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INCREASE IN HISTIDINE DECARBOXYLASE ACTIVITY IN MOUSE SKIN AFTER APPLICATION OF THE TUMOR PROMOTER TETRADECANOYLPHORBOL ACETATE

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SUMMARY

A tumor promoter in two-step carcinogenesis, 12-O-tetradecanoylphorbol-13-acetate, was painted on the dorsal skin of C57BL/6 mice. Within 12 hr after a single application of TPA the activity of histidine decarboxylase increased 10 times. The activity of ornithine decarboxylase also increased, but its increase was significantly earlier than that of histidine decarboxylase. Preincubation of a crude extract of the skin with α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, completely inhibited the increase in histidine decarboxylase activity, indicating the distinct natures of the two decarboxylases.

A tumor promotor in two-step carcinogenesis, 12-O-tetradecanoylphorbol-13-acetate (TPA), has various biological and biochemical effects on susceptible tissues (1,2). One marked biological effect of application of TPA to the skin of mice is increase in ornithine decarboxylase (ODC) activity (3,4). Increase in putrescine concentration is supposed to initiate a series of events leading to synthesis of DNA and protein, and then proliferation of cells (5). Since histamine has also been suggested to be involved

Abbreviations : TPA, 12-O-tetradecanoyl-phorbol-13-acetate ; HDC, histidine decarboxylase, L-histidine carboxyl-lyase, [EC 4.1.1.22]; ODC, ornithine decarboxylase, L-Ornithine carboxy-lyase, [E.C. 4.1.1.17] ; α-FMH, [S]-α-fluoromethylhistidine.

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in some types of cell proliferation, such as development of rat and mouse embryos, wound healing and growth of some experimental tumors (6), we examined the effect of TPA application on the activity of histidine decarboxylase (HDC), the key enzyme for histamine production. We found that the HDC activity in mouse skin increased about 10 times after a single application of TPA.

MATERIALS AND METHODS

Painting TPA on the Skin of Mice : Male C57BL/6 mice, raised in our laboratory, were used at 2-3 months of age. TPA (17 nmoles in 0.2 ml-acetone) was painted with a brush on the dorsal skin of the mice. Control mice were painted with 0.2 ml-acetone alone. Mice were killed at various times after this treatment and pieces of the dorsal and ventral skin (2x2 cm, each) were removed, weighed and stored at -80°C until use.

Analytical Procedures: Pieces of skin (2x2 cm, 100-400 mg) were cut up and homogenized in 2-ml of Solution A in a Polytron (Kinematica) operated three times for 10-sec periods in an ice bath. Solution A consisted of 0.1 M potassium phosphate buffer, pH 6.8, 0.01 mM pyridoxal 5'-phosphate, 0.2 mM dithiothreitol, 1% polyethylene glycol (average molecular weight 300), and 2 μg/ml each of pepstatin, leupeptin, chymostatin, and antipain (Protein Research Foundation, Minoh, Osaka, Japan). The homogenate was centrifuged at 15,000 x g for 20 min. One-tenth of the supernatant was mixed with 9 volumes of 3 % perchloric acid-5 mM EDTA and the supernatant obtained by brief centrifugation was used for histamine analysis as described previously (7). Histamine was purified by Bio-Rad AG-50 column chromatography and high performance liquid chromatography (TSK-IEX 510 SP Toyo Soda Ltd., Tokuyama, Japan) and was measured fluorophotometrically by the o-phthalaldehyde method of Shore et al. (8) using an autoanalyzer developed in this laboratory (7).

The remaining nine-tenths of the supernatant was dialyzed three times against 50 volumes of Solution A and used for HDC and ODC analyses and protein measurement. HDC was assayed with 0.25 mM histidine, and histamine formed during the reaction was measured fluorophotometrically as described previously (9). The activity of ODC was assayed by measuring the release of $^{14}CO_2$ from DL-(1-^{14}C)-ornithine monohydrochloride as described previously (10). Protein was measured by the method of Lowry et al. (11) with bovine serum albumin as a standard.

