Receptor Specificity of Retinoid-Induced Epidermal Hyperplasia: Effect of RXR-Selective Agonists and Correlation with Topical Irritation

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ABSTRACT

Retinoid induction of epidermal hyperplasia was investigated in hairless mice with synthetic ligands for the retinoic acid (RAR) and retinoid X (RXR) nuclear receptors. Induction of hyperplasia by all-trans retinoic acid and the RAR-specific retinoids TTNPB, tazarotene and AGN 190121 varied over a wide range (ED$_{50}$ = 0.2–100 nmol/animal in three daily applications). Potency of induction was not directly correlated to receptor-binding affinity, but specificity of action could be demonstrated by inhibition with the high-affinity antagonist of the RARs, AGN 193109. Although RAR is functionally complexed with RXR in vivo, RXR-selective compounds have only weak potency in induction of hyperplasia. The ED$_{50}$ value of the RXR-selective AGN 191701 was 600 nmol/animal compared with an ED$_{50}$ value of 0.2 nmol for the structurally similar RAR-selective TTNPB. SR11237 and SR11217, also RXR-selective, each have an ED$_{50}$ value of >1000 nmol. Unlike RAR-specific retinoids, RXR-selective retinoids cause only very mild skin flaking at high doses. Relative potencies for cumulative topical irritation (flaking and abrasion) of both RAR and RXR ligands were well correlated with epidermal hyperplasia. These data are consistent with RXR as a silent partner in the RAR-RXR heterodimer in skin.

Topical all-trans RA treatment causes an increase in the number of living cell layers in the epidermis of both human and mouse (Connor et al., 1986; Fisher et al., 1991; Griffiths et al., 1993). Hyperplasia results from an induction of cell proliferation in the basal layer of epidermis within 24 hr, as measured by DNA synthesis (Lützow-Holm et al., 1994; Connor and Lowe, 1983). In the hairless mouse, this effect is induced both by active metabolites of all-trans RA and by synthetic retinoid analogs (Connor et al., 1986; Reynolds et al., 1993). Hyperplasia can also be induced by treatments that compromise the epidermal barrier or interfere with integrity of the stratum corneum (Denda et al., 1996). Several studies suggest that the induction of epidermal hyperplasia by all-trans RA and, by implication, other retinoids may be a nonspecific or irritant effect (Fisher et al., 1991; Lützow-Holm et al., 1995; Marks et al., 1990) analogous to that induced by topical abrasives and detergents. Paradoxically, retinoids also have well-characterized antiproliferative effects in epidermis. Retinoids inhibit epidermal ODC activity and an initial phase of thymidine incorporation after treatment with TPA in mouse (Gendimenico et al., 1989; Verma et al., 1979), and in humans, retinoids are effective both topically and systemically in the treatment of psoriasis, a hyperproliferative disease (Ellis and Voorhees, 1987; Esplugues-Ribot et al., 1994).

The primary targets for retinoid regulation of gene expression are nuclear receptors from the steroid receptor superfamily. Based on sequence homology and ligand binding specificity, the receptors fall into two groups: the RARs, which bind both all-trans RA and 9-cis RA with high affinity, and the RXRs, which bind 9-cis but not all-trans RA (Mangelsdorf et al., 1994). Synthetic retinoids now also distinguish the two receptor classes (Beard et al., 1995; Lehmann et al., 1992). RAREs in retinoid-inducible genes interact with a heterodimer of RAR and RXR. Both RAR and RXR are in direct contact with DNA (Kurokawa et al., 1994), and the heterodimer appears to be the ultimate nuclear target of all-trans RA induction of gene expression (Mangelsdorf et al., 1994). In addition to heterodimerizing with the RARs, the RXRs are required partners for several other nuclear receptors, including the thyroid hormone, vitamin D$_3$ and PPAR receptors (Mangelsdorf et al., 1994). Mouse and human skin

ABBREVIATIONS: ODC, ornithine decarboxylase; RA, retinoic acid; RAR, retinoic acid receptor; RARE, retinoic acid response element; RXR, retinoid X receptor; SDS, sodium dodecyl sulfate; TPA, 12-O-tetradecanoylphorbol-13-acetate.
express a subset of the retinoid nuclear receptors, the RARs α and γ and RXRα (Elder et al., 1991; Fisher et al., 1994; Kumar et al., 1994). For all-trans RA induction of epidermal hyperplasia, several mechanisms seem to be possible: (i) binding to RARs, (ii) binding to RXRs after isomerization to 9-cis RA and (iii) nonspecific disruption of epidermal integrity.

