Metabolism of adenine nucleotides by ectoenzymes of vascular endothelial and smooth-muscle cells in culture

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1. Pig aortic endothelial and smooth-muscle cells in culture rapidly catabolize exogenous ATP, ADP or AMP. 2. In both cell types catabolism is due to Mg^{2+} -stimulated ectoenzymes. 3. Inhibition and substrate-specificity studies suggest that both cell types possess three distinct ectonucleotidases, namely nucleoside triphosphatase (EC 3.6.1.15), nucleoside diphosphatase (EC 3.6.1.6) and 5'-nucleotidase (EC 3.1.3.5), as well as nucleoside diphosphate kinase (EC 2.7.4.6). 4. These ectonucleotidase systems could be of importance in the regulation of neurotransmission, blood platelet function and vasodilation.

Homogenates of vascular tissue can metabolize ADP to produce AMP and adenosine (Heyns *et al.*, 1977; Lieberman *et al.*, 1977). More recent work has suggested that ADPase and 5'-nucleotidase activities are present as ectoenzymes on intact cultured vascular endothelial and smooth-muscle cells (Hablison *et al.*, 1978; Cooper *et al.*, 1979), and that cultured endothelial cells can also catabolize exogenous ATP to adenosine (Dieterle *et al.*, 1978; Dosne *et al.*, 1978). Preliminary work in our own laboratory has confirmed that intact endothelial and smooth-muscle cells in culture can sequentially degrade ATP to ADP, then to AMP and finally to adenosine (Pearson *et al.*, 1979; Pearson & Gordon, 1979). There has, however, been little attempt to characterize these ectoenzymic activities in terms of their substrate specificities or kinetics.

ADP is a powerful inducer of blood platelet aggregation, whereas adenosine is inhibitory, and it has therefore been suggested that the ADPase activity of endothelial cells is related to the resistance of endothelium to thrombogenesis (Lieberman *et al.*, 1977; Glasgow *et al.*, 1978). Additionally, ATP and adenosine are powerful vasoactive agents (for review see Burnstock, 1979). It is well established that ATP and ADP are secreted by stimulated blood platelets (Mills *et al.*, 1968). Other cell types, including mast cells (Cockcroft & Gomperts, 1979), endothelium and vascular smooth muscle (Pearson & Gordon, 1979) selectively release

adenine nucleotides when exposed to stimuli *in vitro*. Thus substrates for vascular ectonucleotidases will be present whenever platelets or vascular cells are stimulated, or after more extensive tissue damage, and the net biological effect of released nucleotides will depend on the relative activities of the nucleotidases present locally.

We have now characterized the ectonucleotidase activities of cultured aortic endothelial and smooth-muscle cells. Inhibition and substrate-specificity studies suggest that both cell types possess distinct nucleoside triphosphatase (EC 3.6.1.15), nucleoside diphosphatase (EC 3.6.1.6) and 5'-nucleotidase (EC 3.1.3.5) ectoenzymes as well as ecto-(nucleoside diphosphate kinase) (EC 2.7.4.6).

Materials and methods

Cell culture

Endothelial cells and smooth-muscle cells were isolated from aortas of 1–10-day-old pigs of the Babraham herd and cultured as previously described (Pearson *et al.*, 1978) in Dulbecco's modification of Eagle's medium containing heat-inactivated (56°C, 30 min) foetal bovine serum [20% (v/v) for endothelium and 10% (v/v) for smooth muscle]. Cells were used for experiments after one to ten passages.

Chemicals

[2-³H]ATP, [2-³H]ADP and [2-³H]AMP (15–20 Ci/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Dipyrindamole (Persantin injection) was from Boehringer Ingelheim Ltd., Bracknell, Berks., U.K., and

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; p[CH₂]₂pA, adenosine 5'-[α,β-methylene]diphosphate; p[NH]ppA, adenosine 5'-[β,γ-imido]triphosphate.

all other chemicals were from Sigma (London) Chemical Co.

