The effects of vanadate and epidermal growth factor on the specific activities of phospholipase A_2 and phosphatidylinositol-specific phospholipase C in human amnion cells

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Summary: We have evaluated the specific activity of phospholipase A_2 (PLA_2) and phosphatidylinositol-specific phospholipase C (PLC) in human amnion cells in monolayer culture that had not (CTL) or that had been treated with vanadate (VAN) and/or mouse epidermal growth factor (mEGF) for 4 h. It is known that both agents stimulate prostaglandin (PG) E_2 synthesis in these cells. Prospholipase enzyme activities were determined in the $750 \times g$ supernatant fraction of amnion cell homogenates under optimal in vitro conditions. The specific activity of PLA_2 ranged from 1.1 to 1.25 nmol/mg protein/0.5 h and that of phosphatidylinositol-specific PLC from 1.04 to 1.2 μmol/mg protein/h. Treatment of amnion cell cultures with VAN and/or mEGF had no statistically significant effect on the specific activi ies of either phospholipases. Thus, we conclude that the stimulation of PGE_2 production by VAN and mEGF is not due to an increase in the release of arachidonic acid from glycerophospholipid storage forms in human amnion cells.

Key words: Vanadate; EGF; Phospholipases; Human amnion.

INTRODUCTION

The liberation of arachidonic acid from tissue lipids is a requisite for the biosynthesis of prostaglandins (PGs) of the "2-series") ([1]). In human fetal membranes and in most other tissues this is accomplished by the action of phospholipase A_2 (PLA_2) and phosphatidylinositol-specific phospholipase C [PLC, ([1])]. Recently, we have assessed and discussed the role of vanadate (VAN) on PGE_2 production by human amnion cells in monolayer culture ([2]). Evidence has ben provided that VAN provokes a stimulation of PGE_2 formation by a mechanism(s) that involves de novo protein synthesis. Specifically, VAN effected an increase in the specific activity of PGE_2 synthase and this was prevented by cycloheximide. In addition, we have demonstrated that these effects of VAN were further enhanced by mouse epidermal growth factor [mEGF, ([3])].

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The purpose of this investigations was to evaluate the possibility that VAN and/or mEGF may act additionally on the specific activities of PLA_2 and phosphatidylinositol-specific PLC in human amnion cells in culture.

MATERIALS AND METHODS

Materials

1-palmitoyl-2-[1-^{14}C] arachidonoyl sn-glycero-3-phosphoethanolamine (59 mCi/mmol) was purchased from Amersham, Arlington Heights, IL, USA. Myo [2-^{3}H] inositol (12.5 mCi/mmol) was from NEN, Boston, MA, USA. PG standards were obtained from Cayman Chemical Company, Ann Arbor, MI. USA, and lipid standards were from Sedary, London, Ontario, Canada. Sodium orthovanadate and co-factors were purchased from Sigma, St. Louis, MO, USA. Mouse epidermal growth factor was from Collaborative Research, Lexington, MA, USA, and all culture media and supplies were obtained from Gibco, Grand Island, NY, USA.

Amnion cells in primary monolayer culture

Human amnion tissues were obtained from three normal pregnancies at the time of elective cesarean section conducted prior to the onset of labor. Amnion cells were dispersed enzymatically and placed in culture dishes (60 mm diameter) as described (4). For each tissue specimen eight dishes were incubated with VAN (32 μM) and/or mEGF (15 ng/ml) for 4 h. Thereafter, the cells were collected, sonicated, and centrifuged at 750 × g for 10 min. at 2° C. The supernatant fraction was used as the enzyme source.

Assay of phospholipase A_2 and phosphatidylinositol-specific phospholipase C

PLA_2 activity was assayed by use of the method described previously (5). Incubation mixtures consisted of Tris-HCL (77 mM, pH 8.5), $CaCl_2$ (5 mM), EGTA (1 mM), 1-palmitoyl-2-[1-^{14}C] arachidonoyl sn-glycero-3-phosphoethanolamine (80 μM, 59 mCi/mmol) as substrate and aliquots of the 750 × g supernatant (0.1 - 0.2 mg protein). The assay mixture were maintained in a shaking water-bath at 37° C for 30 min., and the reactions were terminated by the addition of acetic acid.

The activity of phosphatidylinositol-specific PLC was determined by the method of Di Renzo *et al.* (6). The standard assay mixture consisted of Tris-HCL (20 mM, pH 7.0), phosphatidyl [2-^{3}H] inositol (2 mM, 0.05 μCi/μmol), mercaptoethanol (4 mM), $CaCl_2$ (4 mM), and enzyme (approximately 0.1 mg protein).

