Effect of Vitamin A on Plasminogen Activator Synthesis by Chick Embryo Fibroblasts

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Abstract

Low concentrations of Vitamin A stimulated plasminogen activator synthesis (PA) in chick embryo fibroblasts (CEF). It caused a dose dependent and reversible increase in PA synthesis in both normal CEF and CEF infected with a temperature sensitive mutant of Rous Sarcoma virus (RSV-Ts68). Both induction and deinduction of PA could be inhibited by Actinomycin D.

Vitamin A also accentuated the morphological changes associated with transformation in the Rous Sarcoma virus infected cells. The effects of Vitamin A on PA synthesis were essentially similar to those of the known tumour promoter, phorbol myristate acetate (PMA).

Both Vitamin A and PMA were found to act synergistically with sarcoma gene expression as far as PA synthesis was concerned.

Introduction

Vitamin A has been shown to inhibit squamous metaplasia in organ cultures (Sporn et al., 1976) and to promote the development and growth of experimental tumours (Polliack and Sasson, 1972; Polliack and Levij, 1969). It has also been shown to induce proteolytic activity in chick embryo limb buds cultured in vitro (Lucy et al., 1961; Fell et al., 1962). Wigler and Weinstein (1976) and Wilson and Reich (1979) have shown that the potent tumour promoting agent phorbol myristate acetate (PMA) acts as a strong inducer of the serine protease, plasminogen activator (PA).

In this paper we present the results of experiments that document the stimulatory effect of Vitamin A on PA synthesis in vitro and define certain aspects of the inductive response in normal chick embryo fibroblasts (CEF) and CEF infected with a temperature sensitive mutant of Rous Sarcoma virus (Ts68).

Materials and Methods

Primary cultures of chick embryo fibroblasts (CEF) were prepared as described by Rifkin and Reich (1971) and maintained and propagated in Eagle's minimal medium supplemented with 5% or 10% fetal bovine serum (FBS).

For experiments CEF were seeded at 10^6 cells/60 mm petri dish as tertiary cultures in Eagles medium containing 5% FBS. The cells were allowed to adhere overnight before the experiment was started and drugs were added to the cultures in 5 ml of fresh medium.

Cells were lysed by the addition of 0,1 ml of 0,1% Triton X-100 per 10^6 cells. Lysate aliquots containing 0,3–6 μg of protein were assayed for PA activity using the ^{125}I-fibrin method (Strickland and Beers, 1976). One unit of PA activity is defined as the amount of enzyme that will catalyse the release of 5% of the total trypsinizable radioactivity in 1 hr at 37°C.

Results

a) Morphology and growth

The addition of 10^{-6} M retionoic acid (RA) to Ts-68 infected cells accentuated the morphological changes of transformation normally observed when such cells are shifted from the restrictive temperature (41°C) to the permissive temperature (37°C). The clustering and loss of cell adherence observed were similar to the changes described by Ossowski et al. (1973) and Ossowski et al. (1974) in SV40-transformed hamster fibroblasts.

The addition of RA to normal or Ts-68-infected CEF at 37°C or 41°C had little effect upon the rate of cell growth.

b) Plasminogen activator

The addition of RA to cultures of normal CEF stimulated a rapid, dose dependent rise in intracellular PA activity (Table 1). In the presence of 10^{-6} M RA this rise was evident 1,5 hr after addition and increased to a maximum at 6 hr that was approximately ten-fold higher than in corresponding RA-free cultures, after which intracellular levels fell gradually. Lesser concentrations

Table 1. Effect of retinoic acid on plasminogen activator induction in chick embryo fibroblasts

	Addition	Plasminogen activator (U/mg protein)
	None	60
Retinoic acid	10^{-6} M	795
Retinoic acid	10^{-7} M	698
Retinoic acid	10^{-8} M	594
Retinoic acid	10^{-9} M	430
Retinoic acid	10^{-10} M	60

Normal chick embryo fibroblasts were seeded at 10^6/60 mm petri dish at 37°C. 24 hr later medium alone or medium containing retinoic acid was added to the cultures. 6 hr later duplicate petri dishes were removed and assayed as described in Methods.

