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EFFECT OF SOME DRUGS ON THROMBOPLASTIN ACTIVITY IN MOUSE TROPHOBLAST CELLS *IN VITRO* AND *IN VIVO*

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Abstract—Mouse trophoblast cells are constitutive producers of the thromboplastin apoprotein *in vitro*. The effects on thromboplastin activity of the three transmethylation inhibitors 3-deazaadenosine (DZA), 3-deazaaristeromycin (DZAri) and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA), the four calcium antagonists TMB-8, verapamil, nifedipine and felodipine, the prostaglandin E_2 (PGE_2), the phosphodiesterase inhibitor 1-methyl 3-isobutylxanthine (MIX) and monensin have been studied.

No cytotoxic effects were detected when trypan blue exclusion, release of lactic dehydrogenase, incorporation of ^{14}C-leucine into protein and cell morphology were monitored. TMB-8, felodipine, nifedipine and verapamil all abolished the increase in thromboplastin when added after 68 hr or 90–96 hr in culture. EHNA and DZAri had the same effect (but were only added at 90–96 hr). DZA had a similar effect when added at 68 hr and an even more marked inhibitory effect when added at 90–96 hr. Monensin prevented the increase in thromboplastin activity at 68 hr as well as at 90–96 hr.

The combination of DZA and l-homocysteine thiolactone (Hcy) further increased the inhibition, indicating that in these cases synthesis as well as degradation of thromboplastin were altered. The combination of DZA/Hcy and one of the four calcium antagonists gave no additional inhibitory effect. PGE_2 had a biphasic dose-dependent effect. The increased thromboplastin activity at low concentrations of PGE_2 (10 ng/ml) was inhibited by addition of one of the compounds verapamil, felodipine, nifedipine or DZA/Hcy. PGE_2 at higher levels (10 μg/ml) significantly inhibited thromboplastin synthesis. Combination of PGE_2 (10 μg/ml) and one of the calcium antagonists, DZA/Hcy or MIX gave no significant additive inhibitory effect.

Blood coagulation and fibrinolysis are balanced through sensitive systems of activators and inhibitors. During normal pregnancy this hemostatic balance is disturbed. There is an overall increase in the activity of coagulation factors and a suppression of fibrinolysis [1]. Serial observations on the coagulation and fibrinolytic systems in healthy women in labour have shown striking changes consistent with an activation of the coagulation system during and immediately following placental separation [2].

Placenta is particularly rich in thromboplastin (tissue factor) [3, 4]. The physiological changes of plasma coagulation during labour may be caused by contact of the maternal blood with placental thromboplastin or by release of thromboplastin (e.g. by shedding) from placental into the maternal circulation [1, 3, 5, 6]. In several pregnancy complications such as abruptio placentae, retained dead fetus or missed abortion, such release may cause disseminated intravascular coagulation. Modulation of the thromboplastin synthesis of placenta therefore has potential clinical importance.

We have previously described an *in vitro* system for thromboplastin synthesis in mouse trophoblast cells [7]. In contrast to human monocytes [8] and endothelial cells [9], trophoblasts are apparently constitutive producers of thromboplastin and do not respond to the usual inducers of thromboplastin synthesis such as endotoxin and phytohaemagglutinin. Nor is the complement system necessary for this synthesis to take place, but the intracellular level of cAMP clearly had a regulatory function [10, 11]. The increase in thromboplastin activity was reduced or abolished by inhibitors of protein and RNA synthesis [7]. Cells dispersed with collagenase made slightly more thromboplastin than cells dispersed with trypsin-EDTA, but the differences were not significant [11]. About 90–95% of the total cellular thromboplastin activity was available for inactivation by particle-bound trypsin when whole cells were treated and thus probably located on the cell surface [7]. We have previously described inhibition of the thromboplastin response in inducible cells by various pharmacological agents [12–14]. This paper deals with the effect of these compounds on the thromboplastin activity of murine trophoblast cells *in vitro* and *in vivo*.

