Expression of Ovarian 20α-Hydroxysteroid Dehydrogenase in Rat Thymus

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Abstract. Ovarian 20α-hydroxysteroid dehydrogenase (20α-HSD), which converts progesterone to a derivative devoid of biological activity, plays a crucial role in achieving the short estrous cycle in rats. Although 20α-HSD activity has also been demonstrated in the thymus, its molecular nature, function, and regulation of expression have yet to be determined. In the present study we investigated if 20α-HSD activity in the thymus originates in a transcript identical to that expressed in the ovary. RT-PCR analysis indicated the expression of 20α-HSD mRNA in rat thymus, and sequencing of the PCR product showed 100% identity to ovarian 20α-HSD cDNA. Immunohistochemical study using anti-rat ovarian 20α-HSD antibody demonstrated the expression of 20α-HSD protein in the thymus. The 20α-HSD-expressing cells in the thymus seemed to be some type of lymphocyte by their morphology. These results suggest that the same molecular species as ovarian 20α-HSD is expressed in thymic lymphocytes. Therefore, 20α-HSD may play a role in T-lymphocyte proliferation and differentiation processes.

Key words: 20α-Hydroxysteroid dehydrogenase, Gene expression, Immunohistochemistry, Thymus, Ovary (Endocrine Journal 48: 557–563, 2001)
different genes, as several enzymes such as human placental 17β-HSD [12] and bovine testicular aldose reductase [13] have been shown to possess 20α-HSD activity. Concerning 20α-HSD activity in the thymus, although its apparent Km (progesterone to 20α-OHP) was reported to be close to the value of ovarian 20α-HSD [14, 15], it has not been well characterized at the molecular level. Recently, we have cloned cDNA encoding ovarian 20α-HSD in both rats [16] and mice [17], and the promoter region of the gene up to 8 kb (our unpublished data). The nucleotide sequence and its deduced amino acid sequence have revealed that the enzyme belongs to the aldoketo reductase superfamily. These studies have enabled us to analyze the regulatory mechanisms for tissue-specific expression of 20α-HSD at the transcription level. In the present study, we attempted to determine whether 20α-HSD activity in the thymus is derived from a transcript identical in genetic origin to that expressed in the ovary. Total RNAs from rat thymic tissue were subjected to RT-PCR using primer sets designed from rat ovarian 20α-HSD cDNA. Immunohistochemical analysis with specific antibody against rat ovarian 20α-HSD was also carried out.

Material and Methods

Animals and tissue preparation

Wistar-Imamichi rats were housed in a temperature- and light-controlled room (23°C; lights on, 0500–1900 h) and fed ad libitum. Rats were sacrificed by decapitation, and tissue samples including those of the cerebrum, liver, lung, kidney, adrenal gland, spleen, thymus, testis, uterus, and ovary were collected and immediately frozen by liquid nitrogen for further analyses.

RNA extraction and RT-PCR analysis

Tissue RNAs were extracted using TRIzol Reagent (Life Technologies, Rockville, MD) according to the manufacture's instructions, and the concentrations were calculated from an absorbance of 260 nm (OD260). A 5-mg sample of total RNA was used for the reverse-transcription (RT) reaction using Ready-To-Go You-Prime First-Strand Beads (Amersham Pharmacia, Buckinghamshire, England), and a 1/33 to 1/10 volume of RT solution was used for PCR with Taq Gold Polymerase (Perkin Elmer Applied Biosystems, Branchburg, NJ). Primers for rat ovarian 20α-HSD (Table 1) were designed on the basis of rat ovarian 20α-HSD cDNA [16]. PCR was performed under the following conditions: 10 min of activation at 95°C, 1 min of denaturation at 94°C, 1 min of annealing at 63°C, and 1 min of extension at 72°C followed by 10 min of extension at 72°C. As an internal control, PCR with serially diluted templates using primers for rat β-actin (Table 1) was carried out, and the template amounts were confirmed to be equal between samples. Following 40 cycles of amplification with 20α-HSD-specific primers, electrophoreses were carried out on a 1% agarose gel and stained with ethidium bromide. The nucleotide sequences of the PCR products were determined by the dideoxy chain-termination method with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit and the ABI PRISM 377 DNA Sequencer (Perkin Elmer Applied Biosystems).