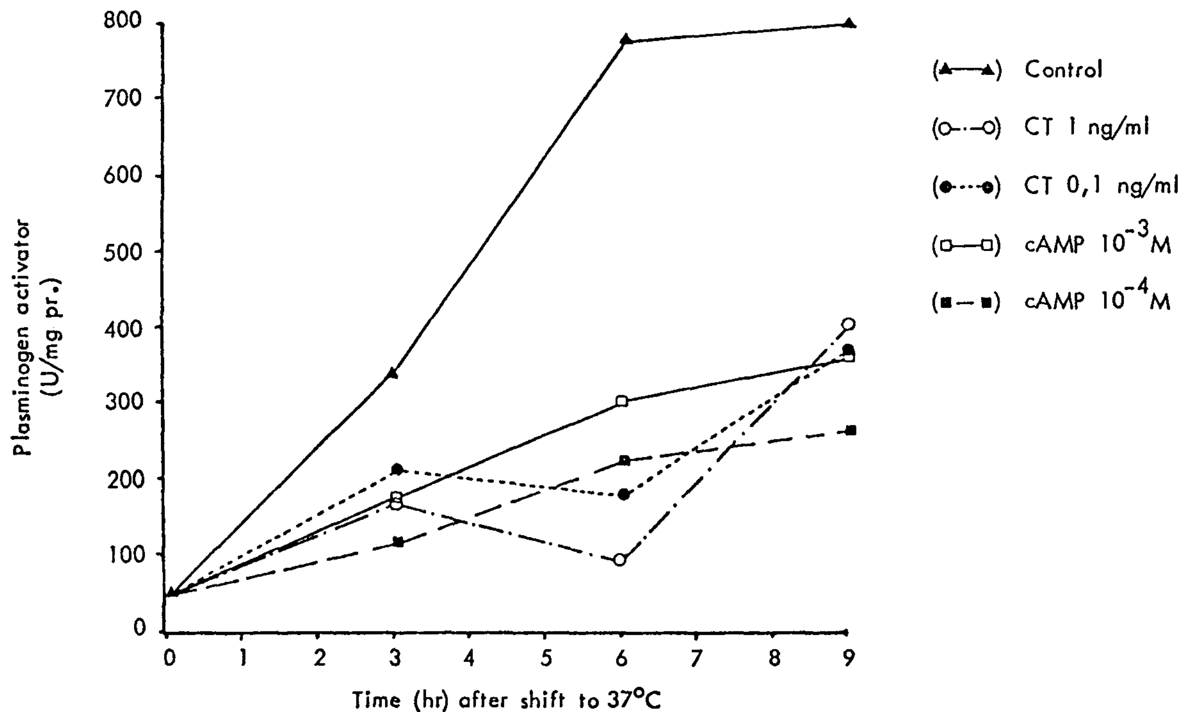

Fig. 1. The effect of cholera toxin and cyclic nucleotides on plasminogen activator induction in Ts-68-infected cells after shift to the permissive temperature for transformation. Ts-68-infected cells were seeded at 10^6/60 mm petri dish at 41°C. 6 hr later cholera toxin at 0,1 ng/ml or 1,0 ng/ml was added to some of the cultures. 18 hr later the medium was removed and replaced with fresh medium at 41°C containing no addition (▲——▲); cholera toxin 0,1 ng/ml (●----●); cholera toxin 1,0 ng/ml (○-·-○); cyclic AMP 10^{-3} M (□——□) or cyclic AMP 10^{-4} M (■——■). The dishes were replaced at 41°C for 1hr, after which some of the cultures were shifted to 37°C. 3, 6 and 9 hr later duplicate plates were removed from 41°C and 37°C and assayed for intracellular plasminogen activator as described in "Methods"

of this compound were also effective, with a significant rise in PA apparent after 3 hr exposure to 10^{-9}M RA (Wilson and Reich, 1978).

Addition of RA to Ts-68-infected cells at both permissive (37°C) and non-permissive (41°C) temperatures also caused a substantial increase in enzyme activity. The magnitude of the increase in enzyme activity observed when RA and viral transformation were acting together at 37°C was far greater than that observed when either was acting alone. The effects of RA and sarcoma gene expression on PA induction were therefore synergistic rather than additive, in analogy with the pattern previously found for phorbol esters (Weinstein et al., 1977; Wilson and Reich, 1979).

Removal of RA from the medium was followed by a progressive decline in PA activity. This fall was significantly inhibited by treatment with Actinomycin D indicating that, as in the case of de-induction after PMA withdrawal (Wigler and Weinstein, 1976) or following temperature shift to non-permissive conditions (Rifkin et al., 1975) the suppression of PA synthesis following RA withdrawal is an active process requiring synthesis of new RNA. Essen-

tially similar reversibility was observed when RA was removed from Ts-68-infected cultures incubated at 41°C or 37°C.

When normal CEF were treated with cycloheximide (10 μg/ml) or Actinomycin D (1 μg/ml) for 30 min prior to addition of RA, the rise in cellular enzyme activity was completely inhibited. Similar results were obtained with Ts-68-infected cultures at 37°C or 41°C. Thus both RNA and protein synthesis are required for enzyme induction by Vitamin A.

Retinoic acid, retinol and Vitamin A acetate, in that decreasing order, induced PA synthesis in cultures of normal CEF. The action of all three compounds was inhibited by Actinomycin D.