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MATERIALS AND METHODS

Chemicals. MEM spinner medium, Eagle's MEM with Earle's salts and 20 mM Hepes, and RPMI 1640 were obtained from Gibco-Biocult, Paisley, Renfrewshire, Scotland, U.K. Iscove's medium, fetal calf serum (FCS) and trypsin-EDTA were obtained from Flow, Irvine, Scotland. 8-(*N*,*N*-diethylamino)-octyl 3,4,5-trimethoxybenzoate hydrochloride (TMB-8), 1-methyl-3-isobutylxanthine (MIX), l-homocysteine thiolactone (Hcy), adeno-

sine and collagenase type I were obtained from Sigma, St. Louis, MO. Nifedipine was kindly given by Bayer AG, Leverkusen, Germany. 3-Deazaadenosine (DZA) and 3-deazaaristeromycin (DZAri) were obtained from Southern Research Institute, Birmingham, AL. Felodipine was a gift from Hassle, Mølndal, Sweden. Verapamil (Isoptin) was from Knoll AG, Ludwigshafen, Germany, and prostaglandin E_2 (PGE_2) from Upjohn Limited, Crawley, West Sussex, U.K. Erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA) was kindly given by Dr McHale, Wellcome Research Laboratories, Beckenham, Kent, U.K. Monensin was obtained from Calbiochem-Behring, La Jolla, CA, and monophyllin (proxiphyllin) from AFI, Oslo, Norway.

For stock solutions, felodipine and nifedipine were dissolved in ethanol, TMB-8 and monensin in RPMI-1640 medium, and DZA, DZAri, Hcy, adenosine and MIX in isotonic saline. Solvent controls were included in all experiments.

Isolation and culture of trophoblast cells. Pregnant CBA/JCr mice (12–20 days) were obtained from Bomholt, Ry, Denmark and killed by cervical dislocation. The trophoblast cells were isolated, cultured and exposed to inhibitors as described previously [11].

Harvesting of cells. The cells of each dish were scraped into ice-cold 0.15 M NaCl using a rubber policeman, washed twice and finally resuspended in 0.5 ml of barbital-buffered saline (BBS) [15]. Complete removal of the cells was ensured microscopically.

In vivo *experiments.* Pregnant mice on day 18–20 were injected intraperitoneally (8 and 4 hr before sacrifice) or intravenously in the tail (24 and 16 hr before sacrifice) with the compounds to be tested, dissolved in 0.5 ml of saline.

Placenta was removed immediately after killing the animal, the trophoblast layer was dissected out, washed three times with a solution of sodium citrate dihydrate (31 g/l) and three times with 0.15 M NaCl and minced to 1–2 mm pieces. Thromboplastin was prepared according to Hjort [15]. Uteri/placenta showing lesions caused directly by the intraperitoneal injection were discarded.

Assays. The cell suspensions were frozen, thawed and gently homogenized using a Teflon–glass homogenizer. The thromboplastin activity was assayed in triplicate as described previously [16] using citrated mouse plasma as substrate. BBS was used as the buffer control. Plasma samples with a recalcification time under 90 sec were not used. Crude thromboplastin was prepared from mouse brain according to Hjort [15]. The thromboplastin clotting times were converted to units of activity per ml cell homogenate by using reference curves established with dilutions of crude mouse thromboplastin arbitrarily chosen to contain 100 U/ml when undiluted [16]. The thromboplastin activity was finally normalized on the basis of the concentration of cell protein determined according to the modification of Markwell *et al.* [17] of the method of Lowry *et al.* [18] using bovine serum albumin as standard.

Protein synthesis in the trophoblasts was measured by incorporation of L-(1-^{14}C)-leucine (New England Nuclear, Boston, MA) (0.5 μCi/ml) into trichloroacetic acid (TCA) precipitable proteins in the absence and presence of the examined compounds. Cell protein was precipitated in 1.2 ml of ice-cold 10% TCA with 1.0 ml BSA (5 mg/ml) as carrier. The precipitates were washed four times with 10% TCA, solubilized in 0.5 ml 0.5 M NaOH and their radioactivity measured in a liquid scintillation counter.

Lactate dehydrogenase (LD) was assayed according to the recommendations of the Scandinavian Committee on Enzymes [19].

