Retinoic Acid Receptor-γ in Human Epidermis Preferentially Traps All-*Trans* Retinoic Acid as its Ligand Rather Than 9-*cis* Retinoic Acid

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The biologic activity of retinoids is mediated through nuclear retinoic acid receptors (RAR), which are ligand-activated transcription factors. RAR directly bind and are activated by two naturally occurring isomers of retinoic acid (RA), all-*trans* retinoic acid (*t*-RA) and 9-*cis* retinoic acid (9*c*-RA). Human skin predominantly expresses RAR-γ (≈87%) and RAR-α makes up the remainder. Recombinant RAR-γ preferentially binds *t*-RA over 9*c*-RA in cell-free assays containing mixtures of the two retinoic acid isomers. We have investigated the ligand-binding properties of RAR in human epidermis. [^{3}H]All-*trans* retinol (*t*-ROL) added to suspensions of intact epidermal cells was metabolically converted to [^{3}H]*t*-RA, which bound to RAR. No binding of [^{3}H]9*c*-RA to RAR was detected. Binding of [^{3}H]*t*-RA, formed from [^{3}H]*t*-ROL, was abolished by adding unlabeled *t*-RA, but was unaffected by adding unlabeled 9*c*-RA. Intact epidermal cells were incubated with mixtures of [^{3}H]9*c*-RA and [^{3}H]*t*-RA in varying ratios, and the amount of each labeled retinoid bound to RAR was measured. At ratios of 9*c*-RA to *t*-RA of 3:1 or lower, only [^{3}H]*t*-RA was bound by RAR. Incubation of cells with [^{3}H]9*c*-RA alone resulted in substantial (38%) binding of [^{3}H]*t*-RA to RAR, in addition to binding of [^{3}H]9*c*-RA, due to isomerization of [^{3}H]9*c*-RA to [^{3}H]*t*-RA. RAR in nuclear extracts from epidermal cells also displayed strong preferential binding of *t*-RA over 9*c*-RA. Competition studies revealed that 9*c*-RA was 6-fold less effective than *t*-RA at displacing [^{3}H]*t*-RA bound to RAR in nuclear extracts. At ratios of 9*c*-RA to *t*-RA of 4:1 or lower, RAR in nuclear extracts bound *t*-RA exclusively. At higher ratios, [^{3}H]9*c*-RA binding increased steeply. RAR-α in nuclear extracts bound both 9*c*-RA and *t*-RA without preference, whereas RAR-γ displayed strong preferential binding of *t*-RA over 9*c*-RA. The level of endogenous *t*-RA exceeds that of 9*c*-RA in human skin *in vivo*, and significant isomerization of topically applied 9*c*-RA and 13*c*-RA to *t*-RA occurs. The relative abundance of *t*-RA in human skin, and preferential binding of *t*-RA by RAR-γ, indicate that *t*-RA is the primary ligand mediating RAR-dependent responses in human skin under physiologic conditions, and under pharmacologic conditions when *t*-RA, 9*c*-RA, or 13*c*-RA are applied to skin. *Key words: isomerization/RAR-α/RAR-γ/retinoids.*

J Invest Dermatol 110:297–300, 1998

Retinol (vitamin A) plays a critical role in the growth and differentiation of mammalian skin in both adult and fetal tissues (Thomson *et al*, 1964; Roberts and Sporn, 1984; Smith *et al*, 1987; Thaller and Eichele, 1987; Chambon, 1996). In adult human skin, retinol is metabolized to its biologically active form, retinoic acid (RA) (Thomson *et al*, 1964; Kurlandsky *et al*, 1994; Kang *et al*, 1995). The physiologic actions of retinoids are mediated through nuclear receptors that regulate transcription of target genes (Evans, 1988; Green and Chambon, 1988; Zelent *et al*, 1991; Chambon, 1993). Two families of nuclear receptors, belonging to the steroid/thyroid hormone superfamily (Evans, 1988), have been identified: these are the retinoid X receptors (RXR) and the retinoic acid receptors (RAR). Each family includes three subtypes: RXR-α, RXR-β, and RXR-γ, and RAR-α, RAR-β, and RAR-γ, respectively (Evans, 1988; Zelent *et al*, 1991; Allenby *et al*, 1993; Mangelsdorf *et al*, 1994).

