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Effect of shear stress on the release of soluble ecto-enzymes ATPase and 5′-nucleotidase along with endogenous ATP from vascular endothelial cells

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1 Stimulation of endothelial cells from human umbilical vein by shear stress induced release of endogenous ATP which was accompanied by an extracellular increase in the activity of enzymes degrading both ATP (ATPases) and AMP (5′-nucleotidases).

2 The activity of soluble ATPase was progressively increased from 1.62 ± 0.27 to 12.7 ± 1.0 pmoles ml^{-1} h^{-1} after 60 min of stimulation by shear stress.

3 The rate of [^{3}H]-ATP hydrolysis in the medium was inhibited by the purinergic agents suramin, Reactive blue 2 and pyridoxalphosphate-6-azophenyl-2′4′-disulphonic acid, and remained insensitive to the classic inhibitors of ion-pumping and intracellular ATPases.

4 Shear stress also increased the activity of 5′-nucleotidase in the medium from 2.0 ± 0.5 to 27.2 ± 2.8 pmoles ml^{-1} h^{-1}. When shear stress was applied after removal of ecto-5′-nucleotidase by phosphatidylinositol-specific phospholipase C, the release of 5′-nucleotidase was drastically reduced.

5 These results show that soluble ATPase and 5′-nucleotidase which are released during shear stress are not released from an intracellular compartment together with ATP but have an extracellular origin.

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Abbreviations: DPBS, Dulbecco's phosphate buffered-saline; GPI, glycosyl-phosphatidylinositol; HUVECs, human umbilical vein endothelial cells; LDH, lactate dehydrogenase; PI-PLC, phosphatidylinositol-specific phospholipase C; PPADS, pyridoxalphosphate-6-azophenyl-2′4′-disulphonic acid; RB2, Reactive blue 2 (Cibacron blue 3GA); TLC, thin-layer chromatography

Introduction

Vascular tone in blood vessels is controlled by both perivascular nerves and endothelial cells (Burnstock & Ralevic, 1994). The role of extracellular adenine nucleotides in this dual control mechanism is prominent (Abbracchio & Burnstock, 1994; Burnstock, 1997). ATP, released as a co-neurotransmitter by sympathetic nerves, acts on ligand-gated P2X receptors located on vascular smooth muscle cells to produce vasoconstriction. ATP released from endothelial cells by shear stress and hypoxia acts on G protein-coupled P2Y receptors on endothelial cells to release nitric oxide and produce vasodilatation.

Under normal physiological conditions, the concentration of adenine nucleotides in the blood is maintained at very low values (Coade & Pearson, 1989). Therefore, substantial levels of extracellular ATP occur only transiently and in response to certain physiological and/or pathological conditions. Ecto-nucleotidases located on the plasma membranes of endothelial cells together with soluble ATPases in the plasma are the major effector system for rapid inactivation of circulating adenine nucleotides (Coade & Pearson, 1989; Meghji *et al*., 1995).

Compared to the ecto-nucleotidase pathway, the mechanism of extracellular ATP degradation by soluble nucleotidases has so far received little attention. It has recently been shown that, upon electrical stimulation of the guinea-pig vas deferens, the sympathetic nerves released not only ATP, but also soluble ATPases that can act in conjunction with membrane ecto-nucleotidases to inactivate ATP (Kennedy *et al*., 1997; Todorov *et al*., 1997). The corelease of specific metabolic enzymes along with ATP may represent a novel mechanism for terminating neurotransmitter function.

During stimulation by shear stress, endothelial cells are able to release endogenous ATP (Milner *et al*., 1990; Bodin *et al*., 1991; Bodin & Burnstock, 1998). Until now, however, no attempt has been made to study the release of nucleotide-metabolizing enzymes under similar conditions. In this study we investigated the possibility of a concomitant release of endogenous ATP and soluble ATPase and 5′-nucleotidase from vascular endothelial cells under experimental conditions of shear stress.

