all-*trans*-Retinoic acid inhibits the appearance of two phorbol ester-induced ornithine decarboxylase mRNAs in mouse epidermis

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Two ornithine decarboxylase mRNA species are seen in mouse epidermis in response to the topical application of the phorbol ester tumor promoter, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). This induction occurs in a time- and dose-dependent fashion and appears to be relatively specific as α-actin mRNA reveals no change with treatment. Both mRNA species are inhibited in a dose-dependent manner by treating mouse skin with all-*trans*-retinoic acid prior to TPA. These results indicate that these two compounds are active at the transcriptional level.

Ornithine decarboxylase mRNA *Phorbol ester* *Retinoic acid*

1. INTRODUCTION

The activity of ornithine decarboxylase (EC 4.1.1.17), the rate-limiting enzyme in polyamine biosynthesis, can be induced in a variety of tissues and cell lines by numerous stimuli [1]. Among the most extensively studied inducers are the phorbol ester tumor promoters, in particular 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), which induces the enzyme in mouse skin [2]. This induction in mouse skin has been implicated in tumor promotion [3]. Furthermore, topically applied vitamin A derivatives (retinoids), such as all-*trans*-retinoic acid (RA), inhibit this induction and are known to inhibit tumor promotion [4] with a degree of effectiveness related to their potency of ornithine decarboxylase inhibition [5,6]. At the protein level, it is now known that RA inhibits the synthesis of ornithine decarboxylase protein caused by TPA [7]. At the mRNA level, a single TPA-inducible mRNA species has been reported in mouse skin [8] using a radiolabeled cDNA probe, pOD49 [9]. Here, utilizing a new method of obtaining RNA from the epidermis, we report the presence of two TPA-inducible mRNA species both of which are inhibited in a dose-dependent manner by retinoic acid. The data described here support the hypothesis that the effects of TPA and RA on epidermal ornithine decarboxylase are, at least in part, mediated transcriptionally.

2. MATERIALS AND METHODS

2.1. *Tissue source and preparation*

All animals were 7–9-week-old SKH/hr 1 female hairless mice housed 5 per cage. Animals were killed by cervical dislocation, and dorsal skins excised and placed in a 55°C water bath containing freshly prepared 0.2% diethylpyrocarbonate (DEP) for 30 s. The skins were then immediately placed in 0–4°C water containing 0.2% DEP. The epidermis was scraped from the dermis using a scalpel and then quickly frozen in liquid nitrogen.

2.2. *Experimental treatments*

A stock solution of TPA (LC Services, Boston)

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in acetone was stored at −20°C. RA (Sigma) was made up fresh in acetone in a darkened room prior to use. 200 μl of a given treatment were applied by micropipette to the dorsal skin. All animals treated with RA were kept in the dark prior to killing. RA was always applied 1 h prior to TPA; TPA was applied for 4 h unless otherwise indicated.

2.3. *Preparation of RNA for blotting*

Total RNA from mouse epidermis was extracted with guanidine thiocyanate (Fluka, Switzerland) by the method of Chirgwin et al. [10]. For Northern blots, 15 μg total RNA was denatured with glyoxal, electrophoresed on a 1% agarose gel, and blotted as described [11,12]. RNA slot blots were prepared with 15 μg total RNA by the method of Thomas [13]. Both Northern and slot blots were prehybridized, hybridized, and washed as described [14]. Autoradiographs were scanned on a densitometer for quantification and expressed as densitometric units.

2.4. *cDNA probes*

Ornithine decarboxylase mRNA was detected with a mouse cDNA clone, pOD48, a gift from Dr P. Coffino (University of California, San Francisco). An α-actin cDNA probe, also from mouse, was a gift from Dr N. Davidson (California Institute of Technology). Both probes were ^{32}P-labelled by nick-translation [16].

