Induction of ornithine decarboxylase in mouse skin by tumor-promoting agents and their specificity

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Abstract

A single topical application of a potent tumor-promoting agent, 12-O-tetradecanoylphorbol-13-acetate (TPA; 17 nmol/mouse) to mouse skin resulted in a large and rapid induction of skin ornithine decarboxylase (ODC) activity. The induction reached a peak at 4 to 5 hr after TPA treatment and returned to control level by 16 hr. Induction of ODC by TPA was significantly inhibited by prior treatment of skin with indomethacin (inhibitor of prostaglandin synthesis, 10 mg/kg body weight) or vitamin A analogs (retinoids, 50 μg/kg body weight). Phenobarbital (liver tumor promoter, 100 mg/kg body weight), bile acids (colonic tumor promoter, 100 mg/kg body weight), and saccharin (bladder tumor promoter, 100 mg/kg body weight) did not affect ODC activity in mouse skin. The skin tumor-promoting phorbol esters (17 nmol/mouse) induced mouse skin ODC in the following order: TPA > 4β-phorbol-12, 13-didecanoate (4β-PDD) > phorbol-12, 13-dibenzoate (PDB). The non-tumor promoting phorbol esters, 4α-phorbol, 4β-phorbol, 4α-phorbol-12, 13-didecanoate (4α-PDD) and phorbol-12, 13-diacetate (PDA), did not affect ODC activity.

These results indicate that phorbol ester-induced skin ODC may be an important component of the mechanism of skin tumor promotion. Furthermore, it is suggested that tumor promoters induce ODC in their target tissues.

Introduction

Tumors can be effectively produced in mouse skin by treatment with a single, topical application of a subcarcinogenic dose of a compound such as dimethylbenz [a] anthracene followed by repetitive applications of a promoting agent such as 12-O-tetradecanoylphorbol-13-acetate (TPA)[2,3]. This 2-stage system called initiation and promotion has been utilized as a model of chemical carcinogenesis in intact animals, the biochemical events of which might be important for the neoplastic process in mouse skin.

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Ornithine decarboxylase (ODC, L-ornithine carboxy-lyase; E. C. 4.1.1.17), the rate-limiting enzyme in the polyamine-biosynthetic pathway is increased in various proliferative cell systems and implicated in neoplastic growth[11,21]. In mouse epidermis, a rapid, transient induction of ODC is observed by application of TPA[13,14].

The present experiments were carried out to evaluate ODC as a marker of tumor promotion on tumor promoters of various tissues.

Materials and Methods

Animals: Female Charles River CD-1 mice, 7 to 9 weeks of age, were housed in wire cages (5 per cage) in a controlled environment at 23°C with a 12 hr light/dark cycle. They were given food and water ab libitum until the day of experimentation. The hair on the dorsal aspect of the body of each mouse was shaved with clippers at least 2 days before use, and only those mice showing no hair regrowth were chosen. Chemicals were topically applied to the shaved areas and the mice were routinely killed between 1 and 3 p. m. to avoid circadian rhythm variations.

Chemicals: Chemicals applied topically to mouse skin were dissolved in reagent grade acetone and delivered in a volume of 0.2 m*l*. Chemicals administered i. p. were dissolved in 40% ethanol and 60% 0.15 M NaCl and delivered in a volume of 0.1 or 0.2 m*l*.

Phorbol esters, phenobarbital, cholic acid, chenodeoxycholic acid, saccharin, indomethacin, retinoic acid and retinol were obtained from Sigma Chemical Co. (St. Louis, MO); DL-[1-^{14}C] ornithine hydrochloride (specific activity 58 mCi/mmol), from the Radiochemical Centre (Amersham, UK).

All other chemicals were reagent grade and purchased from commercial sources.