Statistics. The data are given as mean ± SD. Statistical significance was calculated using Student's *t*-test.

RESULTS

Trophoblast cells

Within a few hours after plating, the trophoblast cells adhered firmly to the culture dish. The cells spread out and a monolayer of trophoblasts with overlapping cytoplasmic processes developed, reaching confluence usually 4–5 days after plating (for details see ref. 11). We usually chose to expose the cells to the various inhibitors after 90–96 hr in culture, i.e. when the cells nearly or just had reached confluency. The effects of most inhibitors and some of the possible combinations were also studied before confluency was reached, i.e. after 68 hr in culture. Judged by microscopy, uptake of trypan blue, release of LD to the medium and the incorporation of ^{14}C-leucine into total protein, none of the inhibitors used in our experiments had any effect on cell viability at the concentrations employed (Table 1).

Synthesis of thromboplastin in vitro

The thromboplastin activity in the cell cultures increased markedly for at least 5 days after plating, especially when Iscove's medium was used (Fig. 1). In homogenates of cells cultured for 116 hr the thromboplastin activity was 117 ± 23 (SD) units/mg cell protein when Iscove's medium was used (N = 16) and 93 ± 33 (SD) units/mg when the cells were grown in RPMI medium (N = 46). The actual clotting time with normal mouse plasma was 16–20 sec. In Iscove's medium the cell protein per dish doubled from day 2 to day 5, whereas in RPMI 1640 there was a very low net increase in cell protein. There was thus in both culture conditions a preferential synthesis of thromboplastin compared to the bulk of cellular proteins, and the inhibitors had no effect on the total protein per dish. The inhibitors had no effect on the synthesis of total protein as judged by ^{14}C-leucine incorporation (Table 1).

Effect of transmethylation inhibitors

DZA caused a dose-dependent inhibition of the thromboplastin activity increase in the trophoblast cells (Table 2). At a final concentration of 50–100 μM DZA, when added at about 90 hr, reduced the activity by 40–50% ($P < 0.001$) during 24 hr. The combination of DZA at various concentrations and Hcy (100 μM) further increased the inhibition (Table 2). These combinations reduced the thromboplastin activity beyond the 90–96 hr level and thus enhanced degradation of thromboplastin as well as inhibited

Table 1. Effect of some drugs on ^{14}C-leucine incorporation into total cell protein and release of lactate dehydrogenase (LD) to the culture medium

Addition (final concentration)	^{14}C-leucine incorporation (% of control)	Lactate dehydrogenase in the medium (% of control)
DZA (50 μM) + Hcy (100 μM)	105 ± 5	95 ± 12
DZA (10 μM) + Hcy (100 μM)	110 ± 2	
PGE_2 (10 μg/ml)	109 ± 0	98 ± 8
PGE_2 (10 ng/ml)	115 ± 2	
PGE_2 (10 μg/ml) + MIX (100 μM)	118 ± 15	
TMB-8 (20 μM)	111 ± 8	
TMB-8 (100 μM)	102 ± 1	90 ± 0
Verapamil (20 μM)	105 ± 7	
Verapamil (100 μM)	107 ± 3	
Monensin (10 μg/ml)	114 ± 2	98 ± 0
Nifedipine (20 μM)	119 ± 5	
Nifedipine (100 μM)	107 ± 5	87 ± 20
MIX (500 μM)	94 ± 13	103 ± 5
MIX (10 μM)	106 ± 4	

Each value is the mean ± SD of duplicate experiments. The inhibitors were added 68 hr after plating and were present for 24 hr before harvest of cells. The mean ^{14}C-leucine incorporation in the control cultures (N = 6) was 16.2 ± 2.7 (SD) cpm/mg cell protein × 10^3, and the mean LD content in the culture medium (N = 4) was 89 ± 11 (SD) U/l.

production. When added at 68 hr a dose-dependent but somewhat smaller inhibition was observed (Table 2).

EHNA at a final concentation of 20 μM caused a moderate reduction in thromboplastin activity ($P < 0.001$), and even lower doses had a significant effect (Table 2). Combinations of EHNA (1–20 μM) with Hcy (100 μM) and adenosine (100 μM) had no additional effect (Table 2).

