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INDUCTION OF HISTIDINE DECARBOXYLASE ACTIVITY IN MOUSE SKIN AFTER APPLICATION OF INDOLE ALKALOIDS, A NEW CLASS OF TUMOR PROMOTER[1]

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The effects of various promoters in two-step carcinogenesis on the induction of histidine decarboxylase in the skin of mice was investigated. The potencies of various phorbol esters in inducing histidine decarboxylase activity were parallel with their tumor-promoting activities. Indole alkaloids such as dihydroteleocidin B and lyngbyatoxin A, which induced ornithine decarboxylase and promoted tumor development in the skin of mice with the same potency as 12-O-tetradecanoylphorbol-13-acetate (TPA), also induced histidine decarboxylase activity. These results suggest that histamine produced by this inducible histidine decarboxylase may play some role in tumor promotion.

The tumor promoter in two-step carcinogenesis 12-O-tetradecanoylphorbol-13-acetate (TPA) increased ornithine decarboxylase (ODC) activity (1,2). The potencies of various phorbol esters in

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Abbreviations used: TPA, 12-O-tetradecanoylphorbol-13-acetate; ODC, L-ornithine decarboxylase, L-ornithine carboxy-lyase, E.C.4.1.1.17; HDC, histidine decarboxylase, L-histidine carboxylyase, E.C.4.1.1.22; PDD, phorbol-12,13-didecanoate; PDB, phorbol-12-,13-dibenzoate; PDBu, phorbol-12,13-dibutyrate; 4-O-Me-TPA, 4-O-methyl-12-O-tetradecanoylphorbol-13-acetate.

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ODC induction are parallel with their tumor-promoting activities (1,3). Recently, Fujiki et al. showed that some indole alkaloids, such as dihydroteleocidin B, teleocidin and lyngbyatoxin A, induce ODC activity and promote tumor development in the skin of mice (4-7). The increase of putrescine concentration, which results from increase of ODC activity, is related to cell proliferation (8). Since histamine is also thought to be involved in some types of cell proliferation (9), Watanabe et al. examined the effect of TPA on the activity of histidine decarboxylase (HDC), and found a marked increase of HDC activity in the skin of mice after a single application of TPA (10). In the present study, we investigated the effects of various chemicals, including the new tumor promoters found by Fujiki et al. (4-7), on HDC activity to clarify the relation between the production of histamine and tumor promotion.

MATERIALS AND METHODS

Chemicals: Teleocidin B was isolated from mycelia of Streptomyces mediocidicus (11) and dihydroteleocidin B was prepared by its catalytic hydrogenation. TPA and mezerein were purchased from Consolidated Midland Corp., Brewster, NY, and CCR Inc., Eden Prairie, Minn, respectively. Lyngbyatoxin A, isolated from the blue-green alga, Lyngbya majuscula (12), was a gift from Dr. R. E. Moore, University of Hawaii. Palytoxin, isolated from the coelenterate, Palythoa tuberculosa (13), was a gift from Dr. D. Uemura, Shizuoka University. Other compounds were obtained from Sigma Chemical Co., St. Louis, Mo.

Experimental Procedures : Male C57BL/6 mice, raised in our laboratory, were used at 2-3 months of age. Chemicals were dissolved in 0.2 ml of acetone and painted on the skin of the back of mice. Mice painted with acetone only served as controls. Since the activity of HDC in the skin of mice has been shown to reach a peak 8 to 18 hr after a single application of TPA (10), mice were killed 12 hr after the application of chemicals. A piece of skin (2 x 2 cm) was removed, weighed and stored at -80°C until its HDC activity was determined by the method described previously (10). Briefly, pieces of skin were homogenized in a solution of 0.1 M potassium phosphate buffer, pH 6.8, 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate and 1 % polyethylene glycol (molecular weight 300) in a Polytron. The homogenate was centrifuged, and the supernatant was obtained and dialyzed against the same solution. HDC activity of

the supernatant was assayed by adding 0.25 mM L-histidine, and the histamine formed was measured by the o-phthalaldehyde method described previously (10,14).

RESULTS

The activities of HDC after single applications of various chemicals are shown in Table 1. TPA induced a marked increase in HDC activity. However, phorbol-12,13-didecanote (PDD), phorbol-12,13-dibenzoate (PDB) and phorbol-12,13-dibutyrate (PDBu), which are weak synthetic tumor pormoters, caused less increase in HDC activity than TPA. The HDC-inducing activities of phorbol esters

Table 1 HDC Activities in the Skin of Mice 12 hr after Single Application of Various Chemicals

Chemicals	Dose (nmoles)	No of mice	HDC activities (pmoles/min/mg protein) (mean ± SE)
None	–	6	0.30 ± 0.04[a]
TPA	17	6	5.35 ± 0.35[b]
PDD	17	4	1.27 ± 0.13[a,b]
PDB	17	5	0.51 ± 0.09[a]
PDBu	17	5	0.37 ± 0.04[a]
Phorbol	17	4	0.15 ± 0.01[a]
4-O-Me-TPA	17	4	0.19 ± 0.01[a]
A23187	17	5	0.65 ± 0.07[a,b]
Mezerein	17	5	4.03 ± 0.35[b]
Dihydroteleocidin B	17	5	6.96 ± 0.39[a,b]
Lyngbyatoxin A	17	6	12.66 ± 1.24[a,b]
Palytoxin	2	6	0.25 ± 0.04[a]
Roridin A	17	6	0.28 ± 0.04[a]
Verrucarin A	17	6	0.39 ± 0.04[a]
Diacetoxyscirpenol	17	6	0.58 ± 0.05[a,b]
Acetic acid	15[c]	5	0.72 ± 0.12[a,b]