To determine the role of receptor binding in induction of epidermal hyperplasia, retinoids with defined pharmacological activity (Beard et al., 1995; Lehmann et al., 1992; Nagpal et al., 1995) were evaluated for potency in hyperplasia assays in the hairless mouse. Mutation of the hairless gene on chromosome 14 leads to hair loss after the first cycle of hair growth (Begona et al., 1994), but the hairless mouse has a normal retinoid receptor complement in skin (Beehler et al., 1995). We find that retinoid-induced epidermal hyperplasia is primarily an effect of binding to the RAR class of receptors and is unrelated to retinoid detergent-like character as measured by cell culture cytotoxicity (Oda et al., 1996). In addition, synthetic ligands selective for the RXRs have a greatly reduced potency for induction of hyperplasia and topical irritation compared with RAR-specific retinoids. Finally, we find that retinoid potencies for the induction of hyperplasia are closely correlated to induction of overall topical irritation.

**Methods**

**Animals.** Female hairless mice (6–8 weeks old) were obtained from the Charles River Laboratories and maintained in an environment of controlled temperature and humidity at Allergan. Mice had access to Purina Chow and reverse-osmosis water ad libitum; animals were housed in the Allergan vivarium for ≥1 week before use. Protocols for animal treatment were approved by the Allergan Animal Use and Care Committee.

**Materials.** All-trans RA was obtained from Sigma Chemical (St. Louis, MO). Other retinoids (table 1) were synthesized at Allergan. AGN 190168 (tazarotene), AGN 190299 (the free acid of tazarotene), AGN 190121 and TTNPB [(E)-4-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthyl)propen-1-yl][benzoic acid] are specific for the RAR and not the RXR family of receptors on the basis of both binding and transactivation (Beard et al., 1995; Nagpal et al., 1995). AGN 190727 is the meta-carboxy analog of AGN 190121 and is not active in inhibition of ODC activity (Oda et al., 1996) or in studies of receptor transactivation (data not shown). AGN 193109 is an RAR antagonist (Johnson et al., 1995). AGN 191701 [(E)-2-[2-(5,6,7,8-tetrahydro-3,5,5,8-pentamethyl-2-naphthyl)propen-1-yl]-4-thiophenecarboxylic acid], an analog of TTNPB, binds and transactivates the RXRα (ED₅₀ = 20 nM) but appears inactive at the RARs (ED₅₀ > 10 μM) (Beard et al., 1995). SR11237 and SR11217 (Lehmann et al., 1992) are also RXR-selective retinoids with minimal activity in assays of RAR function (Nagpal et al., 1996). ¹⁴C-Ornithine was obtained from Amersham and TPA from Chemsyn (Lenexa, KS).

**Hyperplasia.** Mice were randomized by weight, and 3 to 6 mice were included in each treatment group. Retinoids were applied daily for 3 days to the dorsal skin of hairless mice in 100 μl of acetone, and animals were killed 72 hr after the last dose of retinoid unless otherwise noted. Epidermal thickness was measured on hematoxylin and eosin-stained paraffin-embedded sections from three separate sites on mouse dorsal skin in a blinded fashion. An ED₅₀ value for each retinoid was estimated by comparison with maximal hyperplasia induced by a retinoid active at the RARs that was run in the same experiment (eg, all-trans RA in table 3 or TTNPB in fig. 4). In some of the experiments, dosing of RA was performed under dim light, and mice were maintained in darkness for the subsequent 10 to 12 hr to prevent light-induced photoisomerization of all-trans RA. Statistical significance was determined using analysis of variance and Dunnett’s test for multiple comparisons or the paired Student’s t test as appropriate.

