Phorbol esters stimulate spermidine/spermine N^1-acetyltransferase activity in mitogen-stimulated bovine lymphocytes

Isao Matsui-Yuasa, Shuzo Otani, Zhao Wu Shu* and Seiji Morisawa

Department of Biochemistry, Osaka City University Medical School, Asahimachi, Abenoku, Osaka 545, Japan

Received 11 October 1984

Phorbol 12-myristate-13-acetate (PMA) is shown to induce spermidine/spermine N^1-acetyltransferase, a rate-limiting enzyme of polyamine biodegradation, in bovine lymphocytes. When PMA and phytohemagglutinin (PHA) were added simultaneously, the enzyme activity was stimulated synergistically. The ability of phorbol esters to stimulate the enzyme activity was consistent with their tumor-promoting ability. Phorbol, which is not a tumor promotor, was incapable of stimulating the enzyme activity. Phorbol diacetate weakly stimulated the activity of the acetylase. Phorbol dibutyrate had a similar stimulatory effect to PMA. These results suggest that the spermidine/spermine N^1-acetyltransferase may play an important role in changes in polyamine levels in phorbol ester-treated cells and that the increase in the enzyme activity may have some relationship to the control of cell growth and differentiation by phorbol esters.

Phorbol ester *Spermidine/spermine* N^1*-acetyltransferase* *Phytohemagglutinin* *Bovine lymphocyte*

1. INTRODUCTION

Phorbol 12-myristate-13-acetate (PMA) and related phorbol diesters, which are plant diterpenes, promote the formation of skin tumors after initiation by a low dose of a carcinogen [1]. Studies on the effects of tumor-promoting phorbol esters in several cells have revealed that the induction of ornithine decarboxylase and the subsequent accumulation of polyamines is one of the earliest and most pronounced responses of cells to tumor promotors [2–4]. Authors in [2,3] suggested that the alterations in polyamine metabolism could be an essential if not sufficient component of the 2-stage mechanism of tumor promotion.

However, some reports have demonstrated that promotor-mediated cellular hyperplasia and ornithine decarboxylase induction could be independent rather than related events [5,6]. Authors in [7] also indicated that promotor-induced changes in polyamine levels can occur without alterations of activities of ornithine decarboxylase and *S*-adenosylmethionine decarboxylase which are rate-limiting enzymes of polyamine biosynthesis in the PMA-treated human myeloid leukemia cell line HL-60.

Therefore, the mechanism of alterations in polyamine metabolism is not clear. Here, we investigated the effect of phorbol esters on spermidine/spermine N^1-acetyltransferase, which is a rate-limiting enzyme of polyamine biodegradation, in bovine lymphocytes stimulated with mitogen (PHA), PMA or both.

2. MATERIALS AND METHODS

2.1. *Reagents*

PMA was obtained from L.C. Service Corp., Woburn, MA. 4-Phorbol, phorbol 12,13-diacetate, phorbol 12,13-dibutyrate, cycloheximide

* Present address: Department of Biochemistry, Institute of Clinical Medicine, Sino-Japan Friendship Hospital, Peking, China

Published by Elsevier Science Publishers B.V.

and actinomycin D were purchased from Sigma, St. Louis, MO. Phytohemagglutinin P was obtained from Difco, Detroit, MI. Horse serum was obtained from Commonwealth Serum Laboratories, Victoria, Australia. [*acetyl*-1-^{14}C]Acetyl CoA (53.5 mCi/mmol) was from New England Nuclear, Boston, MA.

2.2. *Lymphocytes*

Bovine pharyngenous lymph nodes were kindly provided by Osaka City Meat Inspection Laboratory, Osaka. The lymphocytes were separated from other cells as in [8] and suspended in Eagle's minimum essential medium containing 1% (v/v) horse serum at a concentration of 1×10^7 cells/ml. Three ml of the cells was cultured in a sterile glass Petri dish (45 mm) in a humidified atmosphere of 5% CO_2 in air at 37°C. The cells were preincubated overnight prior to the additions of PHA, PMA and other drugs. Stock solutions of phorbol esters were dissolved in dimethyl sulfoxide and kept at 4°C in the dark until use. The final dimethyl sulfoxide concentration in culture was always 0.5% or less.