DZAri at 100–200 μM had a moderate inhibitory effect on the increase of thromboplastin activity ($P < 0.001$), whereas the effect of 10 μM was barely significant ($0.01 < P < 0.05$) (Table 2). Combination of DZAri (100 μM) and Hcy (100 μM) had essentially no additional effect.

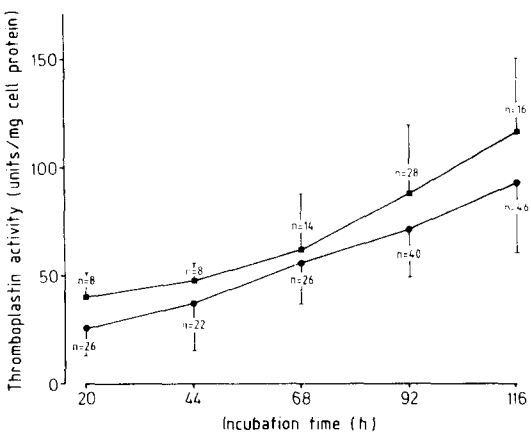

Fig. 1. Thromboplastin activity of mouse trophoblast cells *in vitro*. The cells were cultured in RPMI 1640 + 20% FCS (●——●) or Iscove's medium + 20% FCS (■——■).

Hcy (100 μM) alone or in combination with adenosine (100 μM) reduced the thromboplastin activity by about 15%.

Effect of calcium antagonists

The effects of two intracellular calcium blockers, TMB-8 and felodipine, and two calcium channel blocking agents, verapamil and nifedipine, are illustrated in Table 3. At 20 μM all the calcium antagonists abolished further increase in thromboplastin activity, but their effect on the total thromboplastin activity was modest compared to that of the transmethylation inhibitors. The effect was essentially the same when the inhibitors were present from 68 hr to 92 hr as when they were added at 90–96 hr and present for 24 hr (Table 3).

Combination of one of the four calcium antagonists (each at 20 μM) with DZA/Hcy (10 μM/100 μM) gave no additional inhibitory effect (Table 3).

Effect of PGE_2

The effect of PGE_2 on the thromboplastin activity increase was biphasic ([10] and Table 4). Trophoblast cells subjected to low levels of PGE_2 (10 ng/ml) for 24 hr (added 90–96 hr after plating) showed a thromboplastin activity significantly above that of controls ($P < 0.001$) (Table 4). This increase was inhibited by addition of one of the calcium antagonists verapamil, felodipine, or nifedipine (all at 20 μM) or DZA/Hcy (10 μM/100 μM). Even lower levels of PGE_2 had no effect (data not shown), whereas higher levels (10 μg/ml) reduced the thromboplastin activity levels to an extent showing that both synthesis and turnover were altered (Table 4). Combination of PGE_2 (10 μg/ml) and one of the calcium antagonists (20 μM) or DZA/Hcy (10 μM/

Table 2. Effect of inhibitors of transmethylation on thromboplastin activity in mouse trophoblast cells *in vitro*

Addition (final concentration)	Number of cultures A	Number of cultures B	Thromboplastin activity (% of control ± SD) A	Thromboplastin activity (% of control ± SD) B
DZA (100 μM)		4		46 ± 6‡
DZA (50 μM)		4		63 ± 12‡
DZA (10 μM)		4		71 ± 15‡
DZA (100 μM) + Hcy (100 μM)		4		29 ± 14‡
DZA (50 μM) + Hcy (100 μM)	4	4	66 ± 9‡	42 ± 10‡
DZA (10 μM) + Hcy (100 μM)	4	8	96 ± 5	68 ± 12‡
EHNA (20 μM)		4		71 ± 7‡
EHNA (10 μM)		4		90 ± 12
EHNA (1 μM)		4		85 ± 9‡
EHNA (20 μM) + Hcy (100 μM) + Adenosine (100 μM)		4		69 ± 21‡
EHNA (10 μM) + Hcy (100 μM) + Adenosine (100 μM)		4		78 ± 12‡
EHNA (1 μM) + Hcy (100 μM) + Adenosine (100 μM)		4		89 ± 14
DZAri (200 μM)		4		60 ± 11‡
DZAri (100 μM)		4		68 ± 10‡
DZAri (10 μM)		4		78 ± 22†
DZAri (100 μM) + Hcy (100 μM)		4		64 ± 16‡
Hcy (100 μM) + Adenosine (100 μM)		4		82 ± 3‡
Hcy (100 μM)		8		87 ± 10

