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Communication is Key: Mechanisms of Intercellular Signaling in Vasodilation

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Abstract

Thirty years ago Robert F. Furchgott concluded that nitric oxide, a compound traditionally known to be a toxic component of fuel exhaust, is in fact released from the endothelium, and in a paracrine fashion, induces relaxation of underlying vascular smooth muscle resulting in vasodilation. This discovery has helped pave the way for a more thorough understanding of vascular inter- and intracellular communication that supports the process of regulating regional perfusion to match local tissue oxygen demand. Vasoregulation is not only controlled by endothelial release of a diverse class of vasoactive compounds such as nitric oxide, arachidonic acid metabolites, and reactive oxygen species, but also via physical forces on the vascular wall and through electrotonic conduction through gap junctions. Although the endothelium is a critical source of vasoactive compounds, paracrine mediators can also be released from surrounding parenchyma such as perivascular fat, myocardium, and cells in the arterial adventitia to exert either local or remote vasomotor effects. The focus of this review will highlight the various means by which intercellular communication contributes to mechanisms of vasodilation. Paracrine signaling and parenchymal influences will be reviewed as well as regional vessel communication via gap junctions, connexons, and myoendothelial feedback. More recent modes of communication such as vesicular and microRNA signaling will also be discussed.

Introduction

The key function of the microcirculation is to supply oxygen (O₂) and nutrients to parenchymal tissue while removing metabolic waste. A vascular network comprised of microvessels, including small arterioles (30-150µm in diameter), capillaries, and venules is primarily responsible for these tasks. The microcirculation is ubiquitous, required to be in contact with each living cell in the human body.⁽¹⁾ The majority of flow regulation in the heart and other tissues is provided by adjustments in arteriolar vascular smooth muscle tone with only a small contribution from larger conduit arteries. Due to its high basal level of oxygen extraction, the heart is particularly sensitive to adjustments in perfusion, thus it is not

surprising that multiple mechanisms exist for fine-tuning arteriolar resistance to tightly control coronary flow. To achieve this degree of control, vessels are capable of sensing signals within the vessel lumen, as well as external environment cues (e.g. neural, metabolic, and mechanical activity) to adjust vascular tone.(2) The overall control of vascular tone relies heavily on proper synchronization via an organized network of communication within the vascular wall.(3)

Furchgott is credited with defining the endothelium as a critical regulator of vascular tone. Being the innermost layer of the vessel wall, the endothelium can quickly sense and respond to changes in circulating humoral factors as well as physical effects of blood flow itself, resulting in signal transmission to the underlying smooth muscle cells to adjust vascular tone.(4) While the list of endothelial-derived diffusible vasoactive agents continues to expand, the paradigm is shifting from one where autocrine factors released from the endothelium act on vascular smooth muscle cells, to a more globally coordinated model involving electrotonic and mechanical communication along and through the vascular wall. Control of vascular tone operates in a larger arena with multiple inputs from adjacent vascular segments. An example is “ascending dilation,” which electrically propagates longitudinally up and downstream from a local dilator response via gap junctions.(5) Likewise, electrical communication occurs in the radial direction between endothelial and smooth muscle cells via myoendothelial projections, extensions of endothelial cells which protrude through holes in the internal elastic lamina and abut smooth muscle cell membranes.(6)

All layers of the cell wall participate in regulating arteriolar vasomotion. Recent evidence suggests that paracrine mediators released from neighboring adipose tissue, myocardium, and skeletal muscle also contribute to the control of vascular tone. This review will highlight the various means of vascular intercellular messaging and discuss how each mechanism specifically contributes to the regulation of microcirculatory tone and thereby tissue perfusion.