Methods

Cell culture

Umbilical cords from normal vaginal deliveries were processed as already described (Bodin & Burnstock, 1996). HUVECs were harvested by flushing after incubation of the cord in an enzymatic solution of collagenase (1 mg ml^{-1}) for 10 min at 37°C. After centrifugation (7 min, $180 \times g$), the cells were suspended in M199 medium supplemented with 15% foetal calf serum, and plated in to 6-well plates (Costar, U.K.). The culture medium was changed after 24 h and every 48 h

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thereafter. The primary cultures were used when confluence was reached (3–4 days). The integrity of the cultures at the end of each experiment was tested by light microscopy observations, Trypan blue test and lactate dehydrogenase (LDH) assay (Sigma, procedure No 500).

Shear stress conditions

HUVECs were subjected to shear stress using a cone and plate apparatus designed in our laboratory (Bodin & Burnstock, 1998). This device is positioned above a 6-well plate and is comprised of 6 cones ($\alpha = 0.005$ rd) which fit inside the 6 wells of the culture plate. The rotation of these cones generates a non-turbulent shear stress on the confluent monolayer of endothelial cells. This shear stress (Γ in dyn cm^{-2}) was calculated using the equation $\Gamma = \mu\omega/\alpha$ (Sdougos *et al*., 1984), where μ = kinematic viscosity of the medium in dyn s cm^{-2}, ω = angular velocity of the cone in rd s^{-1} and α = angle of the cone in rd. The cells were rinsed several times with Dulbecco's phosphate buffered-saline (DPBS). The wells were filled with 2 ml of DPBS and positioned on the cone and plate apparatus. The plate was left to rest for 30 min before a shear stress of 25 dyn cm^{-2} ($\cong$120 r.p.m.) was applied. Samples of the bathing medium were then collected at timed intervals, centrifuged ($180 \times g$ for 7 min) and assayed for ATP concentration and nucleotidase activities.

Pretreatment of the cells

In a series of experiments, HUVECs from the same cultures were incubated with either DPBS or phosphatidylinositol-specific phospholipase C (PI-PLC, 500 mU ml^{-1}) for 30 min at 37°C, prior to their stimulation by shear stress. After incubation, the medium was collected and changed for fresh DPBS. When the cells were pretreated with suramin (200 μM), Reactive blue 2 (RB2, 200 μM), PPADS (400 μM) or other agents the incubation was for 15 min at 37°C, and shear stress was applied immediately after treatment, without changing the medium.

ATP measurement

The ATP concentration in the medium was quantitated using a luciferin-luciferase assay. The samples (50 μl) were pipetted into the wells of a white (non-phosphorescent) microplate. The plate was placed in a luminometer (Lucy1, Anthos Labtec, Austria) and processed automatically by injection of 100 μl of luciferin-luciferase reagent (ATP monitoring reagent, Bio-Orbit, Finland) into each well and measured during 10 s. ATP concentrations were calculated from a calibration curve constructed at the same time using standard ATP dissolved in the appropriate solution in which the experiment was performed.

Enzyme assays

Using [^{3}H]-ATP and [^{3}H]-AMP as respective substrates, the activities of ATPase and 5′-nucleotidase were assayed as described previously (Yegutkin & Burnstock, 1998). Briefly, aliquots (45 μl) collected during application of shear stress were incubated at 37°C for 100–120 min in a final volume of 55 μl DPBS with either 100 nM [^{3}H]-ATP or 120 nM [^{3}H]-AMP. For measurement of membrane ecto-ATPase the endothelial cells were detached, resuspended with DPBS and incubated at 37°C for 20–30 min in a final volume of 80 μl DPBS containing 10^5 cells, 500 μM ouabain, 1 mM NaN_3 and 500 μM ATP with tracer [^{3}H]-ATP (5×10^5 d.p.m.). Incubation times were chosen to ensure the linearity of the reaction with time and concentration of the cells. In the case of ATPase inhibitory studies the cells or bathing medium were incubated with various inhibitors at 37°C for 15 min prior to the addition of [^{3}H]-ATP. The reactions were terminated by applying aliquots of the mixture (2×7 μl) to TLC sheets, and adenine nucleotides were separated by use of an appropriate solvent system (Norman *et al*., 1974). [^{3}H]-ATP and [^{3}H]-AMP and products of their hydrolysis were extracted from the silica by 0.1 N HCl and quantified by scintillation counting on a β-spectrometer (Packard, Minaxi Tri-carb 4000 series).

