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EDITORIAL

The vascular endothelium: a survey of some newly evolving biochemical and physiological features

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Summary

The morphological, biochemical and functional characterization of the vascular endothelium has become possible through the broad use of electron microscopic methods, the successful elaboration and application of techniques for the isolation and cultivation of endothelial cells in vitro and through sophisticated studies on vessel and organ preparations, both in vitro and in vivo. In this survey emphasis is placed on certain methodological aspects of endothelial cell culture as well as on biochemical, physiological and pathophysiological features of the vascular endothelium.

Endothelial cells can be propagated in culture dishes, the most commonly applied method, on suspended microbeads (dextrane, polyacrylamide), a technique giving large yields, or on thin porous membranes, a procedure suited for the study of transport processes across the endothelial layer.

Different structural, biochemical and functional properties of the luminal (apical) and abluminal (basal) cell membrane determine important polarity features of the endothelium. Endothelial cells exhibit a variety of biochemical pathways and are characterized by high metabolic activities. Of particular interest is the large content of ATP in endothelial cells of different vascular origin. The rapid intracellular degradation of adenine nucleotides to nucleosides and bases, which are constantly released, is balanced by synthesis, mainly via salvage pathways. In endothelial cells of microvascular origin uric acid predominates by far as the final purine degradative because of the presence of xanthine dehydrogenase in these cells; in the macrovascular endothelium purine breakdown proceeds only to hypoxanthine, since xanthine dehydrogenase is lacking. In this connection interrelations between nucleotide catabolism in myocardial tissue and in coronary endothelial cells are discussed, also with respect to the participation of endothelial xanthine oxidase in the formation of oxygen radicals during post-ischemic reperfusion of the heart.

Vascular endothelial cells of different origin are also capable of a rapid extracellular degradation of ATP, ADP and AMP to adenosine by means of specific ecto-nucleotidases. The subsequent fate of extracellularly formed adenosine appears to be different for endothelial cells of microvascular (preferential adenosine uptake) and macrovascular origin (preferential extracellular adenosine accumulation), thus implying functional consequences for platelet aggregation.

Experimentally well supported aspects of endothelial functions under physiological and pathophysiological conditions include:

- the involvement of metabolic properties of the endothelium in the separation of the intra- and extravascular space (barrier function, e.g. intraendothelial trapping of adenosine, active participation in leukocyte emigration);
- the facilitation of CO_2-release in the lung (endothelial carboanhydrases);

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- the participation in the regulation of vascular resistance (formation of angiotensin II and degradation of bradykinin by means of angiotensin converting enzyme, formation of not yet identified endothelium derived relaxing factor(s) [EDRF] in response to various intraluminally present vasodilating substances);
- the establishment of an antithrombogenic luminal surface of the vessel wall (release of PGI_2, adenosine, antithrombin III and plasminogen activator, intravascular degradation of adenine nucleotides to adenosine by endothelial ecto-nucleotidases, activation of protein C by endothelial thrombomodulin, heparan and antithrombin III containing endothelial glycocalyx).

Key words: adenosine, antithrombogenic properties, angiotensin converting enzyme, cell culture, EDRF, ecto-nucleotidases

Introduction

Until the middle of this century the vascular endothelium was generally regarded as a homogenous, more or less unstructured cell lining of blood vessels, serving rather passive functions, such as separation of the intravascular from the extravascular space and the provision of a smooth gliding surface for the streaming blood. Physiologists and biochemists did not pay particular interest to the endothelium, partly because of a lack of experimental procedures suitable for functional and analytical studies on these cells in situ and partly because endothelial cells were not at all expected to exhibit particular functional or metabolic activities. Moreover, the very small amount of endothelium within the blood vessels of an organ made it seem unlikely that this tissue could contribute significantly to global organ function and metabolism.

Two major developments initiated a complete reappraisal of the role of the vascular endothelium. Electron microscopical investigations of the vessel wall, first carried out by Palade in 1953 on capillaries (32), revealed the great variety and ultrastructural complexity of the endothelial cells. Isolation and cultivation of endothelial cells, first successfully performed by Jaffe et al. in 1973 (23), opened up new perspectives for research, since endothelial cells had thus become accessible to detailed studies of their specific metabolic and functional properties. Today it is possible to cultivate endothelial cells from almost every type of vessel and species including human and it has been repeatedly demonstrated that these cells retain their essential morphological, biochemical, immunological and functional characteristics over several passages. In view of the methodological progress it is not surprising that the number of publications dealing with various features of the vascular endothelium increases from year to year.

Endothelial cells from arterial and venous vessels and from capillaries are now recognized to differ not only in their morphology and ultrastructure, but also in biochemical respects. Furthermore, they display organ-specific characteristics. On the other hand, the individual cells of the entire vascular endothelium exhibit some common features, such as a typically high surface to volume ratio and an intense metabolism. Therefore, the endothelium as a whole does not only represent a very large surface area for transport and exchange processes but must also be regarded as an important tissue compartment of substantial metabolic activity. For a person of 70 kg body weight for instance, one can estimate that the total luminal surface of the vascular bed amounts to about 700 m^2 (41) and the total endothelial weight to 1000–1500 g (9, 16) which is comparable to that of the liver. As suggested by this rough estimate, the above-mentioned former concept concerning a negligible contribution of endothelial to organ metabolism needs to be completely revised.