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Oligonucleotide primers used for RT-PCR analysis</th>
<th>Sequences</th>
<th>Nucleotide number</th>
<th>Expected fragment length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20α-HSD</td>
<td>forward-1</td>
<td>5'-CTAGGGAAGAGCAGCATCTGA-3'</td>
<td>8–28</td>
<td>675</td>
</tr>
<tr>
<td></td>
<td>reverse-1</td>
<td>5'-CACCATAGGCAACGAGAAGA-3'</td>
<td>662–682</td>
<td></td>
</tr>
<tr>
<td></td>
<td>forward-2</td>
<td>5'-ACTCTCTCTAGGGAAGAGACAG-3'</td>
<td>2–21</td>
<td>1141</td>
</tr>
<tr>
<td></td>
<td>reverse-2</td>
<td>5'-CGATGTACAGACAGACAGCTCA-3'</td>
<td>1122–1142</td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td>forward</td>
<td>5'-CCTAGACTTCTGGAGCAGAAGA-3'</td>
<td>666–685</td>
<td>368</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td>5'-CACCAATCCACACAGAGTAC-3'</td>
<td>1014–1033</td>
<td></td>
</tr>
</tbody>
</table>
Immunohistochemical staining

Rat thymus, adrenal gland, or ovary was immersed in 4% paraformaldehyde/PBS, dehydrated in 30% sucrose, and embedded in O.C.T. compound (Sakura, Tokyo, Japan). Tissues were sectioned at 5 mm, and placed on silane-coated slides. The slides were pretreated with 3% H₂O₂ for 15 min, followed by treatment with skim milk for 60 min at room temperature. The slides were then incubated with mouse polyclonal anti-rat 20α-HSD antibody, which was generated in our laboratory [18], diluted with PBS/1% BSA (1:50), at 4°C overnight. The slides were washed three times with PBS and then incubated with HRP-labeled goat anti-mouse IgG antibody (1:1000; American Qualex Antibodies, San Clemente, CA) diluted with PBS/1% BSA at room temperature for 60 min. Finally, the slides were treated with DAB (Vector Laboratories, Burlingame, CA) for 5 min, and after color development, the slides were covered with coverslips.

Results

Tissue distribution of 20α-HSD mRNA

Expression of rat ovarian 20α-HSD mRNA was examined in various tissues by RT-PCR with forward-1 and reverse-1 primers (Table 1). As shown in Fig. 1A, 20α-HSD mRNA was detected specifically in the cerebrum, thymus, and ovaries of rats at the age...
of 9 weeks. In the cerebrum, the amount of mRNA was at a barely detectable level by RT-PCR. In contrast, the PCR product from the thymus was readily visible, and maximal amplification was seen with the ovarian cDNA sample. Using thymus RNA without a reverse transcription reaction as a negative control, no signal was detected by PCR (data not shown). Amplification of a DNA fragment containing the entire coding region from thymus cDNA using forward-2 and reverse-2 primers (Table 1) was also carried out, and a DNA fragment of the predicted size at around 1140 bp was obtained (Fig. 1B). The nucleotide sequences of the PCR products amplified with both primer sets were determined and found to perfectly match that of the ovarian 20α-HSD cDNA, indicating that the thymus expressed the same molecular species as ovarian 20α-HSD mRNA. Expression of 20α-HSD mRNA in the thymus of 3- and 13-week-old rats was also examined by RT-PCR. As shown in Fig. 1C, expression of 20α-HSD mRNA was seen in the thymus at both ages. 20α-HSD mRNA expression in the thymus of 3-week-old rats seemed to be greater than that in the thymus of 13-week-old rats with minor variations between animals. 20α-HSD mRNA expression in another lymphatic organ, the spleen, was also examined, but 20α-HSD mRNA expression was not detected in the spleen of either 3- or 13-week-old rats (Fig. 1C).

Expression and localization of 20α-HSD protein in rat thymus

To further confirm the expression of ovarian 20α-HSD in the thymus, the expression of 20α-HSD protein in the thymus was examined by means of immunohistochemical staining using a specific antibody for rat ovarian 20α-HSD. Fig. 2 shows the immunoreactivity in the ovary (A) and thymus (B and C) of 10-week-old rats. In ovarian sections used as a positive control, immunoreactivity was localized exclusively in the corpora lutea (Fig. 2A), confirming the specificity of the antibody together with our previous report that Western blotting analysis coincides with the rise in 20α-HSD activity in ovarian cytosol [19]. Immunoreactivity was not observed at all in the adrenal gland, which is another steroidogenic organ (data not shown). In the thymus, immunoreactivity was widely distributed in both cortex and medulla, and the immunoreactive cells were of round shape.

