Growth factor-induced release of placental alkaline phosphatase from human syncytiotrophoblast membranes

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The ability of epidermal growth factor, insulin or guanosine thiotriphosphate to induce the release of two glycosyl-phosphatidylinositol-linked proteins from isolated human placental syncytiotrophoblast plasma membrane vesicles was investigated. Epidermal growth factor induced the ATP-dependent release of a fraction of syncytiotrophoblast plasma membrane placental alkaline phosphatase, whereas no release was detected following insulin treatment. This effect of epidermal growth factor was apparent at 30 min but not at 5 min. Guanosine thiotriphosphate stimulated the release of a small amount of syncytiotrophoblast plasma membrane placental alkaline phosphatase and appeared to have an additive effect when applied together with epidermal growth factor. Guanosine thiodiphosphate did not induce phosphatase release, but partially inhibited the epidermal growth factor response. 28.7% of syncytiotrophoblast plasma membrane 5'-nucleotidase was solubilized using glycosyl-phosphatidylinositol-specific phospholipase C. However, unlike placental alkaline phosphatase, no detectable release of 5'-nucleotidase was observed following treatment of syncytiotrophoblast plasma membrane vesicles with epidermal growth factor or guanosine thiotriphosphate. These results indicate (i) the presence of at least two placental alkaline phosphatase-releasing pathways in syncytiotrophoblast plasma membrane vesicles, and (ii) the presence of subpopulations of glycosyl-phosphatidylinositol-linked proteins sensitive to growth factor-induced release.

Introduction

Placental alkaline phosphatase is a 130 kDa dimeric sialoglycoprotein expressed on the cell surface of human placental syncytiotrophoblast as well as certain tumours, notably of the reproductive tract (McLaughlin *et al.*, 1983; McDicken *et al.*, 1985). Placental alkaline phosphatase, like several other cell-surface proteins, is anchored to the plasma membrane by a glycosyl-phosphatidylinositol (GPI) linkage, a structure sensitive to cleavage by phosphatidylinositol-specific phospholipase C (PI-PLC) (Low and Saltiel, 1988; Webb and Todd, 1988). Placental alkaline phosphatase is released, by an unknown mechanism, into the serum of pregnant women and patients with tumours expressing placental alkaline phosphatase (Vergote *et al.*, 1992). The significance of release of placental alkaline phosphatase for the progress of either gestation or cancer is unknown. It has been suggested recently that it can function as an IgG-Fc receptor (Makiya and Stigbrand, 1992). It is possible that liberated placental alkaline phosphatase can bind to a cell surface inositol receptor via its inositol-glycan moiety (as has been demonstrated for hepatocyte heparan sulfate proteoglycan) and is subsequently internalized together with bound IgG (Ishihara *et al.*, 1987).

A potential mechanism for the release of placental alkaline phosphatase is suggested by the liberation of GPI-linked

proteins from the cell surface following treatment of cells with insulin (Chan *et al.*, 1988; Romero *et al.*, 1988; Lisanti *et al.*, 1989; Romero, 1991). In experiments in which the human laryngeal carcinoma cell line, HEp-2, was treated with epidermal growth factor (EGF), insulin or a non-hydrolysable analogue of guanosine triphosphate, guanosine thiotriphosphate (GTP γ S, a GTP analogue that can persistently activate GTP-binding proteins), a small but reproducible release of placental alkaline phosphatase was induced by each agent (Neer and Clapham, 1988; Roberts *et al.*, 1990). Thus, growth factor activation of tumour cells, utilizing a mechanism that may involve a guanosine triphosphate (GTP)-binding protein, could be responsible for the appearance of placental alkaline phosphatase in the serum of cancer patients.

Isolated syncytiotrophoblast plasma membrane vesicles were shown to express receptors for EGF and insulin together with components of several signalling pathways, such as adenylyl cyclase, phospholipase C, and G proteins (Figgs, 1988; Roberts *et al.*, 1991; Jacobs *et al.*, 1992; Johnson, 1992). Some of these components were shown to be coupled to functioning signalling pathways in these membranes (Carpenter *et al.*, 1980; Webb and Mahadevan, 1987; Figgs, 1988; Kenton *et al.*, 1989, 1991). To determine whether growth factor stimulation of syncytiotrophoblast induces release of placental alkaline phosphatase, we treated syncytiotrophoblast plasma membrane vesicles with EGF, insulin or guanine nucleotide analogues and measured the release of placental

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alkaline phosphatase and another GPI-linked protein, 5'-nucleotidase (CD73) expressed on the extracellular surface of cells (Vogel *et al.*, 1992). This enzyme has been identified on plasma membranes prepared from placental homogenates (Thompson *et al.*, 1987; Klemens *et al.*, 1990).

