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COMMENTARY

FUNCTIONAL HETEROGENEITY OF VASCULAR ENDOTHELIAL CELLS

MARY E. GERRITSEN
Department of Physiology, New York Medical College, Valhalla, NY 10595, U.S.A.

The observation that the endothelial lining of the vascular tree consists of cells exhibiting diverse functional and ultrastructural characteristics dates back to early discussions of the embryonic origins of the endothelium (for a recent review see Ref. 1). The detailed and comprehensive ultrastructural studies of Majno [2] led to the conclusion that "there are almost as many varieties of capillaries as there are organs and tissues". Well-defined and characteristic differences in the continuity of endothelial cells in capillaries led to their subclassification into three major divisions: continuous, discontinuous and fenestrated. Capillaries with continuous endothelium are found in the brain, retina, muscle, lung and adipose tissue. Adjacent endothelial cells in these capillary beds are connected by continuous intercellular tight junctions with no breaks in the vessel wall. The degree of junctional permeability in this subclass varies from the characteristic tight blood–brain barrier junctions of brain and retinal capillaries to the relatively more permeable junctions of muscle, lung and adipose tissue. Discontinuous endothelium, characteristic of the liver, spleen and bone marrow, has gaps between adjacent endothelial cells. In these vascular beds the endothelium is not thought to play a major barrier role in impeding blood-borne substances to the surrounding tissue. Fenestrated endothelium exhibits specific ultrastructural features called "fenestrae" which are specialized intracellular pores that have been proposed to play a major role in transport and filter functions. Fenestrated endothelium can be found in the capillaries of all endocrine glands, the renal glomeruli and renal tubules, and in the villi lining the intestine.

The most striking feature of endothelial variability is the apparently specific adaptation(s) of structure and function of the tissue in which it is found. Studies of the embryologic development of vascular cells have suggested a progression in development from an early primitive yet pluripotent cell exhibiting relatively undifferentiated cytoplasmic features and tenuous junctional structures. Such cells are relatively thick, with a low surface to volume ratio. As vascular development proceeds, the endothelial cells of a given tissue acquire specialized cytological characteristics, an increased surface to volume ratio, permanent junctions, and thickened basement membrane [1].

In recent years, with the advent of a variety of methods to isolate and culture endothelial cells from arteries, veins, lymphatics, and microvessels, investigators have identified many sources of heterogeneity that can be studied *in vitro*. These include age, sex, species of origin, organ, artery versus vein, large versus small vessel, growth factor and substrate requirements, stage of growth cycle, and number of population doublings. However, despite these sources of heterogeneity, several basic functions of healthy endothelial cells appear to transcend their diverse origins, including (1) the provision of a nonthrombogenic surface, (2) the mediation of the passage (and in some cases the conversion) of nutrients, solutes, and hormones from the lumen to the interstitium, (3) the maintenance of a patent lumen, and (4) the regulation of vascular tone, through the production of mediators and the activation and inactivation of circulating prohormones, hormones and autocoids.

In this article, I will address these basic functions by examining several areas of research in which endothelial heterogeneity has been documented and will discuss the possible significance of this variability. Although the phenotypic expression of various characteristics of vascular endothelial cells may be altered by the culture of these cells *in vitro*, studies of cultured endothelial cells have provided many clues as to the conditions that promote their growth and expression of biochemical and functional features. Therefore, in this discussion I will review studies of both cultured cells and relevant studies of intact vessels or *in vivo* observations.

ARACHIDONIC ACID METABOLISM

Arachidonic acid (AA) is a polyunsaturated fatty acid, localized predominantly to the sn-2 position of phospholipids, although AA is also found in the triglyceride component of endothelial cell lipids [3]. Arachidonic acid is released from membrane lipids by one or more pathways. The two major pathways appear to be phospholipase A_2 (PLA_2) (Fig. 1) and phospholipase C (PLC) (Fig. 2). PLA_2 liberates unsaturated fatty acids, including AA, from the sn-2 position of membrane phospholipids, resulting in the formation of lysophospholipids. Phospholipase C, specific for phosphatidylinositol (PI), converts PI to diacylglycerol and inositol phosphate. The diacylglycerol can be converted subsequently to a monoacylglycerol and further to glycerol, by diacylglycerol and monoacylglycerol lipase, respectively, resulting