Fig. 2. Immunohistochemical staining against anti-20α-HSD antibody in the ovary (A) and thymus (B and C) of a 10-week-old rat. A. In the ovary, corpora lutea are immunostained. In contrast, follicles were completely devoid of staining. B. In the thymus, immunoreactive cells are widely scattered, and the cytosol was positively stained. C. Immunoreactive cells were not detected without the 1st antibody. Scale bars= 200 μm (A), and 20 μm (B and C).
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Discussion

In the present study, we demonstrated by RT-PCR that ovarian 20α-HSD mRNA is expressed in the thymus as well as the ovary, and, to a much lesser extent, in the brain of the rat. In the 1970s, there were reports that 20α-HSD activity is present in the thymus [10, 20], but the molecule from which the enzyme activity is derived has yet to be determined. The present study suggests that 20α-HSD activity in the thymus originates in a molecule identical to that expressed in the ovary, though it remains possible that some of the enzyme activity is due to other molecules possessing 20α-HSD activity such as 17β-HSD [12] and aldose reductase [13]. As far as we know, there has been no study demonstrating 20α-HSD activity in the brain, but a specific population of cells in the brain may express 20α-HSD mRNA and be involved in steroid metabolism.

Immunohistochemical study using specific antibody against rat ovarian 20α-HSD showed that immunoreactive cells are widely distributed in the thymus and are of round shape. According to these observations, 20α-HSD-positive cells seem to be some type of T-lymphocyte. Considering the fact that CD3-negative immature T-lymphocytes localize in the thymic cortex, and that CD3-positive mature T-lymphocytes distribute in the medullary region, it is unlikely that a correlation exists between the expression of 20α-HSD and the stage of T-cell differentiation. However, whether 20α-HSD expression is limited to a specific developmental stage of T-lymphocytes or not must be determined by further analyses such as double staining experiments.

We found in the present study by RT-PCR that the expression of 20α-HSD in the thymus is apparently stronger in 3- than in 13-week-old rats. Considering that thymic atrophy occurs with age, it is conceivable that the decrease in the expression is correlated with the structural or functional involution of the thymus by a certain mechanism.

Sex steroids as well as corticosteroids are known to modulate thymic function [21]. According to a study using progesterone receptor knockout mice, progesterone receptors in thymic epithelial cells are required for the involution of the thymus during pregnancy [22]. It has also been reported that age-dependent thymic involution is prevented by castration in males [23, 24]. These findings suggest that steroid hormones have an inhibitory effect on immune function. Expression of 20α-HSD may be important for protecting immune cells from these negative effects of progesterone by converting it into a biologically inactive steroid.

In several reports on the regulation of members of the aldoketo reductase superfamily, the expression of human prostaglandin F synthase (AKR1C3) in peripheral blood lymphocytes and HAKR e (AKR1C3) in the human myeloid leukemia-derived cell line HL60 are downregulated by con A [25] and upregulated by all-trans-retinoic acid [26], respectively. In a more recent report, a novel family member of aldoketo reductase genes in myeloid cells whose expression is regulated by IL-3 was cloned [27]. These reports suggest that other members of the aldoketo reductase family, including ovarian 20α-HSD, can be regulated by various factors known to affect the proliferation and differentiation of lymphocytes. Recently, both cDNA encoding mouse ovarian 20α-HSD [17] and the 5'-flanking region of mouse ovarian 20α-HSD gene (mouse 20α-HSD gene promoter) have been cloned in our laboratory. Since all the previous studies on 20α-HSD activity in lymphatic organs and myeloid cells have been conducted using mice, the cloned mouse cDNA and the promoter region could be useful tools for analyzing the gene expression and the mechanism of transcription in mouse cells.

Since the expression of 20α-HSD and related genes has also been confirmed in human lymphocytes [28], the involvemtent of 20α-HSD in lymphocyte differentiation seems common among mammalian species. However, because human 20α-HSD (AKR1C1) can also be regulated by PGF2α and oxytocin in porcine luteinized granulosa cells [28], it is possible that the gene functions in both the immune and reproductive systems. In several rodent species, including mice and rats, the 20α-HSD gene is expressed in newly formed corpora lutea and shortens the estrous cycle; it thus is profoundly involved in reproductive strategy. In these animals, 20α-HSD gene expression is suppressed by prolactin following copulation, with a resultant introduction of functional luteal phase [29], indicating that the promoter of the 20α-HSD
gene in these animals responds to a prolactin signaling pathway. Comparative analyses of the 20α-HSD promoter determining the tissue-specific expression and prolactin-responsiveness might provide interesting evolutionary evidence.

In conclusion, the present study has demonstrated that 20α-HSD mRNA and a protein identical to that expressed in the ovary are expressed in the thymus of rats, and therefore 20α-HSD may play a role in the function of thymic T-lymphocytes. Further studies on the genome structure are needed to elucidate the regulation of tissue-specific expression and the molecular evolution of the gene.

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