Statistical analysis

Pooled data are presented as means $\pm$ s.e.mean where n represents the number of experiments performed with separate cell suspensions in duplicate well plates. Statistical significance was evaluated by Student's t-test.

Drugs and materials

Materials used included M199 medium (Gibco, U.K.), foetal calf serum (Flow Laboratories, U.K.), collagenase (type CLS1, Worthington, NJ, U.S.A.), and Dulbecco's phosphate buffer saline supplemented with calcium (0.133 g l^{-1}) and magnesium (0.1 g l^{-1}) (Sigma, MI, U.S.A.). TLC plates were 20×10 cm silica gel 60 F_{254} type supplied by Sigma-Aldrich. PPADS was obtained from Tocris Cookson. Liquid scintillation cocktail, Wallac OptiPhase 'HiSafe'-3, was from Fisher Chemical. [2,8-^{3}H]-ATP and [8-^{3}H]-AMP with specific activities of 40 and 44 Ci mmole^{-1}, respectively, were purchased from ICN Biomedicals (Belgium). Organic solvents were from Analar. All other chemicals were purchased from Sigma (Poole, U.K.).

Results

Release of endogenous ATP

In the absence of any imposed shear stress on the endothelial cells, the concentration of ATP in the medium was very low and stable (7.2 $\pm$ 1.9 nM, $n = 7$). When a shear stress of 25 dyn cm^{-2} was imposed on the cells, there was a transient increase of extracellular ATP (105.3 $\pm$ 13.6 nM, $n = 7$) which then rapidly decayed to a steady-state ATP concentration of $<$10 nM (Figure 1). The measured efflux represents the net balance between the release of endogenous ATP and the rate of its hydrolysis by membrane-bound ATPases on the cell surface. When ecto-ATPase activity was partially inhibited by 200 μM suramin (Table 1) the concentration of ATP in the medium and the time course of its decline were both significantly increased (Figure 1). It may be concluded that the total amounts of ATP released under shear stress conditions significantly exceed the concentrations measured because of the immediate hydrolysis of extracellular nucleotides by corresponding ecto-nucleotidases.

Release of soluble enzymes

In order to examine whether stimulated endothelial cells are able to release soluble enzymes along with ATP, samples of the bathing solution were assayed for nucleotidase activity in the absence of cell culture. Hydrolysis of [^{3}H]-nucleotides was estimated by TLC assay, which has proved to be one of the most sensitive and versatile methods for screening the

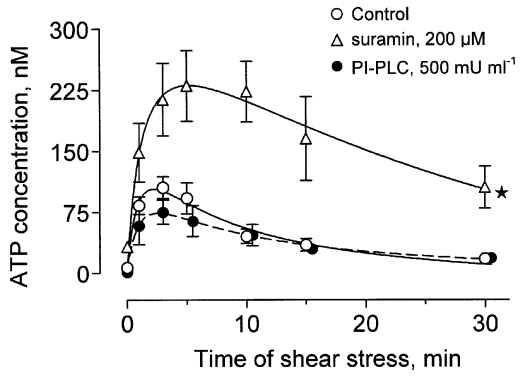

Figure 1 ATP release from endothelial cells under conditions of shear stress. HUVECs in culture were incubated without (Control, $n=7$) and with 200 μM suramin ($n=3$) or 500 mU ml^{-1} phosphatidylinositol-specific phospholipase C (PI-PLC, $n=5$). The treated cells were subjected to a shear stress of 25 dyn cm^{-2} and aliquots of the bathing medium were periodically collected and assayed for ATP concentration. Values are expressed as mean$\pm$s.e.mean (*$P<0.05$).

