The Barrier Within: Endothelial Transport of Hormones

Hormones are involved in a plethora of processes including development and growth, metabolism, mood, and immune responses. These essential functions are dependent on the ability of the hormone to access its target tissue. In the case of endocrine hormones that are transported through the blood, this often means that the endothelium must be crossed. Many studies have shown that the concentrations of hormones and nutrients in blood can be very different from those surrounding the cells on the tissue side of the blood vessel endothelium, suggesting that transport across this barrier can be rate limiting for hormone action. This transport can be regulated by altering the surface area of the blood vessel available for diffusion through to the underlying tissue or by the permeability of the endothelium. Many hormones are known to directly or indirectly affect the endothelial barrier, thus affecting their own distribution to their target tissues.

Dysfunction of the endothelial barrier is found in many diseases, particularly those associated with the metabolic syndrome. The interrelatedness of hormones may help to explain why the cluster of diseases in the metabolic syndrome occur together so frequently and suggests that treating the endothelium may ameliorate defects in more than one disease. Here, we review the structure and function of the endothelium, its contribution to the function of hormones, and its involvement in disease.

Evidence of a Barrier

Many studies investigate cellular responsiveness to hormones to determine the presence of receptors and signaling cascades, but cell reaction to the functional barrier separating blood from many organs and cells is the endothelium, which lines blood vessels throughout the body. This barrier regulates the access of hormones and nutrients to underlying target tissues. The endothelium can be modified in many ways to increase or decrease access to the underlying tissue. Many hormones are themselves able to induce changes in the endothelial barrier to alter their own access. To investigate the ability of a hormone to improve its own access, we must first understand that changes can be made to modify the endothelial transport and access to the interstitium. We will provide evidence that a barrier exists, review the structure of the endothelium, and then investigate transport of hormones across the endothelium and functions of the endothelium that modify access to essential organs.
hormone in vitro may not be indicative of what happens physiologically in vivo. For example, glucose and amino acid concentrations in muscle and adipose tissue were found to be substantially different from circulating levels using microdialysis (54). Thus there is a barrier preventing the equilibration of the plasma with the interstitial space. Similar results have been found for other hormones; angiotensin, involved in regulation of blood pressure and fluid volume, is 1,000-fold higher in the renal interstitium than in plasma (81), suggesting a role as a paracrine rather than an endocrine hormone.

Studies have shown that the concentration of insulin at the cell surface is very different from that in plasma (1, 15, 82). This difference could contribute to impaired insulin response independent of cellular effects. This insulin gradient has been confirmed using microdialysis (40, 82) as well as by direct interstitial access (7) and lymph measurements (1, 15). Based on insulin extraction across a limb, transendothelial transport is more likely to limit interstitial concentrations than hormone uptake or disposal (93). This disequilibrium between the plasma and interstitium is suggestive of a barrier preventing equilibration between the two compartments.

Although this barrier can affect the ultimate concentration reached at the cell surface in relation to the plasma levels, it can also have a temporal effect, delaying the increase in hormone concentration, a representation of which is shown in FIGURE 1. It was noted that insulin-mediated glucose uptake lagged behind the increase in plasma insulin but matched well with insulin receptor kinase activation in muscle (35). In cultured skeletal muscle cells, insulin-mediated glucose uptake occurs almost immediately (47, 74), and the uptake rate is constant during exposure. Thus the time delay seen in vivo (35) suggests an intrinsic delay in insulin access to the myocyte. Glucose disposal in dogs exposed to venous insulin infusion corresponded with the time for lymph insulin increase, yet the increase in arterial insulin was much quicker (57). This temporal relationship confirms the concept that the time required for insulin to reach the interstitial space is the limiting factor for insulin-mediated glucose uptake: only once insulin is present at the muscle cell surface can it bind to receptors to cause glucose uptake. The correlation between insulin concentration and glucose uptake is strongest with interstitial (lymph) insulin concentrations ($r = 0.92; P < 0.001$) rather than vein ($r = 0.77; P < 0.001$) or arterial ($r = 0.19; P > 0.2$) concentrations (15). Therefore, the concentration of insulin at the surface of the target cell, rather than in the blood, is the best predictor for the rate of insulin-mediated glucose uptake. We have shown that diffusion throughout the interstitium is rapid in healthy tissue (15); as such, it seems that the endothelium provides the main barrier to equilibration with the plasma (4). Sampling the fluid at the cell surface is thus essential to understanding the ability of the cell to respond to its environment, and the ability of hormones to access the target tissue is essential for their action.