All-*trans* RA (*t*-RA) and 9-*cis* RA (9*c*-RA), two naturally occurring, biologically active stereoisomers of RA, function as ligands for nuclear retinoid receptors (Heyman *et al*, 1992; Levin *et al*, 1992). RXR bind 9*c*-RA with high affinity (Levin *et al*, 1992), but bind *t*-RA only very weakly (Allenby *et al*, 1993). RAR bind *t*-RA and 9*c*-RA with similar high affinity (Allenby *et al*, 1993). Under ambient conditions, *t*-RA, 9*c*-RA, and 13-*cis* RA (13*c*-RA) (which does not bind either RAR or RXR) are interconverted through isomerization. Mangelsdorf *et al* (1994) have proposed that 9*c*-RA may be functionally distinct from *t*-RA and that interconversion of the two isomers could provide a basis for differential, cell-specific regulation of the activity of the two retinoid pathways.

Using transfected COS cells (a transformed green monkey kidney cell line), Allenby *et al* (1994) have recently shown that, in the presence of a mixture of *t*-RA and 9*c*-RA, recombinant RAR-γ, but not RAR-α or RAR-β, preferentially binds *t*-RA. Competitive binding assays conducted with both nucleosol fractions and intact COS-1 cells, transiently transfected with human RAR subtypes,

Manuscript received June 13, 1997; revised September 17, 1997; accepted for publication November 4, 1997.

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Abbreviations: RAR, retinoic acid receptor; *t*-RA, all-*trans* retinoic acid; 9*c*-RA, 9-*cis* retinoic acid; *t*-ROL, all-*trans* retinol.

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showed that unlabeled 9*c*-RA was much less potent than unlabeled *t*-RA in displacing bound [^{3}H]*t*-RA from RAR-γ.

In human epidermis, RAR-γ is the predominant RAR, accounting for ≈87% of RAR protein. RAR-α makes up the remainder. RAR-β is not detectable (Fisher *et al*, 1994). In this study, we examined binding of *t*-RA and 9*c*-RA by RAR in human epidermis. This study represents an important departure from previous studies: we focus on human skin under *in vivo* conditions, instead of using transformed, transfected cell lines of nonhuman, nonskin origin. We demonstrate that RAR-γ in human epidermis preferentially binds *t*-RA over 9*c*-RA when presented with mixtures of the two isomers. It has recently been demonstrated that endogenous levels of *t*-RA exceed those of 9*c*-RA in human skin, and that substantial amounts of 9*c*-RA and 13*c*-RA isomerize to *t*-RA within 2 d of topical administration to human skin (Duell *et al*, 1996). Taken together, these data indicate that *t*-RA is the primary ligand that mediates RAR-dependent responses in human skin *in vivo*, under physiologic and pharmacologic conditions, when *t*-RA, 9*c*-RA, or 13*c*-RA are topically applied.

MATERIALS AND METHODS

Materials [11,12^{3}H(N)]9*c*-RA and unlabeled 9*c*-RA were generously provided by Drs. P.F. Sorter, J.F. Grippo, and A. A. Levin (Hoffmann-La Roche, Nutley, NJ). *t*-RA was obtained from Sigma (St. Louis, MO). [11,12^{3}H(N)]*t*-RA and [11,12^{3}H(N)]*t*-ROL were obtained from Dupont NEN (Boston, MA). SRI 11237, originally synthesized by Dr. M.I. Dawson (Lehmann *et al*, 1992), was provided by B. Janssen (BASF, Ludwigshafen, Germany). Protein A Sepharose CL4B was obtained from Pharmacia LKB (Piscataway, NJ). PD-10 columns for size-exclusion chromatography were also obtained from Pharmacia.

Procurement of human skin biopsies Keratome biopsies were obtained from healthy adult human volunteers, as previously described (Fisher *et al*, 1991). All procedures involving human subjects were approved by the University of Michigan Institutional Review Board, and all subjects provided written informed consent.

Preparation of epidermal cell suspensions Keratome biopsies were placed in 0.25% trypsin, 0.1% ethylenediamine tetraacetic acid for 40 min at 37°C. Trypsinization was stopped by the addition of Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Cells were released from the tissue by scraping. Cell suspensions were passed through a nylon filter to remove residual tissue, and washed twice in serum-free Dulbecco's modified Eagle's medium.

