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THE TUMOR PROMOTER 12-O-TETRADECANOYL-PHORBOL-13-ACETATE ELEVATES SERUM PROGESTERONE LEVELS

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SUMMARY: The phorbol ester 12-O-tetradecanoyl-13-acetate (TPA), the most potent promoter of two-stage carcinogenesis in mouse skin, simulates some of the early effects of several carcinogens in immature chicks. Actinomycin D, ethionine, thioacetamide (non-mutagenic in the Ames Salmonella test) and kepone are known to increase serum progesterone concentrations and induce synthesis of ovalbumin in chicks. Intraperitoneal administration of TPA to chicks caused a 5- to 7-fold elevation of serum progesterone levels and induced ovalbumin synthesis. Phorbol ester, 4α-phorbol-12,13-didecanoate, which is inactive as a mouse skin tumor promoter, did not increase serum progesterone levels nor did it induce ovalbumin synthesis.

Estrogen administration to immature female chicks results in cyto-differentiation of tubular gland cells in the primitive oviduct which synthesize the egg white proteins ovalbumin, conalbumin, ovomucoid and lysozyme. The continuous presence of estrogen is required for sustained synthesis of these proteins in immature chicks. Discontinuation of estrogen administration results in a gradual decline in cell-specific protein synthesis. Ovalbumin synthesis is no longer detected after 3 to 4 weeks of estrogen withdrawal. A rapid reinduction of oviduct-specific protein synthesis results when either estrogen or progesterone is administered as a secondary stimulating agent to chicks withdrawn from primary estrogen stimulation (1-3).

We have reported earlier a novel effect in immature chicks of the administration of three carcinogens which are non-mutagenic in the Ames Salmonella test (4-6). Ethionine, thioacetamide and actinomycin D induce

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concomitantly cell-specific ovalbumin synthesis in the oviduct as well as an increase in serum progesterone in withdrawn chicks. Palmiter and Mulvihill (7) have observed induction of ovalbumin synthesis and elevation of serum progesterone levels in immature chicks by another carcinogen, kepone.

Chemical carcinogenesis can be demonstrated to be a two-stage process in some systems, consisting of an initiation step by subeffective doses of carcinogen followed by promotion with a compound, such as croton oil, which by itself is not carcinogenic. Such a system was originally described by Berenblum (8) for the chemical induction of mouse skin tumors. Fractionation of croton oil revealed the component most potent as a promoter to be 12-O-tetradecanoyl-phorbol-13-acetate (TPA) (9). TPA itself is neither carcinogenic nor mutagenic (10).

Two-step carcinogenesis appears to be operative in tissues and organs other than mouse skin (see Reference 11). While the mechanism of tumor potmotion is not known, some sequalae of exposure of cells to such compounds have been noted. Tumor promoters induce phenotypic changes in mouse skin or cells in culture which are characteristic of cells transformed by viral or chemical carcinogens. Exposure to TPA alters cell morphology (12), increases ornithine decarboxylase activity (13, 14), DNA synthesis (14), phospholipid synthesis (15), protease activity (16, 17), and enhances cell transformation by chemical carcinogens in culture (18, 19). TPA also induces the shedding of viral genomes from cells transformed by viruses (20) and inhibits the differentiation of Friend erythroleukemic cells (21, 22), neuroblastoma (23), myoblasts (24) as well as the conversion of 3T3 cells to adipocytes (25).

As a result of our observations on the effects of a number of carcinogens on ovalbumin induction in immature chicks (4-6), we proceeded to determine whether a tumor promoting agent might exhibit similar effects. We report here that the administration of TPA to immature chicks previously challenged by estrogen results in the elevation of serum progesterone levels and the induction of ovalbumin synthesis.