Assay of ODC activity: At 4 hr after treatment, mice were killed by cervical dislocation. The skin from individual mouse was excised and homogenized in 3 volumes of ice cold buffer consisting of 50 mM sodium phosphate, pH 7.2, 0.1 mM pyridoxal phosphate and 0.1 mM EDTA. The homogenates were centrifuged at 20,000×g for 30 min at 4°C. The resulting supernatant was used as the source of ODC activity. The ODC activity of soluble skin extracts was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C] ornithine hydrochloride. The concentration of reagents in the assays for the determination of ODC activity was the same as reported by O'BRIEN et al.[13]. Assays were routinely carried out at 0.3 mM L-ornithine in a final volume of 1 m*l*. After incubation for 1 hr at 37°C, the reaction was stopped by the addition of 0.5 m*l* of 4 N sulfuric acid. The incubation was continued for another 1 hr and radioactivity in each sample was measured in 18 m*l* of toluene scintillation fluid with 20% ethanol in a Beckman Model LS 8000 scin-

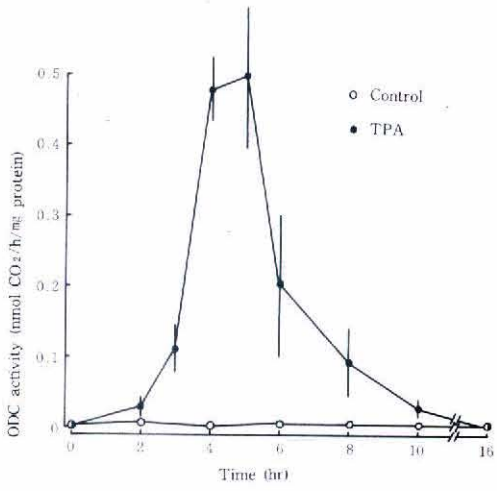

Fig. 1. Time course of mouse skin ODC activity after a single topical application of TPA. Mice received a single topical application of TPA 17 nmol/mouse and were killed at the times indicated. Each point represents the mean ± S. E. M. of 5 individual mouse skin supernatants in which ODC activity was determined in duplicate.

tillation counter at 80% efficiency.

Protein determination: The protein concentration of the mouse skin extracts was measured by the method of LOWRY et al.[12], with bovine serum albumin as standard.

Results

A single topical application of TPA 17 nmol/mouse led to a rapid and remarkable increase in ODC activity in the mouse skin (Fig. 1). The maximal increase in the activity of ODC occurred 4–5 hr after TPA application and reached a level 240-fold above the value obtained from animals receiving the application vehicle alone. TPA-stimulated ODC activity had returned to control levels by 16 hr. The application vehicle alone had no effect upon ODC activity. The increase of skin ODC activity by TPA was inhibited either by the pre-treatment of actinomycin D 2 mg/kg body weight and cycloheximide 5 mg/kg body weight (data not shown). This suggests that the increase of TPA-stimulated ODC activity is the induction of ODC by TPA.

Table 1. Effects of pre-treatment with vitamin A analogs and indomethacin on the induction of mouse skin ODC by TPA.

Treatment	ODC activity (nmol CO_2/h/mg protein)
Vehicle+Vehicle	0.002 ± 0.001
Vehicle+TPA	0.489 ± 0.065
Retinoic acid+TPA	0.082 ± 0.007*
Retinol+TPA	0.187 ± 0.026*
Indomethacin+TPA	0.067 ± 0.004*

Mice were treated topically with TPA 17 nmol/mouse. One hour prior to TPA application, vitamin A analogs (retinoic acid and retinol) 50μg/kg body weight and indomethacin 10 mg/kg body weight were applied topically. Four hours after application of TPA, mice were killed for the immediate preparation of skin homogenates. ODC activity was measured in 20,000 × g, 30 min supernatants in duplicate. Each value represents the mean± S. E. M. of individual determinations from 5 mice. *$P < 0.05$ versus TPA . Vehicle: Acetone.

Table 2. Effects of phenobarbital, bile acids and saccharin on ODC activity in mouse skin.

