INDUCTION OF ORNITHINE DECARBOXYLASE ACTIVITY IN EPIDERMIS BY A TUMOR PROMOTER: DECREASED RESPONSE IN MICE MAINTAINED ON A VITAMIN B_6-DEFICIENT DIET[1]

Andrew W. Murray and Mario Froscio

School of Biological Sciences, Flinders University of South Australia, Bedford Park, South Aust. 5042.

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Summary. Epidermal ornithine decarboxylase (EC 4.1.1.17; L-ornithine carboxy-lyase) activity was rapidly induced after the treatment of mouse skin with 12-O-tetradecanoyl phorbol-13-acetate. Induction was significantly decreased in animals maintained for 10-14 days on a vitamin B_6-deficient diet, and could be completely restored by the injection of deficient animals with pyridoxine. As predicted, decarboxylase activity in epidermal extracts was sensitive to proteolytic attack (trypsin) in the absence of pyridoxal 5'-phosphate, and was protected from attack by the presence of the cofactor.

Considerable interest has recently centered on the proposed identification of a biochemical change specific for tumor promotion. Treatment of mouse skin (1) or cultured epidermal cells (2) with TPA[2] or other tumor promoters resulted in the rapid, transient induction of ornithine decarboxylase (EC 4.1.1.17; L-ornithine carboxy-lyase), the rate-limiting enzyme for the synthesis of polyamines in mammalian tissues (3). Enzyme activity was induced by every promoter tested, but not by a range of non-promoting, hyperplastic agents (4). Turnover of the enzyme ornithine decarboxylase is normally very rapid in mammalian cells (about 17 min in mouse epidermis (3)), and it has been proposed (1) that initiation of cells may result in a defect in the systems responsible for turnover. Promotion

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[2]Abbreviation : TPA, 12-O-tetradecanoyl phorbol-13-acetate.

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may then lead to a prolonged elevation of the decarboxylase in initiated cells providing them with a selective growth advantage. In support of the model, it was reported (1) that epidermal tumors had both a high level of ornithine decarboxylase activity and a decreased turnover of the enzyme.

In view of the potential importance of ornithine decarboxylase in tumor promotion, information is required on factors which may influence the activity and induction of the enzyme in animal tissues. The enzyme requires pyridoxal 5'-phosphate as a cofactor (5) and it was consequently predicted that the TPA-inducibility of ornithine decarboxylase would be affected under conditions of vitamin B_6 (pyridoxine) deficiency. This communication summarizes data from experiments designed to test this prediction.

MATERIALS AND METHODS

The animals used were female Swiss albino mice maintained under reversed light and dark periods as described before (6). The dorsal skins of animals were shaved, and only those which did not show a regrowth of hair over 7 days were used for experimentation. TPA (18 nmoles) was applied to the shaved area as a solution in acetone (0.2 ml).

Animals were maintained on either the complete or vitamin B_6-deficient diets described by Gershoff (7).

Soluble extracts for the assay of ornithine decarboxylase were made as described (3), but were prepared in 50 mM tris-chloride (pH 7.4); centrifugation was carried out for 30 min at 100,000 g. Extracts were prepared from the epidermis of individual mice; the skin was not pretreated with a depilatory agent. Reaction mixtures for the assay of ornithine decarboxylase activity contained 0.4 µmole pyridoxal 5'-phosphate, 1 µmole dithiothreitol, 0.2 µmole L-ornithine, 100 µmole tris-chloride (pH 7.4), 0.5 ml of epidermal extract (310-820 µg protein) and 0.5 µCi DL[1-^{14}C]ornithine (specific activity 59 mCi/mmole) in a final volume of 2 ml; assays were carried out as described (3).

Red blood cell aspartate transaminase was assayed as described before (8).

To determine the susceptibility of epidermal ornithine decarboxylase to proteolysis, pooled extracts from several mice were used. Decarboxylase assays (minus ornithine but containing various amounts of trypsin) were incubated at 37°C. After appropriate incubation periods, proteolysis was stopped by the addition of 200 µg Soybean trypsin inhibitor. Residual ornithine decarboxylase activity was determined after the addition of