Materials and Methods

Materials

Clostridium perfringens phosphatidylcholine-specific phospholipase C (PC-PLC; EC 3.1.4.3), human placental alkaline phosphatase (EC 3.1.3.1), murine EGF, bovine insulin, adenosine, adenosine monophosphate, ATP, GTP γ S, GDP β S, *p*-nitrophenylphosphate (pNPP; as 'phosphate substrate' tablets), β -glycerol phosphate, Ba(OH) $_2$, ZnSO $_4$, pepstatin A, bromelain and phenylmethylsulfonyl fluoride (PMSF) were obtained from Sigma (Poole, Dorset). *Bacillus cereus* PI-PLC (EC 3.1.4.10) and E64 (N-[N-(L-3-*trans*-carboxyoxiran-2-carbonyl)-L-leucyl]-agmatine) were obtained from Boehringer-Mannheim (Lewes). [3 H]5'-AMP (> 30 Ci mmol $^{-1}$) was obtained from New England Nuclear (through Du Pont (UK) Ltd, Stevenage). Triton X-100 was obtained from BDH (Liverpool).

Preparation of syncytiotrophoblast plasma membrane vesicles

Syncytiotrophoblast plasma membrane vesicles were isolated from human term villous placental tissue within 1 h of delivery as described by Webb and Mahadevan (1987).

Placental alkaline phosphatase release assay

Specific conditions for each placental alkaline phosphatase release assay are given with the appropriate figure. Incubations (except those using bromelain) were carried out in the presence of the protease inhibitors PMSF (0.2 mmol l $^{-1}$), pepstatin A (1 μ mol l $^{-1}$) and E64 (1.4 μ mol l $^{-1}$). Since tyrosine kinase activity may be essential for any effect of EGF or insulin, incubations using these growth factors were carried out in the presence or absence of ATP. Divalent cations were also added, as most phosphorylation that occurs in syncytiotrophoblast plasma membrane vesicles requires the presence of these ions (Kenton *et al.*, 1989). After incubation, the membranes were centrifuged at 11 000 *g* for 10 min (in an MSE Micro-Centaur) and an aliquot of the supernatant was gently decanted and dispensed into a fresh tube. This supernatant was centrifuged again (10 min at 11 000 *g*) and an aliquot gently removed and dispensed into a fresh tube. This supernatant, 50 μ l, was then assayed for placental alkaline phosphatase or 5'-nucleotidase, as detailed below. Alternatively, membrane suspensions of syncytiotrophoblast plasma membrane vesicles were centrifuged at 133 000 *g* for 30 min in a Beckman airfuge and the supernatants assayed for placental alkaline phosphatase activity.

Placental alkaline phosphatase and 5'-nucleotidase assays

Typically, placental alkaline phosphatase was measured by its ability to use pNPP (dissolved in 100 mmol ethanolamine

l $^{-1}$ (pH 9.4) containing 5 mmol MgCl $_2$ l $^{-1}$) as a substrate at room temperature, generating a yellow solution absorbing light at 405 nm. Placental alkaline phosphatase was also measured by a specific enzymeimmunoassay using the murine IgG1 monoclonal antibody, H317 (a gift of P. J. McLaughlin, Department of Immunology, University of Liverpool), as previously described (McLaughlin *et al.*, 1983, 1984a, b). H317 specifically recognizes placental alkaline phosphatase but not germ cell placental-type alkaline phosphatase. Assays were standardized using purified human placental alkaline phosphatase of known enzymatic activity per mg protein. Absorbance was measured using a Titertek Multiscan Plus.

