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Differential expression of the α and β retinoic acid receptors in tissues of the rat

Jonathan L. REES, Ann K. DALY and Christopher P. F. REDFERN

Medical Molecular Biology Group and Department of Dermatology, Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, U.K.

We have compared the expression of α and β retinoic acid receptors (RAR-α and RAR-β) in different rat tissues. The two cDNA probes for RAR-α and RAR-β each specifically detect two different transcripts. RAR-α was expressed in all tissues examined, but, in contrast, the expression of RAR-β was undetectable in some tissues. The data do not support the idea that RAR-β is specific to epithelial tissues.

INTRODUCTION

Vitamin A (retinol) and its metabolite retinoic acid have important effects on cell development and differentiation both *in vivo* and *in vitro*. Until recently, the mode of action of retinoic acid has been obscure. The discovery of cellular retinoid-binding proteins led to suggestions that these proteins mediate an interaction of retinoids with the cell nucleus [1]. Nuclei of retinoic acid-responsive F9 embryonal-carcinoma cells contain a retinoic acid-binding protein which is distinct from the cytoplasmic cellular retinoic acid-binding protein [2]. The cloning of cDNAs coding for two retinoic acid receptors, RAR-α [3,4] and RAR-β [5,6], has since been reported, and the protein encoded by the RAR-β cDNA co-sediments with the nuclear retinoic acid-binding activity present in F9 cells (A. K. Daly & C. P. F. Redfern, unpublished work). The predicted primary structures of these retinoic acid receptors have considerable sequence similarity with each other and with the steroid-hormone/thyroid-hormone family of DNA-binding proteins [5]. Although it has been suggested that RAR-β shows considerable specificity for epithelial tissues [6], the biological roles of RAR-α and RAR-β are unknown. In an attempt to clarify the physiological roles of the two retinoic acid receptors, we have investigated patterns of expression of RAR mRNA in tissues of the rat.

MATERIALS AND METHODS

RNA preparation

RNA was extracted from Wistar rat tissues by the guanidinium thiocyanate/CsCl method [7]. Poly(A)$^+$-enriched RNA was prepared by oligo(dT)–cellulose chromatography [8] and quantified by absorbance at 260 nm. RNA was also extracted from HeLa cells for use as a reference sample.

Northern blotting and hybridization

RNA samples (15 μg per track) were size-fractionated on 1.5% agarose/0.2 M-formaldehyde gels and capillary-blotted on to nylon membranes (Schleicher and Schuell, Dassel, Germany) [9]. Membranes were hybridized at 42 °C with ^{32}P-labelled probe essentially as described by Thomas [10] using 25 ng of probe [(1–2) × 10^6 c.p.m./ml]. Membranes were washed twice in 2 × SSC (1 × SSC is 0.15 M-NaCl/15 mM-trisodium citrate)/0.1% SDS for 15 min each at room temperature and twice in 2 × SSC at 68 °C for 30 min each. In addition, blots probed with RAR cDNA were washed at 68 °C in 1 × SSC/0.1% SDS. Autoradiographs were exposed for 1–3 days with intensifying screens at −70 °C. Transcript sizes were estimated relative to rat and yeast rRNA markers.

Probe preparation

The human RAR-α and RAR-β c-DNA clones were gifts from Dr. M. Petkovich and Professor P. Chambon (CNRS, Strasbourg, France) and Dr. A. Dejean (Institut Pasteur, Paris, France) respectively. The RAR-α probe was a 0.5 kb endonuclease-*Kpn*I/*Sac*I fragment from the 5′ end of the human RAR-α cDNA [3]. The RAR-β probe was a 1.4 kb fragment of the RAR-β cDNA clone pCOD20 and contained the complete RAR-β-coding region [11]. The actin probe was a 1.15 kb endonuclease-*Pst*I fragment of a mouse actin cDNA clone, pAM [12], and was used to detect β-actin. Probes were labelled with [^{32}P]dCTP (Amersham International; 3000 Ci/mmol) [13] to a specific radioactivity of 10^9 c.p.m./μg of DNA (RAR probes) or 10^8 c.p.m./μg of DNA (α-actin probe).

RESULTS AND DISCUSSION

RAR-α and RAR-β probes detect different transcripts

Northern blots of poly(A)$^+$ RNA from adult rat liver were probed successively with RAR-α and RAR-β probes. the RAR-α probe hybridized to transcripts of approx. 3.6 kb (RAR-α1) and 2.8 kb (RAR-α2), whereas the RAR-β probe hybridized to transcripts of approx. 3.4 kb (RAR-β1) and 3.1 kb (RAR-β2) (Fig. 1). Similar results were obtained with poly(A)$^+$ RNA extracted from a range of rat tissues (see Fig. 2). However, RAR-α transcript lengths, particularly RAR-α1, for the human epithelial-carcinoma HeLa-cell line were slightly longer than those in rat tissues (see Fig. 2*a*), and this is apparently a species difference, as similar results were obtained with human fetal skin (results not shown). The data show that, under the conditions of stringency used,

Abbreviations used: RAR-α and RAR-β, retinoic acid receptors α and β; poly(A)$^+$, polyadenylated.