Preparation of nuclear extracts Epidermal cells (about 2×10^8) were washed twice in phosphate-buffered saline and resuspended in 1 ml of buffer (20 mM Tris, pH 8, 20 mM NaCl, 6 mM $MgCl_2$, 0.2% Triton X-100, 1 mM dithiothreitol, 200 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 0.02 mg leupeptin per ml, and 0.02 mg pepstatin per ml). Nuclear extracts containing RAR were prepared as described previously (Fisher *et al*, 1994).

Incubation of epidermal cells and nuclear extracts with retinoids Intact epidermal cells (5×10^7 cells per ml) suspended in serum-free Dulbecco's modified Eagle's medium (3 ml) were incubated with [^{3}H]*t*-ROL (50 nM and 2×10^6 cpm) (the metabolic precursor of RA), [^{3}H]*t*-RA, or [^{3}H]9*c*-RA for 3 h at 37°C. In addition, cells were incubated with mixtures of labeled *t*-RA and 9*c*-RA in varying ratios. In these mixtures, total ligand concentration (i.e., *t*-RA plus 9*c*-RA) and specific radioactivity remained constant at 10 nM and 5×10^5 cpm. When cells were incubated with [^{3}H]*t*-RA or [^{3}H]9*c*-RA alone, the ligand concentration and specific radioactivity were 10 nM and 5×10^5 cpm, respectively, in each case. All incubations were performed in the presence of a saturating concentration (50 μM) of SRI 11237, a synthetic retinoid that binds exclusively to RXR (Lehmann *et al*, 1992; Fisher *et al*, 1994). Inclusion of SRI 11237 served to occupy all ligand-binding sites on RXR in intact epidermal cells, thereby enabling measurement of [^{3}H]*t*-RA and [^{3}H]9*c*-RA binding to RAR without interference from RXR binding. Following incubation, nuclear extracts were prepared as described above. Nuclear extracts (300 μg) from epidermal cells were incubated for 3 h at 4°C, with varying ratios of [^{3}H]*t*-RA and [^{3}H]9*c*-RA, in the presence of an excess of unlabeled SRI 11237, as described above.

Immunoprecipitation of RAR-α and RAR-γ Nuclear extracts (300 μg protein per 100 μl) were incubated with 50 μl (5 μg) polyclonal antibody to RAR-α or RAR-γ for 2 h at 4°C, with constant shaking. Protein A Sepharose (100 μl) was added and the resultant mixture was incubated for 1 h at 4°C. Immune complexes bound to protein Sepharose were pelleted by centrifugation at $10,000 \times g$ for 10 min. The resulting supernatants were used for binding analysis.

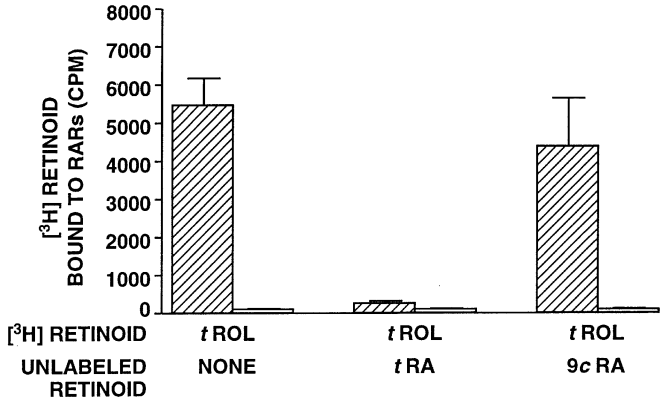

Figure 1. Human epidermal cells metabolize *t*-ROL to *t*-RA, which binds to RAR. Epidermal cells (5×10^7 cells per ml) were prepared from keratome biopsies and incubated with [^{3}H]*t*-ROL (50 nM and 2×10^6 cpm), alone or in the presence of unlabeled *t*-RA (2 μM) or 9*c*-RA (2 μM), for 3 h at 37°C. Fifty micromoles SRI 11237 was added to all assays to saturate RXR. Nuclear extracts were prepared, and [^{3}H]retinoids bound to RAR were quantitated by HPLC, as described in *Materials and Methods*. ▨, [^{3}H]*t*-RA bound to RAR; □ (barely visible to the right of each hatched bar), [^{3}H]9*c*-RA bound to RAR. Results are means ± SEM of six experiments.