Experiments

Confluent cell layers growing adherent to 16 mm-diameter wells (approx. 10^5 cells/well) of Falcon Multiwell tissue-culture plates (Scientific Supplies Co., London E.C.1) were used, after removal of growth medium and rinsing in serum-free medium. Unless otherwise stated, replicate incubations were carried out at 37°C by adding 0.2 ml of 20 mM-Hepes-buffered Dulbecco's modification of Eagle's medium (pH 7.2) (Dulbecco & Freeman, 1959) per well, containing ^3H -labelled adenine nucleotide (200 000–500 000 c.p.m. per well) and any other test compounds. At the end of the incubation period the reaction was stopped by transferring each 0.2 ml sample to a separate tube on ice, and the mixtures were subsequently stored at -20°C until required. Subsamples were taken for liquid-scintillation counting and t.l.c.

Measurement of metabolites

The products of ^3H adenine nucleotide metabolism were separated by t.l.c. in the solvent system of Norman *et al.* (1974) on silica-gel-coated plastic sheets impregnated with fluorescent indicator, essentially as described previously (Pearson *et al.*, 1978), except that no extraction procedure was necessary. Subsamples (20 μl) from incubation samples were chromatographed directly, with 5 μl of a standard marker mixture, for 3–4 h or overnight. Because adenosine and hypoxanthine were poorly separated by this solvent system, we occasionally also chromatographed subsamples for 90 min at room temperature in the solvent system of Pull & McIlwain (1972). In this system R_F values were as follows:

ATP, ADP, AMP, and IMP 0.00; inosine 0.22; hypoxanthine 0.39; adenosine 0.50. Spots were located under u.v. light, outlined in pencil, cut out and transferred to scintillation vials. Distilled water (1 ml) and scintillation fluid (Packard Emulsifier Scintillator 299; 8 ml) were added to each vial and radioactivity was determined in a Packard Tri-Carb liquid-scintillation spectrometer. Counting efficiencies, as determined by the addition of internal standards, did not vary significantly between vials. The radioactivity in each spot was expressed as a fraction of the total on each t.l.c. plate and converted into pmol (if appropriate) by reference to the specific radioactivity found in non-chromatographed subsamples. Recovery of radioactivity from t.l.c. plates was over 90%.

Results

Pattern of adenine nucleotide catabolism

The rates of catabolism of ^3H ATP, ^3H ADP or ^3H AMP were initially studied by incubating $1\text{ }\mu\text{M}$ substrate with intact cells (Fig. 1). Each substrate was unchanged when incubated in the absence of cells. Smooth-muscle cells catabolized each substrate at a similar rate, with a half-life of about 5 min under these experimental conditions. In experiments with endothelial cells under the same conditions, the rate of breakdown of ATP was greater than that of ADP, and the catabolism of AMP was considerably slower than that of ATP or ADP; half-lives were approx. 5, 10 and 40 min respectively. When ^3H ATP was incubated with endothelial cells, and the pattern of metabolites determined, there was an initial transient peak of ^3H ADP, followed by a more prolonged peak of ^3H AMP and a progressive increase in ^3H adenosine, suggesting

Fig. 1. Time course of catabolism of exogenous adenine nucleotides by cultured vascular cells. ATP, ADP or AMP ($1\text{ }\mu\text{M}$) was incubated with vascular cells as described in the text; $10\text{ }\mu\text{M}$ -dipyridamole was present in all incubations. The ordinate axes show the amount of unchanged substrate remaining: ●, ATP; ▲, ADP; ■, AMP.

sequential dephosphorylation. When similar experiments were performed with smooth-muscle cells, the peaks of [^3H]ADP and [^3H]AMP were much smaller, never exceeding the production of [^3H]adenosine (Fig. 2).

