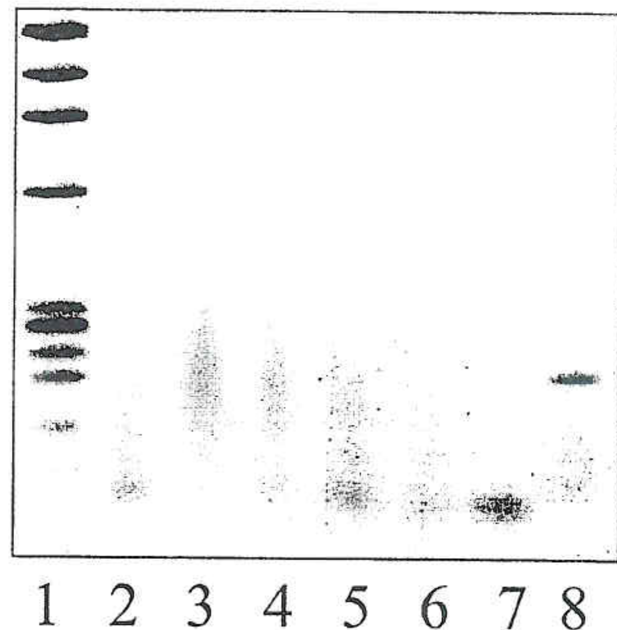

Figure 2. Myometrial tissue. Ethidium bromide–stained gel (image color reversed) demonstrating the results of the RT-PCRs performed with CAR primers and total RNA from myometrial tissues at various gestational days (*lane 2 = day 12; lane 3 = day 16; lane 4 = day 20; lane 5 = day 21; lane 6 = day 22; lane 7 = postpartum day 1*), along with *HaeIII* cut ϕX 174 DNA molecular size marker (*lane 1*) and liver from day 16 pregnant female (*lane 8*).

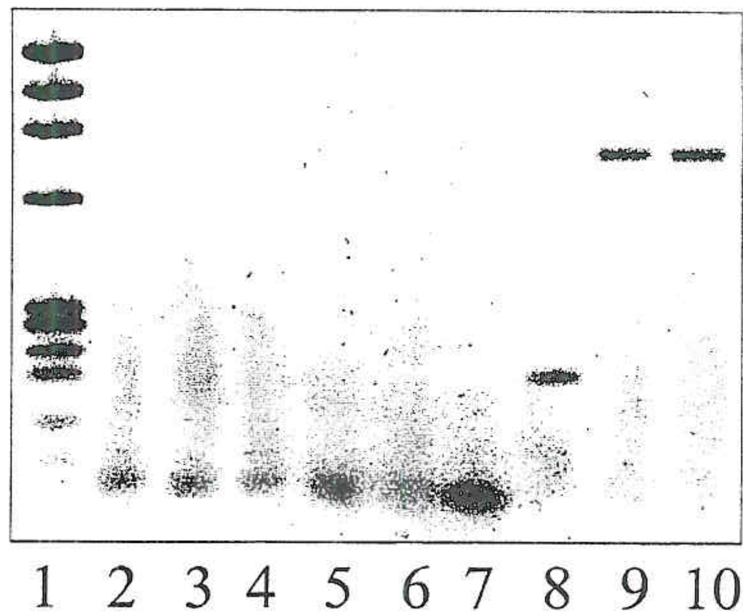

Figure 3. Cervical tissue. Ethidium bromide–stained gel (image color reversed) demonstrating the results of the RT-PCRs performed with CAR primers and total RNA from cervical tissues at various gestational days (*lane 2 = day 12; lane 3 = day 16; lane 4 = day 20; lane 5 = day 21; lane 6 = day 22; lane 7 = postpartum day 1*), along with *Hae*III cut ϕX 174 DNA molecular size marker (*lane 1*) and gestational day 16 liver (*lane 8*). RT-PCRs were also performed using β-actin primers along with cervical and myometrial total RNA as positive controls: *lane 9* = day 20 cervix; *lane 10* = day 20 myometrium.

band was not observed in kidney, heart, skeletal muscle, or lung tissue (Figure 1). RT-PCR studies performed using cervix and myometrium failed to demonstrate CAR expression in either of these uterine tissues during the second half of gestation in the timed-pregnant Sprague-Dawley rat (Figures 2 and 3). The RT-PCR studies performed using cervical and myometrial tissue RNA along with β-actin primers resulted in the generation of the anticipated 764-bp amplicon, thereby confirming the integrity of the RNA isolated from these uterine tissues.

DISCUSSION

5-α-Reduced androgens have been demonstrated to reverse the parturition defect in the 5-α-reductase type-1–deficient transgenic mouse.[2] Mahendroo et al[3] hypothesized that the parturition defect in these transgenic mice is related to the inability of the cervix to dilate properly during labor. The exact mechanism of action of the 5-α-reduced androgens in reversing this defect is unknown. CAR, a member of the orphan nuclear receptor family whose ligand leads to receptor inactivation, has recently been found to bind 5-α-reduced androgens. This is the first study to examine CAR expression in the cervix during gestation and its possible link to parturition. Because of the 5-α-reduced androgen binding properties of CAR, we sought to characterize its expression in cervical and myometrial tissue during the second half of gestation in the rat. These studies confirmed CAR expression in the rat liver; however, we were unable to demonstrate CAR expression in cervical and myometrial tissue from pregnant rats. These data suggest that expression of CAR may not occur in the pregnant rat uterus or that the level of expression is very low, ie, below the level of detection by RT-PCR. Another possibility is that alternative splicing of the CAR sequence could have occurred in the rat, resulting in deletion of the sequence we amplified; however, this is unlikely because the region chosen for amplification is highly conserved in both the human and the mouse CAR isoforms. Thus, although the orphan receptor CAR is known to bind 5-α-reduced androgens as a ligand, its expression cannot be demonstrated in rat cervical tissue using RT-PCR techniques. This evidence suggests that the currently reported CAR1 and CAR2 isoforms are not expressed in the cervix or myometrium; therefore, they would appear not to have a role in the cervical response to 5-α-reduced androgens during parturition. It still needs to be determined whether other CAR isoforms or similar androgen receptors exist, which might be important during 5-α-reduced androgen–mediated cervical ripening.

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