Micellaneous : HDC and ODC were partially purified from rat embryos and rat liver, respectively, as described previously (9, 10). A specific suicide inhibitor of HDC, α-fluoromethyl histidine (α-FMH) was a gift from Dr. Kollonitsch of Merck Sharp and Dohme, Rahway, N.J., USA (12). Purified fetal rat HDC or crude extract of mouse skin was treated with 0.1 mM α-FMH for 30 min at 37°C and then dialyzed extensively against Solution A. The remaining HDC activity was measured with untreated HDC as a control.

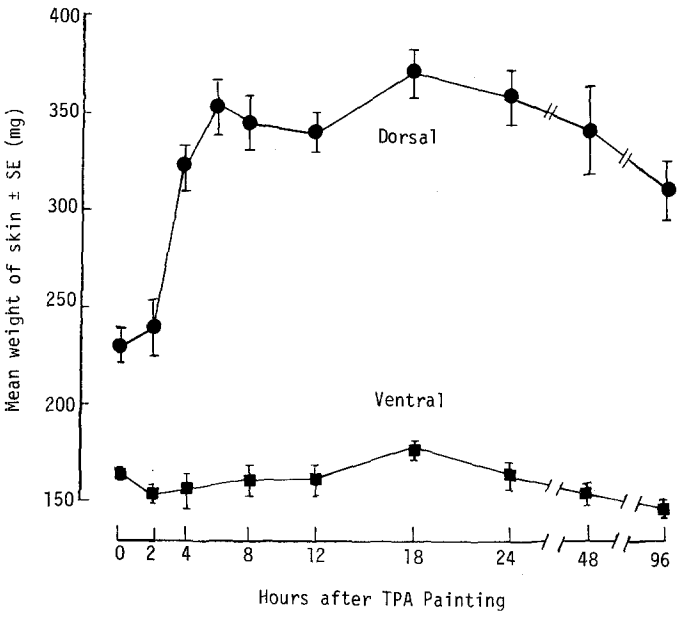

Fig. 1. Changes in weight of pieces of dorsal and ventral skin (2x2 cm, each) after a single painting of TPA on the back of C57BL/6 mice. Mice were killed at various times after TPA application. Points are means ± SE for 4-13 mice.

RESULTS

The weight of the dorsal skin (2x2 cm) started to increase 4 hr after a single application of TPA, and in 6 hr it became about 1.5 times that before treatment (Fig. 1). The activity of HDC in the dorsal skin also started to increase after 4 hr, reached a peak between 8-18 hr, and then decreased gradually for 4 days to the level before treatment (Fig. 2). No increase in weight (Fig. 1) or HDC activity (data not shown) was detected in the ventral skin of the animals that were not treated with TPA. Moreover, a single application of acetone alone did not affect either the weight or HDC activity of the dorsal skin of mice. Despite the marked increase in HDC activity, the concentration of histamine did not increase significantly (Fig. 2).

Since increase of ODC activity after painting TPA on mouse skin has been reported by many authors (3,4), we compared the