In the following it is intended to briefly outline principal steps and some techniques of endothelial cell culture. Then a survey of important biochemical properties of the

endothelium, particularly with respect to its adenine nucleotide metabolism, will be given, followed by a discussion of recent findings pertaining to the regulatory or contributory role of the endothelium in some physiological and pathophysiological phenomena.

I *Culture of endothelial cells* (22, 25, 27)

Generally endothelial cells are isolated from vascular tissues by enzymatic dissociation; the most commonly used proteases are collagenase, trypsin and pronase. The exact procedures differ depending on the vessel type and whether isolated vessels or isolated organs are used as sources.

Usually the harvested endothelial cells need to be separated from contaminating smooth muscle cells, fibroblasts and cell debris. The most appropriate technique for this purpose is density gradient centrifugation (percoll, ficoll, albumin), which takes advantage of the low density of endothelial cells in comparison to contaminating cells.

The purified endothelial cells are grown in culture media of complex composition (M 199; Eagle's minimal essential medium and others), mostly supplemented with fetal calf serum and antibiotics/antimycotics and in certain cases with growth factors. Cell propagation in culture dishes is the most frequently applied procedure. Depending on the initial number of cells seeded a confluent monolayer is established on the bottom of the dishes within several days to weeks. According to our experience, endothelial cells of microvascular origin, such as cells from the coronary system of guinea-pig hearts, grow faster than those of larger

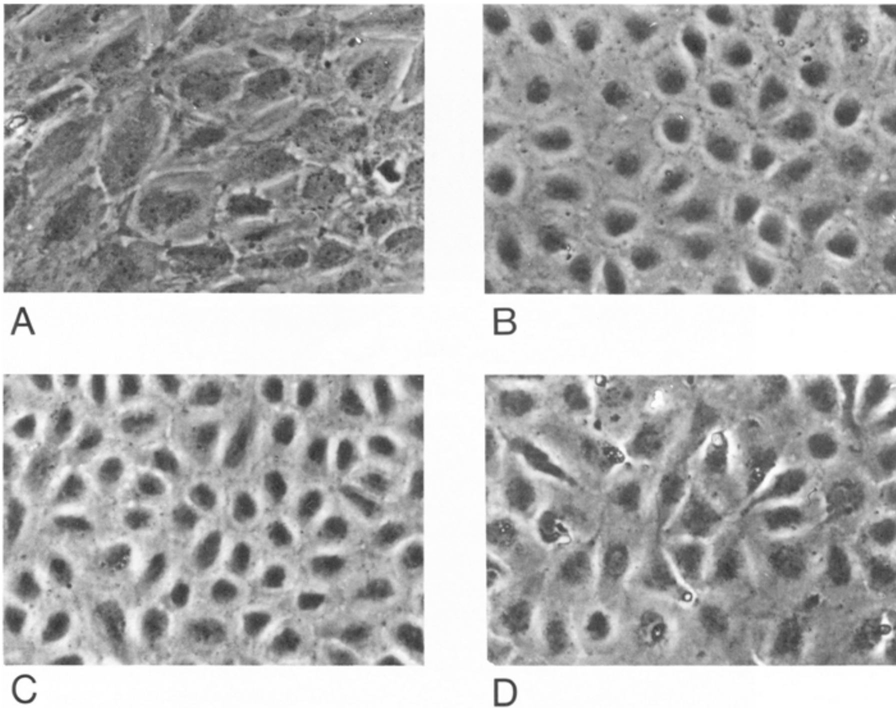

Fig. 1. Phase contrast microscopic views of confluent monolayers of endothelial cells grown in culture dishes. A: Microvascular endothelial cells from the coronary system (guinea-pig). B: Aortic endothelial cells (pig). C: Pulmonary artery endothelial cells (pig). D: Vena cava endothelial cells (pig).

(aorta and pulmonary artery, pig); venous endothelial cells (vena cava, pig) display the slowest rate of propagation (unpublished observations).

Figure 1 depicts confluent monolayers of the above mentioned endothelial cell types grown in culture dishes under identical conditions. Obviously, there are rather striking differences in size and shape between cells of microvascular and those of macrovascular origin. The establishment of monolayers is in all cases the result of the well-known phenomenon of contact inhibition. It is interesting to note that a destruction of single cells of the monolayer relieves contact inhibition, initiating limited divisions of the neighbouring cells and thus a quick repair of the lesion. In this respect endothelial cells in culture behave like the vascular endothelium in situ.