Endothelial-Derived Paracrine Mediators

The endothelium is the largest continuous layer of cells in the body and serves as a critical interface between flowing blood and the static vascular wall. The endothelial lining is responsive to shear stress, and through mechanotransduction releases vasomotor factors or hyperpolarizes sarcolemmal membranes to actively modulate contractile activity of underlying smooth muscle in regulating flow.(7) This phenomenon, now referred to as endothelial-dependent vasodilation, was first identified by Robert F. Furchgott through a now classic series of elegant experiments demonstrating that acetylcholine (ACh) elicits vasodilation in an endothelium-dependent manner in rabbit aortic rings.(8) That study launched a massive search for and identification by innumerable laboratories of many other endothelium-dependent dilator stimuli. In many cases the endothelial-dependent smooth muscle relaxation was due to a transferrable substance released from the endothelium acting on the underlying smooth muscle, however in some cases direct hyperpolarization (via potassium channel opening) was responsible. The identification of a diffusible dilator factor was established using a bioassay or “sandwich” model where ACh was applied to a vessel with endothelium intact in close proximity to one denuded of endothelium.(8) Dilation of

the denuded vessel ring in this situation but not when tested in isolation is indicative of a transferrable mediator of vasodilation.(8) These experiments confirmed the presence of an endothelium-derived relaxing factor, or EDRF which is now known to be nitric oxide (NO), a mediator that diffuses out of the endothelial cell and communicates with underlying smooth muscle in a paracrine manner to regulate vascular tone and ultimately organ perfusion.

Nitric Oxide

Thirty years later we now understand that multiple endothelial-derived vasodilatory compounds exist with the most prototypical substance being nitric oxide (NO) formed from the endothelial isoform of nitric oxide synthase (eNOS) by a variety of pathways resulting in phosphorylation (S1799) or an elevation in endothelial cell calcium.(9),(10) This gaseous mediator diffuses to the smooth muscle cell where it activates soluble guanylyl cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP).(11) Increases in cGMP activate specific cGMP-dependent protein kinases (PKGs)(12), and initiates smooth muscle cell relaxation via multiple proposed mechanisms including alteration of membrane potential and intracellular calcium levels, activation of myosin light chain phosphatases, and even regulation of smooth muscle cell contraction through thin filament regulation.(13) The development of nitric oxide synthase (NOS) inhibitors such as N^G-monomethyl L-arginine (L-NMMA) not only helped to verify the contribution of NO in endothelial-dependent vasodilation(14), but also laid the groundwork for experimental observations that hyperpolarization and dilation can occur with mediators other than NO.(15)

In addition to eNOS, other forms of NOS contribute to smooth muscle relaxation and subsequent vasodilation. Neuronal nitric oxide synthase (nNOS) initially was thought to only play a role in the cerebral vasculature. S-methyl-L-thiocitrulline (SMTC), a selective inhibitor of nNOS has been shown to decrease blood flow in the human forearm as well as the coronary circulation.(16) It is now known that vascular smooth muscle cells express nNOS and interestingly can contribute to vasodilation when eNOS is dysfunctional. (17) Also located in the vascular wall, inducible nitric oxide synthase (iNOS) contributes to the significant vasodilation observed during inflammatory processes such as septic shock which is primarily due to the overproduction of NO.(18)

Prostacyclin

In 1976, four years before Furchgott's pioneering report, Moncada et al. isolated an enzyme from rabbit and pig aortas that catalyzed the conversion of endoperoxides to an unstable substance they referred to as PGX that was capable of inhibiting platelet aggregation and relaxing mesenteric arteries.(19) PGX, known today as prostacyclin (PGI₂), is an eicosanoid derivative of arachidonic acid (AA), and like NO has vasodilator properties which are mediated by activation of adenylyl cyclase leading to formation of cAMP and activation of PKA.(20),(21) Even though two Nobel prizes were awarded for the discovery of prostaglandin- and NO-mediated vasodilation (1982 and 1998, respectively), the critical role of the endothelium was first reported with NO and only later recognized for PGI₂ by Moncada.(19) Through microscopic observations of the rabbit aorta, Moncada et al concluded that the endothelial surface of the arterial wall generates the majority of PGI₂.(22)

Following release from cell membranes via phospholipase A₂ (PLA₂), AA is metabolized by cyclooxygenase (COX) enzymes to generate PGI₂ along with other eicosanoids.(23) Initial studies demonstrated that PGI₂ is capable of activating the adenylyl cyclase/cyclic-AMP transduction system. It was then later discovered that PGI₂ can also induce (19) hyperpolarization and relaxation of surrounding smooth muscle cells.(24) Although the most abundant prostaglandin in the vasculature is PGI₂, additional vasodilator prostaglandins are derived from endothelial cells including PGD₂ and PGE₂, (25) which may compensate for loss of endothelial-dependent dilation. For instance it has been shown that the mediator of ACh-induced vasodilation transitions from NO to PGD₂ in patients with inflammatory bowel disease. (26)