5'-Nucleotidase was measured using the method of Stanley *et al.* (1980). Briefly, a sample of syncytiotrophoblast plasma membrane vesicle supernatant (typically 50 μ l) was incubated with 450 μ l of a solution containing 50 mmol Tris-HCl l $^{-1}$ (pH 7.4), 0.1% Triton X-100, 2 mmol MgCl $_2$ l $^{-1}$, 20 mmol β -glycerol phosphate l $^{-1}$, 0.268 mg adenosine ml $^{-1}$, 0.2 mmol 5'-AMP l $^{-1}$ and 0.45 μ Ci (15 pmol) [3 H]5'-AMP. Incubation was carried out at 37°C for 15 min and the reaction terminated by addition of 300 μ l 150 mmol ZnSO $_4$ l $^{-1}$ followed by 200 μ l of 0.3 mol Ba(OH) $_2$ l $^{-1}$. After centrifugation for 1 min at 11 000 *g*, aliquots of the terminated reaction supernatant were counted in Optiphase Hisafe 3 (LKB) using an LKB 1216 RackBeta liquid scintillation counter.

Results

Syncytiotrophoblast plasma membrane vesicles were incubated with increasing concentrations of either EGF or insulin to determine whether these growth factors induce the release of placental alkaline phosphatase. EGF or insulin alone, or insulin with ATP, showed no effect on placental alkaline phosphatase release (Figs 1 and 2). EGF with ATP, however, induced a small but reproducible increase in the release of placental alkaline phosphatase.

Some reports have suggested that, following insulin treatment of whole cells, GPI-linked proteins are released into the medium rapidly (within 5 min) with extracellular concentrations returning to near basal values by 30 min (Chan *et al.*, 1988). Syncytiotrophoblast plasma membrane vesicles were incubated with EGF or insulin (in the presence of ATP) for 5 and 30 min to test whether this is also the case for syncytiotrophoblast membrane preparations. No effect on release of placental alkaline phosphatase was seen with EGF at 5 min or with insulin at 5 or 30 min (Table 1). After incubation with EGF for 30 min, however, a significant increase in release of placental alkaline phosphatase was seen.

Syncytiotrophoblast plasma membrane vesicles were incubated with increasing concentrations of either GTP γ S or guanosine thiodiphosphate (GDP β S) to test whether a GTP-binding protein is involved in the release of placental alkaline phosphatase. GTP γ S induced the release of a small proportion of the placental alkaline phosphatase of syncytiotrophoblast plasma membrane vesicles, whereas GDP β S (which inactivates GTP-binding proteins) did not affect the amount of placental alkaline phosphatase in syncytiotrophoblast plasma membrane vesicles supernatants (Fig. 3).

Fig. 1. Effects of epidermal growth factor (EGF) on activity of placental alkaline phosphatase in syncytiotrophoblast plasma membrane vesicles. 384 μg syncytiotrophoblast plasma membrane vesicle protein was incubated for 30 min at 37°C with EGF, (●) with or (○) without $0.1 \text{ mmol ATP l}^{-1}$, in $10 \text{ mmol Tris-HCl l}^{-1}$ (pH 7.4) containing protease inhibitors. The incubation buffer contained $1.0 \text{ mmol MnCl}_2 \text{ l}^{-1}$, $1.0 \text{ mmol CaCl}_2 \text{ l}^{-1}$ and $1.0 \text{ mmol MgCl}_2 \text{ l}^{-1}$. The total volume was $100 \mu\text{l}$. Supernatants were assayed for placental alkaline phosphatase activity ($n=4$). Data are shown with buffer control value subtracted. Vertical bars represent standard errors. * $P < 0.05$ tested by Mann-Whitney test (EGF plus ATP versus EGF alone).

Fig. 2. Effects of insulin on activity of alkaline phosphatase of syncytiotrophoblast plasma membrane vesicles. 384 μg syncytiotrophoblast plasma membrane vesicle protein was incubated for 30 min at 37°C with insulin, (■) with or (□) without $0.1 \text{ mmol ATP l}^{-1}$, in $10 \text{ mmol Tris-HCl l}^{-1}$ (pH 7.4) containing protease inhibitors. The incubation buffer contained 1.0 mmol l^{-1} of MnCl_2 , CaCl_2 and MgCl_2 . The total volume was $100 \mu\text{l}$. Supernatants were assayed for placental alkaline phosphatase activity ($n=4$). Data are shown with buffer control value subtracted. Vertical bars represent standard errors.