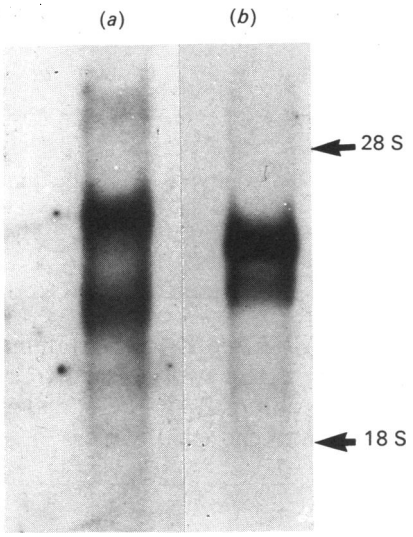

Fig. 1. Northern blot of poly(A)$^+$ RNA from adult rat liver probed successively with RAR-α (*a*) and RAR-β (*b*) probes

The positions of 18 S and 28 S rRNA are given to the right of the Figure.

there was no cross-hybridization between RAR-α and RAR-β sequences. Our finding of two transcripts for RAR-α and RAR-β in most tissues examined is similar to data reported by Petkovich *et al.* [3], Giguere *et al.* [4] and de The *et al.* [11], although published estimates for transcript sizes vary. These results for RAR-β differ markedly from those of Benbrook *et al.* [6], who reported up to four transcripts ranging in size from 2.4 to 9.5 kb in some tissues.

Tissue specificity in expression of RAR-α and RAR-β transcripts

To assess relative levels of RAR-α and RAR-β transcripts in different tissues, Northern blots of poly(A)$^+$-enriched RNA (15 μg per sample) from a range of rat tissues were hybridized successively with the RAR-α and RAR-β probes (Fig. 2). Samples of poly(A)$^+$-enriched RNA from HeLa cells were included for reference. Although the same amount of RNA was loaded per gel track, the degree of enrichment of poly(A)$^+$ RNA may vary from sample to sample, leading to differences in the amount of poly(A)$^+$ RNA loaded in each gel track. To check the loading of poly(A)$^+$ RNA, filters were subsequently proved for β-actin mRNA. However, the expression of β-actin varies between tissues [14], and, in order to account for tissue-specific variation in β-actin expression, Northern blots of total RNA from the various tissues were also probed for β-actin. In these control experiments, the variation between tissues in the intensity of the β-actin signal on blots of poly(A)$^+$-enriched RNA could be accounted for by tissue-specific variation in β-actin expression, as shown by Northern blots of total RNA (Fig. 3).

In all tissues examined, two transcripts were detected using the RAR-α probe, and these were most abundant in poly(A)$^+$ RNA from HeLa cells, lung, heart and kidney (Fig. 2*a*) and least abundant in muscle, where the intensity of the hybridization signal was 9-fold lower than for lung. The relative intensities of the RAR-α1 transcripts also varied between tissues: in spleen and HeLa cells the RAR-α1 transcript was more abundant than RAR-α2, whereas the converse was true for heart and testis (Fig. 2*a*).

In contrast with RAR-α, RAR-β transcripts were undetectable, or present at low levels, in some tissues, such as muscle, small intestine, duodenum and HeLa cells, but relatively abundant in heart, lung, liver, spleen and skin (Fig. 2*b*). The expression of RAR-β1 relative to RAR-β2 varied between tissues: RAR-β1 was the predominant form in some tissues such as heart, in which RAR-β2 was barely detectable, whereas in others the two transcripts were of similar intensity (Fig. 2*b*). Both transcripts were clearly detectable in rat liver and were expressed at a higher level than in kidney. This result differs from the report by de The *et al.* [11] for human tissues, where expression in kidney was considerably

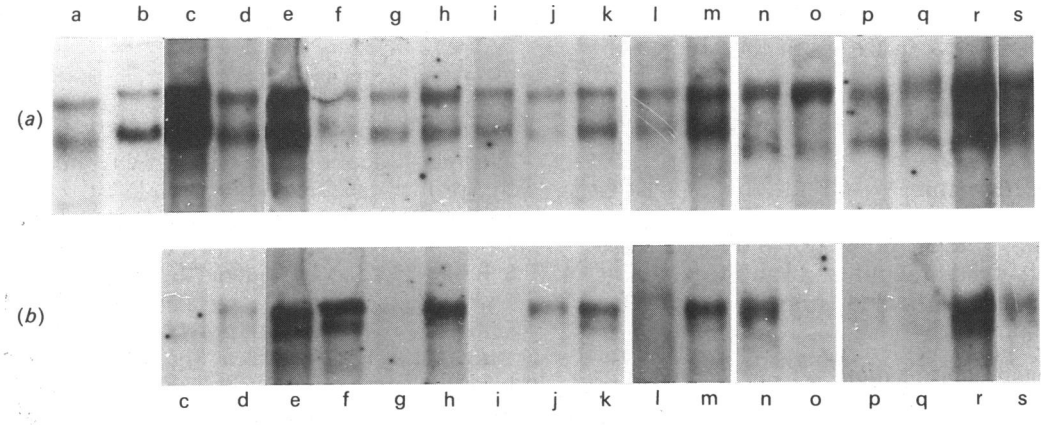

Fig. 2. Northern blots of RNA from different rat tissues and HeLa cells probed successively with RAR-α (*a*) and RAR-β (*b*) probes