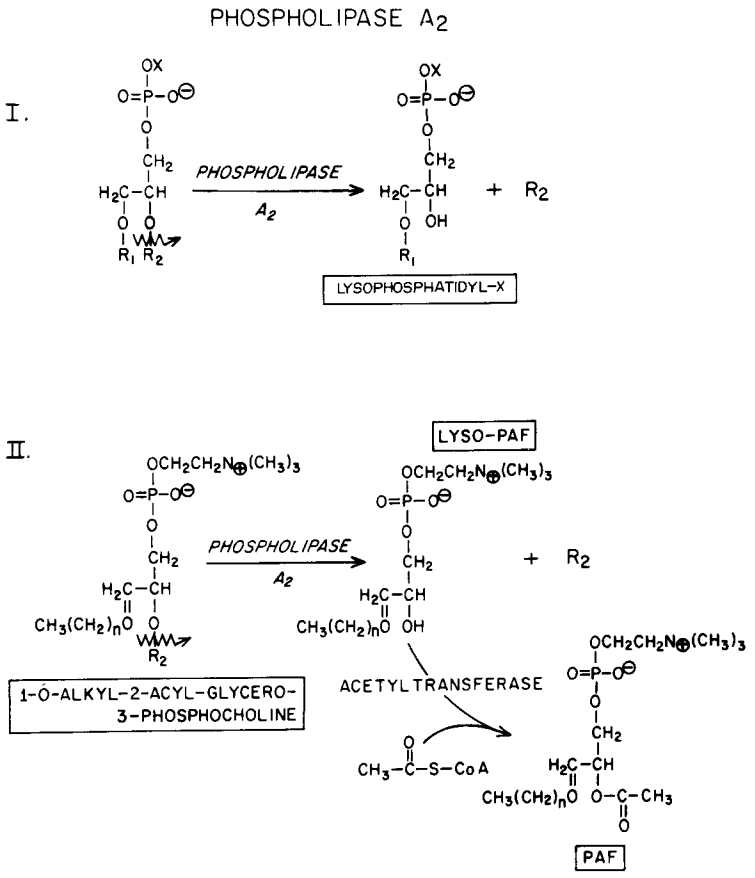

Fig. 1. Phospholipase A_2 action phospholipids (I) and 1-0-alkyl-2-acyl-glycero-3-phosphocoline (II). Abbreviations: R_1, fatty acid esterified at the sn-1 position, usually saturated; R_2, fatty acid esterified at the sn-2 position, usually unsaturated (e.g. arachidonic acid); X, polar head group (e.g. choline, ethanolamine, serine, inositol); and PAF, platelet activating factor.

in the liberation of AA. Alternatively, diglyceride kinase can phosphorylate diacylglycerol to phosphatidic acid (PA), and arachidonic acid can be liberated from PA to PLA_2. In vascular endothelial cells, thrombin, A23187 bradykinin, and histamine, directly or indirectly, appear to activate both PLC and PLA_2 [4, 5]. Hong and coworkers [4, 5] noted an interesting species difference in the phospholipid pools from which the arachidonic acid was released following stimulation. In human endothelial cells, the major sources of AA were phosphatidylcholine and PI, whereas in porcine aortic endothelial cells the major sources were PI and phosphatidylethanolamine. It is difficult to evaluate the significance of these observations, but an additional metabolic pathway should be considered. In most solvent systems, the 1-0-alkyl-2-acyl-3-glycerophosphatidylcholine (Gro-PCHO) comigrates with phosphatidylcholine. Gro-PCHO is also substrate for PLA_2, resulting in the formation of lyso-platelet activating factor (lyso-PAF). Lyso-PAF can be acetylated by an acetyltransferase [6] giving rise to PAF. The recent reports that human vascular endothelial cells can synthesize PAF in response to the same stimuli that evoke PGI_2 production and with a similar time course suggest that there may be some coupling mechanism between prostaglandin synthesis and PAF production [7, 8]. Stimulation of PLA_2-mediated liberation of AA from Gro-PCHO might be such a mechanism. Thus, apparent differences in phospholipid turnover upon challenge with various stimuli may suggest an additional area of endothelial variability, i.e. variation in the capacity to synthesize PAF.

When released from membrane lipids by acylhydrolase activity, AA can be converted by at least three possible enzymatic pathways. The prostaglandin synthase pathway, localized to the plasma membrane and microsomal fraction [9], leads to the formation (via cyclooxygenase) of the unstable prostaglandin endoperoxide intermediate, PGG_2. The PGG_2 is converted to PGH_2 by a peroxidase activity that copurifies with the cyclooxygenase enzyme [10]. The PGH_2 can be enzymatically transformed to PGI_2 (prostacyclin synthase), PGE_2 (PGE_2 isomerase), PGD_2 (PGD isomerase), $PGF_{2\alpha}$ ($PGF_{2\alpha}$ reductase) or thromboxane A_2 (TXA, TXA_2 synthase) or, in the absence of appropriate enzymes, break down to a mixture of $PGF_{2\alpha}$, PGE_2 and PGD_2 and HHT (12-hydroxyheptadecaenoic acid), the proportions of which are dependent on protein concentration and the presence of thiols [11]. PGI_2 and TXA_2 are not formed non-enzymatically but in aqueous solution undergo spontaneous hydrolysis to the