Treatment	ODC activity (nmol CO_2/h/mg protein)
Vehicle	0.006 ± 0.004
Phenobarbital	0.005 ± 0.003
Cholic acid	0.003 ± 0.002
Chenodeoxycholic acid	0.004 ± 0.003
Saccharin	0.007 ± 0.004

Mice were treated topically with phenobarbital 100 mg/kg body weight, bile acids (cholic acid and chenodeoxycholic acid) 100 mg/kg body weight or saccharin 100 mg/kg body weight. Four hours after each application, mice were killed for the immediate preparation of skin homogenates. ODC activity was measured in 20,000 × g, 30 min supernatants in duplicate. Each value represents the mean± S. E. M. of individual determinations from 5 mice. Vehicle: Acetone.

Table 3. Effects of phorbol esters on ODC activity in mouse skin.

Treatment	ODC activity (nmol CO_2/h/mg protein)	Skin tumor-promoting ability
Vehicle	0.002 ± 0.001	−
4α-phorbol	0.003 ± 0.002	−
4β-phorbol	0.006 ± 0.004	−
4α-PDD	0.005 ± 0.002	−
PDA	0.008 ± 0.005	−
PDB	0.174 ± 0.026*	+
4β-PDD	0.253 ± 0.053*	++
TPA	0.481 ± 0.049*	++++

Mice were treated topically with 17 nmol each of the phorbol esters. Four hours after application, mice were killed for the immediate preparation of skin homogenates. ODC activity was measured in 20,000×g, 30 min supernatants in duplicate. Each value represents the mean ± S. E. M. of individual determinations from 5 mice. *$P<0.01$ versus vehicle.

Vehicle: acetone, 4α-PDD: 4α-phorbol-12, 13-didecanoate, PDA: phorbol-12, 13-diacetate, PDB: phorbol-12, 13-dibenzoate, 4β-PDD: 4β-phorbol-12, 13-didecanoate, TPA: 12-O-tetradecanoylphorbol-13-acetate. Promoting ability is taken from Ref. 7.

The effects of pre-treatment with indomethacin (inhibitor of prostaglandin synthesis) and vitamin A analogs (retinoic acid and retinol) on TPA-stimulated mouse ODC activity are shown in Table 1. Indomethacin 10 mg/kg body weight and vitamin A analogs 50 μg/kg body weight were applied to the skin 1 hr prior to the application of TPA 17 nmol/mouse. The increase of skin ODC activity by TPA was inhibited either by indomethacin or vitamin A analogs.

In Table 2 the effects of phenobarbital (liver tumor promoter), bile acids (cholic acid and chenodeoxycholic acid, colonic tumor promoter) and saccharin (bladder tumor promoter) on mouse skin ODC activity are shown. Skin ODC activity was determined 4 hr after each topical application of phenobarbital 100 mg/kg body weight, bile acids 100 mg/kg body weight and saccharin 100 mg/kg body weight to mouse skin. Phenobarbital, bile acids and saccharin did not affect ODC activity in mouse skin.

The effects on skin ODC activity by a number of phorbol esters were examined and compared as to their ability of ODC induction and tumor promotion. Skin ODC activity was determined 4 hr after a single topical application of phorbol esters 17 nmol/mouse. As shown in Table 3, the tumor-promoting phorbol esters, PDB, 4β-PDD and TPA increased ODC activity in skin in the following order: TPA > 4β-PDD > PDB. The non-tumor-promoting phorbol esters, 4α-phorbol, 4β-phorbol, 4α-PDD and PDA, caused no detectable increase in skin ODC activity.

Discussion

The induction of ODC by tumor promoter, TPA, has already been known in mouse epidermis[13,14] and rat liver[4,18,22]. We also observed that a single topical application of TPA to mouse skin caused the induction of ODC in skin. The increase of ODC activity by TPA was transient. This kinetic pattern was similar to what we had already observed in rat liver[18].

Prostaglandins are naturally occurring cyclic metabolites of unsaturated fatty acids that have numerous physiological functions. A number of prostaglandins in tissues of various

species are biosynthesized from their precursor, unsaturated fatty acids present in cell membranes in the form of phospholipids. Elevated levels of prostaglandins are found in various tumors[10]. It has also been reported that TPA enhanced the synthesis of epidermal phospholipids[17] and the accumulation of epidermal PGE and PGF levels, and the activities of various phorbol esters for increasing PGE and PGF levels in mouse epidermis paralleled their tumor-promoting activities[1]. Since indomethacin, inhibitor of prostaglandin synthesis, applied 1 hr prior to TPA, inhibited the induction of mouse skin ODC by TPA significantly, it is suggested that prostaglandins may play an important role in the induction of ODC as well as skin tumor promotion. The exact biological role of prostaglandins in the process of skin tumor promotion remains speculative.