Another technique for growing endothelial cells in vitro makes use of polyacrylamide or dextrane microbeads (diameter about 100 μm). By suspending a great number of these microcarriers in a rather small volume of culture medium an enormous surface area can be created, to which endothelial cells adhere easily. After their attachment the cells proliferate until confluency is reached on each bead (Fig. 2). The major advantage of this method is the

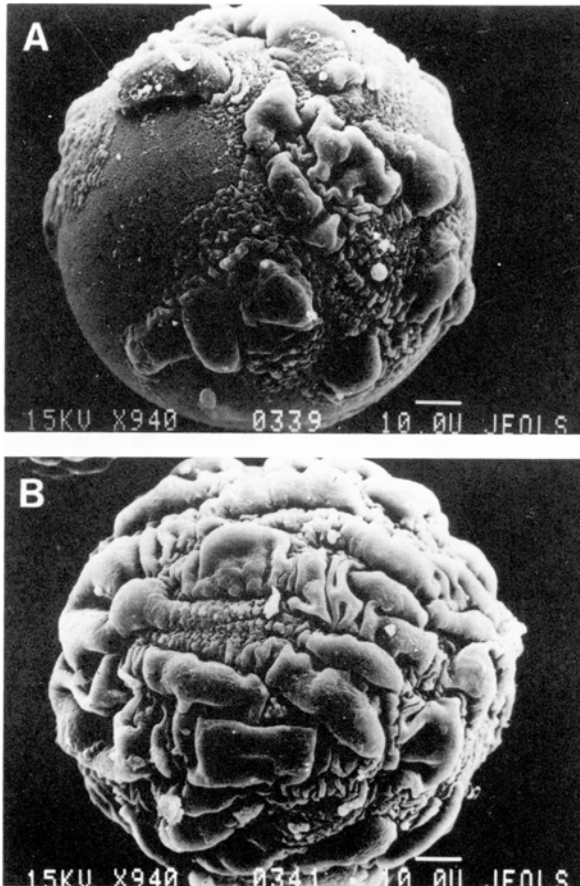

Fig. 2. Electron microscopic scanning view of microbeads (CYTODEX, Pharmacia) with either a partly or fully confluent monolayer of aortic endothelial cells (pig).

high yield of cells. In our laboratory an apparatus has been developed which allows endothelial cells to be grown on a 1 g batch of microbeads with a total surface of about 6000 cm^2. The final harvest of approx. 0.3 g of cells corresponds to the yield from at least 600 culture dishes. Endothelium-covered microcarriers packed into glass capillaries (like filling a column for chromatography) can be superfused with rather small volumes of suitable media. The high ratio of cell surface to perfusate volume facilitates the determination of minute amounts of substances taken up or released by the endothelial cells.

Endothelial cells can also be grown to confluency on thin microporous membranes immersed in culture dishes. These membranes can then be mounted in a double chambered perfusion apparatus (ZPS, Firma E. Bühler, Tübingen) to separately analyze uptake or release processes at the apical (= luminal) and basal (= abluminal) surface of the endothelial layer (see also this volume, p. 522).

II *Some biochemical features of the vascular endothelium*

It is now well established that endothelial cells exhibit, besides a structural polarity, also a variety of biochemical and functional polarity characteristics (Table 1). The luminal surface, covered with a typical glycocalyx, is rich in certain ecto-enzymes of functional significance (see below) and possesses recently recognized receptors or binding sites for many vasoactive substances. Several compounds of physiological importance produced within the endothelial cells are released at the luminal side. A particular feature of the abluminal side of endothelial cells concerns the formation and liberation of components for the basement membrane. Myoendothelial contacts, mainly observed in resistance vessels, are a further characteristic of the basal side; still uncertain is the presence of receptors and the actual release of EDRF(s) (endothelium-derived relaxing factor(s)) on this side.

Another important insight concerns the fact that the vascular endothelium exhibits a high metabolic activity. Numerous routes of metabolism have been demonstrated to be present in endothelial cells in culture as well as in the endothelium in situ. These include the general

Table 1. Biochemical polarity characteristics of the vascular endothelial cell.

Luminal (apical) side		Abluminal (basal) side	
Glycocalyx:	Special glycoproteins and proteoglycanes	Glycocalyx:	Special glycoproteins and proteoglycanes
Ecto-enzymes:	ATPase, ADPase, 5′-nucleotidase, carboanhydrase, angiotensin converting enzyme, lipoprotein lipase	Basal membrane:	Collagen IV and V, fibronectin, laminin, entactin, von Willebrand factor etc.
		Myoendothelial contacts: (tight junctions)	Electronic coupling (?)
Receptors:	Acetylcholine, histamine, serotonin, catecholamines, bradykinin, thrombin, ATP, ADP, AMP, adenosine etc.	Receptors:	?
Release:	Adenosine, PGI_2, von Willebrand factor, antithrombin III, plasminogen activator, fibronectin, EDRF(s) of unknown structure	Release:	EDRF(s) of unknown structure (?)

pathways of carbohydrate, lipid, protein and nucleic acid metabolism, the latter being especially pronounced in accordance with the regenerative capacity of these cells. Interestingly, aerobic glycolysis and not oxydative phosphorylation seems to be the major route for energy production (11, 28). In addition to rather specialized pathways involved in the synthesis of several protein compounds released at the luminal or basal side, endothelial cells possess the machinery to specifically metabolize arachidonic acid to prostacyclin (PGI_2), a compound of great physiological significance.