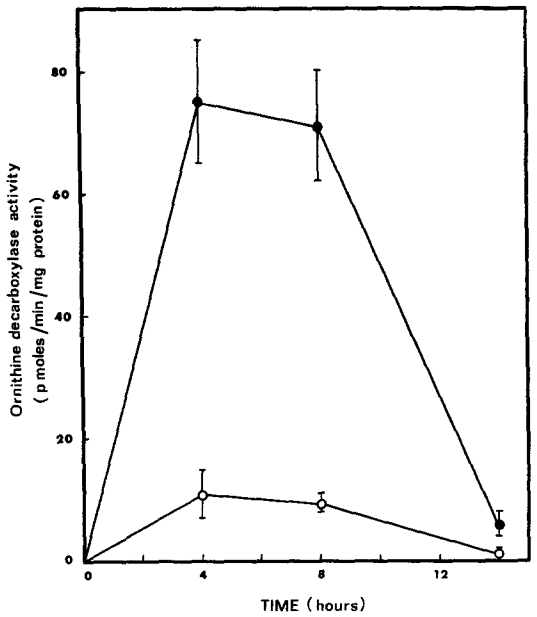

Figure 1. Induction of epidermal ornithine decarboxylase by TPA in vitamin B_6-deficient mice.

Epidermal ornithine decarboxylase was measured at various times after the application of 18 nmoles TPA. Mice were maintained for 14 days on the complete diet (●) or the vitamin B_6-deficient diet (○).

Each point is the mean ± S.E.M. of determinations carried out on 5 separate animals.

[1-^{14}C]ornithine and incubation for a further 60 min (3). In some experiments proteolysis was carried out in the absence of pyridoxal 5'-phosphate, which was then added to reaction mixtures after the addition of trypsin inhibitor. In separate experiments it was established that the addition of the trypsin inhibitor completely prevented the further inactivation of ornithine decarboxylase.

Protein was determined as described by Lowry *et al.* (9).

L-Ornithine, pyridoxine and all vitamin components of the diets were obtained from the Sigma Chemical Co., St. Louis, U.S.A. Vitamin free casein and pyridoxal 5'-phosphate were obtained from Calbiochem (Australia) Ltd.; TPA was from Cambrian Chemicals Ltd., Croydon, England. Soybean trypsin inhibitor and trypsin-TPCK were obtained from Worthington Biochemical Corp., New Jersey. DL[1-^{14}C]Ornithine (specific activity 59 mCi/mmole) was obtained from The Radiochemical Centre, Amersham, England. All other biochemicals were of analytical reagent grade.

RESULTS AND DISCUSSION

The induction of ornithine decarboxylase activity by TPA in mice maintained for 14 days on the complete diet or on the vitamin B_6-deficient diet is shown in Figure 1. Clearly, induction of ornithine decarboxylase

Table 1. Effect of pyridoxine injection on the induction of ornithine decarboxylase in vitamin B_6-deficient mice.

Mice were maintained on either the complete or the vitamin B_6-deficient diets for 14 days. Animals were injected i.p. with 500 µg pyridoxine in 0.2 ml 0.9% NaCl 18 h before TPA treatment and again with 50 µg pyridoxine 2 h before TPA treatment. All animals were treated with 18 nmoles TPA and extracts for ornithine decarboxylase assay prepared after 4 h.

Each point is the mean ± S.E.M. of determinations carried out on 5 separate animals.

Treatment	Ornithine decarboxylase activity (pmoles/min/mg protein)
Complete diet	82.8 ± 21.8
B_6-deficient diet	3.8 ± 2.5
Complete diet + pyridoxine	94.1 ± 23.4
B_6-deficient diet + pyridoxine	103.8 ± 22.5

was decreased in the B_6-deficient group. Enzymic activity in extracts prepared from untreated or acetone-treated animals on either diet was very low and could not be accurately measured with the relatively insensitive assay used; in a number of experiments the mean activity was 0.6 ± 0.4 pmoles/min/mg protein (n = 14).

In the experiment reported in Figure 1, the animals maintained on the deficient diet showed a slight weight loss. The average weight per mouse decreased from 24.8 g to 22 g over the 14 day period; no such loss occurred in the group fed the complete diet. Consequently, it was necessary to show that the decreased ornithine decarboxylase inducibility was a result of vitamin B_6-deficiency and was not due to a decreased caloric intake. Table 1 summarizes experiments in which it was shown that the i.p. injection of vitamin B_6-deficient mice with pyridoxine restored the TPA-inducibility of ornithine decarboxylase to control values.