**ODC inhibition.** ODC inhibition by retinoids was determined in TPA-treated female hairless mice. Retinoids were applied dorsally in 100 μl of acetone 1 hr before TPA treatment (40 nmol/mouse) in 100 μl of acetone. Animals were killed 4 hr later, and epidermis was scraped from the dermis after treatment at 55°C for 30 sec. The supernatant of epidermal homogenate (30 min at 20,000 × g) was stored at −70°C, and ODC was assayed essentially as previously described (Gendimenico et al., 1990) except that benzenthionium hydroxide was used as a trapping agent for released ¹⁴CO₂. Protein concentration in the epidermal extracts was determined by the Coomassie blue binding method (BioRad, Oakland, CA), and ODC activity was normalized to total epidermal protein content.

### TABLE 1

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Receptor specificity</th>
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</thead>
<tbody>
<tr>
<td><img src="image1" alt="structure" /></td>
<td>all-trans RA</td>
<td>RAR&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image2" alt="structure" /></td>
<td>tazarotene</td>
<td>RAR&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image3" alt="structure" /></td>
<td>AGN 190121</td>
<td>RAR&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><img src="image4" alt="structure" /></td>
<td>AGN 190727</td>
<td>Inactive</td>
</tr>
<tr>
<td><img src="image5" alt="structure" /></td>
<td>TTNPB</td>
<td>RAR</td>
</tr>
<tr>
<td><img src="image6" alt="structure" /></td>
<td>AGN 193109</td>
<td>RAR (antagonist)</td>
</tr>
<tr>
<td><img src="image7" alt="structure" /></td>
<td>AGN 191701</td>
<td>RXR</td>
</tr>
<tr>
<td><img src="image8" alt="structure" /></td>
<td>SR 11237</td>
<td>RXR</td>
</tr>
</tbody>
</table>

<sup>a</sup> The 9-cis isomer of all-trans RA binds and transactivates the RXRs.<br>
<sup>b</sup> Tazarotene and AGN 190121 show relative selectivity (~10-fold) for RAR<sub>β</sub> and RAR<sub>γ</sub> in comparison with RAR<sub>α</sub> (Nagpal et al., 1995) in receptor transactivation.
Results

Hyperplasia induced by RAR-specific retinoids. Retinoid induction of epidermal hyperplasia by TTNPB has been reported to require 3 to 5 days for maximal effect (Connor et al., 1986). In initial studies, we observed that all-trans RA and tazarotene, applied daily for 3 days, induced hyperplasia that was stable for an additional 3 days after cessation of treatment, with a significant decline in epidermal thickness observed on the fifth day (table 2). Retinoid potencies for epidermal hyperplasia in dose-response studies were therefore compared at 72 hr after the last of three daily treatments. Figure 1 compares all-trans RA and tazarotene, which have ED50 values of 20 and 100 nmol/mouse, respectively.

To address the question of retinoid nuclear receptor involvement, we compared an additional RAR-specific retinoid, AGN 190121, with its inactive meta-carboxy analog, AGN 190727. AGN 190121 has significant activity in the hyperplasia assay (ED50 ~ 100 nmol), but AGN 190727 appears to induce modest hyperplasia (48% above control) only at the highest dose tested (3000 nmol) (fig. 2). The hypothesis that epidermal hyperplasia is RAR mediated was further supported by the effects of a specific antagonist of the RARs, AGN 193109, which binds isolated RARs with an affinity comparable to all-trans RA but does not induce gene transcription from an RARE in cell culture (Johnson et al., 1995). At a dose of 3 nmol/mouse, TTNPB induces substantial, near-maximal epidermal hyperplasia; however, simultaneous application of an equal dose of the RAR-receptor antagonist, AGN 193109, inhibits the hyperplasia by 36%, whereas a 10-fold excess of the antagonist blocks the TTNPB-induced hyperplasia by ~90% (fig. 3). Observable TTNPB-induced skin flaking was also inhibited by the lower dose of antagonist, as previously described (Standeven et al., 1996). The antagonist had no effect on epidermal thickness by itself at the same doses (3 and 30 nmol) at which it inhibited TTNPB-mediated hyperplasia (fig. 3). The antagonist has a similar effect on hyperplasia induced by all-trans RA (data not shown).