The Endothelium as a Structural Barrier

The endothelium lines the blood vessels throughout the body, from the larger arteries and veins down to the small capillaries that branch through organs. Transport of nutrients and hormones across this barrier is essential for the appropriate functioning of the body. Originally, the capillary endothelium was thought to be an inert semipermeable barrier; however, the concept of a purely physical endothelial barrier has been replaced by our understanding of a more complex and functional one.

The endothelium consists of endothelial cells on the luminal side of the blood vessel, with an underlying layer of smooth muscle cells (FIGURE 2). Isolated arterioles show that endothelial cells can prevent small water-soluble molecules diffusing from the luminal surface to the smooth muscle cell layer (52), whereas lipid-soluble compounds diffuse rapidly from either side of the arteriole, suggesting a selectivity of the endothelial cell (52). Early work used the molecular sieving ability of capillaries to estimate pore size (70). Studies that showed a change in the resistance of the capillary wall due to plasma volume expansion concluded
that the pores can stretch (78). Although the larger arteries and arterioles contain vascular smooth muscle cells, much of the exchange of molecules occurs across the capillary wall where there are usually no vascular smooth muscle cells. Some exchange can also occur at the postcapillary venules, which often do not possess smooth muscle cells.

The glycocalyx is a permeable endothelial surface layer consisting of glycoproteins that extend into the blood vessel. The glycocalyx is involved in the regulation of vascular permeability, mediation of shear stress, and decreasing adhesion [reviewed by Becker et al. (5)], and can regulate the ability of various molecules to access the endothelium based on size and charge (95), thus limiting access to the endothelial cells.

It has long been known that the structure of the endothelium can vary among tissues (34). Depending on the tissue, the junctions between the endothelial cells can be tight or open to large pores. Because of this, the ability of hormones or other nutrients to access the target tissue will depend on the structure of the endothelium within that particular tissue (reviewed in Refs. 2, 56). The skeletal muscle, heart, lung, and brain each have continuous endothelium, where there are tight junctions between cells, and most transport must occur through the cell. Even within this class, there are differences. The blood-brain barrier is a highly specialized endothelial barrier with very tight control, as reviewed by Deli et al. (24). The pancreas and intestine are examples of tissue that contain fenestrated endothelium, with pores allowing more access to the underlying tissue than would be permitted by continuous endothelium. However, the liver, spleen, and bone marrow have discontinuous endothelium with large holes, which allow rapid equilibration of plasma with the underlying tissue. This exposure of the liver to plasma changes may explain its earlier susceptibility to insulin resistance, for example (44). The role of the endothelium in hormone transport varies between organs and can depend on the physical ability of the endothelium to block transport.

The Endothelium as a Functional Barrier

The location of the endothelium is such that it is often the first structure to be exposed to any changes in blood and is, therefore, the first to be able to respond to such changes. Although the endothelium can function as a physical barrier, endocytosis and transcytosis (66, 79) have been observed in endothelial cells, suggesting a more active capacity of the endothelium to modulate access to the underlying tissue. In that sense, the endothelium can act as a “gateway” by which hormones or other signals can reach target cells. Interestingly, many hormones can have direct effects on the endothelium and as such may be able to modify the endothelium to alter their effects.