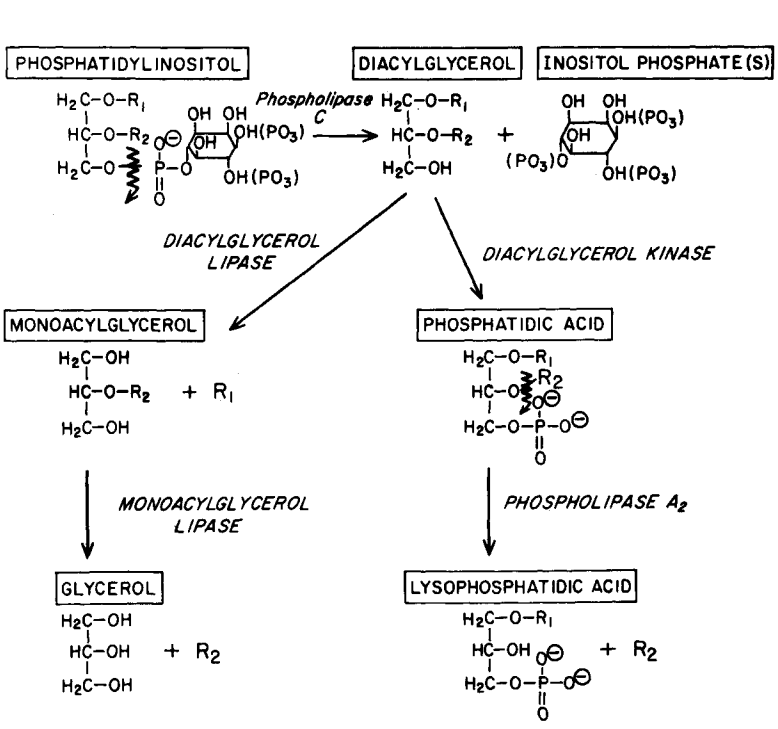

Fig. 2. Generation of arachidonic acid via the phospholipase C pathway. Abbreviations: R_1, fatty acid (unsaturated) esterified at the sn-1 position; R_2, arachidonic acid.

stable metabolites, 6-keto-$PGF_{1\alpha}$ and TXB_2 respectively. The lipoxygenase pathway(s) localized predominantly to the cytosol (although membrane bound forms have been described) leads to the formation of hydroperoxy and hydroxy derivatives of arachidonic acid. The 5-lipoxygenase pathway results in the formation of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which can be converted by leukotriene synthase to the unstable epoxide, leukotriene A_4 (LTA_4). LTA_4 can be further transformed by a hydrase with the addition of water to the dihydroxymetabolite LTB_4. Alternatively, in the presence of GSH, LTA_4 can be converted by GSH-*S*-transferase to the peptidoleukotriene, LTC_4. Subsequent removal of the terminal amino acids gives rise to LTD_4 and LTE_4. The epoxygenase pathway, catalyzed by the cytochrome P-450 system, can transform AA into various epoxides and diols. As the structures and biological activities of the epoxygenase metabolites are poorly defined at this time, we will not elaborate further on the metabolic routes leading to these metabolites (for further information, see Ref. 12).

Most reviews of vascular endothelial cell arachidonic acid metabolism have stated unequivocally that "PGI_2 is the major product of all endothelial cells". Such a statement is an overgeneralization, based primarily on studies with endothelial cells derived from large blood vessels, generally arterial in origin (Fig. 3). The idea that vascular endothelial cells may differ in their capacity to produce PGI_2 dates back to a study by Skidgel and Printz [13] who demonstrated that veins exhibited a reduced capacity to transform PGH_2 to PGI_2 when compared with

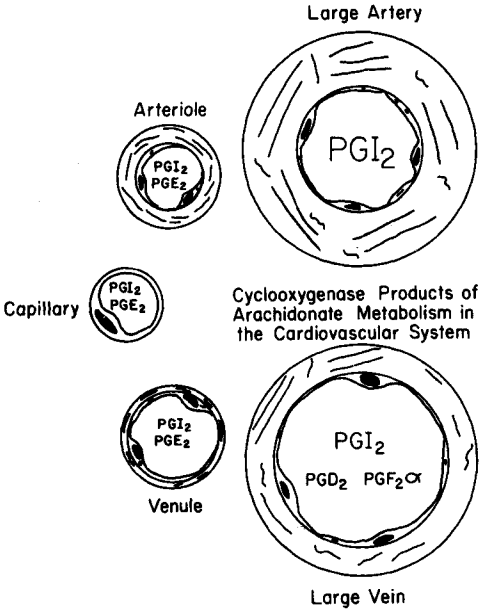

Fig. 3. Generalized summary of the distribution of prostaglandin synthetic pathways present in blood vessels in different regions of the vascular tree.

arteries from the same animal. An earlier observation by Moncada *et al.* [14] provided strong evidence that the PGI_2 synthetic capacity of blood vessels was derived predominantly from the vascular endothelium. This localization was further studied by Smith and coworkers [15] who demonstrated that the PGI_2 synthetic capacity of endothelial cells was most likely due to the preferential localization of the cyclooxygenase enzyme. Using monoclonal antisera against cyclooxygenase and PGI_2 synthase, these authors demonstrated that PGI_2 synthase was found distributed uniformly across the vessel wall, whereas cyclooxygenase was localized predominantly to the intimal layer. Further evidence that the difference in the PGI_2 synthetic capacity between arteries and veins was due to differences in endothelial cell PGI_2 came from studies by Johnson [16], using cultured endothelial cells derived from human arteries and veins. This author demonstrated that arterial cells exhibited a greater ability to produce PGI_2 than their venous counterparts.