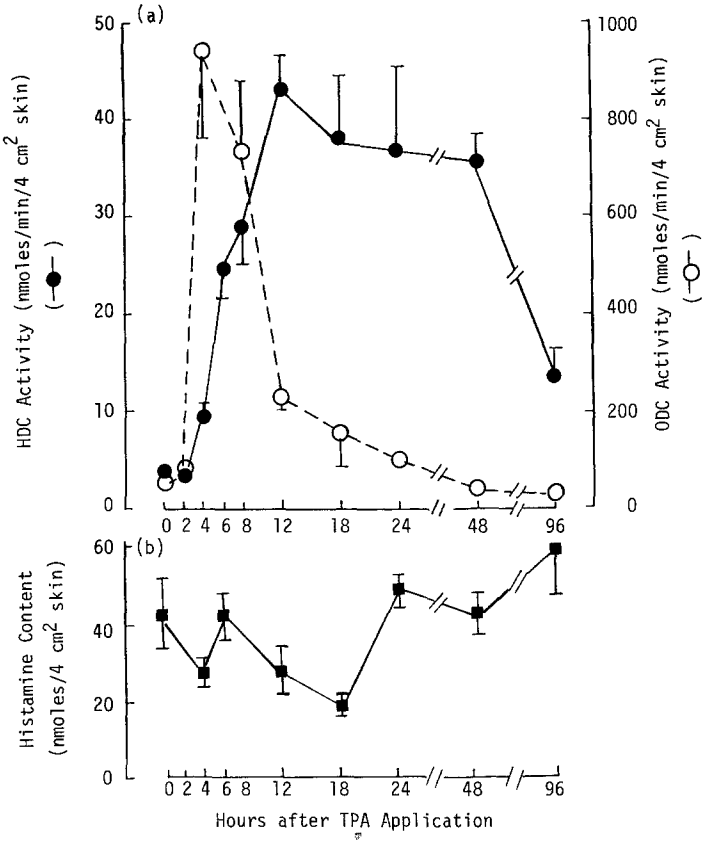

Fig. 2. Effect of a single application of TPA on the activities of HDC and ODC (a) and on the concentration of histamine (b) in the skin of C57BL/6 mice. Points are means ± SE for 4-11 mice.

changes in HDC activity and ODC activity after treatment using the same skin extract. As shown in Fig. 2, the increase and subsequent decrease in ODC activity occurred earlier than those of HDC activity. Therefore, the HDC measured in the present study seems to be different from ODC. Moreover, although the absolute activity of HDC was much less than that of ODC, the observed HDC activity was that of HDC itself, and was not due to ODC, as shown by the following results. 1) The addition of 0.25 mM L-ornithine (twice the Km value for ODC) to the assay mixture for HDC, which contained the same concentration of histidine, did not inhibit the HDC activity at all. Moreover, addition of 2.5 mM L-ornithine

Table 1.
Effects of Ornithine and α-FMH on HDC Activities of Fetal Rat Enzyme and Crude Extract of Mouse Skin Treated with TPA

Compound added	HDC activity (% of control)[a]	
	Fetal rat enzyme[b]	Mouse skin extract[c]
0.25 mM ornithine	100	100
2.50 mM ornithine	51	40
0.10 mM α-FMH	1	2

a) HDC activity was measured with 0.25 mM histidine in the presence of ornithine in the assay mixture or after pretreatment with α-FMH as described in MATERIALS and METHODS. Fetal rat enzyme was purified as described previously (9). Crude skin extract was prepared from mice 12 hrs after TPA painting.
b) 100 % represents 20 pmoles/min/ml of activity
c) 100 % represents 40 pmoles/min/ml of activity.

did not completely suppress the HDC activity (Table 1). 2) Pre-incubation with 0.1 mM α-FMH completely inhibited the activity of both partially purified HDC and a crude extract from the skin (Table 1). 3) A purified preparation of ODC did not catalyze the decarboxylation reaction of histidine under the assay conditions used for HDC (data not shown).

DISCUSSION

The present study showed for the first time that the activity of HDC in the skin of mice increased after painting TPA on the skin. The change in HDC activity occurred significantly later than that of ODC activity, but the magnitudes of change of the two enzymes were comparable. Schayer (13) and Kahlson and Rosengren (6) suggested that the rapid increase in HDC activity was associated with "inducible HDC" in non-mast-cells and that histamine synthesized by the inducible HDC was not stored in cells but was released immediately. Since the histamine concentration of the skin did not increase after painting TPA, the increase in HDC activity after this treatment may be due to induction of HDC in non-mast-cells. However, mouse skin is a tissue which contains

many mast cells (14), and so another explanation is that mast cells may produce histamine after its depletion by TPA painting. (WB x C57BL/6)F_1-W/W^V mice should be useful for distinguishing between these two possibilities because these mice are genetically depleted of mast cells (14). We are now testing the effect on HDC activity of painting TPA on the skin of W/W^V mutant mice.

The mechanisms of induction of HDC and ODC activities after a single application of TPA are unknown. The effect of other tumor promoters, such as phorbol diacetate and phorbol dibenzoate (4), and inflammatory drugs, such as carrageenin, acetic acid, cantharidine and ethylphenylpropionate (4) should also be tested.

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