Hormones Can Increase Surface Area for Diffusion

Increasing the surface area for diffusion can increase access to underlying cells; an increased area for exchange to underlying tissue allows more rapid equilibration.

FIGURE 2. Structure of typical blood vessel
The endothelial cell lines the blood vessel with an underlying basement membrane, and vascular smooth muscle cells are present on arteries and larger venules. Capillaries do not contain vascular smooth muscle cells and are the major site of exchange for nutrients, hormones, gases, and macromolecules. Any molecule for exchange must 1) move through the blood, 2) pass through the glycocalyx, 3) cross the endothelial barrier, and 4) diffuse through the interstitial space to the target tissue where it can 5) interact with the target tissue.
Some hormones can regulate the formation of new blood vessels, which will increase the surface area available for diffusion. These include angiotensin II, endothelin, growth hormone, insulin, and vasopressin, among others (18); hormones can induce angiogenesis either directly through actions on endothelial cells or indirectly by regulating proangiogenic factors like vascular endothelial growth factor [reviewed by Clapp et al. (18)]. There are also anti-angiogenic hormones, including somatostatin and ghrelin.

Aside from this chronic effect on surface area, acute manipulations of the vascular system can alter surface area much more dynamically.

**Vasodilation/Vasoconstriction**

As shown in Figure 2, the general structure of the endothelium includes vascular smooth muscle cells. These are able to induce vasoconstriction or vasodilation, thus modulating the diameter of the vessel (Figure 3). Known vasoactive hormones include serotonin, norepinephrine, insulin, and endothelin, among others. Nitric oxide (NO) is the major effector of vasodilation; its release from endothelial cells by nitric oxide synthase (NOS) leads to relaxation. The sex steroid estradiol rapidly activates cells by nitric oxide synthase (NOS) leads to relax-

**Capillary Recruitment**

Although vasodilation can increase the surface area of each vessel for nutrient exchange, vasoconstriction and vasodilation can also alter the surface area throughout the tissue in a process known as capillary recruitment. At rest, only a small portion of the capillaries through tissue are exposed to blood flow, but this can be altered by dilating or constricting the feed arterioles (Figure 3). For example, exercise increases the number of perfused capillaries in skeletal muscle to improve delivery of oxygen for muscle activity (69). Norepinephrine causes vasoconstriction, leading to the perfusion of more small vessels than under control conditions, and has a stimulatory effect on muscle metabolism as measured by oxygen uptake (61). If more of the vessels are perfused, the distance that molecules must diffuse to access the underlying tissue decreases: some hormones have this ability, thus increasing access to the underlying tissue. Although the ability of the hormone itself to access the tissue is important, it can also have a much more general effect on nutrient supply and even metabolism.

Techniques to measure capillary recruitment can investigate the breakdown of molecules by endothelium-bound enzymes; the rate of breakdown gives an indication of the endothelial surface area exposed to flow (67) and has shown microvascular effects of insulin (69,102) and serotonin (67); microdialysis (60,62) and Laser Doppler (19) have also been used to show insulin-mediated capillary recruitment. Also, contrast-enhanced ultrasound has demonstrated that the microvascular effects of insulin are an early effect regulating insulin-mediated glucose uptake (92,94). NO blockade decreases insulin-mediated capillary recruitment and also impairs glucose uptake in humans (91). Since NO antagonism should only prevent the vascular effects of insulin, it reinforces the concept that
endothelial function has an impact on the metabolism of the target tissue.