Syncytiotrophoblast plasma membrane vesicles were incubated with either GTP γ S or GDP β S, in the presence of EGF and ATP, to determine whether EGF and GTP-binding proteins interact in the release of placental alkaline phosphatase. GTP γ S enhanced the EGF-induced release of placental alkaline phosphatase but, compared with earlier results (Figs 1 and 2), the

effect was additive rather than synergistic (Table 2). GDP β S, however, partially inhibited the EGF-induced release of placental alkaline phosphatase. The possibility that any effect of insulin on release of this enzyme might be GTP dependent was tested, but insulin failed to stimulate any measurable increase in soluble placental alkaline phosphatase in the presence of GTP γ S ($17.2 \pm 16.4 \text{ mU mg}^{-1}$ syncytiotrophoblast plasma membrane vesicle protein, $P = 0.37$, $n = 4$).

The results obtained with EGF/ATP or GTP γ S were shown not to be due to contamination by microvesicles and the released alkaline phosphatase activity was shown to be placental alkaline phosphatase by incubating syncytiotrophoblast plasma membrane vesicles with EGF and ATP or GTP γ S. The membranes were removed by ultracentrifugation and the placental alkaline phosphatase activity in the resulting supernatant was assessed by enzymeimmunoassay (Table 3). EGF with ATP induced a small but significant release of the enzyme. GTP γ S also induced significant release of placental alkaline phosphatase. The precise amounts of placental alkaline phosphatase released in these experiments were different from those obtained in earlier experiments. However, quantitative data obtained in the enzymeimmunoassay are not necessarily comparable with those obtained from the enzyme assay. The enzymeimmunoassay for placental alkaline phosphatase is approximately threefold less sensitive than the enzyme assay and is more variable, having an intra-assay coefficient of variation of 5.82%, compared with 1.79% for the enzyme assay.

Although 5'-nucleotidase is GPI-linked to whole placental membranes, the extent to which this enzyme is GPI-anchored to syncytiotrophoblast plasma membrane vesicles is unknown (Thompson *et al.*, 1987; Klemens *et al.*, 1990). The mean 5'-nucleotidase activity detected in syncytiotrophoblast plasma membrane vesicles was $76.6 \pm 2.3 \text{ U 5'-nucleotidase mg}^{-1}$ protein (data not shown). Treatment of syncytiotrophoblast plasma membrane vesicles with PI-PLC (from *Bacillus cereus*) gave a dose-dependent increase in the release of 5'-nucleotidase up to $1 \text{ mU PI-PLC ml}^{-1}$. Maximum release with PI-PLC accounted for 28.7% of total 5'-nucleotidase in the vesicles. Phosphatidylcholine-specific phospholipase C (from *Clostridium perfringens*) had no effect on 5'-nucleotidase release (Table 4).

Treatment of syncytiotrophoblast plasma membrane vesicles with EGF or insulin, in the presence or absence of ATP, did not result in the release of a significant amount of 5'-nucleotidase. Similarly, treatment with GTP γ S gave no detectable rise in soluble 5'-nucleotidase activity (Table 4).

Treatment of syncytiotrophoblast plasma membrane vesicles with EGF or insulin, in the presence or absence of ATP, did not result in the release of a significant amount of 5'-nucleotidase. Similarly, treatment with GTP γ S gave no detectable rise in soluble 5'-nucleotidase activity (Table 4).

Discussion

Insulin induces the release of GPI-linked proteins from the surface of cells (Chan *et al.*, 1988; Romero *et al.*, 1988; Lisanti *et al.*, 1989; Romero, 1991). EGF and GTP γ S influence the release of a GPI-anchored protein, placental alkaline phosphatase, from the human laryngeal carcinoma cell-line, HEp-2 (Roberts *et al.*,

Table 1. Effect of short incubation with epidermal growth factor (EGF) or insulin on release of placental alkaline phosphatase from human syncytiotrophoblast plasma membrane vesicles

Treatment	Time (min)	Placental alkaline phosphatase release ^a	
		(mU mg ⁻¹ SPMV protein)	P
Buffer alone		77.0 ± 3.0	
EGF	5	79.3 ± 3.0	n.s.
EGF	30	97.6 ± 3.0	<0.05
Insulin	5	74.6 ± 2.9	n.s.
Insulin	30	84.1 ± 1.4	n.s.