The discovery of PGI_2 by Moncada and coworkers [17] and the elucidation of its potent vasodilator [18] and anti-platelet properties [17, 19] resulted in a profusion of literature describing the production of PGI_2 by vascular cells [20–24]. These exciting findings left relatively few investigators interested in the question: Are other AA metabolites produced by endothelial cells?

Evidence that additional enzymatic pathways of PGH_2 metabolism were present in specific blood vessels was first provided by Gerritsen and coworkers [25]. In this study, microvessels isolated from bovine cerebral cortex were found to exhibit two principal pathways of PGH_2 metabolism: GSH-requiring PGH_2-PGE_2 isomerase and PGI_2 sythase. In a later study by this group [26] the same enzymes were also described in bovine coronary microvessels. Large cerebral and pial arteries, large coronary arteries and aorta did not exhibit the GSH-requiring PGH-PGE isomerase [25, 26].

Studies of isolated rat vessels have indicated two additional pathways of PGH_2 metabolism. Isolated cerebral cortex microvessels exhibit a soluble GSH-independent PGH_2-PGD_2 synthase [27] with properties similar to the rat brain PGH_2-PGD_2 synthase reported by other investigators [28]. However, Gerritsen and Printz observed that the PGH_2-PGD_2 synthase was not found in microvessel-free cerebral homogenates or cytosol, suggesting that this enzyme was preferentially localized to the cerebral microcirculation. Studies of the peripheral rat vasculature revealed a variant of the cerebral enzyme, a GSH-requiring PGH_2-PGD_2 isomerase [29, 30]. This enzyme was localized preferentially to rat venous tissue.

There are a few reports of thromboxane synthesis by vascular tissue. Salzman *et al.* [31] reported conversion of PGH_2 to TXA_2 in the rabbit pulmonary artery, but the cellular origins of the TXA_2 synthase were not evaluated. Several reports of endothelial cell TXA_2 (as measured by radioimmunoassay or HPLC) release have been published and, curiously, these reports were from bovine aortic endothelial cells [32, 33]. Attempts to measure TXB_2 release from most other sources of endothelium have yielded numbers generally at or below the limit of detection (i.e. < 0.2 ng/10^6 cells).

The relevance of PGE_2 and PGD_2 synthesis to the physiology of the vessel wall has been generally discounted because PGE_2 and PGD_2 are less potent than PGI_2 in most bioassays. Measurements of endogenous production of PGD_2 by blood vessels and endothelial cells have proven extremely difficult due to both the lack of a sensitive, selective and commercially available radioimmunoassay for PGD_2 and the discovery of an enzyme that actively converts PGD_2 to an isomer of $PGF_{2\alpha}$, 9α-11β-PGF [34]. Although the distribution of this enzyme has not been well documented, such an enzyme may explain the apparently high levels of "$PGF_{2\alpha}$" reported for some vascular tissue [35]. No evidence for enzymatic conversion of PGH_2 directly to $PGF_{2\alpha}$ has been reported in vascular tissues, and it is likely that the usually small amounts of $PGF_{2\alpha}$ formed by vascular tissues may be accounted for by non-enzymic decomposition of PGH_2. The release of PGE_2 by intact cerebral and coronary microvessels [36, 37] was reported to be at a ratio of roughly 1:3 to PGI_2. This compares to a ratio of 1:10 (PGE_2:PGI_2), which is often reported for larger vessels [38–40]. In one report [41], the release of PGI_2, $PGF_{2\alpha}$, TXA_2, and PGE_2 from bovine cerebral microvessels was measured by radioimmunoassay. In this communication, 6-keto $PGF_{1\alpha}$ and $PGF_{2\alpha}$ constituted about 92% of the AA metabolites found, with only trace amounts of PGE_2. However, the method of microvessel isolation, i.e. homogenization, a discontinuous sucrose density gradient, followed by collection of the pellet over a 153 μm sieve, would result in the collection of many vessels in the size range of 60–80 μm [42] and relatively few vessels within the size range of true capillaries. The observations of our laboratory [25, 26, 29] as well as others [43] indicate that the contaminating larger vessels (and any pial vessels not removed by initial dissection) exhibit a greater capacity to produce PGI_2 and, thus, could obscure (the relatively smaller) output by small arterioles, capillaries and venules.

The development of techniques for the cultivation of microvascular endothelial cells led to the discovery by our group [37] that PGH-PGE isomerase is present in the coronary microvascular endothelial cell and that these cells are capable of releasing PGE_2 in excess of PGI_2. Confirmation of microvascular PGE_2 synthesis came from the group of Charo *et al.* [44] who found that PGE_2 was the principal product of human foreskin capillary endothelium. Other investigators have also reported the significant production of PGE_2 by rat cerebral endothelial cells [45] and the diminished production of PGI_2 by bovine cerebral endothelial cells [43]. However, it remains to be determined if PGE_2 synthesis is a characteristic feature of microvascular endothelial cells or is perhaps specific to certain organs or species. The majority of culture descriptions for microvascular endothelial cells have measured PGI_2, but have not compared the levels of PGI_2 synthesis to that of PGE_2. This is an important point that should be addressed by various investigators who attempt to characterize microvascular endothelial cell arachidonic acid metabolism.