**Transport of Hormones Across the Endothelium**

The main function of the endothelium is as a semi-permeable barrier between plasma and the tissue; therefore, there are limited ways for hormones or other solutes to leave the vasculature. As discussed, in some tissues, hormone transport can occur through large pores, in other cases even tight junctions may permit paracellular "leak" (FIGURE 4). This involves passage through the junctions between the cells and is usually restricted to small molecules, such as urea, glucose, and ions; some have proposed that insulin may also use this method (73), although it is thought to be too big for this

**FIGURE 3.** Vascular smooth muscle cells lining the arteriole can modulate blood flow through capillaries

The vascular smooth muscle cells lining the arteriole can modulate blood flow through capillaries (A). Vasoconstriction in response to certain stimuli can prevent blood flow through the vessel (B). Other factors can dilate the vessel, allowing more blood flow through the vessel (C). Capillary recruitment refers to the perfusion of previously unperfused vessels. In a tissue bed, the arteries supply a network of capillaries, yet at rest not all vessels are perfused (D). Vasoactive hormones can increase the number of perfused vessels (E), thus increasing the ability of the hormone to access more of the tissue.
process. Paracellular transport would be considered a more passive role of the endothelium, yet it is increased specifically in venules during inflammation by opening the junctions between endothelial cells and can be modified by hormones such as ghrelin (49). Hormones can directly affect the permeability of a vessel, for example, angiotensin II increases vascular permeability factor gene expression (99), and angiotensin II-induced endothelial cell hyperpermeability can be inhibited by the hormone adiponectin (100).

Since endothelial permeability is dependent of the size of the solute, the pathway for transport of larger molecules such as hormones is different from that for smaller molecules (56). Aside from paracellular transport or movement through pores, transport through the endothelial cell can also occur.

**Transendothelial Transport**

Transcytosis, or the pathway that couples endocytosis to exocytosis on the opposite plasma membrane, is a function of endothelial cells (66, 79). Tracers are transported through the endothelial cell via vesicles (FIGURE 4), but vascular permeability varies depending on the vessel (23). Studies following the transport of the hormone human chorionic gonadotropin (hCG) through the vessel demonstrated internalization by coated pits and vesicles on the luminal side of the endothelium and showed in a time-dependent manner the transfer through tubulovesicular endosomes and large smooth vesicles to the abluminal space (37). Vesicular transport has also been shown for albumin (56) through albumin binding proteins on the endothelial cell surface. Leptin transport across the capillary endothelium was shown to be transcellular rather than paracellular and associated with plasmalemmal vesicles (12).

Caveolae are invaginations of the plasma membrane that occur on endothelial cells, and are involved in endocytosis and signal transduction. Hormones can be dependent on caveolae for their eNOS effects, such as estrogen (43), or for altering signaling cascades, such as leptin, an adipokine involved in energy homeostasis (80). Caveolae defects alter microvascular permeability (76) through a NOS-dependent pathway and may be compensated for by increased paracellular transport through larger interendothelial junctions (66). Although the contribution of caveolae includes signaling and trafficking within the cell (71), their role in vesicular trafficking and transcytosis has been much studied (reviewed in Ref. 66). Studies have suggested that caveolae are required for insulin transport through endothelial cells (97); however, much of the transported fluid is not receptor bound (87). Therefore caveolae can be involved in hormone signaling cascades, changes in endothelial permeability to alter access to the underlying tissue, or hormone transcytosis, which may or may not be bound to receptors.

**Receptor-Mediated Transport**

Although many hormone receptors can be expressed on endothelial cells, the receptor may contribute more to signaling and vascular effects as described above than to transport across the endothelium. Transport of hormones across the endothelial barrier is specific to the hormone. Insulin is possibly the most studied of these and has been shown to be concentrated in the endothelial cells relative to both plasma and muscle (96). Some studies have concluded that transport is saturable (26, 40) and, as such, must be receptor mediated; however, others have shown no saturation even at concentrations in the pharmacological range (18 µU·min⁻¹·kg⁻¹) (85), claiming that there is no evidence for receptor-mediated transport (10, 85) or...
that any receptor-mediated transport is not quantitatively significant for insulin transport (73). Interestingly, inactivation of the insulin receptor on endothelial cell has no effect under basal conditions but alters expression of vasoactive mediators and may play a role in maintaining vascular tone and regulation of insulin sensitivity (89). Perhaps the most likely explanation is that the insulin receptors are involved, but at higher doses the IGF-1 receptors also contribute to insulin transport (96).