Action of RXR-selective retinoids. The RXR-selective compound AGN 191701 does not inhibit TPA-induced ODC activity in epidermis of the hairless mouse (Beard et al., 1995). We also tested SR 11217 and SR 11237, two additional RXR-specific synthetic retinoids (Lehmann et al., 1992; Nagpal et al., 1996), for inhibition of TPA-induced ODC activity. At a dose of 300 nmol/mouse, neither compound produced >20% inhibition of TPA-induced ODC activity. In contrast, all-trans RA, tazarotene and AGN 190121 cause 80% inhibi

Table 2

<table>
<thead>
<tr>
<th>Compound and dose</th>
<th>Day 1b</th>
<th>Day 2b</th>
<th>Day 3b</th>
<th>Day 5b</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 nmol all-trans RA</td>
<td>112 ± 24% (3)</td>
<td>115 ± 7% (4)</td>
<td>56 ± 20% (3)*</td>
<td></td>
</tr>
<tr>
<td>25 nmol all-trans RA</td>
<td>90 ± 16% (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 nmol tazarotene</td>
<td></td>
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*Relative to concurrent acetone control. n, Number of animals per test group.

Days after cessation of retinoid treatment.

Mice were treated for 5 consecutive days.

Significantly different from day 2 after end of treatment (p < .05).

Significantly different from days 1 and 3 after end of treatment (p < .05).
tion of TPA-induced ODC activity at doses of ~1 nmol/mouse (Nagpal et al., 1995; Oda et al., 1996). Induction of hyperplasia by an RXR-selective compound, AGN 191701, was then compared with the structurally similar RAR-specific molecule, TTNPB (fig. 4). This analysis revealed that the RAR-specific compound was substantially more potent than AGN 191701, by a factor of $1000$, but that AGN 191701 exhibited some activity at the highest concentration tested (1000 nmol/mouse). The approximate ED50 value of TTNPB is 0.2 nmol/mouse, whereas that of AGN 191701 is ~300 nmol/mouse.

We also tested the RXR-specific compounds SR 11217 and SR 11237 in the hyperplasia assay. SR 11237 produced statistically significant induction of hyperplasia at both 100 and 1000 nmol/mouse. The magnitude of the effect of SR 11237 was greater than that of SR 11217 and showed a clear dose response (table 3). Both compounds were much less active than all-trans RA, tested simultaneously as a positive control in the same experiment. All-trans RA at 100 nmol increased epidermal thickness by 156% compared with a 73% increase for SR 11237 at 1000 nmol (table 3).

Two of the RXR-selective compounds were also characterized in the hairless mouse for indications of overt topical irritation (e.g., skin flaking), during a 12-day extended treatment, as shown in figure 5. The flaking observed was minimal in response to both AGN 191701 and SR 11237, whereas AGN 190121 produced substantial flaking at a dose of 1000 nmol/mouse. In contrast to AGN 190121, its inactive congener, AGN 190727, produced no perceptible change in the gross appearance of hairless mouse skin. The findings suggest that induction of skin flaking, like hyperplasia, is most sensitive to ligands for the RARs.

We further investigated the correlation between retinoid-induced hyperplasia and overall retinoid-induced skin irritation, including both epidermal flaking and abrasion. Mice were treated topically for 5 consecutive days with retinoid and observed for an additional 3 days. The potencies of AGN 190121, all-trans RA and TTNPB in producing topical irritation were compared. TTNPB is ~100-fold more potent than all-trans RA and >500-fold potent than AGN 190121 (fig. 6).

Relative retinoid potencies for induction of topical irritation are well correlated with induction of hyperplasia (table 4).