Vascular endothelial cells also contain lipoxygenase enzymes, although these pathways have been poorly characterized and not compared across different endothelial cell types. Identified lipoxygenase products synthesized by endothelial cells include 11- and 15-HETE [46] as well as an unidentified lipoxygenase product "LOX" [47]. The production of AA metabolites inhibited by lipoxygenase blockers has been described for rat cerebral microvessels [48] and rabbit coronary microvessel endothelial cells [38]. At this time, there is no evidence for a 5-lipoxygenase pathway in vascular endothelial cells [46].

There is ample evidence from the literature that the capacity of vascular endothelial cells to release arachidonic acid metabolites can be affected by culture conditions. Many authors have described [23, 41, 49] variability in PGI_2 production among identically treated cultures in the same passage. PGI_2 production decreases with time in senescent endothelial cells [49], and in porcine aortic endothelial cells it declines rapidly with subcultivation [40]. The release of PGI_2 is greatest during the logarithmic phases of endothelial growth, declining with confluency [50, 51]. Enrichment of the growth medium with certain fatty acids (linoleic or dihomo-γ-linoleic) decreases PGI_2 synthesis [52, 53] as does addition of low density lipoprotein to the culture medium [54, 55]. Thus, variation in both the proportion and content of the serum added to the culture medium can exert profound effects on the subsequent ability of these cells to synthesize PGI_2 and possibly other arachidonic acid metabolites.

The significance of differences in the capacity of the vascular endothelial cell to produce AA metabolites other than PGI_2 with respect to blood vessel function is poorly understood. The profound biological activities of PGI_2 have strongly suggested roles for this eicosanoid in the regulation of hemostasis and vascular tone and in the inhibition of the adhesion of platelets and other blood-borne cells to the vascular endothelial surface. PGE_2 is generally less potent than PGI_2, although in aqueous solution PGE_2 is considerably longer lived than PGI_2. *In vivo*, however, PGE_2 is rapidly cleared by the lung [56]. Studies with various cell types have demonstrated that there are distinct membrane receptors for PGE_2 and PGI_2 [57]. PGE_2 does appear to exhibit some activities in which it is more potent than PGI_2, including modulation of vascular reactivity to norepinephrine and angiotensin II [58] and inhibition of LTB_4 release from neutrophils [59]. However, there are many assays in which the activities of PGE_2 and PGI_2 are not routinely compared. Given the apparent propensity of microvascular endothelial cells from several sources to enzymatically generate PGE_2, the possibility of elucidating a unique role for PGE_2 in the microcirculation is intriguing.

The function of lipoxygenase products in endothelial cells is at best speculative. Possible roles for HETEs and HPETEs generated by endothelial cells include activation of leukotriene synthesis in mast cells [60], inhibition of lipoxygenase activity in leukocytes and platelets [60, 61], inhibition of PGI_2 synthase in vascular endothelial cells [62], and stimulation of vascular smooth muscle migration [63].

VASOACTIVE PEPTIDE METABOLISM

The endothelial uptake and metabolism of vasoactive peptides and autocoids can exert a powerful influence on the biological effects of these substances. The role of the pulmonary endothelial cell in the processing of vasoactive materials is well recognized, yet certain substances, including angiotensin II, substance P, oxytocin, vasopressin, and vasoactive intestinal peptide, are unaffected by circulation through the lung [64, 65]. However, these substances can be processed by peptidases present on endothelium in other vascular beds.

The distribution and activity of one of these peptidases, angiotensin I converting enzyme (ACE, Kininase II), have been well documented. ACE serves two principal functions: inactivation of kinins and activation of angiotensin I by conversion to angiotensin II [66]. This is accomplished by the removal of the C-terminal dipeptide of those substrates. ACE has been reported to be asymmetrically distributed in vascular endothelial cells, i.e. it is localized to the luminal surface [67]. In cultured endothelial cells, ACE is localized to the plasma membrane [68].

It is well recognized that the pulmonary vascular bed is a major site for the metabolism of angiotensin I and bradykinin, yet this enzyme can be found in most vascular beds [67, 69–72]. A question that reasonably arose from these observations is: Is there more ACE activity in pulmonary vascular endothelium than other endothelial cells or can the extensive surface area of the pulmonary endothelium account for the apparent efficiency of the lung in vasoactive peptide metabolism? In an attempt to address this question, Johnson and coworkers [16, 73] cultured endothelial cells from different human vessels. In these studies, cells from arteries had three to five times as much ACE activity as cells from veins. However, pulmonary endothelial cells were no more active than other endothelial cells in metabolizing bradykinin and angiotensin I.

Caldwell and coworkers [67] used fluorescein-labeled goat antibodies to pure rabbit lung ACE to study the distribution of ACE in the rabbit. These authors found ACE localized to the endothelial cells of lungs, spleen, adrenal gland and liver. However, the capillary endothelial cells of the rabbit renal glomerulus were not reactive to antibodies to ACE. All other endothelial cells thus far examined seem to possess ACE.