Intracellular metabolism of adenine nucleotides in coronary endothelial cells

Studies on various features of endothelial adenine nucleotide metabolism, which is intimately related via the ATD-ADP system to the processes of energy consumption and production, have been performed in our laboratory mainly on microvascular coronary endothelial cells grown in culture (27). Data concerning contents of adenine nucleotides and their dephosphorylated degradatives in these cells and in myocardial tissue of guinea-pigs are listed in Table 2. Obviously the levels of adenine nucleotides – in particular ATP – are far greater in the endothelium than in the myocardium. To our knowledge, such high contents have not been reported for any other mammalian tissue except for platelets, in which, however, a large fraction of the adenine nucleotides is stored in metabolically inactive pools (20). Adenine nucleotide degradatives, such as adenosine, inosine and hypoxanthine are also present in greater amounts in the endothelium.

A further finding in support of a high adenine nucleotide turnover concerns the continuous release of dephosphorylated purine metabolites from cultured coronary endothelial cells. As shown in Figure 3a, not only adenosine, inosine and hypoxanthine, but foremost uric acid, the end-product of purine metabolism in most mammalian tissues (Fig. 4), are liberated at high rates. A qualitatively very similar pattern of purine release occurs in isolated perfused guinea-pig hearts (Fig. 3b), where uric acid is also the major degradation product (3). Quantitative differences in the respective release rates can be readily ascribed to the additional formation of purines in the myocyte compartment of isolated hearts.

The fact that uric acid is the predominating purine released from both coronary endothelial cells and isolated hearts deserves particular interest. Xanthine dehydrogenase, the enzyme responsible for the formation of uric acid from hypoxanthine and xanthine, is

Table 2. Contents of adenine nucleotides and their degradatives in cultured coronary endothelial cells and in ventricular tissue of isolated perfused guinea-pig hearts. Mean values, n = 5–9. Data taken from (28).

	nmol/g wet weight	
	Endothelial cells	Myocardium
ATP	11,900	4,280
ADP	2,100	1,050
AMP	230	160
$\sum$ ATP, ADP, AMP	14,230	5,490
Adenosine	72	1.9
Inosine	85	1.3
Hypoxanthine	50	0.8

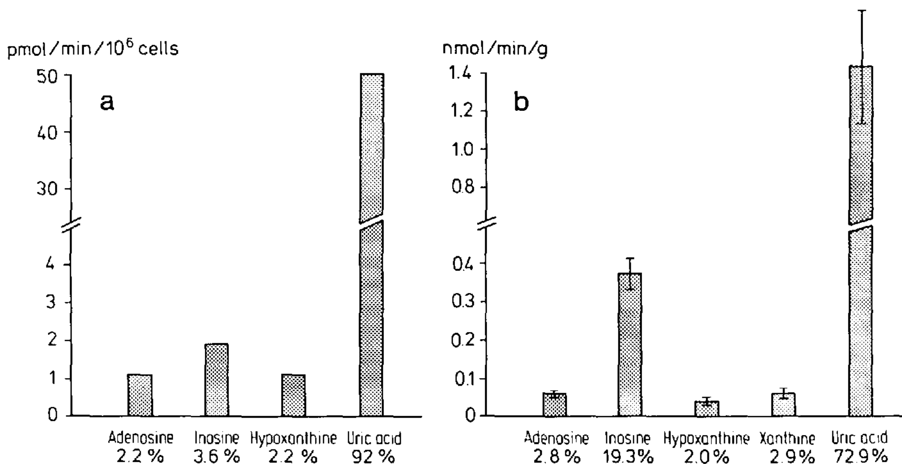

Fig. 3. a) Release of purine metabolites from cultured coronary endothelial cells (guinea-pig) into the culture medium. Mean values of 5 experiments (unpublished results). b) Coronary venous release of purine metabolites from isolated guinea-pig hearts perfused under normoxic conditions. Mean values ± SEM, n = 23. Data taken from (3).

localized within the myocardial tissue solely in the endothelium of the capillaries (24). According to our own observations cardiomyocytes of guinea-pig hearts are devoid of this enzyme. Therefore, uric acid formation can only occur in the microvascular endothelium, regardless of whether precursor substances are derived from the endothelium itself or from the cardiomyocytes. In the latter situation the precursors must obviously be taken up by the capillary endothelium, from which – after metabolization – they are then released into the intravascular space, mainly in the form of uric acid. From our experimental results and the interpretation given it thus appears that in myocardial tissue the nucleotide metabolism of the two compartments, vascular endothelium and cardiomyocytes, is intimately coupled.

The localization of xanthine dehydrogenase exclusively in the microvascular endothelium of the heart bears an additional pathophysiological implication. As has been shown for different organs, xanthine dehydrogenase becomes rapidly converted into xanthine oxidase under conditions of ischemia (2, 34). Xanthine oxidase also catalyzes the formation of uric acid from hypoxanthine and xanthine, but in this case toxic oxygen radicals are concomitantly formed. Such oxygen species are assumed to be causally related to myocardial damage on reperfusion (26). It thus seems possible that a xanthine oxidase-induced formation of oxygen radicals in the coronary microvascular system is one of the primary events eliciting the impaired postischemic reperfusion.

Since xanthine dehydrogenase is regarded to be a general marker enzyme of the capillary endothelium (24), one can presume that the catabolism of adenine nucleotides proceeds to uric acid not only in the coronary microvascular endothelium. On the other hand, it was recently shown in our laboratory (5, 6) that endothelial cells from large vessels such as aorta, pulmonary artery, and vena cava are devoid of xanthine dehydrogenase. In these cells degradation accordingly ceases with the formation of hypoxanthine (cf. Fig. 4).