Table 1 Effect of various compounds on [^{3}H]-ATP hydrolysis by HUVEC suspension and in bathing medium collected after 45 min stimulation by increased flow

Compounds	*Concentration*	*% of ATPase activity remaining* *HUVECs*	*Bathing medium*
None	Control	100	100
NaN_3	5 mM	86.1	93
Ortho-vanadate	100 μM	87.9	73.6
β-glycerophosphate	20 mM	100	100
Concanavalin A	0.3 mg ml^{-1}	100	100
Dithiothreitol	1 mM	100	100
Ouabain	500 μM	76.6	84.5
EDTA	10 mM	12.3	3.0
Suramin	200 μM	42.5	9.3
RB2	200 μM	48	2.5
PPADS	400 μM	51.7	4.9

Standard incubation mediums and experimental conditions are described in Methods. Ouabain and NaN_3 were not included in the HUVEC suspension unless their effects were being studied. Actual ATPase activities were 315$\pm$45 nmoles 10^6 cells^{-1} h^{-1} for HUVECs and 11.7$\pm$0.5 pmoles ml^{-1} h^{-1} in the case of bathing medium. The data are the mean of duplicate assays from a representative experiment which was repeated twice.

catabolism of adenine nucleotides (Pearson, 1985). Without any shear stress imposed on the cells, the rate of [^{3}H]-ATP hydrolysis in the medium was 1.62$\pm$0.27 pmoles ml^{-1} h^{-1} ($n=10$). Similar values were obtained after incubation of [^{3}H]-ATP with DPBS alone (2 h, 37°C) indicating that the observed level of hydrolysis of the nucleotide was caused by its spontaneous decay and not by any specific enzyme released during cell incubation. The rate of [^{3}H]-ATP hydrolysis in the medium was progressively increased to 12.7$\pm$1.0 pmoles ml^{-1} h^{-1} after 60 min of stimulation by increased flow and remained unchanged for at least a further 30 min of stimulation (see Figure 2).

The observed [^{3}H]-ATP hydrolysis was abolished after chelation of cations with 10 mM EDTA (Table 1) as well as by heating the medium for 10 min at 65°C, suggesting the presence in the medium of specific Ca^{2+}-Mg^{2+}-dependent 'factor(s)', presumably soluble enzyme ATPase. Addition of exogenous ATP (1–10 μM) to the endothelial cells did not induce any release of soluble ATPase (data not shown) indicating that the observed release of the enzyme occurs directly as a result of shear stress and not as a result of ATP-induced autocrine stimulation.

Figure 3 shows the time course of hydrolysis of 100 nM [^{3}H]-ATP by the bathing medium taken from endothelial cell cultures after 45 min of shear stress. The decrease in ATP concentration was accompanied by a small increase in ADP and AMP concentration and there was progressive production of adenosine. The levels of metabolites in the medium showed an apparent precursor-product reaction, suggesting sequential catabolism of ATP by distinct soluble nucleotidases: ATP→ADP→AMP→adenosine. It should be noted that the observed pattern of enzymatic [^{3}H]-ATP dephosphorylation was very similar to that described previously for human plasma (Coade & Pearson, 1989) but not for endothelial cells (Meghji *et al*., 1995).

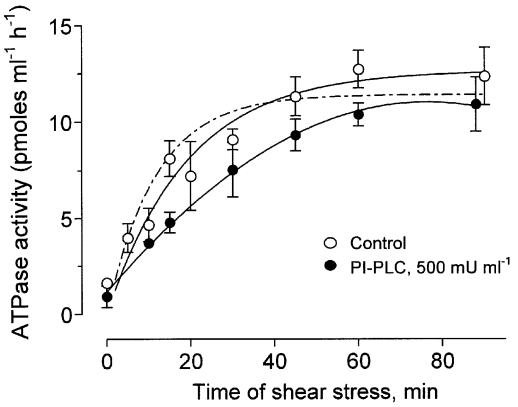

Figure 2 Effects of shear stress on the release of soluble ATPase by endothelial cells. HUVECs in culture were incubated for 30 min without (Control, $n=10$) or with 500 mU ml^{-1} phosphatidylinositol specific phospholipase C (PI-PLC, $n=3$) prior to their stimulation by shear stress. The results show no significant effect of the PI-PLC treatment on ATP hydrolysis. Samples of bathing medium were collected from the stimulated cells at intervals and assayed for ATPase activity. ATPase activity was expressed on the ordinate axis as picomoles of [^{3}H]-ATP hydrolyzed per millilitre of medium per hour (pmoles ml^{-1} h^{-1}). The initial concentration of [^{3}H]-ATP was 100 nM. ATPase activity in the controls was also calculated with regard to concentrations of both exogenous [^{3}H]-ATP and endogenously released ATP and this time course is shown by the dotted line.