Del Vecchio and Lincoln [74] studied the regulation of ACE activity in cultured bovine pulmonary artery cells. These authors found a lateral heterogeneity in ACE expression, i.e. significant changes in the specific activity of ACE at different stages of growth and different population doubling levels. ACE activity was not expressed until several days post-confluence, and ACE specific activity decreased with increasing *in vitro* age. A similar loss in ACE activity of porcine aortic endothelial cells was reported by Gordon and Pearson [75]. A later

study by this group [76] found that, although ACE activity declined rapidly in cultured porcine aortic endothelial cells in the presence of fetal calf serum, ACE activity was sustained during primary culture when the medium contained homologous serum. These authors also found ACE activity was similar in cultures whether harvested enzymatically or mechanically. However, others have claimed that part or all of the phenotypic alteration in ACE activity observed could be due to the use of trypsin to subculture the cells. Ryan and coworkers [77] and others [78] provided strong evidence that bovine pulmonary endothelial cells lose ACE activity after treatment with trypsin, and subcultivation of the cells using microcarriers [77] or by scraping may avoid these apparent heterogeneities due to protease treatment. It is possible that there may be species differences in the susceptibility of ACE to proteinase attack, such that in porcine endothelium the maintenance of ACE activity depends on some constituents present in homologous serum.

The ability of arteriolar, capillary or venular endothelial cells to exert fine control over the concentrations of angiotensins and kinins allowed to reach the interstitial compartment, underlying smooth muscle and venous effluent may provide the vascular endothelium with a principal role in the local control of blood flow, tissue perfusion and capillary permeability. This activity is not necessarily restricted to angiotensins and kinins. For example, ACE and other vascular peptides that inactivate substance P [79] and transform enkephalins [80, 81] have been described. Although many of these enzymes have not been characterized completely, their specific localization to some vascular beds and the apparent lack of such activity in others is an avenue worthy of future investigation.

HORMONE RECEPTORS AND RECEPTOR RESPONSIVENESS

Insulin

The presence of insulin receptors on vascular endothelial cells was demonstrated by radioautography studies [82, 83] and detailed binding studies using cultured endothelial cells or isolated microvessels [84–91]. Although the characteristics of insulin binding were generally similar, Bar and colleagues [86, 87] demonstrated a striking difference in receptor number between arterial and venous endothelial cells. The human umbilical artery bound 2.5 times more insulin per cell than the corresponding venous endothelial cells. Characterization of insulin receptors has been described for bovine retinal [88, 89] and rabbit coronary microvessel endothelial [90] cells. The characteristics and properties of the microvascular endothelial receptors appeared to be identical to their large vessel counterparts and those of other insulin-sensitive tissues [89]. However, various investigators were unable to demonstrate consistent effects of insulin on glucose metabolism or neutral amino acid uptake [82, 91–93] in large vessel endothelial cells. In contrast, King and coworkers [88] demonstrated marked stimulation by insulin of [^{14}C]glucose into glycogen in retinal microvessel endothelial cells. Pillion *et al.* [94] demonstrated insulin-stimulated glucose metabolism in isolated cerebral microvessels. Insulin stimulated [^{14}C]-glucose incorporation and metabolism to CO_2, lipid, and glycogen, and increased the transport of [^{3}H]-2-deoxyglucose and [^{3}H]-3-*O*-methyl glucose in rabbit coronary microvessel cells [90]. Insulin stimulation of glucose transport in bovine retinal endothelial cells has also been described [95].

The significance of microvascular endothelial receptors for insulin can best be appreciated when one considers the enormous surface to volume ratio at the capillary level. Binding of insulin at the capillary level could, for example, play a role as an extrapancreatic readily releasable pool of insulin [96, 97]. Jailal *et al.* [96] have shown that, although endothelial cells readily internalize insulin, they do not metabolize it, in contrast to most other types (e.g. fibroblasts, adipocytes) which show significant degradation of insulin under the same conditions. An exciting report by King and Johnson [98] suggests that insulin receptors on endothelial cells may function as a specific transport mechanism for insulin from the luminal to the interstitial surface of endothelial cells. Thus, endothelial cells may have an important role in regulating insulin's access to the surrounding tissues. In the past, the endothelium has been viewed as an insulin-insensitive tissue and, therefore, it was thought that insulin played no role in the access of glucose or amino acids to the interstitium. However, in view of the observations of our [90, 95] as well as King's [88] laboratory that serum starvation was required to demonstrate reproducible effects of insulin on cultured cells and that the responses to insulin developed relatively slowly, i.e. requiring 15–50 min to increase glucose uptake compared to a few seconds (adipocytes [99]) or a few minutes (fibroblasts [100]), the *in vivo* responses of endothelial cells to insulin may be tonic rather than phasic in nature.