From the data given in Table 2 and Figure 3a it can be estimated that the total release of purine nucleosides and bases from coronary endothelial cells in culture per minute amounts to about 1% of the adenine nucleotide pool. Since this extraordinarily rapid rate of

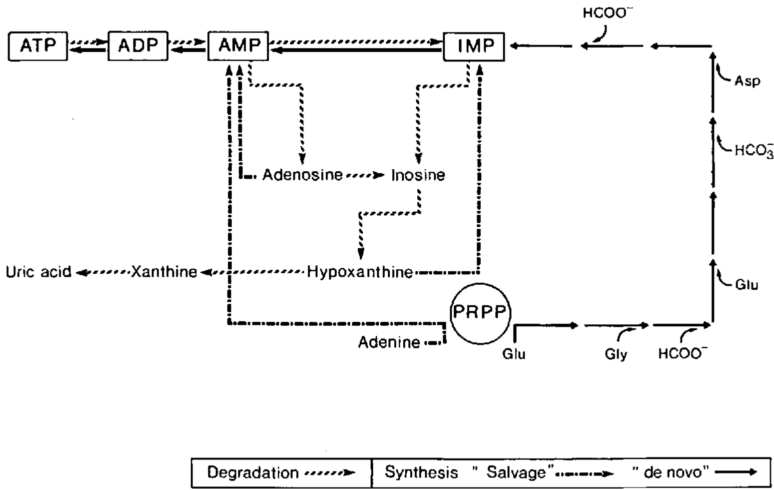

Fig. 4. Pathways of adenine nucleotide degradation and synthesis (PRPP = 5-phosphoribosyl-1-pyrophosphate, Glu = glutamine, Gly = glycine, Asp = aspartate).

nucleotide catabolism does not lead to a reduction of nucleotide levels, the cells must avail themselves of potent mechanisms of nucleotide synthesis.

As depicted in Figure 4, synthesis of nucleotides can be accomplished by utilization of preformed purine precursors such as adenosine, hypoxanthine and adenine ("salvage pathways"), or by a multistep process in which smaller precursors such as amino acids and formate are utilized at considerable energetic cost ("de novo synthesis"). Recently, the rate of de novo synthesis in cultured coronary endothelial cells has been determined by applying specially elaborated microanalytical methods in combination with the use of radioactive formate; in addition, salvage of adenosine and adenine has been measured under comparable conditions (10). As is evident from the data summarized in Figure 5, the capacity of the endothelium for de novo nucleotide synthesis is small (about 10 %) in comparison to the

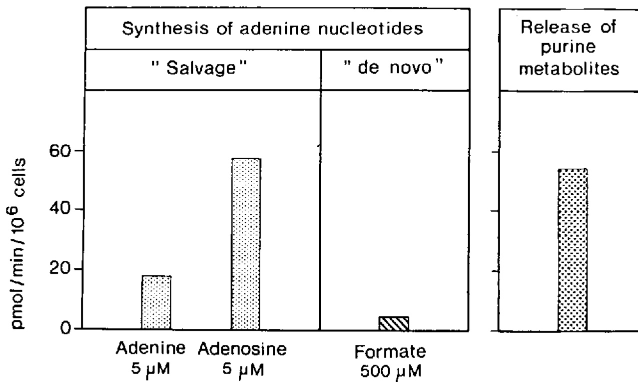

Fig. 5. Rates of salvage and de novo synthesis of adenine nucleotides as well as of purine metabolite release in cultured coronary endothelial cells (guinea-pig heart). Data taken from (10).

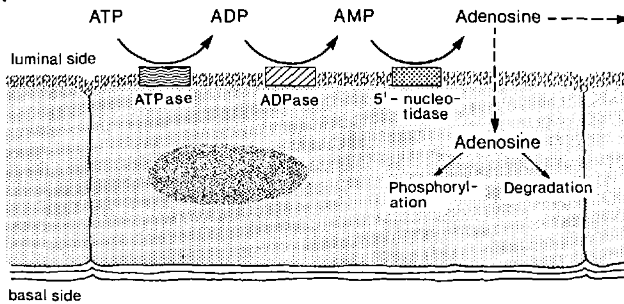

Fig. 6. The ecto-nucleotidase cascade of the luminal endothelial surface. For details concerning the fate of the extracellular end product adenosine see text.

total rate of release of purine metabolites. In contrast, salvage of adenosine appears to be far more efficient, since at a concentration of 5 μM it fully compensates the purine loss. Adenine proved to be less effective at the same concentration. It can thus be concluded that the coronary microvascular endothelium mainly depends on the salvage of purine precursors for the maintenance of constant nucleotide levels.