In these experiments dipyrindamole ($10\mu\text{M}$), a potent inhibitor of adenosine uptake by endothelial cells (Pearson *et al.*, 1978), was added to prevent any uptake of [^3H]adenosine formed. Under these conditions less than 0.5% of the ^3H in the medium above endothelial cells at 60 min was found in inosine, hypoxanthine or IMP, indicating that production (if any) of these components takes place only intracellularly. With 120 min incubations in the absence of dipyrindamole, the amounts of products formed from $1\mu\text{M}$ -[^3H]ATP were identical, except that the amount of [^3H]adenosine found in the medium was decreased (with endothelial cells it was 22% of that found in the presence of dipyrindamole, with smooth-muscle cells it was 73%) because of transport into the cells. The amounts of [^3H]inosine and [^3H]hypoxanthine present extracellularly were still very low (both were less than 0.5% of the total ^3H in the medium above endothelial cells, and the amounts were respectively 10 and 2% of the total above smooth-muscle cells), confirming our earlier finding that adenosine taken up by vascular cells is predominantly converted into adenine nucleotides (Pearson *et al.*, 1978).

To investigate whether the cultured cells released nucleotidase activity into the medium, we compared the catabolism of [^3H]adenine nucleotides ($0.13\mu\text{M}$) in wells containing cells with catabolism in medium that had been previously incubated with cells and then transferred to empty wells. The contribution of this released enzyme activity to the catabolism measured in the presence of cells was only 6% (mean of six experiments), and did not exceed 15% for any nucleotide above either cell type.

Kinetic analysis

Approximate kinetic constants were derived by incubating [^3H]ATP, [^3H]ADP or [^3H]AMP with cultures of each cell type at substrate concentrations ranging from $1\mu\text{M}$ to 1mM . Results obtained in individual experiments are shown in Fig. 3. In smooth-muscle cells, apparent K_m values obtained for all three substrates in several independent experiments were in the range 100 – $150\mu\text{M}$. In endothelial cells, however, apparent K_m values were approx. $350\mu\text{M}$, $125\mu\text{M}$ and 20 – $50\mu\text{M}$ for ATP, ADP and AMP respectively.

Cation-dependence

We found no evidence for Ca^{2+} -dependence of any of the endothelial enzymes. Catabolism of $1\mu\text{M}$ -ATP, -ADP or -AMP in the presence of 0.7mM - Mg^{2+} plus 0.7mM -EGTA was not significantly different from control values (Table 1). Catabolism in the presence of 0.7mM -EDTA, however, was inhibited, with ADP catabolism more affected than that of ATP or AMP in both cell types (Table 1). Similar results were obtained with substrate concentrations of $500\mu\text{M}$; again, metabolism in the presence of Mg^{2+} plus EGTA was similar to control values, whereas in samples containing EDTA it was decreased, with ADP catabolism most inhibited (to 39 and 52% of control values in endothelial cells and smooth-muscle cells respectively).

Substrate specificity

Catabolism of ATP, ADP and AMP by endothelium and smooth muscle was apparently almost entirely due to nucleotide-specific phosphohydrolase activity, and not to non-specific phosphatase activity. There were no differences in catabolism whether experiments were performed in Dulbecco's medium (containing 1.2mM - P_i) or phosphate-buf-

Fig. 2. Time course of product formation from catabolism of exogenous ATP by cultured vascular cells. ATP ($1\mu\text{M}$) was incubated with vascular cells as described in the text; $10\mu\text{M}$ -dipyrindamole was present in all incubations. Key: $\bullet$, ATP; $\blacktriangle$, ADP; $\blacksquare$, AMP; $\blacktriangledown$, adenosine.

Fig. 3. Concentration-dependence of exogenous adenine nucleotide catabolism by cultured vascular cells

Adenine nucleotides ($1\mu\text{M}$ – 1mM) were incubated with vascular cells as described in the text: 1mM -adenosine was present in all incubations to prevent uptake of $[^3\text{H}]$ adenosine formed. ●, ATP (5 min incubations); ▲, ADP (10 min incubations); ■, AMP (20 min incubations with endothelium, 10 min with smooth muscle). The inset shows on a larger scale the concentration-dependence of catabolism of AMP (1 – $100\mu\text{M}$) by endothelial cells.