Analysis of retinoids bound to epidermal RAR Following incubation with labeled retinoids, nuclear extracts were passed through PD-10 size exclusion columns to separate bound from unbound ligands. Bound labeled retinoids, which eluted in the column void volume, were extracted in chloroform:methanol (2:1). Extracted retinoids were resolved and quantitated using reverse phase high performance liquid chromatography (HPLC) analysis, with in-line radioactive detection, as described previously (Duell *et al*, 1996).

RESULTS

Human epidermal cells metabolize all-*trans* retinol (*t*-ROL) to *t*-RA, which binds to RAR Intact epidermal cells were incubated with [^{3}H]*t*-ROL, and labeled retinoids bound to RAR were determined by HPLC. As **Fig 1** illustrates, the only labeled retinoid that was detected bound to RAR was [^{3}H]*t*-RA. No binding of [^{3}H]9*c*-RA or [^{3}H]13*c*-RA to RAR was detected. Incubation of cells with equal concentrations (1 μM) of [^{3}H]*t*-ROL and unlabeled *t*-RA resulted in substantial (>90%) reduction in [^{3}H]*t*-RA binding to RAR, and no detectable binding of [^{3}H]9*c*-RA (**Fig 1**). In contrast, incubation of cells with equal concentrations (1 μM) of [^{3}H]*t*-ROL and unlabeled 9*c*-RA resulted in a minor (20%) and variable reduction in [^{3}H]*t*-RA binding to RAR. These data demonstrate that human epidermal cells metabolize [^{3}H]*t*-ROL to [^{3}H]*t*-RA, which binds to RAR, without any detectable binding of [^{3}H]9*c*-RA or [^{3}H]13*c*-RA.

Failure of unlabeled 9*c*-RA, but not *t*-RA, to effectively diminish binding of [^{3}H]*t*-RA, formed from [^{3}H]*t*-ROL, to RAR could have reflected either preferential binding of *t*-RA over 9*c*-RA by epidermal RAR, or low uptake of 9*c*-RA, relative to *t*-RA, by epidermal cells. To decide between these possibilities, we incubated intact epidermal cells with mixtures of [^{3}H]*t*-RA and [^{3}H]9*c*-RA in varying ratios. [^{3}H]retinoids bound to RAR were quantitated by HPLC. Addition of [^{3}H]*t*-RA alone resulted in exclusive (100%) binding of [^{3}H]*t*-RA, with no detectable binding of [^{3}H]9*c*-RA (**Fig 2**, *first pair of bars*). Incubation of cells with 1:1 and 1:3 mixtures of [^{3}H]*t*-RA:[^{3}H]9*c*-RA also resulted in exclusive binding of [^{3}H]*t*-RA (**Fig 2**, *second and third pairs of bars, respectively*). Remarkably, even when cells were incubated with 100% [^{3}H]9*c*-RA, 38 ± 3.1% of the bound ligand was still [^{3}H]*t*-RA, whereas 62 ± 3.1% was [^{3}H]9*c*-RA (**Fig 2**). In these experiments, total cell-associated [^{3}H]retinoid was similar, irrespective of the ratio of [^{3}H]*t*-