Extracellular metabolism of adenine nucleotides

The presence of ecto-nucleotidases on the luminal surface of the endothelial cells has been briefly mentioned (cf. Table 2). As is shown in Figure 6, the so-called ecto-nucleotidase cascade, consisting of an ATPase, an ADPase and a 5'-nucleotidase, catalyses the stepwise rapid extracellular degradation of ATP, ADP or AMP to adenosine. The adenosine thus formed can either accumulate extracellularly or it can be taken up by the endothelium, where it is phosphorylated to build up intracellular nucleotides (salvage pathway) or degraded stepwise to hypoxanthine and uric acid, respectively (cf. Fig. 4). Both extracellular accumulation and intracellular metabolism of adenosine originating from the nucleotidase cascade are realized, depending on whether endothelial cells of micro- or macrovascular origin are involved (5, 6). According to results of representative experiments depicted in Figure 7, adenosine accumulates extracellularly in substantial amounts only when ATP is

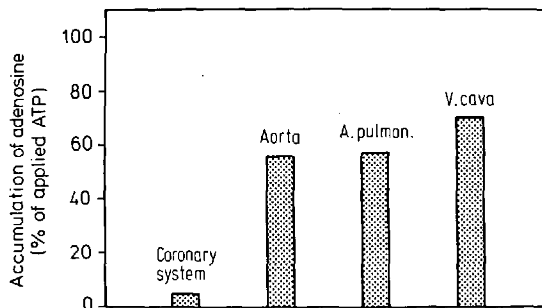

Fig. 7. Extracellular accumulation of adenosine upon addition of ATP to the culture medium of microvascular coronary endothelial cells (guinea-pig) and macrovascular endothelial cells from aorta, pulmonary artery and vena cava (pig). Initial ATP concentration 10^{-5}M = 100%; incubation time 60 minutes. Mean of 2 experiments each. Data taken from (5).

dephosphorylated by endothelial cells from large vessels. Principally the same results were obtained on ATP-perfused isolated segments of the aorta and vena cava from pigs (unpublished results). Neither in cultures of microvascular coronary endothelial cells, nor in isolated perfused hearts of guinea-pigs was the extracellular dephosphorylation of ATP accompanied by a comparable accumulation of adenosine. Hence, it seems justified to assume that dephosphorylation of adenine nucleotides in contact with the luminal endothelial surface of large vessels will also in situ lead to a pronounced increase in the concentration of adenosine in the immediate vicinity of the vessel wall. The possible functional importance of this particular endothelial characteristic is discussed below.

III *Certain physiological and pathophysiological aspects of the endothelium*

The following discussion of endothelial functions is limited to some experimentally well-founded newer aspects. These encompass the involvement of metabolic properties of the endothelium in the separation of the intra- and extravascular space (metabolic barrier), the facilitation of pulmonary CO_2-release, the participation in the regulation of vascular resistance and the establishment of an antithrombogenic luminal surface of the vessel wall.

Barrier function

The endothelium does not only serve as a physical barrier between the vascular and extravascular spaces owing to its well-known membrane and structural properties. On account of its only recently recognized high metabolic activities the endothelium can also create a metabolic barrier. Various substances, e.g. nucleotides or lipoproteins, are degraded by ecto-enzymes at the luminal surface; therefore these substances as such do not reach the extravascular space via the endothelial cells. Other compounds, although taken up by the endothelium, can be trapped intracellularly by metabolic conversion. This holds true for instance for adenosine, which, when applied in vasoactive concentrations to the coronary system, cannot cross the endothelial barrier to directly reach the smooth muscle of the vessel wall (28). Consequently, vasodilation induced by intraluminal adenosine cannot be caused by the nucleoside itself (see below and Nees et al., this volume, pp 515–529). Endothelial metabolism is indirectly involved in the emigration of various types of leukocytes from the blood to the tissue, especially under conditions of inflammation. After an initial adhesion of the leukocytes to the endothelial surface induced by leukotriene B_4 or complement C_{5a} (21, 39), intercellular junctions are opened by active contraction of endothelial actin and myosin filaments in response to mediators released from the leukocytes. This then allows outward passage of leukocytes to the extravascular space.

CO_2 release in the lung

It has long been realized that the brief contact between blood and the gas exchanging surface of the lung cannot allow for sufficient release of CO_2, if the process is only catalyzed by the carboanhydrase/anion exchange system of the red blood cells. In recent years evidence was first obtained for the existence of a carboanhydrase also in the pulmonary vessels (8, 12). The pulmonary carboanhydrase is now known to be localized specifically in the vascular endothelium as a cytosolic enzyme and a membrane-bound ecto-enzyme (35, 36). Both these endothelial enzyme forms are presently considered to significantly add to the formation of CO_2 from plasma bicarbonate within the pulmonary microcirculation and thus to enhance CO_2 equilibration in the lungs.

Participation in the regulation of vascular resistance

Two principal mechanisms by which the endothelium can influence the vascular smooth muscle tone have become apparent. For about 10 years it has been known that angiotensin converting enzyme (kininase II) is localized as an ecto-enzyme on the luminal surface of the vascular endothelium (7), particularly high activities of this endothelial marker enzyme being found in the pulmonary vessels (36, 37, 38). Angiotensin converting enzyme catalyzes the formation of vasoconstrictory angiotensin II from angiotensin I; in addition, it breaks down the vasodilatory peptide bradykinin to vasoinactive degradation products. The net result of these enzymatic processes at the luminal surface of the microvessels in the lung is an increase in systemic arterial tone, predominantly induced by angiotensin II, but possibly also enhanced by the relief of the bradykinin effects. Conversely, inhibition of the converting enzyme by drugs, such as captopril, enalapril or ramipril, leads to a fall in aterial tone, primarily through a reduced formation of angiotensin II, but perhaps also partly as a result of higher bradykinin levels. The other mechanism by which the endothelium may induce relaxation of the vascular smooth muscle was first discovered by Furchgott und Zawadski in 1980 on isolated vessel preparations. Acetylcholine was found to relax rings and strips of rabbit thoracic aorta only if the endothelium was intact and it was concluded that relaxation was brought about by a mediator substance released from the endothelial cells (18). Since then many other vasodilating substances have been shown by numerous authors to exhibit the same or a similar kind of endothelium-dependent relaxation in various types of isolated vessels from different species (see review by Furchgott 1984 (17)).