The role of vitamin A analogs (retinoids) in the modification of skin tumor promotion as well as in the prevention of epithelial cancers is well demonstrated[19,20]. It has also been reported that systemic applications of high doses of retinoids have a prophylactic effect on skin tumor promotion[5] and a therapeutic effect on established skin papillomas and carcinomas[6]. Since retinoid treatment before application of TPA to mouse skin inhibits the induction of skin ODC, it is suggested that TPA-induced ODC activity may be an important component of the mechanism of skin tumor promotion.

BOUTWELL, et al.[7] have reported tumor promoting ability in mouse skin by TPA and its structurally related compounds (phorbol esters) isolated from croton oil. The present experiments demonstrate that the magnitude of ODC activity in mouse skin by phorbol esters correlates well with their skin tumor promoting ability. Its correlation has been known in rat liver[18]. It is suggested that phorbol esters-stimulated skin ODC induction relates to tumor promotion during carcinogenesis in skin.

Phenobarbital is a potent promoter of liver tumors in rats initiated with 2-acetylaminofluorene or other hepatocarcinogens[15]. Saccharin is a promoter of bladder tumor in rats initiated with N-methyl-N-nitrosourea[9]. A number of bile acids promote formation of colon tumors in rats initiated with N-methyl-N'-nitro-N-nitrosoguanidine[16]. These compounds fail to induce ODC in mouse skin. It has been known that compounds that are promoters in one system may be inactive in another[8]. It is suggested that tumor promoters induce ODC and are organ specific.

Further studies concerning the cellular sites of action of phorbol esters in mouse skin are currently being carried out.

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発癌プロモーターによるマウス皮膚のオルニチン脱炭酸酵素誘導とその特異性

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摘　要

マウスの皮膚に発癌プロモーターである12-O-tetradecanoylphorbol-13-acetate (TPA, 17 nmol/mouse) を塗布すると，皮膚のオルニチン脱炭酸酵素 (ODC) 活性は著しく増加した。この活性の増加は，TPA 適用後4～5時間で最大に達し，16時間以内に元のレベルに戻った。さらに，actinomycin D (RNA合成阻害薬，2 mg/kg body weight) および cycloheximide (蛋白合成阻害薬，5 mg/kg body weight) の前投与により抑制されたことから，酵素誘導によるものと思われた。

TPA による皮膚の ODC 誘導は，indomethacin (プロスタグランジン合成阻害薬，10 mg/kg body weight) および vitamin A analogs (レチノイド，50 μg/kg body weight) の皮膚への前適用により抑制された。

phenobarbital (肝発癌のプロモーター，100 mg/kg body weight)，bile acids (結腸発癌のプロモーター，100 mg/kg body weight) および saccharin (膀胱発癌のプロモーター，100 mg/kg body weight) は皮膚の ODC を誘導しなかった。

マウス皮膚発癌のプロモーター作用を有する phorbol esters: TPA, 4β-phorbol-12, 13-didecanoate (4β-PDD) および phorbol-12, 13-dibenzoate (PDB) は皮膚の ODC を誘導した (TPA＞4β-PDD＞PDB) が，プロモーター作用の無い phorbol esters: 4α-phorbol, 4β-phorbol, 4α-phorbol-12, 13-didecanoate (4α-PDD) および phorbol-12, 13-diacetate (PDA) は効果が無かった。phorbol esters による皮膚の ODC 誘導能と皮膚発癌のプロモーター作用とがよく相関することが認められた。

以上の成績から，phorbol esters による皮膚の ODC 誘導は，皮膚発癌のプロモーション過程に重要な役割を果たしていると推察される。さらに，発癌プロモーターによる ODC 誘導には，臓器および細胞特異性があることが示唆される。

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