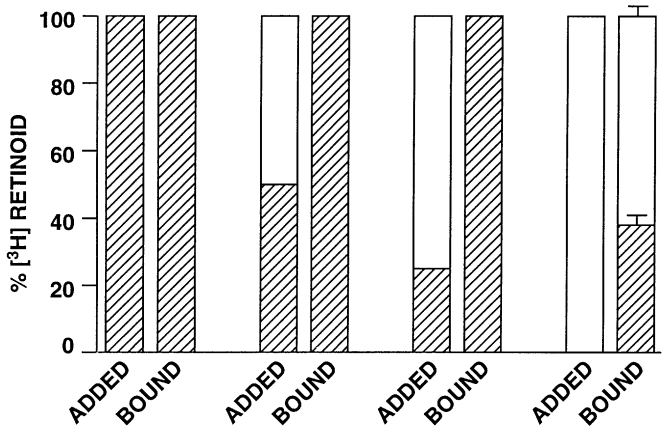

Figure 2. Human epidermal RAR preferentially bind *t*-RA. Epidermal cells (5×10^7 cells per ml) were prepared from keratome biopsies, and incubated with mixtures of [^{3}H]*t*-RA and [^{3}H]9*c*-RA in varying ratios ([^{3}H]*t*-RA alone, 1:1 and 1:3 mixtures of [^{3}H]*t*-RA:[^{3}H]9*c*-RA, and [^{3}H]9*c*-RA alone), for 3 h at 37°C. In these mixtures, total ligand concentration (i.e., [^{3}H]*t*-RA plus [^{3}H]9*c*-RA) and specific radioactivity remained constant at 10 nM and 5×10^5 cpm, respectively. Nuclear extracts were prepared, and [^{3}H]retinoids bound to RAR were quantitated by HPLC, as described in *Materials and Methods*. "Added" refers to the percentage of [^{3}H]*t*-RA (▨) and [^{3}H]9-*cis* RA (□) incubated with epidermal cells. "Bound" refers to the percentage of [^{3}H]*t*-RA (▨) and [^{3}H]9-*cis* RA (□) detected bound to RAR. Results are means ± SEM of four experiments. In each of four experiments, addition of [^{3}H]*t*-RA alone (*left bar of first pair of bars*), a 50:50 ratio of [^{3}H]*t*-RA:[^{3}H]9*c*-RA (*left bar of second pair of bars*), and a 25:75 ratio of [^{3}H]*t*-RA:[^{3}H]9*c*-RA (*left bar of third pair of bars*) resulted in exclusive (100%) binding of [^{3}H]*t*-RA by total RAR (*right bar of first, second, and third pairs of bars*). Addition of [^{3}H]9*c*-RA alone (*right bar of fourth pair of bars*) resulted in binding of both [^{3}H]9*c*-RA and [^{3}H]*t*-RA (*left bar of fourth pair of bars*).

RA to [^{3}H]9*c*-RA, indicating that cellular uptake of the two retinoids was similar (data not shown).

The finding that substantial amounts of *t*-RA bind to RAR in cells incubated with [^{3}H]9*c*-RA suggests that 9*c*-RA isomerizes to *t*-RA in epidermal cells. To determine the extent to which isomerization of 9*c*-RA to *t*-RA occurred during incubation of cells with [^{3}H]9*c*-RA, total cellular and nuclear [^{3}H]retinoids were extracted and quantitated following incubation of cells with [^{3}H]9*c*-RA. The ratio of [^{3}H]9*c*-RA to [^{3}H]*t*-RA in whole cells and nuclear extracts was 61 ± 4:39 ± 4 (n = 2) and 58 ± 1.5:42 ± 1.5 (n = 2), respectively. The [^{3}H]9*c*-RA used for these studies contained less than 1% *t*-RA. These data indicate that significant isomerization of 9*c*-RA to *t*-RA occurred in epidermal cells. No 9*c*-RA was detected in either whole cells or nuclear extracts from cells incubated with [^{3}H]*t*-RA.

Epidermal RAR preferentially bind *t*-RA We next prepared nuclear extracts containing RAR from human epidermal cells, and performed competitive ligand-binding assays. Increasing concentrations of unlabeled *t*-RA effectively displaced [^{3}H]*t*-RA (10 nM) from human skin RAR, with 50% displacement (IC_{50}) occurring at 10 nM (**Fig 3**). In contrast, 10 nM 9*c*-RA displaced only about 10% of [^{3}H]*t*-RA. The calculated IC_{50} of 9*c*-RA for displacement of [^{3}H]*t*-RA was seven times higher (70 nM) than that of *t*-RA. These data are consistent with those obtained for intact epidermal cells, indicating preferential binding of *t*-RA *versus* 9*c*-RA.

RAR-γ in human epidermis preferentially binds *t*-RA *versus* 9*c*-RA Finally, we examined direct binding of mixtures of [^{3}H]*t*-RA and [^{3}H]9*c*-RA, in varying ratios, to total and individual RAR subtypes in epidermal cell nuclear extracts. At ratios of *t*-RA:9*c*-RA between 100:0 and 20:80, total RAR bound exclusively *t*-RA (**Fig 4**). As the proportion of 9*c*-RA increased from 80% to 100%, binding of 9*c*-RA increased sharply, with a concomitant decrease in *t*-RA binding (**Fig 4**). Epidermal nuclear extracts contain both