The inhibitors were added 68 hr (A) or 90–96 hr (B) after plating and were present for 24 hr before harvest of cells. The mean activity of control cultures was (A) 94 ± 41 (SD) and (B) 112 ± 30 (SD) units mg cell protein.

* $0.01 < P < 0.05$, † $0.001 < P < 0.01$, ‡ $P < 0.001$

Table 3. Effect of monensin and calcium antagonists alone and in combination with inhibitors of transmethylation on thromboplastin activity in mouse trophoblast cells *in vitro*

Addition (final concentration)	Number of cultures A	Number of cultures B	Thromboplastin activity (% of control ± SD) A	Thromboplastin activity (% of control ± SD) B
Monensin (10 μg/ml)	6	4	65 ± 19‡	51 ± 18‡
TMB-8 (1 μM)		6		88 ± 11
TMB-8 (10 μM)		9		91 ± 14
TMB-8 (20 μM)	4	4	82 ± 11	78 ± 8‡
TMB-8 (100 μM)	6	9	67 ± 11‡	67 ± 18‡
TMB-8 (20 μM) + DZA (10 μM) + Hcy (100 μM)		4		75 ± 8‡
Verapamil (10 μM)		4		95 ± 11
Verapamil (20 μM)	4	4	83 ± 18	77 ± 8‡
Verapamil (100 μM)	4	8	78 ± 16†	79 ± 17‡
Verapamil (20 μM) + DZA (10 μM) + Hcy (100 μM)		4		83 ± 4‡
Nifedipine (10 μM)		3		83 ± 19‡
Nifedipine (20 μM)	4	4	79 ± 22	67 ± 1‡
Nifedipine (100 μM)	6	9	61 ± 27†	57 ± 10‡
Nifedipine (20 μM) + DZA (10 μM) + Hcy (100 μM)		4		71 ± 16‡
Felodipine (20 μM)		4		74 ± 16‡
Felodipine (20 μM) + DZA (10 μM) + Hcy (100 μM)		4		74 ± 14‡

The inhibitors were added 68 hr (A) or 90–96 hr (B) after plating and were present for 24 hr before harvest of cells. The mean activity of control cultures was (A) 97 ± 36 (SD) and (B) 113 ± 35 (SD) units/mg cell protein.

* $0.01 < P < 0.05$, † $0.001 < P < 0.01$, ‡ $P < 0.001$.

Table 4. Effect of prostaglandin E_2 and methylisobutylxanthine (MIX) alone and in combination with calcium antagonists or transmethylation inhibitors on thromboplastin activity in mouse trophoblast cells *in vitro*

Addition (final concentration)	Number of cultures A	Number of cultures B	Thromboplastin activity (% of control ± SD) A	Thromboplastin activity (% of control ± SD) B
PGE_2 (10 ng/ml)	4	10	124† ± 13	154 ± 40‡
+ DZA (10 μM) + Hcy (100 μM)		2		128 ± 11
+ Felodipine (20 μM)		2		91 ± 20
+ Nifedipine (20 μM)		4		81 ± 20*
+ Verapamil (20 μM)		4		98 ± 11
+ MIX (10 μM)		2		73 ± 2
PGE_2 (1 μg/ml)		8		96 ± 29
+ DZA (10 μM) + Hcy (100 μM)		2		70 ± 2
+ Felodipine (20 μM)		2		83 ± 8
+ Nifedipine (20 μM)		2		70 ± 3
+ Verapamil (20 μM)		2		90 ± 3
PGE_2 (10 μg/ml)	12	12	59 ± 21‡	73 ± 17‡
+ DZA (10 μM) + Hcy (100 μM)		4		84 ± 12
+ Felodipine (20 μM)		2		71 ± 7
+ Nifedipine (20 μM)		4		82 ± 19
+ Verapamil (20 μM)		4		59 ± 11‡
+ Verapamil (20 μM) + DZA (10 μM) + Hcy (100 μM)		2		77 ± 5
+ MIX (100 μM)	4	2	56 ± 18†	63 ± 5
MIX (10 μM)	4	8	116 ± 16	141 ± 53†
+ Nifedipine (20 μM)		2		48 ± 6
+ Verapamil (20 μM)		2		80 ± 3
MIX (100 μM)		4		70 ± 15†
+ Nifedipine (20 μM)		2		82 ± 18
+ Verapamil (20 μM)		2		42 ± 3
MIX (500 μM)	8		53 ± 23‡	