2.3. *Assay of spermidine/spermine N^1-acetyltransferase activity*

Preparation of enzyme solution from the cells has been described [9]. Spermidine/spermine N^1-acetyltransferase was determined by following the incorporation of [*acetyl*-1-^{14}C]acetyl CoA into monoacetylspermidine [9]. The incubation time was 10 min at 37°C.

3. RESULTS

When various concentrations of PMA were added to the lymphocyte suspensions and the activity of spermidine/spermine N^1-acetyltransferase determined 24 h after the addition, the enzyme activity was found to increase in a dose-dependent manner as shown in fig.1. The enzyme activity had increased by 2.5-fold 24 h after the addition of PMA (50 ng/ml). The addition of PHA produced a similar or somewhat smaller increase, but the combined exposure to PMA and PHA resulted in a synergistic increase. The elevation of the enzyme was markedly reduced when cycloheximide or actinomycin D was added to the cell culture simultaneously with PMA and/or PHA (not shown). These results imply that new protein and RNA synthesis are required for the enhancement of spermidine/spermine N^1-acetyltransferase activity in bovine lymphocytes treated with PMA and/or PHA.

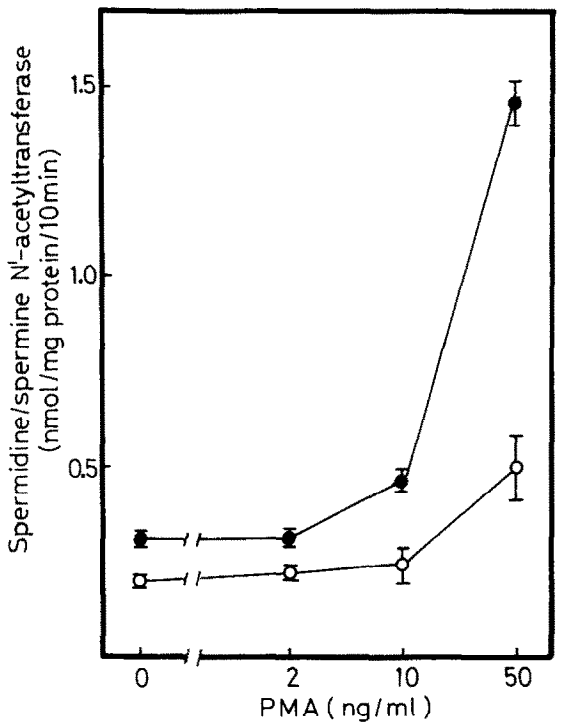

Fig.1. Effect of various concentrations of phorbol 12-myristate-13-acetate on spermidine/spermine N^1-acetyltransferase activity. Bovine lymphocytes (10^7 cells/ml) were treated with various concentrations of phorbol 12-myristate-13-acetate (PMA) and/or phytohemagglutinin (PHA) (5 μg/ml). Spermidine/spermine N^1-acetyltransferase activity was measured as described previously. The results are the means ± SE of 3 experiments. (○) PMA-treated cells, (●) PMA- and PHA-treated cells.

The spermidine/spermine N^1-acetyltransferase activity changed with time after addition of PMA or PHA as shown in fig.2. The enzyme activity in PMA-treated lymphocytes was induced biphasically having peaks at 24 and 48 h after the addition, whereas that of PHA-stimulated lymphocytes gradually increased and reached its maximum at 40 h. In the cells treated with the combination of PMA and PHA, the enzyme activity peaked at 24 and 48 h after the addition of inducers.