Table 1. Effects of Ca^{2+} and Mg^{2+} on vascular-cell ectonucleotidase activities

Cultured vascular cells were incubated for 5 min with $1\mu\text{M}$ -adenine nucleotide, as described in the text. Incubations were in phosphate-buffered saline (pH 7.2) (Dulbecco & Vogt, 1954) with or without the addition shown below (each at 0.7mM). The results are percentages of the amount of metabolism in control samples (containing phosphate-buffered saline alone), and are means of duplicate determinations; the average range about the mean values was ± 4 .

Cells	Addition	ATP	ADP	AMP
Endothelium	Mg^{2+} + EGTA	118	99	100
	EDTA	80	57	64
Smooth muscle	Mg^{2+} + EGTA	103	99	100
	EDTA	63	24	83

Table 2. Incubation of vascular-cell ectonucleotidase activities

Cultured vascular cells were incubated for 5, 15 or 20 min with $500\mu\text{M}$ -adenine nucleotide, as described in the text, with or without the addition of the compounds listed below (each at $500\mu\text{M}$). The results are percentages of the amount of metabolism in control samples (no added compound) and are means of duplicate determinations. The average range about the mean values was ± 8 . Abbreviation: PNPP, *p*-nitrophenyl phosphate.

Cells	Addition	ATP	ADP	AMP
Endothelium	β -Glycerophosphate	88	99	100
	PNPP	97	98	115
	$\text{p}[\text{CH}_2]\text{pA}$	138	99	<4
	Adenosine	97	95	
	AMP + PNPP	124	92	
	ADP + PNPP	120		
Smooth muscle	β -Glycerophosphate	99	95	102
	PNPP	91	76	83
	$\text{p}[\text{CH}_2]\text{pA}$	100	102	<1
	Adenosine	132	101	97
	AMP + PNPP	114	61	
	ADP + PNPP	121		

ferred saline (7.9mM-P_i). β -Glycerophosphate or *p*-nitrophenylphosphate had little or no effect at a concentration of $500\mu\text{M}$ (Table 2). Also, there was very little product inhibition (Table 2). Catabolism

was, however, inhibited by the addition of the other corresponding purine nucleotide (e.g. ATP catabolism was inhibited by GTP or ITP), as shown in Table 3.

Table 3. *Inhibition of vascular-cell ecto-(adenine nucleotidase) activities by other purine nucleotides*

Cultured vascular cells were incubated for 15 min as described in the text with ATP, ADP or AMP, with or without the addition of guanosine phosphates or inosine phosphates as listed below. The results are percentages of the amount of metabolism in control samples (no added compound) and are means of duplicate determinations. The average range about the mean values was ± 5 .

Addition	Endothelium	Smooth muscle
500 μM -ATP + 1 mM-ITP	66	58
500 μM -ATP + 1 mM-GTP	65	36
500 μM -ADP + 1 mM-IDP	48	25
500 μM -ADP + 1 mM-GDP	35	58
100 μM -AMP + 1 mM-IMP	42	28
100 μM -AMP + 1 mM-GMP	30	22

Fig. 4. *Inhibition of vascular-cell ectonucleotidase activities by synthetic adenine nucleotide analogues*

(a) Cultured vascular cells were incubated as described in the text for 20 min with AMP (30 μM for endothelial cells, $\blacksquare$; 100 μM for smooth-muscle cells, $\square$) with or without various concentrations of p[CH₂]pA; 1 mM-adenosine was present in all incubations. Results are means of three observations. The ordinate axis shows the percentage of the amount of catabolism in control samples (no added p[CH₂]pA). (b) Cultured vascular cells (closed symbols, endothelium; open symbols, smooth muscle) were incubated as described in the text for 15 or 20 min with 500 μM -ATP ($\bullet$, $\circ$), -ADP ($\blacktriangle$, $\triangle$) or -AMP ($\blacksquare$, $\square$) with or without various concentrations of p[NH]ppA: 1 mM-adenosine was present in all incubations. Results are means $\pm$ S.E.M. (four observations) where error bars are shown, or means of duplicates. The ordinate axis shows the percentage of the amount of catabolism in control samples (no added p[NH]ppA).