The insulin receptors present on endothelial cells have been shown to be involved in the uptake of insulin (98), and more recent studies have shown that a knockout mouse model of endothelial IRS-2 is insulin resistant, and impaired insulin signaling in endothelial cells reduces insulin-mediated glucose uptake as well as impaired eNOS phosphorylation and capillary recruitment (48). Studies of human chorionic gonadotropin have shown transcytosis in the testicular microvasculature occurs via vesicles from the luminal to large vesicles open on the abluminal surface; this occurs through a receptor-mediated process (37). Receptor-mediated transcytosis of leptin (12) and growth factors (55) in other cell types have been shown, although information specific to the endothelium is scarce.

**Endothelial Dysfunction: The Endothelium in Disease**

Endothelial dysfunction is broadly used to describe any defect in the endothelium; however, it often refers to an impaired NO system, either by reduced NO production or increased breakdown due to oxidative stress. Endothelial dysfunction has been observed in hypertension, dyslipidemia, diabetes, obesity, and inflammation (32), and can be assessed in a variety of ways (reviewed in Ref. 32). Endothelial dysfunction can develop from otherwise healthy adaptive responses that become excessive or inappropriate (3).

**Cardiovascular Disease**

As mentioned, NO is responsible for the vasodilata
tion effects of many hormones. However, NO also prevents platelet adhesion and aggregation, leukocyte adhesion and migration, and smooth muscle cell proliferation, when these abilities are lost, atherosclerosis can develop. Hyperlipidemia leads to increased transcytosis of LDL, leading to accumulation of lipoprotein in the intima, and a cascade of events that lead to plaque formation and atherosclerosis (79). Decreased permeability in an experimental model of hypertension (13) and hypertensive humans with the metabolic syndrome (25) has been detected and may promote atherosclerosis development as well as impair the transendothelial transport of hormones.

**Diabetes**

Although impairments in cellular insulin response have been noted (6, 17, 29, 36), some studies show that signaling is not altered in insulin-resistant individuals (47), suggesting the contribution of a factor other than cellular response to whole body insulin sensitivity. Some studies have shown increased permeability in diabetic microcirculation mediated by hyperglycemia (75) or advanced glycation end products, the result of prolonged hyperglycemia (8), and acute hyperglycemia also increases glycocalyx permeability (103). However, decreased ability of a substance to reach the interstitial fluid was found in Type 2 diabetic subjects (39); since permeability of the endothelium is increased, it becomes more likely that delivery of the substance to the tissue is impaired. Consistent with this, capillary recruitment is inhibited in diabetic states (reviewed in Ref. 68). In a model of experimental insulin resistance, transendothelial transport was impaired, and it was proposed that, although cellular defects could also contribute, transport was responsible for one-third of the insulin resistance observed (58). So far, the correlation between interstitial insulin concentration and local glucose uptake (15) has held in insulin-resistant studies, where there was no significant increase in interstitial insulin when insulin was injected into the muscle and there was no change in local glucose uptake (14, 45). Therefore, there still appears to be a linear correlation between interstitial insulin concentration and local glucose uptake, suggesting the importance of transendothelial transport in insulin action.

**Obesity**

Decreased permeability (83) and microvascular function (84) have been detected in experimental obesity and also in obese humans (42). A high-fat diet reduces the distribution volume of macromolecules without altering the vascular volume, suggesting reduced delivery to tissues (27). Obese humans had impaired endothelium-dependent vasodilation in visceral adipose tissue, whereas nonendothelium-dependent vasodilation was unaffected (31), and the concurrent increase in inflammation suggested a potential link to atherosclerosis.