**Discussion**

Based on similarities to the action of SDS and other irritants in skin, the possibility has been raised that epidermal hyperplasia induced by all-trans RA may be the result of nonspecific effects that are independent of receptor binding (Fisher et al., 1991; Lützow-Holm et al., 1995; Marks et al., 1990). Physical disruption of epidermal barrier function can explain the effect of nonspecific irritants in inducing epidermal hyperplasia (Proksch et al., 1991). The potential of retinoids for nonspecific irritant activity has been approximated...
among RAR-specific retinoids do not correlate directly with relative potencies in regulation of gene expression through the individual RARs transfected in cell culture. The potency of all-trans RA for induction of gene expression at each of the RARs is greater than or equal to TTNPB, and binding affinities of the two retinoids to the RARs are similar (Agarwal et al., 1996; Beard et al., 1995). On the other hand, TTNPB is far more potent than all-trans RA in induction of hyperplasia (table 4 and see Connor et al., 1986). Because the in vivo stability of TTNPB is significantly greater than all-trans RA (Howard et al., 1989), retinoid bioavailability also appears to be a major determinant of differences in retinoid potency in hyperplasia in addition to differences in receptor specificity. Consistent with a substantially greater stability or bioavailability for TTNPB, Connor et al. (1986) found a peak of TTNPB-induced hyperplasia in hairless mice at 5 days after a single dose of this retinoid. In contrast, we find that induction of hyperplasia by all-trans RA and tazarotene is stable for 3 days but is substantially decreased at 5 days after the cessation of treatment (table 2).

Effects of RXR-selective retinoids. Although heterodimerization with an RXR is critical for RAR transcriptional activity (Mangelsdorf et al., 1994), compounds selective for binding the RXRs induce hyperplasia only very weakly. AGN 191701 is >1000-fold less potent in induction of epidermal hyperplasia than TTNPB, a structurally related RAR-specific compound. The greatly reduced potency of AGN 191701 with respect to induction of hyperplasia most likely reflects its pharmacological properties (i.e., selectivity for the RXR family). Two additional RXR-selective retinoids, SR 11237 and SR 11217, also have an ED<sub>50</sub> value for induction of hyperplasia of >1000 nmol and appear to be >50-fold less potent than all-trans RA. SR 11237, the more active of the two, also has a higher affinity for RXRα (Nagpal et al., 1996), the RXR isoform found in skin. In contrast to all-trans RA and other RAR-specific retinoids, SR 11237 is completely inactive in a separate assay of retinoid in vivo effects, reduction in the diameter of the abnormal hair follicle remnants or utriculi of the rhino mouse, at a topical dose of ~250 nmol/animal (Gendimenico et al., 1994). These data show that RXR-selective compounds are quite distinct from RAR-specific retinoids in their effects on skin pharmacology. Our data support current receptor models in which RXR-selective ligands neither activate the RAR-RXR heterodimer nor bind the RXR component when associated in the heterodimer (Forman et al., 1995; Kurokawa et al., 1994). The limited hyperplasia induced by the RXR-selective compounds can, in principle, be explained by weak interactions with the RARs. However, none of these compounds is active in inhibition of TPA-induced ODC activity, a property common to RAR-specific retinoids (table 4). Liganded RXR can, however, activate gene expression as part of an RXR homodimer or as an RXR heterodimer in association with other receptors. Although RXR homodimers are not detectable in epidermal homogenates (Fisher et al., 1994) and are unlikely to form and have activity unless highly expressed (Mangelsdorf et al., 1991; Nakshatri and Chambon, 1994; Xiao et al., 1995), liganded RXR may regulate gene expression with other heterodimeric partners (Forman et al., 1995). For example, RXR-selective ligands induce the vitamin D<sub>3</sub> responsive gene hydroxysteroid D<sub>3</sub>-24-hydroxylase in kidney (Allegretto et al., 1995). Because 1α,25-dihydroxyvitamin D<sub>3</sub> and its analogs cause