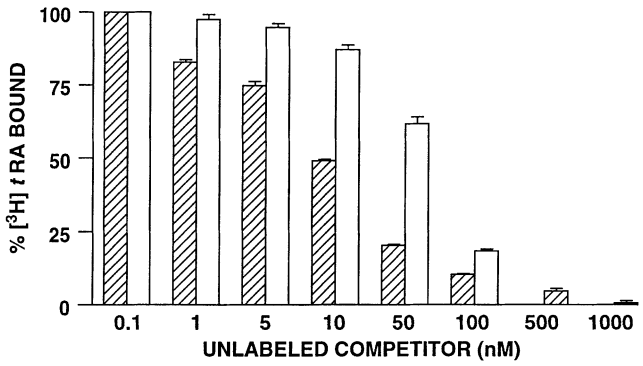

Figure 3. *t*-RA is a better competitor than 9*c*-RA for [^{3}H]*t*-RA binding to RAR in nuclear extracts from human epidermis. Nuclear extracts containing RAR were prepared from human epidermal cells (about 2×10^8). [^{3}H]*t*-RA (10 nM) was incubated with the indicated concentrations of unlabeled *t*-RA or 9*c*-RA for 3 h at 4°C, as described in *Materials and Methods*. The degree of displacement of [^{3}H]*t*-RA from RAR was measured by HPLC, as described in *Materials and Methods*. (▨) [^{3}H]*t*-RA bound to RAR in the presence of unlabeled *t*-RA; (□) [^{3}H]*t*-RA bound to RAR in the presence of unlabeled 9*c*-RA. Results are means ± SEM of three experiments.

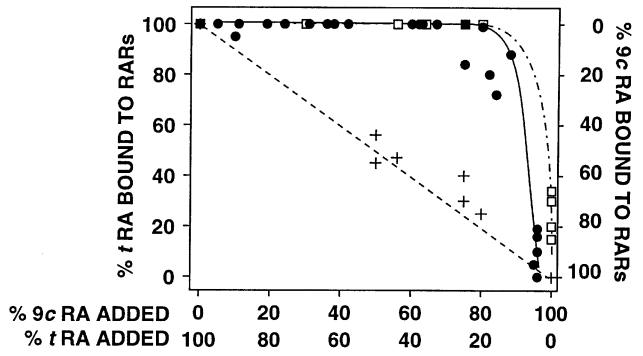

Figure 4. RAR-γ, but not RAR-α in human skin nuclear extracts preferentially binds *t*-RA *versus* 9*c*-RA. Nuclear extracts containing RAR were prepared from human epidermal cells (about 2×10^8). RAR-α and RAR-γ were separately removed by immunoprecipitation to allow measurement of ligand binding to RAR-γ and RAR-α, respectively. Mixtures of [^{3}H]*t*-RA and [^{3}H]9*c*-RA in the varying ratios indicated on the *x*-axis were incubated with total or individual RAR subtypes for 3 h at 4°C, and labeled ligands bound to RAR were determined by HPLC, as described in *Materials and Methods*. - - - - and +, RAR-α; –·–·– and □, RAR-γ; —— and ●, total RAR. Note that ■ indicates overlap between ● and □. Results are means ± SEM of three experiments.

RAR-α and RAR-γ (Fisher *et al*, 1994). RAR-α and RAR-γ were separately removed by immunoprecipitation to enable measurement of *t*-RA and 9*c*-RA binding to the two individual receptor subtypes. RAR-γ displayed a strong preference for *t*-RA *versus* 9*c*-RA (**Fig 4**), similar to that observed in intact epidermal cells and nuclear extracts. No significant binding of 9*c*-RA by RAR-γ occurred until the proportion of 9*c*-RA reached 90%. In contrast, RAR-α displayed no preferential binding of either *t*-RA or 9*c*-RA, binding each ligand in proportion to its relative amount (**Fig 4**). For example, RAR-α incubated with a 1:1 mixture of *t*-RA and 9*c*-RA bound nearly equal amounts of the two ligands.

DISCUSSION

Human epidermis expresses predominately RAR-γ (87%) with lesser amounts of RAR-α (13%) (Fisher *et al*, 1994). Human epidermal nuclear extracts containing these two RAR subtypes separately bind *t*-RA and 9*c*-RA with similar high affinity (Fisher *et al*, 1994). The data presented above, however, demonstrate that intact human

epidermal cells and human epidermal cell nuclear extracts display strong preferential binding of *t*-RA *versus* 9*c*-RA in the presence of mixtures of the two ligands. This preferential binding of *t*-RA was attributable to RAR-γ, the major RAR form in human epidermis. RAR-α in human epidermis exhibited no preference for either ligand, binding *t*-RA and 9*c*-RA in proportion to their relative concentrations in mixtures of the two ligands.