A simplified concept concerning the mediator role of the endothelium for inducing relaxation of the vascular smooth muscle in response to intraluminal application of a variety of agents is presented in Figure 8. Depending on the type and origin of the vessel many

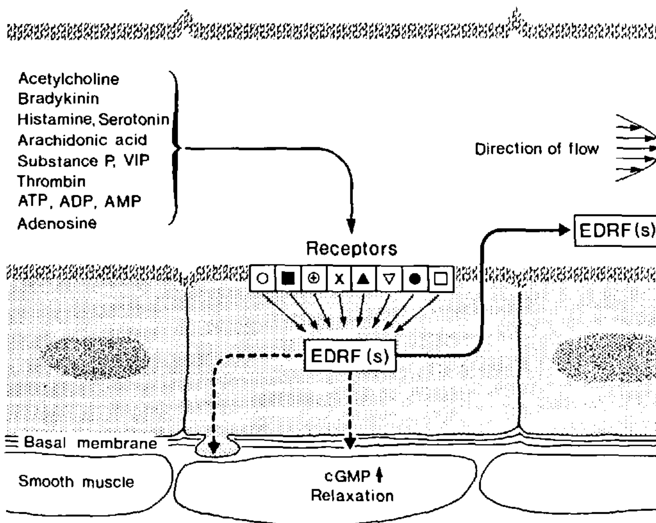

Fig. 8. Schematic illustration of essential steps involved in the endothelium-mediated relaxating of vascular smooth muscle induced by a variety of vasodilating substances at the luminal surface. The endothelium-derived relaxing factor (s) [EDRF(s)] is assumed to act at the abluminal side of the endothelial cell on the smooth muscle (dashed arrows), either directly or via myoendothelial contacts. EDRF(s) release into the intravascular space is well established (thick arrow). For further details see text.

substances, including acetylcholine, bradykinin, histamine, serotonin, thrombin, adenine nucleotides, adenosine and others, interact with receptors on the luminal surface. This somehow results in the formation of a short-lived substance of unknown chemical structure in the endothelial cell, which is termed the endothelium-derived relaxing factor (EDRF). This or these EDRF(s) are supposed to act at the basal side of the endothelial cell onto the smooth muscle cell, in which an increase in cGMP levels and relaxation occurs.

As yet it has not been proven directly that the assumed EDRF release at the abluminal side actually takes place. Conceivable would also be an EDRF-induced electrotonic transmission via myoendothelial contacts (Fig. 8). On the other hand, it has been repeatedly demonstrated that the EDRF is released at the luminal side. The functional significance of a release into the intravascular space, however, appears questionable, especially when taking into account the brief half-life time of the EDRF and its rapid inactivation in the plasma (15, 19).

Further reservations need to be applied with respect to the scheme in Figure 8. First of all, EDRF mediated relaxations have been demonstrated mostly in isolated preparations of large vessels and seldom in situ (1). Comparable studies on small resistance vessels are still lacking, except for endothelium-mediated vasodilating effects of adenosine in the coronary system of guinea-pig hearts (28, 29); see also this volume, (p. 525). Secondly, a generalization of the EDRF mechanism is not at all possible: On the one hand, large vessels from different segments of the vascular tree in one and the same species can react differently, either quantitatively or qualitatively, to a given vasoactive substance. On the other hand, the reactivity of the same vessel type varies considerably from species to species.

In view of the briefly outlined restrictions and quite a number of unclarified details in connection with the EDRF mechanism no estimates can be made in regard to the extent of EDRF mediated changes of the vascular tone in the intact circulatory system under physiological conditions. Nevertheless, endothelium-induced responses of the vasculature, be they angiotensin converting enzyme- or EDRF-dependent, must be seriously taken into account as regulatory mechanisms.

Antithrombogenic properties of the vascular endothelium (4, 30, 31)

The luminal surface of blood vessels is known to exhibit distinct antithrombogenic features. Endothelial antithrombogenic properties include all factors and enzymatic mechanisms capable of interfering with platelet aggregation and blood coagulation, and – in a wider sense – of activating fibrinolysis. The most important facets of these interactions are outlined in Figure 9.

Aggregating platelets are known to release, among other compounds, ATP, ADP and TXA_2 (thromboxane), the latter two being strong inductors and potentiators of the aggregation process. The endothelium, on the other hand, continuously liberates PGI_2 (prostacyclin) and adenosine, which are antiaggregatory and antagonize the effects of TXA_2 and ADP, respectively. In the vicinity of the vessel wall ATP and ADP, released during platelet aggregation or liberated from damaged tissue, can be rapidly degraded to adenosine by the endothelial cascade of ecto-nucleotidases. Thus the aggregating stimulus in the form of ADP becomes diminished and, in addition, the antiaggregatory action of adenosine is fortified.