3. RESULTS AND DISCUSSION

These studies indicate that the induction of ornithine decarboxylase mRNA by TPA is time- and dose-dependent, and that RA inhibits this induction in a dose-dependent manner. Northern blot analysis of epidermal RNA from mouse skin treated with TPA reveals a major mRNA species of about 2.1 kb and a minor mRNA species of 2.4 kb (fig.1A, lane 2; fig.1B, lane 1). The size of the major mRNA species is similar to that reported in mouse kidney [15] and hamster fibroblasts [16]. Multiple ornithine decarboxylase mRNA species have been documented in other tissues [15–17], but in mouse skin, only a single 2.1 kb mRNA species has previously been reported [8]. The existence of two mRNA species in mouse skin is supported by DNA sequence data in mouse kidney in which there appear to be two similar, but separate ornithine decarboxylase genes [18]. The application of RA 1 h prior to TPA inhibits the TPA-mediated increase of both mRNA species in a dose-dependent manner (fig.1B, lanes 2–5). A densitometric scan of this Northern blot showed that both gene products are influenced by the lowest

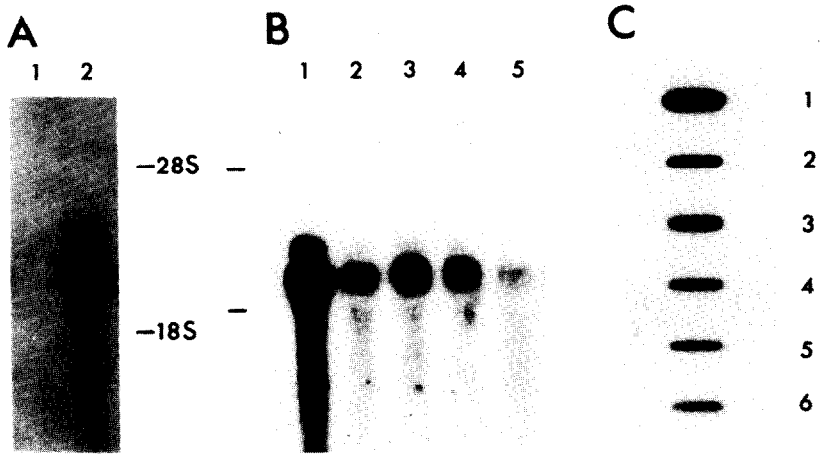

Fig.1. Effects of TPA and RA on ornithine decarboxylase mRNA using Northern (A,B) and slot blot (C) analysis. Acetone control (A, lane 1) and 17 nmol TPA alone (A, lane 2; B, lane 1; C, lane 1). Panels B and C, lanes 2–5 in each, are 17 nmol TPA together with 1, 5, 10, and 25 nmol RA, respectively. Panel C, lane 6 is 17 nmol TPA together with 50 nmol RA.

dose (1 nmol) of RA employed (not shown). Overall quantitation of both gene products by slot blot analysis and a densitometric scan from this same experiment reveal that the low dose of 1 nmol RA inhibits ornithine decarboxylase mRNA accumulation by 64%, while 50 nmol inhibits it by 80% (fig.1C). A comparable low dose of RA (0.2 nmol) has been reported to inhibit 50% of the TPA-induced enzyme activity in mouse skin [5].

In the absence of TPA (acetone control), no detectable levels of ornithine decarboxylase mRNA were seen in Northern blots probed with cDNA (fig.1A, lane 1). This is probably the result of the small amount of this mRNA in our unstimulated controls. This sensitivity problem was overcome by the use of slot blots in which the control can be visualized (fig.2, lane 1); hence, TPA's effect upon ornithine decarboxylase gene expression could now be quantified by a densitometric scan (table 1). As compared to control, a 2-fold increase in titratable mRNA was seen at 1 h, peaking at 4 h (13-fold induction), and declining thereafter. This time course correlates well with that seen for the induction of enzyme activity in mouse skin [5].

