

Nicotinamide Adenine Dinucleotide-dependent 15-Hydroxyprostaglandin Dehydrogenase Activity in Hydatidiform Mole Tissue and Choriocarcinoma Cells¹

M. Linette Casey,² David Gal, John M. Johnston, and Paul C. MacDonald³

Cecil H. and Ida Green Center for Reproductive Biology Sciences, Departments of Biochemistry and Obstetrics-Gynecology, The University of Texas, Southwestern Medical School, Dallas, Texas 75235

ABSTRACT

Prostaglandins may occupy an important role in viral and chemical carcinogen-induced neoplasia. To evaluate the possible role of prostaglandin catabolism in neoplastic cells, we measured nicotinamide adenine dinucleotide-dependent 15-hydroxyprostaglandin dehydrogenase activity in hydatidiform mole tissue and in choriocarcinoma cells maintained in monolayer culture. The specific activity of nicotinamide adenine dinucleotide-dependent 15-hydroxyprostaglandin dehydrogenase in hydatidiform mole tissue (0 to 1.2 nmol 15-ketoprostaglandin E₂ formed × min⁻¹ × mg⁻¹ cytosolic protein) and in choriocarcinoma cells (1.0 nmol 15-ketoprostaglandin E₂ × min⁻¹ × mg⁻¹ protein) was strikingly less than that found in normal placental tissue [11.4 ± 2.3 (S.E.) nmol 15-ketoprostaglandin × min⁻¹ × mg⁻¹ protein].

INTRODUCTION

It is believed that PGs⁴ may be important in metabolic processes involved in neoplastic transformation, tumor growth and metastasis, and metabolic derangements associated with cancer (*cf.* Ref. 5). For example, it has been demonstrated that the papilloma development in mice that is induced by the potent tumor-promoting agent TPA is inhibited by indomethacin treatment (3). However, PGE₂ but not prostaglandin F_{2α} treatment of indomethacin-treated animals restores tumor promotion by TPA (3). Moreover, the increase in ornithine decarboxylase activity that is induced upon TPA treatment of mouse epidermis (8) is inhibited by indomethacin treatment but is restored in indomethacin-treated animals by PGE₂ (12). High levels of PGs are found in a variety of tumors (*cf.* Ref. 5). Moreover, a number of carcinogens and tumor-promoting agents stimulate PG synthesis and release and enhance deacylation of phospholipids (4, 6, 7).

For these reasons, the regulation of tissue levels of PGs may be important in tumor formation and metastasis. The tissue concentration of prostaglandins is believed to be regulated primarily by the rate of release of the polyunsaturated fatty acid precursor from its esterified form and by the rate of prostaglandin catabolism or inactivation. PGDH catalyzes the initial reaction in the catabolism of prostaglandins to inactive 15-keto

metabolites. Whereas the tissue levels and rates of release of PGs in malignant tissues have been examined, little attention has been given to the role of the inactivation of prostaglandins in neoplastic tissues. We considered the possibility that a decrease in PGDH activity may be important in the accumulation of PGs in neoplastic tissues. To address this issue, we examined the activity of PGDH in neoplastic trophoblastic tissue, namely, in hydatidiform mole tissues and in choriocarcinoma cells grown in monolayer culture. We chose this neoplasia as the first to study because the activity of PGDH in the nonmalignant counterpart of this tissue, *i.e.*, placenta, is known to be high. Moreover, it has been reported that the rate of metabolism of PGE₁ in hydatidiform mole tissue was low (1).

MATERIALS AND METHODS

Preparation of Hydatidiform Mole and Placental Tissues and Choriocarcinoma Cells in Monolayer Culture. Hydatidiform mole tissue was obtained by suction curettage of the uteri of 3 women in whom the diagnosis of hydatidiform mole was made by ultrasonography. Immediately following removal, the molar tissue was placed in ice-cold NaCl (0.15 M). Individual vesicles were removed, dissected free of blood clots and decidual tissue, and transferred to a container in ice-cold NaCl (0.15 M). The molar tissue was homogenized in 4 volumes of a solution of ice-cold 50 mM Tris-HCl, buffer (pH 7.2) containing 20% glycerol (v/v), 250 mM sucrose, 1 mM EDTA, and 2 mM dithiothreitol. Human choriocarcinoma cells of fetal trophoblastic origin [BeWO line (9)] were a gift of Dr. R. A. Patillo, Department of Gynecology and Obstetrics, Medical College of Wisconsin, Milwaukee, Wis. These cells were grown in monolayer culture in Waymouth-Gey's (55% Waymouth's MB 752/1 medium and 45% Gey's balanced salt solution) culture medium that contained fetal calf serum (10%) in a humidified atmosphere of air (95%) and CO₂ (5%) at 37°. When the cells became confluent, the medium was removed, and the culture dishes were placed on ice. The cells were scraped from the dish with a rubber policeman into ice-cold 0.15 M NaCl. The cell suspension was centrifuged at 700 × g for 10 min at 4°. The supernatant fluid was decanted, and the cells were resuspended and homogenized in the buffer described.

Term human placental tissue was dissected free of vascular tissue and rinsed repeatedly in ice-cold 0.15 M NaCl. Thereafter, the placental tissue was homogenized, using a Potter-Elvehjem tissue grinder, in 4 volumes of the homogenization buffer. The supernatant fraction obtained following centrifugation at 105,000 × g for 1 hr at 2° was assayed for PGDH activity.