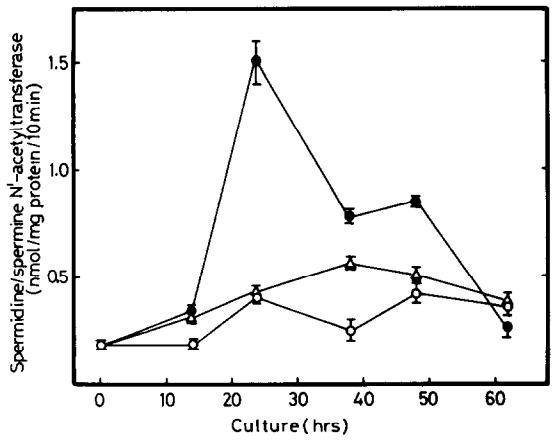

Fig.2. Effect of phorbol 12-myristate-13-acetate and phytohemagglutinin on spermidine/spermine N^1-acetyltransferase activity. Bovine lymphocytes (10^7 cells/ml) were treated with phorbol 12-myristate-13-acetate (PMA) (50 ng/ml) and/or phytohemagglutinin (PHA) (5 μg/ml) and were harvested at the times shown. The results are the means ± SE of 3 experiments. (○) PMA-treated cells, (△) PHA-treated cells, (●) PMA- and PHA-treated cells.

Table 1

Relative increase in the activity of spermidine/spermine N^1-acetyltransferase following treatment with various phorbol compounds

Treatment	Spermidine/spermine N^1-acetyltransferase activity (nmol/mg protein per 10 min)
Control	0.18 ± 0.03
PHA	0.24 ± 0.02
PHA + phorbol	0.25 ± 0.03
PHA + phorbol 12,13-diacetate	0.43 ± 0.03
PHA + phorbol 12,13-dibutyrate	1.07 ± 0.03
PHA + phorbol 12-myristate-13-acetate	1.00 ± 0.08

Bovine lymphocytes (10^7 cells/ml) were treated with various phorbol compounds (50 ng/ml) and/or phytohemagglutinin (PHA) (5 μg/ml) and were harvested 24 h later. The results are the means ± SE of 3 experiments

The relative ability of a series of phorbol esters to stimulate spermidine/spermine N^1-acetyltransferase activity was investigated. Phorbol esters having tumor-promoting activity in mouse skin were also capable of stimulating the enzyme activity. A non-promotor, α phorbol, was incapable of stimulating the enzyme activity. Phorbol diacetate weakly stimulated the activity of the acetylase. Phorbol dibutyrate had a stimulatory effect similar to that of PMA (table 1). Thus the structural requirements of the phorbol diterpenes for tumor promotion are similar to those for the stimulation of spermidine/spermine N^1-acetyltransferase activity.

4. DISCUSSION

The physiologically occurring polyamines, putrescine, spermidine and spermine, have been shown to be involved in the regulation of cell growth and in tumor promotion [10].

When applied to mouse epidermis or cultured cells, PMA induces many phenotypic changes which are associated with the transformed state [6]. One of this series of changes is the induction of ornithine decarboxylase with the subsequent increase in polyamine levels and cellular proliferation [3]. However, some reports indicated that promotor-mediated cellular hyperplasia and ornithine decarboxylase induction could be independent rather than related events [5,6]. Glucocorticoids, which inhibit tumor promotion, failed to inhibit the PMA-mediated induction of ornithine decarboxylase [11]. Authors in [7] also indicated that treatment of HL-60 cells with PMA or phorbol 12,13-didecanoate resulted in increased levels of putrescine without alterations in ornithine decarboxylase activity, suggesting the existence of an alternative pathway for putrescine formation which might be involved in the control of cell growth and differentiation in HL-60 cells.

The mechanism of degradation of spermidine and spermine into putrescine has been established and shown to involve the sequential action of two enzymes, spermidine/spermine N^1-acetyltransferase and polyamine oxidase [12–19]. We have demonstrated that there is a large increase in the activity of spermidine/spermine N^1-acetyltransferase in rat liver [12] and kidney [16], bovine lymphocytes [9] and rabbit costal chondrocytes [20]

after exposure to a number of stimuli and that this enzyme is a rate-limiting enzyme in this acetylase/oxidase pathway for interconversion of the polyamines [15,17].