Table 1

A densitometric scan of the slot blots shown in fig.2

Treatment	Densitometric units	Fold increase
Acetone-acetone	1.2	–
Acetone-1 h TPA	2.2	1.8
Acetone-2 h TPA	13.4	11.1
Acetone-4 h TPA	16.2	13.5
Acetone-6 h TPA	13.0	10.8
1 h RA + 3 h TPA	2.3	1.9

To ascertain the relative cellular specificity of TPA at 4 h, slot blot analysis was performed using RNA from mice treated with increasing doses of TPA. The expected dose-response pattern was seen when the filter was probed for ornithine decarboxylase mRNA (fig.3A); the same filter reprobed with radiolabeled α-actin cDNA demonstrated no change to the highest dose employed (fig.3B). This latter result indicates that TPA in this dose range,

Fig.2. Slot blot analysis of the appearance of ornithine decarboxylase mRNA in response to TPA. Treatment with either acetone alone for 4 h (slot 1) or with 17 nmol TPA for 1, 2, 4, and 6 h (slots 2–5). 50 nmnol RA pretreatment for 1 h followed by 17 nmol TPA for 3 h is also shown (slot 6).

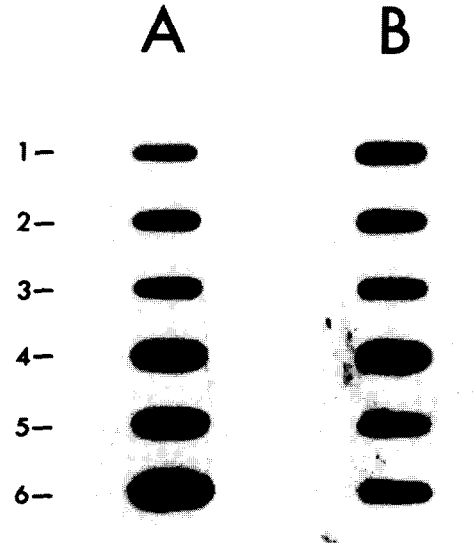

Fig.3. Treatment with increasing amounts of TPA. 15 μg of total RNA was slot blotted and probed with ^{32}P-labeled ornithine decarboxylase cDNA (A); the same filter was stripped and reprobed with ^{32}P-labeled actin cDNA (B). Slots 1–6 are 0.53, 1.06, 2.12, 4.25, 8.5, and 17.0 nmol TPA.

topically applied for 6 h, does not produce widespread gene alterations. All other slot blots were similarly reprobed with α-actin cDNA, and no altered level of mRNA was observed even in untreated control epidermis.

A recent report by Verma and co-workers [8] on ornithine decarboxylase mRNA utilized whole skin. However, prior studies of enzyme induction by TPA and its inhibition by RA have utilized epidermis alone, heat-separated from the dermis [5]. Hence, in order to perform RNA studies on epidermis free of contaminating RNA from the dermis, we utilized heat separation of these two layers in the presence of the RNase inhibitor DEP. This method provides a means to obtain undegraded RNA from epidermis and probably accounts for our ability to detect an additional mRNA species.

Several other systems already exist in which TPA or RA affect gene transcription. In the F9 teratocarcinoma cell line, seven independent RA-inducible gene sequences have been identified [19], and in whole animal feeding studies RA can both activate and inhibit gene expression [20]. In mouse 3T3 cells, TPA treatment results in altered transcription of several genes as measured in nuclear run-off experiments [21]. The strong parallels between the mRNA results described above and the published literature on the dose-response and time course of the induction of the enzyme and enzyme protein by TPA, including RA's dose-dependent inhibition of the induced enzyme, lead us to suggest that these well-characterized changes in enzyme activity are, at least in part, mediated transcriptionally. One aspect of the overall significance of these findings lies in their providing a better understanding of the molecular mechanisms of retinoic acid's antitumor action. As for the phorbol esters, the large increase in enzyme activity (230-fold) observed in mouse skin in response to TPA [17], which greatly exceeds the peak mRNA increase seen in our study (13-fold, table 1), suggests that other mechanisms may still prove to play a role here. Nevertheless, to demonstrate unequivocally that TPA and RA regulate transcription of the ornithine decarboxylase gene(s) in skin will require run-off transcription experiments which are difficult to perform in the epidermis. Cultured keratinocytes should offer more promise here.

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