Allenby *et al* (1993) have reported that recombinant mouse RAR-γ, but not RAR-α or RAR-β, expressed in COS cells preferentially bound *t*-RA over 9*c*-RA in mixtures of the two ligands. This preference for *t*-RA by RAR-γ was found to result from *t*-RA having a slower off-rate, compared with 9*c*-RA. Thus, once *t*-RA binds to RAR-γ, it remains bound for a longer time than 9*c*-RA before it dissociates. This difference in off-rates between *t*-RA and 9*c*-RA is such that, at equilibrium, in the presence of mixtures of *t*-RA and 9*c*-RA, very little 9*c*-RA is bound to RAR-γ, unless the concentration of 9*c*-RA exceeds that of *t*-RA by at least 5-fold.

Preferential binding of *t*-RA *versus* 9*c*-RA by RAR-γ is an inherent property of the structure of the ligand-binding domain of the receptor. RAR subtypes are highly conserved between species (Zelent *et al*, 1991; Leid *et al*, 1992); the amino acid sequence of the ligand-binding domain of mouse RAR-γ exhibits 100% homology with that of human RAR-γ. Thus, the slower off-rate of *t*-RA *versus* 9*c*-RA observed for mouse RAR-γ likely explains the observed preferential binding of *t*-RA by human epidermal RAR-γ. *t*-RA and 9*c*-RA bind overlapping yet distinct sites on RAR (Tate *et al*, 1994). This may contribute to the observed difference in the kinetics of binding of these two ligands.

Human epidermal cells metabolize *t*-ROL to *t*-RA, which binds to RAR. No binding of 9*c*-RA, formed from *t*-ROL, was detected. The lack of detectable 9*c*-RA binding was due not only to preferential binding of *t*-RA by RAR-γ, but also to the very low level of isomerization of *t*-RA, formed from *t*-ROL, to 9*c*-RA. This observation is consistent with recent findings that 48 h after topical application, only 1% of *t*-RA has isomerized to 9*c*-RA in human skin (Duell *et al*, 1996). It has been suggested that formation of 9*c*-RA through biosynthesis from 9*c*-ROL and/or isomerization of *t*-RA may represent a distinct retinoid signaling pathway (Heyman *et al*, 1992; Levin *et al*, 1992; Allenby *et al*, 1993). To date, however, there is no direct evidence for enzymatic formation of 9*c*-RA from either 9*c*-ROL or *t*-RA.

Endogenous levels of *t*-RA exceed those of 9*c*-RA in human skin (Duell *et al*, 1996). In addition, within 48 h of topical application of 9*c*-RA, the ratio of 9*c*-RA to *t*-RA in human skin is 52:36, due to isomerization of 9*c*-RA to *t*-RA (Duell *et al*, 1996). As shown above, at this ratio of the two ligands, RAR-γ would bind *t*-RA exclusively. Topically applied 13*c*-RA isomerizes predominantly to *t*-RA in human skin (Duell *et al*, 1996). Because 13*c*-RA does not bind RAR (Allenby *et al*, 1993), *t*-RA must therefore mediate RAR-dependent responses to 13*c*-RA.

Given that, in human skin, (i) levels of *t*-RA exceed those of 9*c*-RA (Duell *et al*, 1996), (ii) RAR-γ is predominant (87%) (Fisher *et al*, 1994), (iii) topically applied retinol is, to a great extent, metabolized to the all-*trans* isomer (Duell *et al*, 1996), and (iv) RAR-γ preferentially binds *t*-RA over 9*c*-RA (this study), we conclude that *t*-RA is the predominant ligand that binds to RAR and therefore must be the major mediator of RAR-dependent responses in human skin under both physiologic and pharmacologic conditions, when *t*-RA, 9*c*-RA, or 13*c*-RA are applied.

We thank Dr. Elizabeth Duell for HPLC analyses of retinoids, and for her generous help and advice during the writing of this paper. We thank Dr. Anne Chapple for technical editing and manuscript preparation. This work was supported in part by the Babcock Endowment for Dermatological Research and a grant from Johnson & Johnson Corporation.

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