The compounds were added 68 hr (A) or 90–96 hr (B) after plating and were present for 24 hr before harvest of cells. The mean activity of control cultures was (A) 80 ± 40 (SD) and (B) 98 ± 42 (SD) units/mg cell protein.

* $0.01 < P < 0.05$, † $0.001 < P < 0.01$, ‡ $P < 0.001$.

100 μM) gave no additive inhibitory effect except possibly with verapamil. The presence of PGE_2 from 68 hr to 92 hr after plating had essentially the same effects (Table 4).

Effect of MIX

Mix at 10 μM concentration stimulated thromboplastin formation in a way similar to 10 ng/ml of PGE_2. This increase was again (as with PGE_2) effectively inhibited by nifedipine and verapamil (Table 4). At a higher concentration (100 μM) MIX was an effective inhibitor of thromboplastin expression and in this case there was no additional inhibitory effect of the calcium blockers.

Addition of PGE_2 and MIX, at low concentrations where each alone caused stimulation of thromboplastin expression, resulted in inhibition as if a much higher concentration of either compound alone had

Table 5. *In vivo* effect of prostaglandin E_2 and phosphodiesterase inhibitors on thromboplastic activity in murine trophoblast cells

Compound	Dose[a]	Number of experiments	Thromboplastin activity (% of controls ± SD)[b]
MIX	1.5 nmoles	5	78 ± 15†
	1.5 μmoles	5	82 ± 12*
Proxiphyllin	30 μg	5	83 ± 19*
	1.5 mg	5	94 ± 15
PGE_2	1.5 ng	6	92 ± 10
	15 μg	5	92 ± 13
	150 μg	6	73 ± 14‡

[a] Given intraperitoneally in 0.5 ml of saline 8 and 4 hr before sacrifice.

[b] The mean thromboplastin activity of controls was 5.1 ± 3.1 (SD) units/mg protein ($N = 27$).

* $0.01 < P < 0.05$, † $0.001 < P < 0.01$, ‡ $P < 0.001$.

been used. At higher concentrations MIX and PGE_2 had no additive inhibitory effect, consistent with the hypothesis that they both act by raising intracellular cAMP levels, which leads to inhibition of thromboplastin expression in other cell types [20].

Effect of monensin

Monensin at a final concentration of 10 μg/ml inhibited the increase in thromboplastin activity (Table 3) both when added at 68 hr ($0.01 < P < 0.05$) and at 90–96 hr ($0.001 < P < 0.01$) after plating.

In vivo *experiments*

For *in vivo* experiments we selected compounds which are related to compounds already in clinical use and which also are effective inhibitors of thromboplastin synthesis. This left us with prostaglandins and phosphodiesterase inhibitors (PGE_2 and MIX). These compounds were given intravenously or intraperitoneally. Controls received an identical volume of saline. Intravenous injections had essentially no effect. Intraperitoneal injections were more effective (Table 5) in that PGE_2 at 150 μg given twice reduced the mean thromboplastin activity to 73% ($P < 0.001$). MIX and proxiphyllin reduced the activity by about 20%. No abortions were induced by these injections.

DISCUSSION

Placenta thromboplastin may play an important role both in the physiological changes of plasma coagulation during pregnancy and labour, and in the pathogenesis of disseminated intravascular coagulation in obstetrics [2, 3, 5]. Regulation of the thromboplastin level in placenta therefore may be clinically important, and we have established the thromboplastin-synthesizing murine placenta cell cultures for studies.