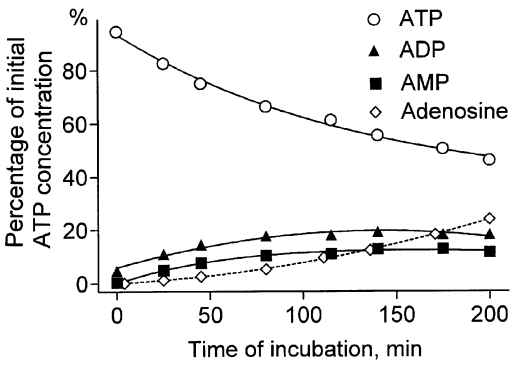

Figure 3 Pattern of [^{3}H]-ATP catabolism in the medium of endothelial cell cultures after 45 min stimulation by shear stress. The bathing medium was incubated with 100 nM [^{3}H]-ATP with subsequent TLC analysis of both [^{3}H]-ATP decrease and formation of its ^{3}H-labeled derivatives (ADP, AMP and adenosine). The relative amounts of nucleotides and adenosine are expressed on the ordinate axis as percentage of the initial concentration of [^{3}H]-ATP. ($n=2$).

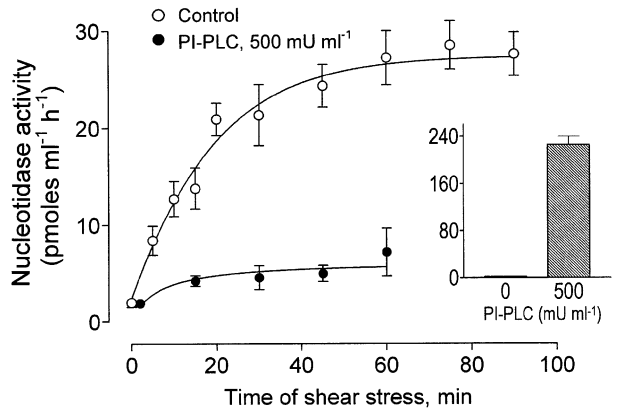

Figure 4 Effects of shear stress on the release of soluble 5′-nucleotidase by endothelial cells. The bathing medium was collected during shear stress of endothelial cells without pretreatment (Control, $n=8$) or pretreated with 500 mU ml^{-1} phosphatidylinositol-specific phospholipase C (PI-PLC, $n=3$). Activity of soluble 5′-nucleotidase was assayed with [^{3}H]-AMP (120 nM) as substrate and expressed on the ordinate axis as picomoles of [^{3}H]-AMP hydrolyzed per millilitre of medium per hour. Inset: release of soluble 5′-nucleotidase after treatment of the cells with PI-PLC. 5′-Nucleotidase activity (pmoles ml^{-1} h^{-1}) was measured in the medium collected from the control and PI-PLC-treated cells prior to their stimulation with shear stress ($n=3$). The initial concentration of [^{3}H]-AMP was 2 μM. The data are presented as means$\pm$s.e.mean.

Evidence for the presence of different soluble nucleotidases was sought by use of AMP, an another exogenous nucleotide. The rate of [^{3}H]-AMP hydrolysis in the medium was increased from 2.0$\pm$0.5 pmoles ml^{-1} h^{-1} at the onset of shear stress to 27.2$\pm$2.8 pmoles ml^{-1} h^{-1} ($n=8$) after 60 min shear stress (Figure 4). Addition to the assay buffer of the lectin Concanavalin A (0.3 mg ml^{-1}), a specific 5′-nucleotidase inhibitor (Pearson, 1985), prevented the hydrolysis of [^{3}H]-AMP (data not shown) without having any effect on the rate of [^{3}H]-ATP hydrolysis (Table 1). No changes of [^{3}H]-ATP and [^{3}H]-AMP hydrolysis were detected in the presence of β-glycerophosphate (20 mM) indicating that non-specific phosphatases are not involved in the measured hydrolysis. Taken together these experiments show that, under experimental conditions of shear stress, endothelial cells are able to release at least two different soluble enzymes, ATPase and 5′-nucleotidase.