Values are means ± SEM (n = 5).

^a417 µg syncytiotrophoblast plasma membrane vesicle protein was incubated at 37°C with either EGF (300 nmol l⁻¹) or insulin (300 nmol l⁻¹), with 0.1 mmol ATP l⁻¹, in 10 mmol Tris-HCl l⁻¹ (pH 7.4) containing protease inhibitors for various times. Total volume was 100 µl. The incubation buffer contained 1.0 mmol l⁻¹ of MnCl₂, CaCl₂ and MgCl₂. Supernatants were assayed for placental alkaline phosphatase activity. Significance of differences between treatment groups and the buffer control assessed by Student's *t* test.

Fig. 3. Effects of guanosine thiotriphosphate (GTPγS) and guanosine thiodiphosphate (GDPβS) on placental alkaline phosphatase activity in syncytiotrophoblast plasma membrane vesicles. 312 µg syncytiotrophoblast plasma membrane vesicle protein was incubated for 30 min at 37°C with either guanosine thiotriphosphate (GTPγS) (●) or guanosine thiodiphosphate (GDPβS) (○) in 10 mmol Tris-HCl l⁻¹ (pH 7.4) containing protease inhibitors. The total volume was 100 µl. Supernatants were assayed for placental alkaline phosphatase activity (n = 6). Data are shown with buffer control value subtracted. Vertical bars represent standard errors. ***P* < 0.01, ****P* < 0.001 assessed by Student's *t* test (GTPγS versus GDPβS).

1990). We have now extended these studies to determine whether similar events occur at the plasma membrane of a normal human cell, placental syncytiotrophoblast. Placental alkaline phosphatase is expressed not only on this cell surface, but also by many solid tumours (McLaughlin *et al.*, 1983; McDicken *et al.*, 1985). This alkaline phosphatase is released into the circulation during pregnancy and in the course of malignancy (Vergote *et al.*, 1992). The physiological significance and the full mechanism of this release remain unresolved. Growth factor-induced release of placental alkaline phosphatase may be a candidate for such a mechanism.

EGF induces the release of a small, but significant proportion of the placental alkaline phosphatase of syncytiotrophoblast

Table 2. Effects of epidermal growth factor (EGF) and guanosine thiotriphosphate (GTPγS) or guanosine thiodiphosphate (GDPβS) on placental alkaline phosphatase activity in human syncytiotrophoblast plasma membrane vesicles

Treatment	Placental alkaline phosphatase release ^a	
	(mU mg ⁻¹ protein)	P
Buffer alone	58.67 ± 2.28	
EGF + buffer	64.83 ± 3.20	<0.01
EGF + GTPγS	69.59 ± 3.20	<0.001
EGF + GDPβS	59.89 ± 2.28	n.s.

Values are means ± SEM (n = 9).

^a312 µg syncytiotrophoblast plasma membrane vesicle protein was incubated for 30 min at 37°C with EGF (300 nmol l⁻¹) and ATP (0.1 mmol l⁻¹) and either GTPγS (300 µmol l⁻¹) or GDPβS (300 µmol l⁻¹) in 10 mmol Tris-HCl l⁻¹ (pH 7.4) containing protease inhibitors. The total volume was 100 µl. The incubation buffer contained 1.0 mmol l⁻¹ of MnCl₂, CaCl₂ and MgCl₂. Supernatants were assayed for placental alkaline phosphatase activity. Significance of differences between treatment groups and buffer control was assessed by Wilcoxon one-sample rank test.

plasma membrane vesicles. The ability of EGF to affect this release depends on the presence of ATP, which suggests that phosphorylation may play a role. Although it has been reported that insulin stimulates the release of GPI-linked proteins, no evidence for such release was seen in syncytiotrophoblast plasma membrane vesicles in the presence or absence of ATP. However, maximal insulin-stimulated release of this alkaline phosphatase in HEp-2 cells amounted to only half of that released following EGF treatment and, therefore, it is possible that the assay conditions were insufficiently sensitive to measure an insulin effect in syncytiotrophoblast plasma membrane vesicles (Roberts *et al.*, 1990). The proportion of total cell-surface placental alkaline phosphatase released by EGF (0.4%) was comparable to (and not significantly different from) that stimulated by EGF in HEp-2 cells (1.6%), suggesting that the low value for release is not an artefact of the membrane system used (Roberts *et al.*, 1990). Chan *et al.* (1988) observed that treatment of cells with insulin resulted in the