The placenta cells which synthesize thromboplastin have been identified as trophoblasts [11]. In contrast to monocytes [8] and endothelial cells [9] trophoblast cells are constitutive producers of thromboplastin [10, 11]. We have previously studied the inhibition of inducible thromboplastin synthesis in monocytes and endothelial cells. It was therefore of great interest to see to what extent the constitutive expression of thromboplastin was influenced by the same inhibitors. We have already demonstrated that cAMP and some cAMP-elevating compounds suppress the expression of thromboplastin in cells inducible as well as constitutive in this respect [8, 10, 12, 20] (although not all effect of methylxanthines are mediated by cAMP [21]). It is interesting to note that the biphasic effect of cAMP and cAMP-elevating compounds [20] also extends to PGE_2 and MIX in constitutive thromboplastin expression ([10] and the present paper). A stimulation of thromboplastin expression was observed at low concentrations, whereas higher doses decreased the activity.

The effect of the inhibitors used in this study is given as the remaining thromboplastin activity in per cent of that in the uninhibited control samples. Since the inhibitors regularly were added at 68 or 90–96 hr, i.e. when all cultures already had synthesized much thromboplastin, this way of reporting the results may lead to an underestimation of the inhibitory effects. We have nevertheless chosen this simpler way of expressing the results because sufficient information about the turnover of thromboplastin and how that is influenced by the various inhibitors is presently unobtainable. A more stringent evaluation of the effects on synthesis versus degradation is therefore not possible at the moment.

Calcium antagonists abolished the thromboplastin activity increase observed in control cultures, but they did not reduce total thromboplastin activity beyond the level observed at the time of inhibitor addition. The data therefore do not allow any conclusions about the relative importance of inhibition of synthesis and increase of degradation or shedding.

Nifedipine (a 1,4-dihydropyridine compound blocking L-type calcium channels) [22] was the most effective, giving 43% inhibition at 100 μM. Only in the case of the increased thromboplastin activity at low concentrations of PGE_2 or MIX a marked inhibitory effect of all the calcium antagonists was observed (Table 4).

The effect of inhibitors of transmethylation on thromboplastin activity was very similar in monocytes, endothelial cells and trophoblast cells. The intracellular mechanisms whereby these compounds exert their inhibitory effect(s) are not established. DZA, DZAri, and EHNA are inhibitors of transmethylation processes in which *S*-adenosyl-1-methionine serves as methyl donor. Methylation of proteins, nucleic acids, and phospholipids have all been shown to be involved in regulation of cellular functions [23–25]. Whether any of these processes are important in relation to thromboplastin synthesis or expression is not yet known. DZA is also reported to inhibit cyclic nucleotide phosphodiesterase [26], leading to increased intracellular levels of cAMP and to induce disorganization of macrophage microfilaments [27]. The question of mechanisms of action therefore remains open. Combinations of transmethylation inhibitors with calcium antagonists (Table 3) gave no additive effects at all, suggesting common mechanism(s) of action for these compounds. Neither did combinations of calcium antagonists with cAMP-elevating compound PGE_2 give any additive inhibitory effect (Table 4) except possibly in the case of verapamil. The Ca^{2+}/cAMP balance may be the unifying mechanism behind these effects, but the question remains unclear. Differences between various calcium antagonists are not unexpected [28, 29].

The mean thromboplastin activity in trophoblast cells *in vitro* increased for at least 5 days. Some of the inhibitors abolished the activity increase seen in control cultures, whereas others reduced the activity beyond this level. This might be due to intracellular degradation of apoprotein III (the protein component of thromboplastin) or to shedding of vesicles containing thromboplastin from the cell surface [6, 30]. Until more detailed measurements of these processes are possible a more stringent description of the effects of various inhibitors is difficult.

Compounds acting via elevation of cAMP seemed to be the most promising candidates for *in vivo* studies on the regulation of placental thromboplastin activity. Using pregnant mice such compounds were

shown to be without significant effect *in vivo* when given intravenously. A significant inhibitory effect was seen after intraperitoneal administration.

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