Since 3′-5′ cyclic nucleotides mediate numerous cellular processes, and these compounds, or substances which modify their metabolism are known to have effects on PA levels (Strickland and Beers, 1976; Vassalli et al., 1976) a number of experiments were performed to examine the effects of cAMP and cholera toxin on the induction of PA synthesis in CEF.

As seen in Fig. 1 cAMP at concentrations of 10^{-3}M and 10^{-4}M and cholera toxin at 0,1 ng/ml and 1,0 ng/ml, inhibited the increase in PA levels associated with a shift from restrictive to permissive temperatures.

In contrast, neither cAMP nor cholera toxin inhibited the induction of PA by retinoic acid (Wilson and Reich, 1978) indicating that the control of RA-induced PA synthesis, in contrast to the virally induced increase in PA levels, is not related to the cellular concentration of cyclic nucleotides.

Discussion

Studies of a series of phorbol derivatives (Weinstein et al., 1977) have shown that the potency of these compounds for inducing PA in CEF cultures closely parallels their potency as tumour promoting agents in mouse skin carcinogenesis. The results presented here suggest that Vitamin A derivatives may have an in vitro tumour promoting action that corresponds with their in vivo action in the RSV-chicken system (Polliack and Sasson, 1972) or the DMBA-hamster cheek pouch system (Polliack and Levij, 1969) but it is difficult to reconcile with studies that have ascribed antineoplastic activity to retinoids (Sporn et al., 1976). These diametrically opposed views and conflicting reports on the action of retinoids in pre-neoplasia may well reflect differences in the way in which different species or tissues respond to Vitamin A.

References

Fell, H. B., Dingle, J. T., Webb, M.: Studies on the mode of action of excess vitamin A. The specificity of the effect on embryonic chick-limb cartilage in culture and on isolated rat liver lysosomes. Biochem. J. **83**, 63–69 (1962)

Lucy, J. A., Dingle, J. T., Fell, H. B.: Studies on the mode of action of excess vitamin A. 2. A possible role of intracellular proteases in the degradation of cartilage matrix. Biochem. J. **79**, 500–508 (1961)

Ossowski. L.. Quigley. J. P., Kellerman. G. M.. Reich. E.: Fibrinolysis associated with oncogenic transformation. Requirement of plasminogen for correlated changes in cellular morphology. colony formation in agar and cell migration. J. Exp. Med. **138**, 1056–1064 (1973)

Ossowski. L.. Quigley. J. P.. Reich. E.: Fibrinolysis associated with oncogenic transformation. Morphological correlates. J. Biol. Chem. **249**, 4312–4320 (1974)

Polliack. A., Sasson. Z. B.: Enhancing effect of excess topical vitamin A on Rous sarcomas in chickens. J. Natl. Cancer Inst. **48**, 407–416 (1972)

Polliack. A., Levij. I. S.: The effect of topical vitamin A on papillomas and intraepithelial carcinomas induced in hamster cheek pouches with 9,10-dimethyl-1,2-benzanthracere. Cancer Res. **29**, 327–332 (1969)

Rifkin. D. B., Beal. L. P.. Reich. E.: Macromolecular determinants of plasminogen activator synthesis. In: Proteases and biological control. Rifkin. D., Reich. E., Shaw. E. (eds.). pp. 841–847. New York: Cold Spring Harbour Press 1975

Sporn. M. B., Dunlop. M. M., Newton. D. L., Smith. J. M.: Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed. Proc. **35**, 1332–1338 (1976)

Strickland. S., Beers. W. H.: Studies on the role of plasminogen activator in ovulation. J. Biol. Chem. **251**, 5694–5702 (1976)

Vassali. J.-D., Hamilton. J.. Reich. E.: Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids. mitotic inhibitors and cyclic nucleotides. Cell. **8**, 271–291 (1976)

Weinstein. I. B., Wigler. M.. Pietropaola. C.: The action of tumor-promoting agents in cell culture. In: Origins of Human Cancer. Vol. 4. Hiatt. H. H.. Watson. J. D.. Winsten. J. A. (eds.). pp. 751–772. New York: Cold Spring Harbour Press 1977

Wigler. M., Weinstein. I. B.: Tumour promoter induces plasminogen activator. Nature **259**, 232 (1976)

Wilson, E. L., Reich, E.: Modulation of plasminogen activator synthesis in chick embryo fibroblasts by cyclic nucleotides & phorbol myristate acetate. Cancer Res. **39**, 1579–1586 (1979)

Wilson, E. L., Reich, E.: Plasminogen activator in chick embryo fibroblasts; induction of enzyme synthesis by retinoic acid and synergism with viral transformation. Cell **15**, 385–392 (1978)