Lanes a and b: expt. 1, total RNA from rat lung (a, 48 μg) and HeLa cells (b, 30 μg). Tracks c–k: expt. 2, poly(A)$^+$ RNA from HeLa cells (c), kidney (d), lung (e), liver (f), placenta (g), heart (h), ovary (i), brain (j) and spleen (k). Tracks l and m: expt. 3, poly(A)$^+$ RNA from abdominal-wall muscle (l) and heart (m). Tracks n and o: expt. 4, poly(A)$^+$ RNA from heart (n) and testis (o). Tracks p–s: expt. 5, poly(A)$^+$ RNA from duodenum (p), small intestine (q), lung (r) and adult skin (s). Tracks of heart and lung samples are repeated to allow comparisons between experiments.

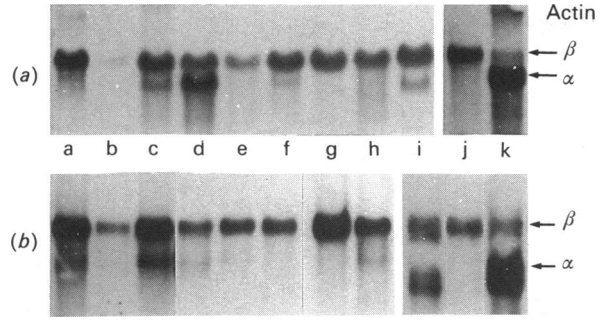

Fig. 3. Northern blots of total RNA (*a*) or poly(A)$^+$ RNA (*b*) from different rat tissues and HeLa cells probed with α-actin probe under low stringency to detect β-actin

a, Lung; b, liver; c, placenta; d, ovary; e, brain; f, spleen; g, HeLa cell; h, kidney; i, testis; j, kidney; and k, heart.

greater than in normal liver, in which only one RAR-β transcript was detectable. This discrepancy is a species difference in expression of RAR-β in liver (J. L. Rees & C. P. F. Redfern, unpublished observations).

The results for the expression of RAR-β in rat tissues are significantly different from those obtained by Benbrook *et al.* [6], who reported a high level of expression in kidney, ovary and testis, but failed to detect RAR-β in liver, spleen and lung. The reasons for this discrepancy are not clear. However, those authors published no controls for loading of RNA and used low-stringency washes. In view of the wide range in RAR-β transcript sizes observed by Benbrook *et al.* [6], it is possible that other sequences related to steroid- and thyroid-hormone receptors were being detected.

Our experiments clearly demonstrate distinct patterns of tissue specificity in expression of the two RARs. RAR-α was expressed in all tissues examined and may be involved in the regulation of general cellular functions. In contrast, the greater variation in expression of RAR-β between tissues, and the fact that this receptor is reported to have a 10-fold greater affinity for retinoic acid than RAR-α [5], suggest a more specific physiological and developmental role. Studies on retinoid deficiency in animals and the effects of retinoic acid on epithelial-tissue development *in vivo* and *in vitro* suggest that retinoic acid is important for epithelial-tissue differentiation and function. In the present study, RAR-β transcripts were present at a low level in duodenum and small intestine, but relatively abundant in skin and lung, two tissues in which the biological effects of retinoic acid are well characterized [15]. Benbrook *et al.* [6] have suggested that, in its pattern of expression, RAR-β displays greater specificity for epithelial tissues. However, the fact that RAR-β transcripts were present in spleen and brain, but particularly abundant in heart, suggests that RAR-β should not be regarded solely as an epithelial-type RAR. The relative abundance of RAR-β in heart is particularly intriguing in view of the low level of expression in muscle and the lack of evidence for effects of retinoic acid on cardiac function.

The RARs have a high degree of sequence similarity to thyroid-hormone receptors [3–5]. Retinoic acid seems to have properties analogous to those of thyroid hormones in that it is important in development [16] and also exerts a variety of effects on different tissues [15]. Furthermore, RAR-α can bind to a thyroid-hormone response element with high affinity [17]. The existence of two distinct transcripts for RAR-α and RAR-β increases the potential for retinoic acid to influence a wide range of cellular functions. As has been shown for thyroid-hormone receptors [18], alternative splicing within the coding region may lead to the generation of different RAR isoforms, each of which may play distinct roles in development.

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