**Metabolic Syndrome**

Many hormones are tied to the actions of others. For example, endothelin is intrinsically involved in the vascular actions of insulin (30), stimulates catecholamine release (epinephrine and norepinephrine) in DOCA-salt hypertension (51), and is also involved in the vascular effects of steroids (progestins and androgens) (65). Norepinephrine and epinephrine are altered by insulin or insulin's
effects (53), yet epinephrine also modulates the renin-angiotensin system (81), which is involved in hypertension. Obesity impairs microvascular responses to insulin before a significant effect on metabolic processes (22, 84), and this has been proposed as a potential cause of the related disorders of insulin resistance and hypertension (90). In this way, the cluster of diseases that are associated with the metabolic syndrome begin to show their interrelatedness in terms of hormone effects. For example, angiotensin-converting enzyme inhibition can restore insulin-mediated capillary recruitment in late-stage diabetes (21). Insulin-resistant hypertensive patients with metabolic syndrome show systemically altered capillary permeability and blunted endothelial-mediated vasodilation (25), and this endothelial dysfunction could affect the delivery of any or all hormones to their target tissue.

**Future Directions**

Many studies on hormones use either plasma concentrations or in vitro preparations to determine hormone action and efficiency, yet the access of the hormone to the target tissue in vivo is a major determinant for action. There is much that is still unknown about how specific hormones cross the endothelial barrier and how this transport rate may be affected in disease. Insulin is possibly the best-studied of the hormones, yet the specific mechanism of transport is still being clarified (97). For many other hormones, the involvement of receptors in transendothelial transport is unknown. Studies of many diseases would benefit from a more complete understanding of the ability of hormones to access the target tissue, a factor that may be manipulated to improve disease.

In conclusion, the endothelium behaves in many tissues as both a structural and a functional barrier, and many hormones can have either a direct or indirect effect on the endothelium. By increasing the capillary surface area available for nutrient and hormone exchange, the access of the hormone and other nutrients to the target tissue, as well as whole body metabolism, can be altered by effects on the endothelium. These effects can be chronic, such as angiogenesis. However, the acute vascular effects such as vasoconstriction and vasodilation, which can potentially lead to changes in the distribution of flow, are more likely to have a rapid effect on access. The method of transport of a particular hormone across the endothelial barrier can also affect the activity of the hormone. This transendothelial transport can occur in a passive form such as leakage or diffusion but can also use a more regulated receptor-mediated transport system. Since many diseases, including hypertension, obesity, diabetes, cardiovascular disease, and atherosclerosis, exhibit some form of endothelial dysfunction, understanding the role of the endothelium in each of these diseases is essential. The interrelatedness of the actions of a range of hormones and their associated diseases suggests that the endothelium could be a good primary target, and in many cases restoring functionality of the endothelium may help in part ameliorate the disease.

**Endothelium as a Target for Treatment**

Since it has been shown that endothelium function is impaired in many diseases, the endothelium can be targeted to improve access to underlying tissue or to restore the functionality, thereby ameliorating some of the effects of the disease. The endothelium has been postulated as a target in chronic kidney disease, using, for example, erythropoietin, which protects endothelial cell function and integrity (33), and the cardiovascular benefits of resveratrol were investigated in retinal vascular disease (59), which is often associated with diabetes. The endothelium has even been suggested as a target for treatment of arthritis (72). Acute hyperglycemia increases glycocalyx permeability and decreases functional capillary density (103) as well as causing endothelial dysfunction and coagulation activation in vivo (63), and as such the glycocalyx is a potential target for treatment (5).

Perhaps the more interesting aspect of endothelial treatment is the ability to ameliorate several aspects of the metabolic syndrome at once. For example, activators of AMPK, such as AICAR (aminimidazole carboxamide ribonucleotide), activate eNOS and recruit microcirculation (9) but also improve glucose tolerance and lower blood pressure in insulin resistance (11). Angiotensin II receptor blockade appears to improve numerous aspects of the metabolic syndrome, including blood pressure and insulin sensitivity (64), and improves microvascular function in hypertension (41). Although it is likely that there are other factors that contribute to disease, the contribution of the endothelium to disease and its potential role as a drug target should be considered.
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