Table 3. Effects of epidermal growth factor (EGF) and guanosine thiotriphosphate (GTP γ S) treatment on placental alkaline phosphatase activity in human syncytiotrophoblast plasma membrane vesicles

Treatment	Placental alkaline phosphatase release	
	(mU mg ⁻¹ protein) ^a	P
Buffer alone	36.21 $\pm$ 1.42	—
EGF and ATP (n = 12)	38.53 $\pm$ 1.26	<0.01
Buffer alone	49.61 $\pm$ 2.43	—
GTP γ S (n = 11)	54.40 $\pm$ 1.99	<0.05

Values are means $\pm$ SEM.

^a360 μ g syncytiotrophoblast plasma membrane vesicle protein was incubated for 30 min at 37°C with either EGF (300 nmol l⁻¹) and 0.1 mmol ATP l⁻¹ or GTP γ S (300 μ mol l⁻¹) in 10 mmol Tris-HCl l⁻¹ (pH 7.4) containing protease inhibitors. The total volume was 100 μ l. For experiments with EGF and ATP, the incubation buffer contained 1 mmol l⁻¹ of MnCl₂, CaCl₂ and MgCl₂. After incubation, the syncytiotrophoblast plasma membrane vesicle suspension was centrifuged at 133 000 g for 30 min and the supernatants were assayed for placental alkaline phosphatase activity by enzyme immunoassays. Significance of differences between treatment groups and buffer control was assessed by Wilcoxon one-sample rank test.

Table 4. Effects of epidermal growth factor (EGF), insulin and guanine nucleotides on 5'-nucleotidase of syncytiotrophoblast plasma membrane vesicles

Treatment	Number of samples	5'-Nucleotidase release
		(mU mg l ⁻¹ protein) ^a
PI-PLC	3	21.42 $\pm$ 0.64
PC-PLC	3	- 0.18 $\pm$ 0.07
EGF/ATP	4	0.020 $\pm$ 0.008
EGF	4	- 0.024 $\pm$ 0.018
Insulin/ATP	4	- 0.007 $\pm$ 0.013
Insulin	4	- 0.012 $\pm$ 0.023
GTP γ S	4	- 0.396 $\pm$ 0.090
GDP β S	4	- 0.716 $\pm$ 0.110

Values are means $\pm$ SEM.

^a125 μ g syncytiotrophoblast plasma membrane vesicle protein was incubated for 30 min at 37°C with either 1 mU PI-PLC ml⁻¹, 1 mU PC-PLC ml⁻¹, 333 nmol EGF l⁻¹ or 333 nmol insulin l⁻¹ (with or without 0.1 mmol ATP l⁻¹), or with 333 μ mol GTP γ S l⁻¹ or 333 μ mol GDP β S l⁻¹ in 10 mmol Tris-HCl l⁻¹ (pH 7.4) containing protease inhibitors. For incubations containing EGF or insulin the buffer also contained 1.0 mmol l⁻¹ of MnCl₂, CaCl₂ and MgCl₂. The total volume was 100 μ l. Data are shown with buffer control value subtracted. Supernatants were assayed for 5'-nucleotidase activity.

PI-PLC: phosphatidylinositol-specific phospholipase C; PC-PLC: phosphatidylcholine-specific phospholipase C; GTP γ S: guanosine thiotriphosphate; GDP β S: guanosine thiodiphosphate.

liberation of soluble alkaline phosphatase with maximal release at 2 min, followed by the return of alkaline phosphatase in the extracellular medium to basal values by 30 min. This effect was considered to be due to either degradation of the released enzyme or internalization. In syncytiotrophoblast plasma membrane vesicles, however, no effect of EGF was noted on

placental alkaline phosphatase at 5 min, but a significant increase in soluble placental alkaline phosphatase was observed at 30 min. This indicates that (i) EGF-stimulated release of placental alkaline phosphatase from syncytiotrophoblast plasma membrane vesicles is substantially slower than insulin-induced phosphatase release in BC₃H1 myocytes, and (ii) the degradation/internalization mechanism is absent from, or inactive in, trophoblast membrane preparations.