METHODS

Four-day-old female Rhode Island Red chicks were injected intramuscularly below the knee with 1 mg of estradiol benzoate in sesame oil daily for ten days (primary estrogen stimulation). After 3 to 4 weeks of subsequent estrogen withdrawal the chicks were used in the experiments; at this stage, ovalbumin synthesis in the oviducts could not be detected. The magnum portion of the oviduct was freed of adjoining tissue, cut into small pieces and placed in 2 ml of medium containing ^{14}C-lebelled amino acids in 25 ml rubber-stoppered Erlenmeyer flasks. The flasks were incubated at 37°C, subjected to constant shaking and were gassed with 95% O_2 and 5% CO_2 at hourly intervals (1). At the end of the incubation period the pieces of tissue were blotted on filter paper, homogenized in a Potter-Elvehjem homogenizer with 2 ml of 15 mM sodium chloride and 10 mM sodium phosphate, pH 7.5, and centrifuged at 100,000 g for 1 hr. The supernatant obtained at high speed was used for the assay of ovalbumin synthesis by specific immunoprecipitation (1, 4).

RESULTS AND DISCUSSION

Intraperitoneal administration of TPA (200 µg) to estrogen withdrawn chicks caused a 5- to 7-fold elevation of serum progesterone levels and induced ovalbumin synthesis reproducibly within 3 to 4 days. The data from one of the experiments are given in Table 1. In other experiments ovalbumin synthesis could be induced by 40 µg of TPA. Phorbol ester, 4α-phorbol-12, 13-didecanoate (4α-PDD) which is inactive as a mouse skin tumor promoter (9), did not increase progesterone levels nor did it induce ovalbumin synthesis even after prolonged administration for a week.

Induction of ovalbumin synthesis by TPA was not observed in the oviduct of 5-6 week-old chicks which had not received primary stimulation with estradiol benzoate.

As mentioned above, progesterone has been shown to be effective as a secondary stimulant for the induction of ovalbumin synthesis in chicks withdrawn from estrogen. Thus, the elevation of serum progesterone levels found after administration of TPA to withdrawn chicks may be sufficient to account for the reinduction of ovalbumin synthesis.

The data presented here reveal yet another example of a tumor promoter simulating some of the early effects of actual carcinogens and reveal a possible mechanism by which a promoter might act in the reversible manner which has been demonstrated for this class of compounds.

TABLE 1. Elevation of Progesterone and Induction of Ovalbumin Synthesis in Chicks by TPA

Treatment	Serum progesterone (ng/100 ml)	Ovalbumin synthesis in oviduct explants, % of total protein synthesis
DMSO-sesame oil (control)		
3 days	83, 109	Not detected
TPA		
3 days	541, 762	2.6 18.6 24.5
4α-PDD		
3 days	92, 87	Not detected
7 days	84, 101	Not detected

TPA and 4α-PDD were procured from Consolidated Midland Corporation, Brewster, New York. The compounds (10 mg) were dissolved in 1 ml of dimethylsulfoxide (DMSO) and diluted with 9 volumes of sesame oil. Chicks withdrawn from estrogen stimulation for four weeks (average weight 400 g) following primary stimulation were injected daily intraperitoneally with TPA, 4α-PDD (200 µg in 0.2 ml DMSO-sesame oil) or with 0.2 ml of DMSO-sesame oil. At specified times blood was collected by heart puncture and the oviduct removed from three chicks in each group. The magnum portion of the oviduct freed from adjoining tissue was cut into small pieces and ovalbumin synthesis was determined separately in the oviduct from each chick. Progesterone was determined in the pooled serum from each group by radioimmunoassay, performed by Laboratory Procedures, Upjohn Company (5). Serum samples were submitted for analysis with code numbers. Values for duplicate progesterone determinations are given.

Progesterone, itself a biologically active steroid hormone, is a precursor in the biosynthetic pathway for all other classes of steroid hormones. Thus an increase in progesterone levels could lead to alterations in the levels of other steroid hormones. Steroid hormones have been implicated in carcinogenesis as well as in the maintenance of certain types of tumors (26). By inducing a hormonal imbalance, a promoter such as TPA might potentiate the effect of the initial carcinogen or provide an environ-

ment conducive to the selective growth of a transformed cell. Such possibilities as well as the basic mechanism by which TPA alters hormonal levels remain to be explored.

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