The present results demonstrate that PMA induced the spermidine/spermine N^1-acetyltransferase activity in bovine lymphocytes. When PMA and PHA were added to the lymphocyte suspensions simultaneously, the enzyme activity increased synergistically. Furthermore, only phorbol ester derivatives which were capable of promoting the formation of tumors in the mouse skin system led to the stimulation of spermidine/spermine N^1-acetyltransferase activity. In preliminary experiments, we measured the intracellular levels of polyamines in PMA- and/or PHA-treated bovine lymphocytes. The level of putrescine was increased and the levels of spermine and spermidine were decreased when spermidine/spermine N^1-acetyltransferase activity increased. These results suggest that the increase in the acetylase activity may have some relationship to the control of cell growth and differentiation by phorbol esters.

ACKNOWLEDGEMENTS

We wish to thank Dr R. Nakayama for his kind supply of bovine pharyngenous lymph nodes and Miss Y. Mimura for her secretarial assistance. This investigation was supported by a grant in Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

REFERENCES

[1] Hecker, E. (1978) in: Mechanisms of Tumor Promotion and Cocarcinogenesis (Slaga, T.J. et al. eds) pp.14–48, Raven, New York.
[2] Mufson, R.A., Fisher, S.M., Verma, A.K., Gleason, G.L., Slaga, T.J. and Boutwell, R.K. (1979) Cancer Res. 38, 4791–4795.
[3] O'Brien, T.G. (1976) Cancer Res. 36, 2644–2653.
[4] Lichti, U., Slaga, T.J., Ben, T., Patterson, E., Hennings, H. and Yuspa, S.H. (1977) Proc. Natl. Acad. Sci. USA 74, 3908–3912.
[5] O'Brien, T.G. and Diamond, L. (1978) Cancer Res. 37, 3895–3900.
[6] Colburn, N.H. (1980) Carcinogenesis 5, 33–56.
[7] Huberman, E., Wecks, C., Solanki, V., Callaham, M. and Slaga, T.J. (1981) Carcinogenesis 7, 405–416.
[8] Böym, A. (1968) Scand. J. Clin. Lab. Invest. 21, Suppl.97, 77–89.
[9] Matsui, I., Otani, S., Kuramoto, A., Morisawa, S. and Pegg, A.E. (1983) J. Biochem. 93, 961–966.
[10] Jänne, J., Pösö, H. and Raina, A. (1978) Biochim. Biophys. Acta 473, 241–293.
[11] Yuspa, S.N., Lichti, U., Hennings, H., Ben, T., Patterson, E. and Slaga, T.J. (1978) Carcinogenesis 2, 245–255.
[12] Matsui, I. and Pegg, A.E. (1980) Biochem. Biophys. Res. Commun. 92, 1009–1015.
[13] Matsui, I. and Pegg, A.E. (1980) Biochim. Biophys. Acta 633, 87–94.
[14] Matsui, I., Wiegand, L. and Pegg, A.E. (1981) J. Biol. Chem. 256, 2454–2459.
[15] Matsui, I. and Pegg, A.E. (1981) Biochim. Biophys. Acta 675, 373–378.
[16] Matsui, I. and Pegg, A.E. (1982) Cancer Res. 42, 2990–2995.
[17] Matsui, I. and Pegg, A.E. (1982) FEBS Lett. 139, 205–208.
[18] Seiler, N., Bolkenius, F.N. and Knodgen, B. (1980) Biochim. Biophys. Acta 633, 181–190.
[19] Bolkenius, F.N. and Seiler, N. (1981) Int. J. Biochem. 13, 287–292.
[20] Matsui-Yuasa, I., Otani, S., Morisawa, S., Takigawa, M., Enomoto, M. and Suzuki, F. (1984) J. Biochem., submitted.