Lipids were extracted by use of the method of Folch *et al.* (7), and radiolabelled arachidonic acid was separated from radiolabelled glycerophospholipids and lysoglycerophospholipids by thin-layer chromatography as described (8). Radioactivity that comigrated with authentic arachidonic acid was quantified by liquid scintillation spectrometry. Enzyme activities of individual tissues were analysed in quadruplicate. Protein was quantified by the method of Lowry and associates (9) with bovine serum albumin as the standard. Statistical analyses were conducted by use of the unpaired Student t test.

RESULTS AND DISCUSSION

Arachidonic acid metabolism has been investigated in a number of cell cultures and is stimulated by a variety of compounds including growth factors, tumor promoters and VAN (2, 10). Among the human tissues studied so far, the amnion is unique in that it synthesizes almost exclusively PGE_2 and there is little or no further metabolism (11). In addition, there is evidence to support the phsysiologic importance of PG production in the initiation and maintenance of labor in women, and it has been proposed that PGE_2 produced in amnion may participate in these processes (11).

Previously, we found that VAN stimulated PGE_2 production in human amnion cell cultures in a time- and dose-dependent fashion (2). Moreover, these effects were further enhanced by the addition of mEGF (3). We employed VAN and mEGF because they are among the very few agents that act to increase PG formation in these cells (3, 12). Evidence has been provided that both compounds stimulate de novo protein synthesis, i.e., the specific activity of PGE_2 synthase was increased and in the presence of cycloheximide these effects were abolished (2). This investigation was designed to evaluate, additionally, the specific activities of PLA_2 and phosphatidylinositol-specific PLC in hu-

Table 1. — *The effect of mouse epidermal growth factor and vanadate on the specific activities of phospholipase A_2 and phosphatidylinositol-specific phospholipase C in human amnion cell cultures.*

	PLA_2 (nmol/mg protein/0.5 h)	PLC (μmol/mg protein/h)
CTL	1.1 ±0.15	1.04±0.06
mEGF	1.0 ±0.09	1.14±0.07
VAN	0.95±0.9	0.9 ±0.1
mEGF+VAN	1.25±0.07	1.2 ±0.15

The data represent the mean±SEM of replicates of 2 tissue specimens analysed in quadruplicate. VAN and/or mEGF had no statistically significant effect on the specific activities of either phospholipase compared with CTL.

PLA_2 = Phospholipase A_2, PLC = Phosphatidylinositol-Specific Phospholipase C, CTL = Control, mEGF = Mouse Epidermal Growth Factor, VAN = Vanadate.

man amnion cell cultures. We did so, because free arachidonic acid, the essential precursor of PGs of the "2-series", is present only in very low concentrations in cells or tissues, but rather has to be liberated by the action of these phospholipases. Both enzymes are Ca^{2+}-dependent and are well characterized in human amnion tissue ([1]).

The specific activities of PLA_2 and phosphatidylinositol-specific PLC, as determined under optimal in vitro conditions, in human amnion cell cultures that had not (CTL) or that had been treated with VAN and/or mEGF for 4 h are presented in Table 1. Neither compound alone or in combination effected a significant change in the specific activities of these enzymes. We employed sn-1-palmitoyl-2-[1-^{14}C] arachidonoyl phosphatidylethanolamine for the determination of PLA_2 activity because this is the prefered substrate in human fetal membranes ([13]). The specific activity of phosphatidylinositol-specific PLC was several orders of magnitude greater than that of PLA_2 (Table 1). However, some reservation must be placed on such a direct comparison since the assays for the two enzymes were optimized to in vitro conditions. Whether a similar difference is expressed in vivo cannot yet be deduced.

Our results were not necessarily expected because others have shown that mitogens, such as EGF and VAN stimulate phosphatidylinositol metabolism in some cell systems and also mobilize intracellular Ca^{2+} ([14]). Moreover, evidence has been obtained that VAN and its intracellular metabolite, vanadyl, inhibits plasma endoplasmic reticulum membrane (Ca^{2+}, Mg^{2+}) ATPase in rat adipocytes, and this would be expected to give rise to an increase in cytosolic Ca^{2+} ([15]). These effects on Ca^{2+} homeostasis may stimulate the activity of phospholipases. In addition, it has been demonstrated that the stimulation of PGE_2 production in human amnion cells by mEGF is dependent on the presence of free arachidonic acid in the culture medium ([12]). This conclusion was based on the observation that mEGF is ineffective in stimulating PGE_2 production in amnion cells maintained in serum-free culture medium. It is, of course, possible that the effects of VAN and/or mEGF on PGE_2 production in these cells are manifest only in concert with another agent in the fetal salf serum that evokes mobilization of arachidonic acid, e.g., a substance that causes an increase in the intracellular free calcium concentration, which in turn leads to increased activities of phospholipases.