Thrombin

Another endogenous substance that exhibits heterogeneity with regard to endothelial responsiveness is the enzyme thrombin. Thrombin is a serine protease that converts fibrinogen to fibrin. Additionally, it exerts dramatic effects on cells. It stimulates platelet activation and the release of eicosanoids. It elicits the formation of small "blisters" on the endothelial cell surface [101] and stimulates PGI_2 release from human endothelial cells [101–104]. However, Hong [104] found that, although thrombin would stimulate PGI_2 release from human endothelial cells, it did not stimulate PGI_2 release from bovine endothelial cells. Goldsmith and Kisker [105] subsequently observed that thrombin stimulated PGI_2 production from the sheep umbilical vein but not from fetal sheep aortic segments. These observations had been interpreted by some authors as evidence for the lack of thrombin receptors on these tissues. However, the lack of PGI_2 release upon addition of thrombin is not conclusive evidence for the lack of thrombin receptors, nor proof that thrombin receptor binding is a necessary prerequisite to prostaglandin release. For example, although addition of thrombin to bovine endothelial cells does not stimulate prostaglandin synthesis, it does induce the loss of intracellular plasminogen activator (PA), inhibits PA secretion [106], increases

calcium influx and stimulates ornithine decarboxylase [107]. Inactivation of thrombin with diisopropylfluorophosphate (DFP) prevents stimulation of PGI_2 synthesis [102, 108] in human umbilical vein endothelial cells. However, blockade of nearly all (98%) of thrombin binding sites with DFP-thrombin does not prevent active thrombin from stimulating PGI_2 synthesis [109]. Studies of thrombin binding sites on vascular endothelial cells have indicated differences in receptor number. For example, in human umbilical vein endothelial cells, 3300 binding sites/cell have been reported [110], yet up to 5.8×10^5 sites/cell have been described for rabbit aortic endothelial cells [109]. In bovine endothelial cells, the number of thrombin binding sites has been reported to be inversely related to cell density [111].

Thus, endothelial cells exhibit considerable heterogeneity with respect to thrombin receptor characteristics and thrombin responsiveness. The release of PGI_2 provoked by thrombin may be a form of negative feedback, dampening for example, the proaggregatory effects of thrombin on the platelet. However, the nature of the coupling between thrombin and prostaglandin release is unclear. It is possible that there is more than one class of binding sites for thrombin and that only one of these receptor classes is linked to prostaglandin production. Lollar and Owen [108] have proposed that low affinity binding sites for thrombin may be linked to prostaglandin release, whereas higher affinity sites may subserve other functions.

Biological substances and prostaglandin release

Many investigators have used the release of PGI_2 (and occasionally other prostaglandins) as a measure of endothelial responsiveness to a given hormone, neurotransmitter, metabolite or enzyme. As indicated above in the discussion of thrombin, the release or lack of release of prostaglandins by endothelial cells incubated with a given substance is not proof of the presence or absence of receptors but does illustrate further the concept of heterogeneity. Summarized in Tables 1 and 2 are substances that have been tested on various endothelial cells for their ability to release prostaglandins. As is clearly evident from these tables, there are several examples of apparently species-specific responses to certain substances, including thrombin, angiotensin II, ATP and f-Met-Leu-Phe. However, in other instances, investigators evaluating endothelial cells derived from the same species and vessel or origin have reported divergent observations. For example, Clark and coworkers [118, 128] demonstrated that LTC_4 and LTD_4 mediated PGI_2 release from bovine aortic endothelial cells, yet Miller *et al.* [33] were unable to stimulate PGI_2 synthesis with the same stimuli in BAE cells. These discrepancies could possibly be explained by the loss of specific endothelial responsiveness following extensive subcultivation. In both studies, the authors used endothelial cell lines that were of high passage number. Leukotriene D_4 does stimulate prostaglandin release from rabbit coronary microvessel endothelial cells [112] and human umbilical vein endothelial cells [119, 129]. Angiotensin II stimulates prostaglandin release from bovine aortic endothelial cells [113, 114] but does not stimulate PGI_2 release from human umbilical vein endothelial cells [51]. Thus far, several agents appear to stimulate prostaglandin release from all endothelial cells: bradykinin, mellitin, the calcium ionophore A23187, and trypsin [23, 51, 112]. In addition, another group of agents appear to exert no effect on prostaglandin release from endothelial cells: acetylcholine, norepinephrine and adenosine. Many other agonists have been reported to stimulate or to have no effect on prostaglandin release. However, the universality of these observations is uncertain as relatively few endothelial cell types have been tested extensively. The conclusion that could be drawn from these divergent observations of substance-stimulated prostaglandin release is that generalizations should not be made as to the role of prostaglandins in the response to a given substance (including extrapolation from large vessel endothelium to the local responses of the microcirculatory compartment) or to account for altered vascular physiology in human disease states such as atherosclerosis and diabetes. This survey of the literature strongly suggests that such suggestions of clinical or physiological significance remain restricted to the vessel and species of origin until demonstrated to be a universal observation.

SUMMARY

This review has highlighted some of the well-described differences in endothelial cells derived from different sites of the vascular tree. In presenting a select group of endothelial properties, there was no intention to imply that these are the only properties of endothelial cells that exhibit heterogeneity. Nonetheless, having described endothelial heterogeneity in regard to a number of defined properties, we are left with persistent questions including: Are these divergent properties of endothelial cells fixed? Alternatively, can we alter the properties of endothelial cells by affecting the signals from the environment?