We tested the inhibitory effects of two synthetic adenine nucleotide analogues in an attempt to discriminate further between the ectonucleotidase activities. p[CH₂]pA did not inhibit the catabolism of ATP or ADP by either cell type, but was an extremely potent inhibitor of AMP catabolism (Table 2; Fig. 4a) with K_i values approx. 0.1 μM . p[NH]ppA had little inhibitory effect on ATP catabolism by endothelial or smooth-muscle cells

($K_i \gg 500 \mu\text{M}$); in contrast, catabolism of ADP was powerfully inhibited ($K_i < 50 \mu\text{M}$ for each cell type). p[NH]ppA also inhibited, with intermediate potency, catabolism of AMP (Fig. 4b).

The following compounds had no effect on adenine nucleotide catabolism in either cell type; P^1 , P^5 -bis-(5'-adenosyl)pentaphosphate (up to 500 μM), ouabain (1 mM), the cell-penetrating thiol reagent *N*-ethylmaleimide (up to 100 μM) and the non-

Fig. 5. Conversion of exogenous ADP into ATP by cultured endothelial cells

[^3H]ADP ($1\mu\text{M}$) was incubated with endothelial cells, as described in the text, either alone (●) or with (Δ) $1\mu\text{M}$ - or ($\circ$) $10\mu\text{M}$ -ATP added at the same time; $10\mu\text{M}$ -dipyridamole was present in all incubations. Each point is the mean of duplicates. Continuous lines show amounts of [^3H]ADP, dashed lines [^3H]ATP.

penetrating reagent *p*-chloromercuribenzenesulphonic acid (up to $100\mu\text{M}$).

Nucleoside diphosphate kinase activity

During our studies on the substrate specificity of nucleotidase activity, the possible inhibitory effect of ATP on ADP catabolism was investigated and yielded an unexpected result. Added ATP stimulated endothelial ADP metabolism, with this stimulation being greater at 5 min than 10 min, presumably because of concomitant catabolism of the added ATP. When the distribution of ^3H was studied, however, it was found that the increment in [^3H]ADP metabolism was exactly balanced by the formation of [^3H]ATP (Fig. 5). Thus endothelial cells possess an ectoenzyme with nucleoside diphosphate kinase (EC 2.7.4.6) activity. We have subsequently obtained similar results with smooth-muscle cells. In contrast, neither cell type possessed detectable ectoenzymic activity capable of converting [^3H]AMP into [^3H]ATP [i.e. adenylate kinase (EC 2.7.4.3) or nucleoside monophosphate kinase (EC 2.7.4.4)]. Nucleoside diphosphate kinase activity was not affected by $100\mu\text{M}$ -hydroxylamine, which has been reported to inhibit a similar

ectoenzymic activity on blood platelets (Mustard *et al.*, 1975).

Discussion

Endothelial cells and smooth-muscle cells possess Mg^{2+} -stimulated ectoenzymes that degrade exogenous ATP, ADP and AMP. We found no evidence that ATP was directly degraded to AMP, which suggests that these cells possess no ecto(ATP-pyrophosphatase) (EC 3.6.1.8). Neither AMP nor adenosine was deaminated extracellularly. Although the apparent affinities of the smooth-muscle ectoenzymic activities were similar for ATP, ADP and AMP (K_m approx. $100\text{--}150\mu\text{M}$), the affinities of the endothelial enzymes differed (K_m for ATP approx. $350\mu\text{M}$, for ADP approx. $125\mu\text{M}$ and for AMP $20\text{--}50\mu\text{M}$) and there was a significantly lower V_{max} for endothelial AMP catabolism than for any other activity. Little of these ectoenzymic activities was due to the presence of non-specific phosphatases. AMP catabolism by both cell types was potently and specifically inhibited by $p[\text{CH}_2]\text{pA}$, which is generally recognized as an inhibitor of 5'-nucleotidase (Burger & Lowenstein, 1975). Also, in both cell types $p[\text{NH}]\text{ppA}$ was a much more potent inhibitor of ADP than of ATP catabolism, and ADP did not inhibit ATP catabolism, strongly suggesting that separate enzymes were responsible for ATP and ADP dephosphorylation. Finally, studies with phosphates of inosine and guanosine demonstrated that the corresponding purine nucleotide inhibited catabolism of the adenine nucleotide. Thus both endothelial and smooth-muscle cells in culture possess, as distinct Mg^{2+} -stimulated ectoenzymes, nucleoside triphosphatase (EC 3.6.1.15, rather than ATPase, EC 3.6.1.3), nucleoside diphosphatase (EC 3.6.1.6) and 5'-nucleotidase (EC 3.1.3.5).

