Detection of retinoic acid receptor mRNA in rat tissues by reverse transcriptase-polymerase chain reaction

Y.-J. Y. Wan, L. Wang* and T.-C. J. Wu*

Department of Pathology, Harbor–UCLA Medical Center, 1000 West Carson Street, Torrance, California 90509, U.S.A.
*Department of Obstetrics and Gynecology, UCLA School of Medicine, 10833 Le Conte Avenue, Los Angeles, California 90024, U.S.A.

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ABSTRACT

The presence of retinoic acid receptor (RAR) α, β and γ mRNA was examined in 16 different kinds of rat tissue using the highly sensitive reverse transcriptase-polymerase chain reaction technique. The data demonstrated that each tissue expressed at least two types of RAR mRNA. Among the three types of RAR mRNA, RAR α was widely expressed in all types of organ and was the dominant form expressed in the gastrointestinal tract. RAR β mRNA was not present in the intestine and spleen. In addition, RAR β mRNA levels were high in the heart, lung, brain, testis and epididymis. RAR γ mRNA was abundant in both male and female reproductive systems, as well as epidermal tissues. The prevalence of each RAR mRNA in the tissues suggests the diverse biological roles of these receptors.

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INTRODUCTION

Retinoic acid (RA) has pleiotropic effects in the regulation of cell growth, differentiation and proliferation (Sporn, 1977; Sporn et al. 1984). RA exerts its biological functions through retinoic acid receptors (RAR). Several RAR cDNAs (α, β and γ) have been cloned in human and mouse (Giguere et al. 1987; Pektovich et al. 1987; de The et al. 1987; Benbrook et al. 1988; Brand et al. 1988; Zelent et al. 1989; Ishikawa et al. 1990). In addition, a class of more distantly related retinoid receptors, RXR, has also recently been described (Mangelsdorf et al. 1992). The presence of multiple receptors for one ligand indicates that each receptor probably has its own unique biological function.

The expression of each receptor gene was examined in some tissues from mouse, rat and human by Northern blot hybridization (de The et al. 1987; Zelent et al. 1989; Ishikawa et al. 1990). However, there has been no study which systemically examines the expression of three types of RAR mRNA in various organ systems. It has been found that RAR α mRNA is widely distributed in many tissues, while the expression of RAR β and γ mRNAs is more tissue-specific (Zelent et al. 1989). RAR β mRNA is abundant in the brain but is not detected in spleen, intestine or testis (Zelent et al. 1989). Other studies, however, showed the presence of RAR β mRNA in Sertoli cells (Kim & Griswold, 1990). The discrepancy between these reports is probably due to the sensitivity of the methods used. RAR γ mRNA was exclusively found in the skin at comparatively high levels (Zelent et al. 1989; Ishikawa et al. 1990). To understand the possible physiological roles of each receptor and to clarify the contradictory reports, we determined the presence of each type of RAR mRNA in rat tissues using the highly sensitive reverse transcriptase-polymerase chain reaction (RT-PCR) and semiquantitated the relative amount of mRNA for each type of RAR expressed in various rat tissues.

MATERIALS AND METHODS

Different kinds of tissue were obtained from adult Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, U.S.A.) or rat fetuses on gestational day 17. Tissues were obtained from a pool of at least three rats. RNA was extracted by the guanidinium thiocyanate–cesium chloride method (Chirgwin et al. 1979). Total RNA was reverse-transcribed using 1 µg random primers (Boehringer

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Mannheim Co., Indianapolis, IN, U.S.A.) or 0.5 μg oligo(dT) (20 mer) primers and 200 U Moloney murine leukemia virus reverse transcriptase (Boehringer Mannheim Co.) in 1× PCR buffer (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 1.5 mM MgCl₂, 0.01% (w/v) gelatin and 0.1% Triton X-100), 20 U RNasin (Promega, Madison, WI, U.S.A.) and 10 mM each of dATP, dCTP, dGTP and dTTP (Boehringer Mannheim Co.). Then the cDNA was divided into four equal portions for amplification of RAR α, β and γ and β-actin cDNAs, using 30 cycles of PCR. According to our experience, limiting the number of PCR cycles to 30 allows the amplification to proceed in the exponential phase, and the assay is linear with respect to the initial amount of RNA.

For PCR, a mixture including 30 pmol primers specific for RARs α, β or γ or β-actin (Table 1), 2.5 U Taq DNA polymerase (Promega) and 10 mM each of dATP, dCTP, dGTP and dTTP was added to the reaction. The total volume was brought to 100 μl with 1× PCR buffer, and then samples were overlaid with light mineral oil. Each PCR cycle included denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s. After PCR, one-tenth of the sample was electrophoresed on a 1.5% agarose gel. Water was used to replace cDNA and served as a negative control in each run of PCR.