Assay of PGDH Activity. PGDH activity was assayed using a spectrofluorometric method in which NADH formation is

¹ This investigation was supported in part by USPHS Grant 5-T01-AG00306.

² Postdoctoral trainee supported in part by USPHS Training Grant 1-T32-HD07190.

³ To whom requests for reprints should be addressed, at Cecil H. and Ida Green Center for Reproductive Biology Sciences, 5323 Harry Hines Boulevard, Dallas, Texas 75235.

⁴ The abbreviations used are: PG, prostaglandin; TPA, 12-O-tetradecanoylphorbol-13-acetate; PGE₂, prostaglandin E₂; PGDH, NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase.

Received December 1, 1980; accepted March 23, 1981.

measured. This method has been described in detail (2). Briefly, assays were conducted in 50 mM sodium carbonate-bicarbonate buffer (pH 9.5) containing 1 mM NAD⁺, PGE₂ (284 μM, added in 10 μl ethanol), and cytosolic protein (10 to 100 μg) at 25°. The final volume of the reaction mixture was 1 ml. The rate of increase in fluorescence was monitored at an excitation wavelength of 340 nm and an emission wavelength of 442 nm. PGDH activity was computed following correction for contributions from (a) endogenous cytosolic enzymatic activities leading to NADH formation in the presence of NAD⁺ but in the absence of PGE₂ and (b) a nonenzymatic reaction that occurs at pH 9.5 in the presence of NAD⁺ and PGE₂.

RESULTS

The specific activity of PGDH in cytosolic fractions of homogenates of hydatidiform mole tissue varied from 0 to 1.2 nmol 15-ketoprostaglandin E₂ × min⁻¹ × mg⁻¹ protein, and the specific activity of PGDH in cytosolic fractions of homogenates of choriocarcinoma cells was 1.0 nmol × min⁻¹ × mg⁻¹ protein (Table 1). These specific activities are strikingly less than those found in the cytosolic fraction prepared from homogenates of normal term human placentae, namely, 11.4 ± 2.3 (S.E.) nmol 15-ketoprostaglandin E₂ × min⁻¹ × mg⁻¹ protein (Table 1).

DISCUSSION

PGDH activity in neoplastic trophoblastic tissues was found to be one-tenth or less of that in normal term human placentae. In fact, these differences are no doubt even greater than computed. This obtains because the specific activity of PGDH in the cytosolic fraction of normal placental tissue homogenates was reduced artifactually due to the presence of a considerable amount of protein that originated in tissues other than the chorionic villi, e.g., blood. On the other hand, the hydatidiform mole tissues and choriocarcinoma cells were not contaminated by blood. Therefore, it is reasonable to suggest that the magnitude of the difference between the specific activities of PGDH in normal and that in neoplastic chorionic trophoblasts is very great. We cannot deduce whether the low specific activity of PGDH in hydatidiform mole tissues is due to the absence of the enzyme (*i.e.*, reduction in enzyme synthesis) or else to the presence of an inhibitor of the enzyme itself. Experiments are in progress to distinguish between these 2 possibilities. Based on the results of the present findings, it can be envisioned that

Table 1

PGDH activity in normal and in neoplastic trophoblastic tissue

PGDH assays were conducted in 50 mM sodium carbonate-bicarbonate buffer (pH 9.5) containing 1 mM NAD⁺, 284 μM PGE₂, and cytosolic protein at 25° in a final volume of 1 ml.

Tissue	Specific activity of PGDH (nmol 15-ketoprostaglandin E ₂ formed × min ⁻¹ × mg ⁻¹ protein)
Normal term placentae (n = 4)	11.4 ± 2.3 ^a
Hydatidiform mole	
1	0
2	1.2
3	0
Choriocarcinoma cells grown in monolayer culture	1.0

^a Mean ± S.E.

failure of PG inactivation may be conducive to neoplastic transformation or tumor growth since PGs appear to serve a critical role in some such processes (*cf* Ref. 5). To explore this possibility further, we have initiated a study of PGDH activity in human cancer tissues. To date, we have found⁵ little or no PGDH activity in cancer tissues that originated in breast, endometrium, urinary bladder, or uterine cervix, whereas considerable PGDH activity frequently was detected in contiguous but normal tissue.

Recently, Pottathil et al. (10) demonstrated a role for fatty acid cyclooxygenase in the establishment of interferon-mediated antiviral state. In these studies, it was shown that inhibitors of fatty acid cyclooxygenase caused suppression of the establishment of the interferon-mediated antiviral state, but PGs did not replace the need for a functional cyclooxygenase. They postulated that the prolonged action of an unstable metabolite(s) of arachidonic acid may be required for the establishment of the interferon-mediated antiviral activity. This possibility seemed particularly attractive since the stable PGs have been shown to suppress interferon-mediated protection (11). Thus, arachidonic acid metabolism as well as the inactivation of PGs may be of signal importance in viral and chemical carcinogen-induced neoplasias.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Sue MacDonald in obtaining tissues, Janette Tuckey in maintaining BeWo cells in culture, and the expert editorial assistance of Rebekah McKinney-Reese in the preparation of this manuscript.

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⁵ M. L. Casey, D. Gal, J. M. Johnston, and P. C. MacDonald, unpublished observations.