The results shown in Figure 1 and Table 1 were predictable in view of the known effects of B_6 deficiency on the activities of other pyridoxal

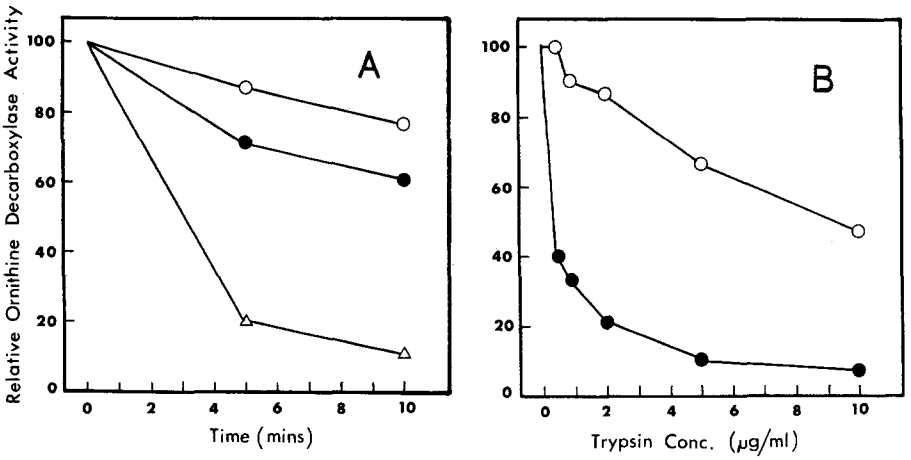

Figure 2. Effect of pyridoxal 5'-phosphate on the inactivation of epidermal ornithine decarboxylase by trypsin.

A. Extract (660 μg protein), prepared 6 h after treatment with TPA, was incubated with trypsin (10 μg/ml) in the presence (●) or absence (△) of pyridoxal 5'-phosphate for the indicated times, and then assayed for residual ornithine decarboxylase activity (see Materials and Methods). Preincubation was also carried out in the absence of both trypsin and pyridoxal 5'-phosphate (○). All decarboxylase assays contained 0.2 mM pyridoxal 5'-phosphate.

B. Extract (708 μg protein), prepared 6 h after treatment with TPA, was incubated for 10 min with varying concentrations of trypsin in the presence (○) or absence (●) of pyridoxal 5'-phosphate. Residual ornithine decarboxylase activity was then assayed; all decarboxylase assays contained 0.2 mM pyridoxal 5'-phosphate.

5'-phosphate requiring enzymes (10,11). In the present experiments, the activity of red blood cell aspartate transaminase was 30.6 ± 2.5 pmoles/min/mg hemoglobin protein (n = 8) in animals fed the complete diet and 16 ± 0.7 pmoles/min/mg hemoglobin protein (n = 7) in animals kept for 10 days on the B_6-deficient diet. Deficient animals given i.p. injections of pyridoxine (50 μg/animal) 22 h and 6 h before the collection of blood had a transaminase activity of 30.8 ± 1.4 pmoles/min/mg hemoglobin protein (n = 8).

All ornithine decarboxylase assays were carried out in the presence of pyridoxal 5'-phosphate. Consequently extracts from B_6-deficient animals contain less ornithine decarboxylase apoenzyme after TPA induction than

extracts from control animals. This could result from decreased protein synthesis, from increased susceptibility of ornithine decarboxylase to proteolytic attack as a consequence of the lowered pyridoxal 5'-phosphate concentration, from increased activity of proteolytic enzymes or from a combination of these factors. The activity of group-specific proteases has been shown to increase in the tissues of rats maintained on a vitamin B_6-deficient diet (10,11). Similarly, the susceptibility of pyridoxal enzymes to proteolytic attack has been reported to be highly dependent on the concentration of pyridoxal 5'-phosphate (10,11). The same may be true of ornithine decarboxylase. Thus, as shown in Figure 2, epidermal ornithine decarboxylase activity was highly susceptible to inactivation by trypsin in the absence of added pyridoxal 5'-phosphate but was significantly protected by the addition of this cofactor.

The possibility that extracts from vitamin B_6-deficient animals contain higher levels of an inhibitor of ornithine decarboxylase activity (e.g. see ref. 12) was eliminated by a series of mixing experiments. Thus assays carried out with a mixture of extracts prepared from control and B_6-deficient animals showed strictly additive ornithine decarboxylase activities (data not shown).

In future experiments it will be of interest to determine the effects of vitamin B_6 deficiency on other molecular changes triggered by TPA (e.g. macromolecule synthesis), on the subsequent progression of tumors developed in an initiation/promotion experiment, and on the effectiveness of TPA as a promoting agent.

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