GTP γ S stimulated the release of a small subpopulation of placental alkaline phosphatase from syncytiotrophoblast plasma membrane vesicles compared with the non-hydrolysable guanosine diphosphate analogue, GDP β S. GTP γ S has been shown to stimulate G proteins, whereas GDP β S inhibits G proteins (Neer and Clapham, 1988). This finding suggests the presence of a GTP-binding protein coupled to the mechanism for placental alkaline phosphatase release. GTP γ S stimulated the release of approximately 0.77% of total enzyme. This is lower than the values for EGF action on syncytiotrophoblast plasma membrane vesicles or for GTP γ S action on HEP-2 cells (4.3%).

The effect on release of placental alkaline phosphatase of treatment of vesicles with EGF and GTP γ S in combination was additive, signifying that the EGF effect on release of this enzyme does not involve a GTP-binding protein, as a GTP-binding-protein-coupled receptor would be expected to exhibit either synergism or uncoupling when treated with both agonist and GTP analogue. Furthermore, this finding indicates that the EGF-induced mechanism operates on a subset of cell surface placental alkaline phosphatase separate from that used by the GTP γ S-dependent mechanism. The observation that GDP β S partially inhibited EGF-stimulated placental alkaline phosphatase release suggests that, although the EGF receptor may not be coupled to placental alkaline phosphatase release by a GTP-binding protein, it is sensitive to downstream modulation involving one or more GTP-binding proteins.

It is possible that the alkaline phosphatase released in these studies resulted from EGF- or GTP γ S-induced microvesiculation. This mechanism appears to be excluded by the observation that placental alkaline phosphatase is still enriched in ultracentrifugation supernatants from EGF/ATP- or GTP γ S-treated syncytiotrophoblast plasma membrane vesicles. Furthermore, since EGF/ATP and GTP γ S lead to the release of the alkaline phosphatase, but not of 5'-nucleotidase, it would appear that fragmentation of the membranes cannot explain release of placental alkaline phosphatase. The same set of experiments also demonstrated that the alkaline phosphatase activity released is recognized by the H317 monoclonal antibody specific for placental alkaline phosphatase (McLaughlin *et al.*, 1983, 1984a, b).

Another GPI-linked protein of the placenta, 5'-nucleotidase, was also studied to determine whether GPI-linked proteins are differentially regulated by growth factors or GTP-binding proteins, as has been suggested by Lisanti *et al.* (1989). 5'-nucleotidase is known to be coupled to the cell surface by a GPI-anchor in several cells (reviewed by Lisanti *et al.*, 1990). A proportion of 5'-nucleotidase from syncytiotrophoblast plasma membrane vesicles was shown to be sensitive to PI-PLC. This finding provides supportive evidence that this protein is GPI-linked to the cell surface of human trophoblast. However, over 60% of 5'-nucleotidase of syncytiotrophoblast plasma

membrane vesicles was resistant to PI-PLC. This finding contrasts with a previous study in which over 80% of 5'-nucleotidase associated with membranes prepared from whole placental homogenates was sensitive to PI-PLC (Thompson *et al.*, 1987). 5'-Nucleotidase was shown to be more resistant than was alkaline phosphatase to release by PI-PLC (reviewed by Turner *et al.*, 1991).

Neither EGF nor insulin treatment resulted in a significant release of 5'-nucleotidase, irrespective of whether ATP was included in the incubation. Similarly, GTP γ S was ineffective. This result supports the contention that subsets of GPI-linked proteins are sensitive to growth factor-induced release, whereas other GPI-anchored proteins are resistant.

In conclusion, these studies showed that small but discrete populations of placental alkaline phosphatase of syncytiotrophoblast plasma membrane vesicles were sensitive to growth factor-induced release and to release by a mechanism that involves GTP-binding proteins. Another GPI-linked protein, 5'-nucleotidase, was resistant to such release. Thus, it seems that different GPI-linked proteins and different populations of the same GPI-anchored protein are partitioned into groups with varying degrees of sensitivity to extracellular signals.

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