Two main questions arise from these observations: how do our results compare with those previously obtained for vascular or other cell types, and what may be the physiological relevance of such ectonucleotidases?

Previous observations on ectonucleotidases

Ecto-ATPase activity has been detected on blood platelets, granulocytes, mast cells and several cell types in culture, including endothelial, smooth-muscle, neural and hepatic cells (Chambers *et al.*, 1967; Tenney & Rafter, 1968; Trams & Lauter, 1974; Dieterle *et al.*, 1978; Dosne *et al.*, 1978; Chakravarty & Echetebe, 1978). Although most of these studies excluded the possibility that ATP was catabolized by non-specific phosphatases, there was little attempt to characterize substrate specificity further; likewise there is little kinetic information, although in the granulocyte, for example, K_m values

of approx. $50\mu\text{M}$ have been reported, where the enzyme activity has been distinguished from non-specific ectophosphatase activity which is also present (De Pierre & Karnovsky, 1974; Smolen & Weissmann, 1978).

There is more information about 5'-nucleotidase; the kinetics and substrate specificity of this ecto-enzyme have been characterized on granulocytes, lymphocytes, cultured neural cells and isolated fat-cells (De Pierre & Karnovsky, 1974; Dornand *et al.*, 1978; Stefanovic *et al.*, 1976; Newby *et al.*, 1975). K_m values of approx. $20\mu\text{M}$ have usually been found, and potent selective inhibition by $p[\text{CH}_2]_p\text{A}$. More recently, Hayes *et al.* (1979) noted, in agreement with our results, that 5'-nucleotidase activity of cultured porcine endothelial cells was markedly lower than in cultured smooth-muscle cells. Because they have found that endothelial 5'-nucleotidase activity was higher and similar to that of smooth muscle in freshly isolated cells, they suggested that the low activity found after culture was due to some effect of the culture environment on endothelium (but not smooth muscle). It seems equally plausible, however, that the enzymic treatment used to isolate their endothelial (but not smooth-muscle) cells transiently stimulated activity, as has been found for prostaglandin synthesis (Ager *et al.*, 1979).

There is surprisingly little information on ecto-ADPase activity; although the degradation of ADP to adenosine by cultured vascular cells and isolated vascular tissue has been noted (Lieberman *et al.*, 1977; Heyns *et al.*, 1977; Habliston *et al.*, 1978; Dosne *et al.*, 1978; Cooper *et al.*, 1979), there has been virtually no attempt to investigate the substrate specificity or other characteristics of this enzyme. Furthermore, we are not aware of any studies of ecto-ADPase activity on other cells.

Physiological role

It was recognized over 10 years ago that circulating ATP, ADP and AMP were inactivated by catabolism, not predominantly by blood cells or plasma enzymes, but possibly by vascular cells (Baer & Drummond, 1968; Brashear & Ross, 1969). Since then it has been shown that in perfused organs (particularly the lung) each nucleotide can be catabolized without cellular uptake (Ryan & Ryan, 1977; Crutchley *et al.*, 1978), and 5'-nucleotidase activity has been cytochemically localized to the plasma membrane of endothelium in lung, heart and skeletal muscle (Nakatsu & Drummond, 1972; Rubio *et al.*, 1973; Ryan & Ryan, 1977). It therefore seems that the ectoenzymes that we have characterized on cultured vascular cells are present *in vivo*.