To understand the antithrombogenicity of the endothelium with respect to the clotting process certain steps in the coagulation system are of particular importance. As shown in Figure 9 thrombokinase from both the extrinsic and intrinsic system catalyzes the formation of thrombin, which serves several functions. It is essential for the formation of fibrin and

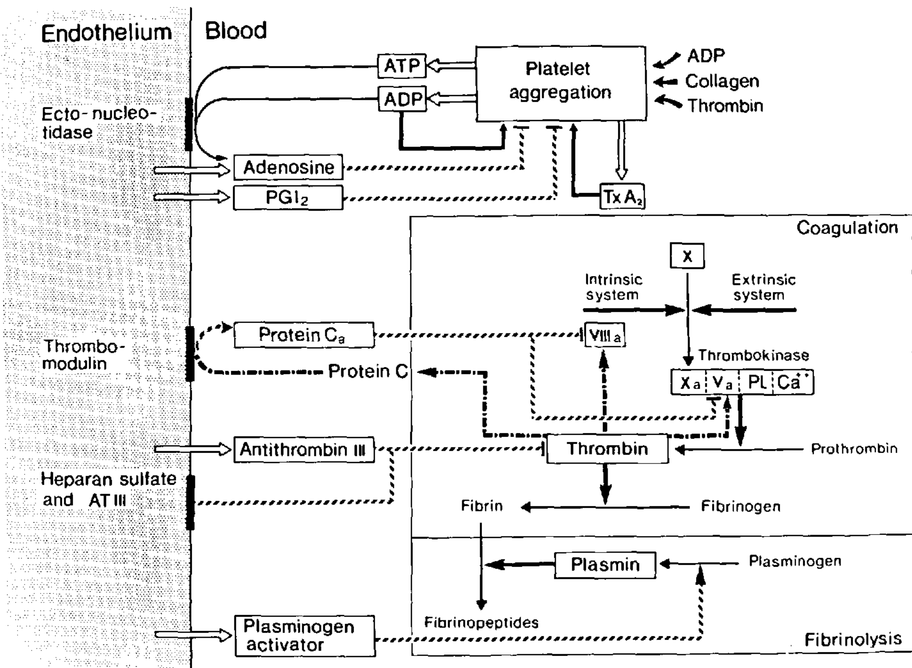

Fig. 9. Antithrombogenic properties of the endothelium displayed at its luminal surface. The major mechanisms for limiting platelet aggregation and coagulation as well as for activating fibrinolysis are schematically outlined. Not indicated are, among others, the thrombin-induced stimulation of PGI_2 release (40) and the enhancement of plasminogen activator release by activated protein C (14, 31). Furthermore, quantitative differences existing in the antithrombogenic properties of different segments of the vascular tree are disregarded. For a detailed discussion see text.

activates factor V, a constituent of the thrombokinase, as well as factor VIII, which accelerates the activation of factor X by the intrinsic system. Furthermore, thrombin in association with protein C is essential for a strong activation of protein C, occurring at the endothelial surface in conjunction with a surface cofactor, termed thrombomodulin (13, 14). The activated protein C (protein C_a), a protease, cleaves specifically the afore-mentioned factors Va and VIIIa and thus limits the further formation of thrombin. A direct inactivation of thrombin as well as of other activated coagulation factors (XIIa, XIa, Xa, IXa) (33) is afforded by antithrombin III liberated from the endothelium. Thrombin is also inactivated in contact with heparan sulfate and antithrombin III constituents of the luminal glycocalyx of the endothelium.

Plasminogen activator, which leads to a stimulation of fibrinolysis, can also be released from the endothelium, in particular from that of veins and capillaries (35). It is interesting to note that recent studies have indicated this release to be enhanced by activated protein C (14, 31).

When all the antithrombogenic properties of the endothelium are considered together, i.e. the release of PGI_2, adenosine, antithrombin III, and plasminogen activator, the functions of the ecto-nucleotidase cascade and the membrane constituents thrombomodulin, heparan sulfate and antithrombin III, then it becomes readily understandable, why platelet

aggregation and coagulation occur in vessel areas with damaged endothelium and do not normally progress intravascularly beyond the lesions, as would be expected from the autocatalytic nature of the hemostatic processes. Further physiological and pathophysiological implications of this vital endothelial interaction with the mechanisms of hemostasis are presently under investigation in many laboratories.

Concluding remarks

Several important aspects emerging from modern research on endothelial cell function could not be covered in this brief survey. In this context one should mention the role of the vascular endothelium in processes of inflammation and immunological defence, the contribution to regeneration and repair of intima lesions and the very important though still controversial involvement of the endothelium in lipoprotein metabolism of the vessel wall and its particular significance with regard to the development of atherosclerosis. This listing, which is by no means complete, in conjunction with those biochemical and functional aspects discussed above may, however, serve to give an appreciation of the complexity and diversity of endothelial cell function in health and disease.

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