[a] $p < 0.01$, significance by t test of difference from value for mice which were painted with TPA.
[b] $P < 0.01$, significance t test of difference from value for mice which were painted with acetone by t test.
[c] mg.

correlated well with their tumor-promoting activities (1,3). Phorbol, which is not a promoter in mouse skin, did not induce HDC at all. 4-O-Methyl-12-O-tetradecanoylphorbol-13-acetate (4-O-Me-TPA) and A23187, first-stage promoters (15), did not induce appreciable HDC. Mezerein, a potent second stage promoter (15), greatly increased HDC activity. As far as tested, the inductions of HDC by various tumor promoters are similar to that of ODC.

We also tested the inductions of HDC by a new class of tumor promoters, indole alkaloids (4-7). Dihydroteleocidin B and lyngbyatoxin A induced HDC more strongly than TPA. Although the structure of dihydroteleocidin B is very similar to that of lyngbyatoxin A, lyngbyatoxin A was much more effective than dihydroteleocidin B in induction of HDC.

Since irritation of the skin seems to be one of the associated effects of skin tumor promotion, we tested several powerful irritants isolated from various sources. Palytoxin is the most powerful known toxin of those isolated from marine animal sources (13). Several mycotoxins are known to be carcinogenic and some of them are strong irritants (16); in other words, they have the skin-necrotizing effects (17). Palytoxin and three mycotoxins, roridin A, verrucarin A and diacetoxyscirpenol, did not induce HDC. Acetic acid, a non-specific irritant, had only weak effect in increasing HDC activity even at a very high dose.

Since 13-cis-retinoic acid has been reported to inhibit the induction of ODC activity by TPA (18), its inhibiting effect on HDC induction was tested: it was painted on the skin of mice before and after the application of TPA. As shown in Table 2, retinoic acid did not significantly inhibit the induction of HDC activity by TPA.

Table 2 Effect of Retinoic Acid on HDC Activity Induced by TPA[a]

Treatment		No. of	HDC activity (pmoles/min/mg protein)
TPA	Retinoic acid	mice	(mean ± SE)
-	No	5	0.25 ± 0.04
-	-1 hr	5	0.30 ± 0.04
+	No	5	5.22 ± 0.44[b]
+	-1 hr	9	5.03 ± 0.32[b,c]
+	-1, 3, 7, 11 hr	5	3.82 ± 0.32[b,c]

[a]Retinoic acid (170 nmoles/0.2 ml acetone) was applied 1 hr before TPA (17 nmoles/0.2 ml acetone) or 1 hr before and 3, 7, 11 hr after TPA application. Mice were killed 12 hr after TPA application.
[b]$p < 0.01$, significance by t test of difference from the value mice not treated etiher TPA or retinoic acid.
[c]$p > 0.05$, significance by t test of difference from the value in mice treated with TPA only.

DISCUSSION

The tumor promoting activities of various phorbol esters have been shown to be parallel to their abilities in inducing ODC activity (1,3). A good correlation was reported through the range of 1 to 10 nmoles of TPA (19). The present results showed that the effects of various phorbol esters in inducing HDC activity were also parallel with their tumor-promoting activities.

Recently, Fujiki and Sugimura have found that dihydroteleocidin B, teleocidin and lyngbyatoxin A, all of which are indole alkaloids, induce ODC activity in mouse skin (4,20). These three compounds form a new class of potent tumor promoters, although they are structurally entirely different from phorbol esters (4-7). Since these indole alkaloids also induced HDC, the increase in HDC activity was also parallel with the increase in ODC activity. The reason why lyngbyatoxin A was more effective in HDC induction than dihydroteleocidin B may be related to the differences in the optimal doses of these compounds. Recently, Suganuma et al. reported that

the optimal dose of dihydroteleocidin B for tumor-promoting activity coincided with the optimal dose for ODC induction (21).

In contrast, palytoxin and three irritant mycotoxins caused little or no induction of HDC activity. Since palytoxin is the most potent known cytotoxic agent and it causes cytolysis preceded by marked potassium loss (R. E. Moore, unpublished data), the effect of palytoxin may be due to ionic disturbance. However, the reasons for the inability of the three mycotoxins to induce HDC may be different. As mycotoxins did not induce ODC either (Fujiki et al., unpublished data), we suppose that their inability to induce the two decarboxylases was due to their effect in inhibiting protein synthesis (17). These compounds should be tested for tumor promoting activity in mouse skin.

In spite of the parallel between the inductions of ODC and HDC by various compounds described above, the applications of retinoic acid, which is known to inhibit the induction of ODC by TPA (18), and by dihydroteleocidin B, teleocidin, lyngbyatoxin A and debromoaplysiatoxin (4,20), did not inhibit the induction of HDC activity by TPA. These results may be explained by the difference in location of these two enzymes: increase in ODC activity occurred in the epidermis (1,2), but that of HDC activity occurred in the dermis (22). Since most neoplasms that develop after application of 7,12-dimethylbenz(a)anthracene (DMBA) and TPA are of epidermal origin, the HDC induced by TPA cannot directly be related to the neoplastic change of target cells. However, histamine produced by HDC may play some promoting roles in tumor formation through epithelial-mesenchymal interaction that have not as yet been clearly defined.

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