Concentrations of both exogenous and endogenous nucleotides should be taken into consideration for a proper estimation of nucleotidase activities. However, the release of ATP was transient and occurred only within the first minutes of shear stress, therefore applying such correction for endogenous ATP did not result in any significant changes in the time-course of the ATPase activity (dotted line on Figure 2). In the subsequent experiments, concentrations of endogenously released nucleotides were not taken into account and only exogenously applied [^{3}H]-nucleotides were considered in calculation of the respective enzymatic activities.

Influence of inhibitors on soluble and membrane-bound ATPases

Several inhibitors of intracellular ATPases and purinergic agents were examined for their ability to inhibit the activity of soluble ATPase in the bathing medium collected from the shear stress-stimulated cells. We also correlated these measurements with the influence of the same compounds on ecto-ATPase activity in HUVEC suspension. The results are summarized in Table 1. Both soluble and membrane-bound ATPases were weakly inhibited by sodium azide (5 mM), ouabain (500 μM) and ortho-vanadate (100 μM) and remained insensitive to dithiothreitol (1 mM) (Table 1). Suramin (200 μM), RB2 (200 μM) and PPADS (400 μM) which have been shown to behave as both P2 antagonists and ecto-ATPase inhibitors (Abbracchio & Burnstock, 1994; Chen *et al*., 1996) caused partial inhibition of ecto-ATPase activity in HUVEC suspension and completely inhibited [^{3}H]-ATP hydrolysis by soluble ATPases in the bathing medium (Table 1). Relative resistance of the measured soluble ATPase to inhibitors of intracellular ATPases and inhibition by P2 antagonists suggest a close relationship between soluble and plasma membrane ATPases.

Effects of phosphatidylinositol-specific phospholipase C

Treatment of HUVECs with the glycosyl-phosphatidylinositol- (GPI-) specific enzyme PI-PLC (500 mU ml^{-1}) induced a marked increase of 5′-nucleotidase activity in the extracellular medium (inset to Figure 4). The observed increase may be explained by specific cleavage of the lipid anchor by PI-PLC and shedding of the enzyme from the cellular surface (Zimmermann, 1992; Airas *et al*., 1997). After subsequent shear stress stimulation, the release of 5′-nucleotidase from the PI-PLC-treated cells was drastically reduced (Figure 4). These results show that the release of 5′-nucleotidase during shear stress originates from the extracellular side of the plasma membrane. Treatment of the cells with PI-PLC (500 mU ml^{-1}) did not cause any significant changes in release of endogenous ATP (Figure 1) and soluble ATPase (Figure 2).

Discussion

This study demonstrates that shear stress stimulation of vascular endothelial cells induces a concomitant release of endogenous ATP and soluble enzymes degrading both ATP (ATPases) and AMP (5′-nucleotidases).

ATP is released from endothelial cells upon stimulation by shear stress (Milner *et al*., 1990; Bodin *et al*., 1991) and by other chemical mediators and P2 agonists (Yang *et al*., 1994). There are other recent reports showing that various mechanical and osmotic stimuli induce ATP release from cultured hepatoma cells (Roman *et al*., 1997), fibroblasts (Grierson & Meldolesi, 1995), myeloblasts, promyelocytes (Clifford *et al*., 1997), bladder epithelial cells (Ferguson *et al*., 1997) and smooth muscle cells (Matsuo *et al*., 1997). It is clear therefore that ATP is released as an autocoid from a variety of cells without cell lysis or irreversible damage of the cell membrane. ATP efflux occurs either through the opening of channel-like pathways, including ATP binding cassette proteins (Higgins, 1995; Roman *et al*., 1997) or *via* vesicular exocytosis (Dubyak & El-Moatassim, 1993). However, the signalling pathways involved and the mechanisms of transmitter release have not been fully defined.

In the present work, we have found that the release of endogenous ATP is accompanied by an extracellular increase in the activity of enzymes degrading both ATP (ATPases) and AMP (5′-nucleotidases). LDH activity was not detectable in the medium and, at the end of our experiments, light microscopic examinations revealed no sign of cell damage in the cultures. This suggests that the observed release of enzymes is not caused by 'leakage' of intracellular enzymes into the medium in the course of cell lysis or loss of membrane integrity.