RESULTS AND DISCUSSION

The RT-PCR conditions were chosen as follows, so that the relative amounts of RAR α, β and γ and β-actin mRNA could be semiquantitated. (1) Both oligo(dT)-primed and random-primed cDNA were used for PCR, to ensure that the efficiency of cDNA synthesis was similar among RAR α, β and γ; (2) the cDNA was divided equally into four portions for the PCR; (3) a quantitation experiment was performed to ensure the linearity of the PCR, therefore the signal increased in proportion to the amount of starting material; (4) all the primers used for PCR had very similar percentages of GC content; (5) the sizes of amplified fragments did not differ much (396–521 bp), so that the efficiency of PCR remained similar even when a different primer set was used.

Figure 1 demonstrates that PCR can reliably semiquantitate the amount of mRNA expressed in the tissue. Various amounts of liver RNA ranging from 0 to 2.5 μg were reverse-transcribed using random primers, then the newly synthesized cDNA was divided equally for amplification of RARs α, β and γ. As shown in Fig. 1, the more RNA used, the more PCR products resulted. Comparing different types of RAR, RAR α mRNA was the most dominant and the level of RAR γ mRNA was the lowest in the liver. Similar results were obtained if Northern blot analysis was employed (Wan et al. 1992).

To examine the presence or absence of RAR α, β and γ transcripts, 16 different types of tissue were used for RT-PCR. RAR α and γ mRNAs were present in all the tissues examined, including heart, lung, fetal and adult brain, liver, spleen, kidney, intestine, epididymis, testis, ovary, uterus, fetal and adult skin, tail and gravid uterus. RAR β mRNA was

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<th>Table 1. The oligonucleotides used for reverse transcriptase-polymerase chain reaction (PCR)</th>
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<tr>
<td><strong>Oligonucleotides</strong></td>
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<td>β-actin</td>
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RAR, retinoic acid receptor.
not found in spleen and intestine but was detected in the rest of the tissues examined, including testis (Fig. 2). The β-actin cDNA was also amplified along with RARs α, β and γ to serve as a positive control. The expected sizes of amplified RAR α, β and γ and β-actin cDNA fragments were 396, 470, 521 and 410 bp respectively. Different sizes of fragments were chosen for easy identification. To ensure that only cDNA was amplified, all the primers chosen were located on separate exons. Bigger fragments are obtained if genomic DNA is used for amplification.

Among the four types of mRNA examined, the intensity of β-actin fragments was the highest, therefore β-actin mRNA was the most abundant one (Fig. 2). Comparing the three types of RAR mRNA, the relative amounts of RAR α, β and γ mRNA were similar in heart and brain. RAR α mRNA was the predominant form expressed in liver, spleen, kidney and intestine when compared with RARs β and γ. In contrast, RAR β mRNA was not detected in spleen and intestine, and only trace amounts of RAR γ mRNA could be detected in these organs. These data suggest that among the three RARs, RAR α probably plays a major role in the gastrointestinal system. In the reproductive system, all three receptor mRNAs could be detected. However, only trace amounts of RAR β mRNA were found in the female reproductive system (ovary, uterus and gravid uterus). In contrast, epididymis and testis expressed higher levels of RAR β mRNA. The amount of RAR β mRNA was also very low in the fetal and adult skin and tail. These tissues predominantly expressed RAR α and γ mRNA. No significant differences in any of the RAR mRNA levels were observed between fetal and adult tissues or between non-pregnant and gravid uteri. All the data were further confirmed by an internal restriction enzyme digestion (HaeIII for RARs α and β, and Sac I for RAR γ, data not shown). The relative amounts of these three types of RAR mRNA in various rat tissues in this study were similar to those shown by Northern blot hybridization (de The et al. 1987; Zelent et al. 1989; Ishikawa et al. 1990; Wan et al. 1992 and the authors’ unpublished data).

**FIGURE 2.** Detection of retinoic acid receptor (RAR) and β-actin mRNAs in rat tissues by reverse transcriptase-polymerase chain reaction (PCR). Total RNA (1 μg) extracted from rat tissues was reverse-transcribed using 0.5 μg oligo(dT). The synthesized cDNA was divided equally into four portions, and used for PCR as described in the legend to Fig. 1. The expected sizes of PCR products for RAR α (lane 1), β (lane 2) and γ (lane 3) and β-actin (lane 4) were 397, 470, 521 and 410 bp respectively. Samples from the intestine are indicated by intest., those from the epididymis by epid. The size markers are MspI fragments of pBR322. The amount of DNA loaded on each lane of the gel was one-tenth of the amplified products.
In summary, each tissue contains at least two kinds of RAR mRNA. RAR α mRNA is highly and widely expressed in all the tissues examined. RAR β mRNA cannot be detected in spleen and intestine, and is low in most tissues examined, with the exception of heart, lung, brain and male reproductive organs. RAR γ mRNA is quite abundant in both male and female reproductive systems, besides the epidermal tissues. The differential expression of RAR β and γ MRNAs in male and female reproductive systems may be related to their distinctively different reproductive functions.

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