A number of reports strongly suggest that endothelial cells are capable of acquiring new properties. Stewart and Wiley [130] investigated the role of the neural tissue environment on the differentiation of brain capillary endothelial cells. These authors transplanted ectopic sites, i.e. vascular segments of brain from very young quail embryos to chick coeliac cavity, and a quail somites to chick brain ventricles. The distinctive morphology of quail cells provided a cell marker to differentiate host from graft. The results of this study demonstrated that mesenteric or somatic vessels growing into grafted brain developed functional, structural and histochemical features specific for neural capillaries. Conversely, capillaries in mesodermal tissue that had been grafted to the brain were devoid of the neural capillary characteristics, indicating that brain vessels do not form a barrier when they are made to vascularize non-neural tissue. Milici and Carley [131] reported that bovine adrenal capillary cells cultured on plastic exhibited occasional diaphragmed fenestrations and no transendothelial channels. However, if these same cells were cultured on a basement membrane (matrix)

Table 1. Substances that increase prostaglandin release from vascular endothelial cells

	Endothelial cell origin*
A. Hormones, autocoids and endogenous substances	
Thrombin	HUVE [51, 102, 103]
Adenine nucleotides (ATP, ADP)	RCME [112], PAE [111]
Angiotensin II	BAE [113, 114]
Bradykinin	PAE [103], HUVE [103], BPAE [115], RCME [112]
Histamine	HUVE [51, 113]
Interleukin 1	HUVE [116], RCME [112]
Platelet-derived growth factor	BAE [117], BCE [117]
Leukotriene C_4, Leukotriene D_4	BAE [118], RCME [112], HUVE [119]
B. Exogenous substances	
Melittin	HUVE [107], RCME [112], BPAE [120], BCE [120]
f-Met-Leu-Phe	RCME [112]
Trypsin	BAE [103], PAE [103], HUVE [102]
A23187	HUVE [102], PAE [121], RCME [112]
Lipopolysaccharide	RCME [112], BAE [122]

* Abbreviations: HUVE, human umbilical vein endothelium; RCME, rabbit coronary microvessel endothelium; BAE, bovine aortic endothelium; PAE, porcine aortic endothelium; BPAE, bovine pulmonary aortic endothelium; and BCE, bovine capillary endothelium (adrenal).

Table 2. Substances that do not increase prostaglandin release from vascular endothelial cells

	Endothelial cell origin*
A. Hormones, autocoids and endogenous substances	
Acetylcholine	HUVE [51], RCME [112]
Adenosine	RCME [112], PAE [123]
Angiotensin II	HUVE [51]
Norepinephrine, epinephrine, isoproterenol	RCME [112], HUVE [51, 102]
Vasopressin	HUVE [51]
Substance P	HUVE [51]
Thrombin	BAE [103], PAE [103]
β-Thromboglobulin	BAE [124, 125]
Leukotriene C_4, Leukotriene D_4	BAE [33]
B. Exogenous substances	
Sodium nitroprusside	HUVE [126]
f-Met-Leu-Phe	BAE [127]
Thrombin	BAE [103]

* Abbreviations: HUVE, human umbilical vein endothelium; RCME, rabbit coronary microvessel endothelium; BAE, bovine aortic endothelium; PAE, porcine aortic endothelium; BPAE, bovine pulmonary aortic endothelium; and BCE, bovine capillary endothelium (adrenal).

laid down by MDCK cells (a canine nephron epithelial cell line), the cells responded by increasing the number of diaphragmed fenestrations and transendothelial channels. This cell culture study supported an earlier whole animal study in which the importance of the epithelium and/or epithelial basal lamina in the maintenance of endothelial ultrastructure was demonstrated in a developmental study of rat intestinal capillaries [132]. In this earlier study, it was noted that epithelial development coincided with the formation of fenestrations by the endothelium.

Enzymatic activities of endothelial cells can also be altered by environmental signals. For example, primary cerebral microvascular endothelial cells exhibit barrier features and are enriched in gamma-glutamyl transpeptidase activity, yet rapidly lose the activity when subcultured [133, 134]. Co-cultivation of cerebral endothelial cells with glial cells, however, restores gamma-glutamyl transpeptidase [135]. Glucocorticoid treatment of vascular endothelium can increase the expression of an inhibitor of plasminogen activator [136, 137], suppress basal and stimulated prostaglandin secretion [138, 139], and induce ACE activity [140]. Other studies have shown that the expression of Ia antigens by vascular endo-

thelial cells is inducible *in vitro* by treatment with gamma-interferon [141–143], or with phytohemagglutinin [144].

Endothelial cells exhibit a range of ultrastructural and biochemical features. Observations from several groups now suggest that these features are not a result of terminal differentiation, but rather of signals from the environment of the endothelial cell. Endothelial cells apparently can modify their activities in response to alterations in the quantity and/or quality of these signals (e.g. hormones, neurotransmitters, subcellular matrix, nutrients, oxygen supply, metabolites). This feature may be of significance in the many facets of endothelial function—in the regulation of fibrin formation and dissolution, injury, wound healing, angiogenesis, and local inflammation. Perhaps the plasticity of endothelial cells' responsiveness enables them to maintain their integrity in a world of constant challenges.

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