### TABLE 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>ODCID&lt;sub&gt;50&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hyperplasia  ED&lt;sub&gt;50&lt;/sub&gt;</th>
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<tbody>
<tr>
<td>all-trans RA</td>
<td>1.4 nmol</td>
<td>20 ng mol</td>
</tr>
<tr>
<td>Tazarotene</td>
<td>0.8 nmol</td>
<td>100 ng mol</td>
</tr>
<tr>
<td>TTNPB</td>
<td>0.12 nmol</td>
<td>0.2 ng mol</td>
</tr>
<tr>
<td>AGN 190121</td>
<td>0.5 nmol</td>
<td>100 ng mol</td>
</tr>
<tr>
<td>AGN 190299</td>
<td>7 nmol</td>
<td>100 ng mol</td>
</tr>
<tr>
<td>AGN 190727</td>
<td>&gt;1000 nmol</td>
<td>&gt;1000&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>AGN 191701</td>
<td>&gt;300 nmol</td>
<td>300 ng mol</td>
</tr>
<tr>
<td>SR 11217</td>
<td>&gt;300 nmol</td>
<td>&gt;1000&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SR 11237</td>
<td>&gt;300 nmol</td>
<td>&gt;1000&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data on inhibition of ODC activity are taken from Oda et al., 1996; Nagpal et al., 1995, and Beard et al., 1995 and from this study.

<sup>b</sup> Compared with 1000 nmol AGN 190121, taken as maximal hyperplasia (fig. 2).

<sup>c</sup> Compared with 100 nmol all-trans RA, taken as maximal hyperplasia (table 2).
epidermal hyperplasia (Kobayasi et al., 1995), it is also possible that liganding of RXR in association with the vitamin D$_3$ receptor induces the weak epidermal hyperplasia observed in this study.

**Epidermal hyperplasia and topical irritation.** Retinoid-induced epidermal hyperplasia in the hairless mouse is well correlated with topical irritation: RXR-selective compounds are very weak in both assays, whereas relative dose responses for irritation and hyperplasia among RAR-specific compounds are very similar (compare table 4 and fig. 6). For example, the ED$_{50}$ value for TTNPB in induction of hyperplasia (0.2 nmol) is ~100-fold lower than all-trans RA, whereas the cutaneous toxicity dose response for TTNPB approximately parallels that for all-trans RA but is shifted again by a factor of ~100-fold to lower doses. AGN 190121 is ~2- to 5-fold less potent than all-trans RA in both assays. Finally, retinoid-induced topical toxicity, like epidermal hyperplasia, is blocked by the RAR receptor antagonist, AGN 193109 (see fig. 3 and Standeven et al., 1996). The retinoid doses at which irritation is readily apparent (composite irritation scores >5) correspond to those at which maximal or near-maximal hyperplasia is observed (e.g., 1 nmol for TTNPB and 50–200 nmol for all-trans RA and AGN 190121). These correlations suggest that retinoid-induced epidermal cell division and hyperplasia may be a critical driving force for topical irritation. In support of this interpretation, epidermal cell division reaches its peak at 3 days after topical all-trans RA treatment, regardless of dose (Lützow-Holm et al., 1992), whereas the first significant macroscopic effects of topical retinoids, such as skin flaking, start to be visible only after ~3 days (fig. 5). More rapid shedding of the dead layer of epidermis, the stratum corneum, during hyperplasia could contribute directly to skin flaking. On the other hand, retinoid treatment of skin is accompanied by changes of gene expression throughout the living layers of epidermis (Lützow-Holm et al., 1994). Although many of these changes are found in response to nonspecific irritants such as SDS, and therefore could be indirect effects of retinoid-induced hyperplasia, others are unique to all-trans RA (Elder et al., 1993; Fisher et al., 1992; Griffiths et al., 1993). Retinoids induce a substantial loss of epidermal barrier function, which is measured as increased transepidermal water loss (Gendimenico et al., 1995). In the hairless mouse, loss of barrier function is accompanied by changes of gene expression characteristic of the living layers of epidermis rather than an indirect effect of the concurrent hyperplasia.

In summary, we have demonstrated that retinoid-induced hyperplasia is an RAR-mediated effect that is unrelated to nonspecific or detergent-like characteristics of the retinoids tested. In contrast to RAR-specific ligands, RXR-selective retinoids have only a weak effect in this assay, which is consistent with RXR being a silent partner in the RAR-RXR heterodimer. Potency in induction of epidermal hyperplasia by both classes of compounds is well correlated to induction of topical irritation, suggesting that there is a close mechanistic relationship between the two processes. Receptor-mediated effects of retinoids in epidermis are dualistic, including not only inhibition of initial DNA incorporation and ODC activity in TPA-treated skin but also induction of hyperplasia in naive skin.

**Acknowledgments**

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**References**


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