Adenosine and adenine nucleotides affect three important biological processes: neurotransmission,

blood-platelet function (and therefore haemostasis and thrombosis) and vasodilation. The ectonucleotidases that we have described could be of importance in regulating each of these three processes. Firstly, both ATP and adenosine have been postulated as neurotransmitters; ATP is released from nerve endings, and both compounds can modulate the response to other transmitters (see Burnstock, 1979; Paton, 1979). The model for purinergic transmission proposed by Burnstock (1972) requires the presence of ectonucleotidases, although he did not envisage these as a complete ectoenzymic system on the effector organ (i.e. smooth muscle).

Secondly, ADP induces platelet aggregation, whereas adenosine is inhibitory (Haslam & Rosson, 1975). The role of the ecto-ADPase activity on endothelium was first envisaged as contributing to its antithrombotic properties (Heyns *et al.*, 1974; Lieberman *et al.*, 1977; Glasgow *et al.*, 1978), but this view has been challenged by Bunting *et al.* (1977), who found that endothelial cells were not antithrombotic in a blood platelet bioassay system if their capacity to synthesize prostaglandins was blocked. We subsequently showed that endothelial cells can be stimulated to release nucleotides (particularly ATP) selectively in such a system, producing sufficient ADP (through ecto-ATPase activity) to aggregate blood platelets (Pearson & Gordon, 1979). Thus the net effect on thrombogenesis of a complete endothelial ectonucleotidase system (and also of nucleoside diphosphate kinase) is difficult to predict, especially as the relative proportions of nucleotides released will vary with their source; platelet degranulation releases ATP and ADP in approximately equal amounts (Mills *et al.*, 1968), whereas ATP is the major nucleotide released from damaged tissue.

Thirdly, both ATP and adenosine are powerful vasodilators (Burnstock, 1978). Adenosine has been proposed as a local hormone with particular reference to coronary vasoregulation, where it appears in the myocardial tissue and blood in response to hypoxia (for review see Berne *et al.*, 1979). ATP is also found (Paddle & Burnstock, 1974), but the sources of ATP and adenosine and the mechanisms by which they are released are not known. It has been supposed that myocardial cells regulate vascular tone by the release of adenosine in response to hypoxia, but the possibility that selective release of ATP by these cells occurs, with subsequent catabolism to adenosine by ectonucleotidases, has apparently not been rigorously tested. The release of adenosine itself from cells (whether cardiocytes or endothelium) in response to a stimulus such as hypoxia would require a dramatic change in intracellular adenine nucleotide metabolism, because the intracellular concentration of adenosine is normally extremely low. Outward

transport of adenosine would then be needed, presumably by the uptake process operating in reverse. At present there is no evidence that strongly supports this; adenosine concentrations in myocardial cells are very low even when extracellular concentrations are high (Mustafa *et al.*, 1975), and inhibitors of adenosine transport do not prevent the appearance of adenosine outside myocardial cells *in vitro* (Mustafa, 1979); also they do not block, but potentiate post-hypoxic reactive hyperaemia *in vivo* (Parratt & Wadsworth, 1972).

The alternative hypothesis merits consideration; Trams (1974) introduced the general concept of ATP translocation across cell membranes, and we have shown that selective release of ATP from vascular cells can occur (Pearson & Gordon, 1979). It is clear that the selective release of a very small proportion (<1%) of ATP from myocardial or vascular cells with catabolism to adenosine by ectonucleotidases could account for the amounts of adenosine found in blood after hypoxia. The vascular cells' ectonucleotidase system together with their adenosine-uptake system could therefore form a complete cycle for the local control of vascular tone by adenosine: release of precursor ATP (which could have short-lived vasodilatory activity itself), catabolism to active hormone, and termination of action by uptake.

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