Ecto-5′-nucleotidase is covalently attached to cell membranes *via* a GPI glycolipid moiety (Zimmermann, 1992) and endogenous phospholipase C might be responsible for shedding this ectoenzyme from the plasma membrane. To test this possibility we depleted the cells of ecto-5′-nucleotidase by treatment with PI-PLC and then subjected them to shear stress. The removal of these ecto-enzymes prevented further release of soluble 5′-nucleotidase from the stimulated cells. These data clearly indicate the extracellular origin of soluble 5′-nucleotidase which is released under shear-stress. Such conclusion is also consistent with the demonstration of the activation of PI-PLC in endothelial cells under conditions of shear stress (Bhagyalakshmi *et al*., 1992).

The mechanisms by which shear stress stimulation leads to release of ecto-ATPase into the extracellular medium are unclear. Since normal exocrine cells are able to release secretory products wrapped into microvesicles which are associated with an ATP-diphosphohydrolase activity (so-called 'exo-ATPases') (Beaudoin *et al*., 1986), it could be an interesting possibility that in our experiments ATPase is also secreted and transferred in the form of vesicles. However, it seems likely that this enzyme arises from the endothelial cell surface. This soluble enzyme displays the general characteristics of a dedicated E-type ecto-ATPase such as Ca^{2+} and Mg^{2+} dependence, insensitivity to common intracellular ATPase inhibitors and inhibition by purinergic agents suramin, RB2 and PPADS. It is interesting to draw some analogy with the recently described release of soluble ATPase from *Tetrahymena*, a single-cell eukaryote. The enzyme was also released into the surrounding culture fluid at a constant rate over time and possessed the main hallmarks of a dedicated membrane-bound ecto-ATPase (Smith *et al*., 1997).

It is also of importance to consider the relationships that are likely to exist between shear stress and the release of soluble ecto-enzymes. Because of their location on the lumenal surface of blood vessels and their contact with the circulating blood, endothelial cells *in vivo* are constantly subjected to shear stress. These haemodynamic forces play an important role in the regulation of vascular tone, in prevention of cellular apoptosis (Kaiser *et al*., 1997) and are associated with the modulation of the synthetic and secretory functions of vascular endothelial cells (Milner *et al*., 1990; Dubyak & El-Moatassim, 1993). Here we have shown that, under conditions of shear stress, vascular endothelial cells are able to release soluble nucleotide-metabolizing ecto-enzymes suggesting that these cells may be one of the major *in vivo* sources of soluble nucleotidases in the blood stream. However, the endothelial catabolism of circulating nucleotides predominates in microvascular beds (Coade & Pearson, 1989) and because of the very slow rate of blood flow in these vessels, the action of membrane-bound ATPases will be prominent. Within large blood vessels or at sites of impaired blood flow, the volume and the flow rate of blood are such that soluble nucleotidases may then act as important regulators of the concentrations of vasoactive nucleotides as ATP flows downstream (Meghji *et al*., 1995). Furthermore, the soluble nucleotidases could also participate in the termination of neurotransmitter function and sensitization of the P2Y receptors on the cells with low ATPase activity (Clifford *et al*., 1997; Kennedy *et al*., 1997; Todorov *et al*., 1997) and prevent recruitment of additional platelets into a growing thrombus (Gayle *et al*., 1998).

Another interesting role for ecto-5′-nucleotidase, otherwise known as CD73 surface antigen (Zimmermann, 1992), is its direct involvement in lymphocyte-endothelial cell interactions and its release from the cell surface as a consequence of triggering by an anti-CD73 monoclonal antibody, mimicking ligand binding (Airas *et al*., 1997). Recently the ecto-ADPase activity of vascular endothelial cells has been attributed to CD39, another surface antigen which acts as an effective endothelial cell inhibitor of platelet reactivity (Marcus *et al*., 1997). Based on these observations, it may be speculated that shedding of these ecto-enzymes from the cell surface under conditions of shear stress might represent an important mechanism of controlling adherence of the blood cells to vascular endothelium. These mechanisms may include either removal of surface antigens or formation of soluble anti-adhesion compounds, as recently shown for soluble recombinant ADPase/CD39 (Gayle *et al*., 1998).

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