From the result of this investigation, we suppose, that VAN and/or mEGF do not act by way of a mechanism that leads to an increase in the release of free arachidonic acid from esterified stores, e.g., by activation of amnion phospholipases. We propose, therefore, that both agents effect an increase in the activities of enzymes involved in the conversion of arachidonic acid to PGE_2.

REFERENCES

1) Bleasdale J. E., Johnston J. M.: "Plostaglandins and human parturition: regulation of arachidonic acid mobilization". *Rev. Perinatol. Med.,* 1985, *5,* 151.
2) Korte K., Marzusch K., Casey M. L.: "The effect of vanadate on arachidonic acid metabolism in human amnion cells". *Clin. Exp. Obst. Gyn.,* in press.
3) Korte K., Casey M. L.: "Vanadate, epidermal growth factor and prostaglandin E_2 production in human amnion cells". *Clin. Exp. Obst. Gyn.,* in press.
4) Okita J. R., Sagawa N., Casey M. L., Snyder J. M.: "A comparison of human amnion cells in primary culture by morphological and biochemical criteria". *In vitro,* 1983, *19,* 117.
5) Okazaki T., Sagawa N., Bleasdale J. E., Okita J. R., McDonald P. C., Johnston J. M.: "Initiation of human parturition. XIII. Phospholipase C, phospholipase A_2, and diacylglycerol lipase activities in fetal membranes and decidua vera tissues from early and late gestation". *Biol. Reprod.,* 1981, *25,* 103.
6) Di Renzo G. C., Johnston J. M., Okazaki T., Okita J. R., McDonald P. C., Bleasdale J. E.: "Phosphatidylinositol-specific phospholipase C in fetal membranes and uterine decidua". *J. Clin. Invest.,* 1981, *67,* 847.
7) Folch J., Lees M., Sloane-Stanley G. H.: "A simple method for the isolation and purification of total lipids from animal tissues". *J. Biol. Chem.,* 1957, *226,* 497.
8) Korte K., Casey M. L.: "Phospholipid and neutral lipid separation by one-dimensional thin-layer chromatography". *J. Chromatogr.,* 1982, *232,* 47.
9) Lowry D. H., Rosebrough N. J., Farr A. L., Randall R. J.: "Protein measurement with folin phenol reagent". *J. Biol. Chem.,* 1951, *193,* 265.
10) Levine L.: "Tumor promoters, growth factors and arachidonic acid metabolism". In: 'Prostaglandins in cancer research'. (Eds.: Garaci E., Paoletti R., Santoro M. G.), Springer-Verlag, Berlin, 1987, 62.
11) Casey M. L., McDonald P. C.: "The initiation of labor in women: regulation of phospholipid and arachidonic acid metabolism and of prostaglandin production". *Sem. Perinatol.,* 1986, *10,* 270.
12) Casey M. L., Mitchell M. D., McDonald P. C.: "Epidermal growth factor-stimulated prostaglandin E_2 production in human amnion cells: specificity and nonesterified arachidonic acid dependency". *Mol. Cell. Endocrinol,* 1987, *53,* 169.
13) Okazaki T., Okita J. R., McDonald P. C., Johnston J. M.: "Initiation of human parturition. X. Substrate specificity of phospholipase A_2 in human fetal membranes". *Am. J. Obst. Gyn.,* 1978, *130,* 432.
14) Yang D. C., Brown A. B., Chan T. M.: "Stimulation of tyrosine-specific protein phosphorylation and phosphatidylinositol phosphorylation by orthovanadate in rat liver plasma membrane". *Arch. Biochem. Biophys.,* 1989, *274,* 659.
15) Delfert D. M., McDonald J. M.: "Vanadyl and vanadate inhibit Ca^{2+} transport systems of the adipocyte plasma membrane and endoplasmic reticulum". *Arch. Biochem. Biophys.,* 1985, *241,* 665.

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