Endothelial derived hyperpolarizing factors (EDHF)

AA can also be metabolized by cytochrome P450 (CYP450) to form a mixture of 4 regioisomers of epoxyeicosatrienoic acid (EET; 5,6-, 8,9-, 11,12-, and 14,15-), EETs are one of the most widely studied endothelial derived hyperpolarizing factors.(27), (28) Responsible for dilation to bradykinin in arterioles from the human heart,(29) EETs contribute significantly to bovine conduit coronary dilation.(30) Importantly EETs can serve as a compensatory dilator mechanism when other endothelium-dependent dilator mediators such as NO, are compromised.(31) Through activation of smooth muscle large conductance Ca²⁺-activated potassium (BK_{Ca}) channels, EETs have been shown to vasodilate rat renal arteries(32) as well as cat cerebral arteries.(33) Activation of BK_{Ca} channels is achieved through increases in intracellular calcium sparks via opening of transient receptor potential (TRP) channels.(34) Interestingly, in human coronary endothelial cells AA but not EETs participate in activating TRP channels.(35)

Hydrogen Peroxide

In 2000, Matoba and colleagues observed that ACh-induced vasodilation and hyperpolarization were well preserved in eNOS-KO mice, however in the presence of catalase, an enzyme that converts hydrogen peroxide (H₂O₂) to oxygen and water, dilation was inhibited.(36) Further studies indicated that H₂O₂ was indeed an EDHF capable of inducing smooth muscle hyperpolarization, relaxation and vessel dilation in human, as well as in pig mesenteric arteries and canine coronary vessels.(37) Our lab has demonstrated that H₂O₂ contributes to FMD in coronary arterioles collected from patients diagnosed with CAD,(38) and further that the mitochondria is the primary source of H₂O₂ as evidenced by the fact that flow-mediated dilation (FMD) is greatly impaired in CAD arterioles in the presence of rotenone, a mitochondrial complex I inhibitor.(39) It is now accepted that H₂O₂, traditionally thought to be a destructive reactive oxygen species (ROS), is also an important endothelial-derived signaling molecule that is prominently active in the human microcirculation and acts as a compensatory mediator in patients with cardiovascular disease.(38) H₂O₂ is crucial in mediating dilation during shear stress, and contributes to the dilation evoked by agonists such as bradykinin (29). H₂O₂ importantly links myocardial metabolism to blood flow.(40) Thus the coronary arteriolar medial layer of smooth muscle is stimulated by H₂O₂ derived both from the endothelium during flow and from the underlying metabolic signal from the myocardium. While the exact mechanism of H₂O₂-induced dilation remains unclear, data supports numerous pathways as capable of mediating H₂O₂-

induced dilation including actions on cGMP(41), cAMP(42), as well as various potassium channels (e.g. BK_{Ca}) channels(43), ATP-sensitive potassium (K_{ATP}) channels, and voltage-sensitive potassium (K_v) channels.(44)

Hydrogen Sulfide

More recently, hydrogen sulfide (H₂S), a gaseous molecule similar to NO, has been identified as a EDHF.(45) Endothelial and smooth muscle H₂S formation is catalyzed by either cystathionine β-synthase (CBS) and/or cystathionine γ-lyase (CSE) by converting L-cysteine to L-serine, and H₂S.(46) Similar to NO, formation of H₂S is dependent on the calcium-calmodulin system for activation of CSE.(47) Despite the controversial evidence that H₂S can act as both an effective vasodilator as well as vasoconstrictor, studies show that the endothelial-derived version hyperpolarizes smooth muscle in a dose-dependent manner via activation of ATP-sensitive potassium (K_{ATP}) channels.(48) Other work has suggested a role for BK_{Ca} channels(49) as well as K_v channels, specifically K_{v7.4}. (50) Additional studies suggest that H₂S-induced vasoconstriction versus dilation is dependent on physiological oxygen (O₂) levels, where rapid contraction has been observed in the rat aorta with high O₂ levels as opposed to vasorelaxation in low O₂ environments. It is further believed that the vasoconstriction is primarily due to oxidation products formed between H₂S and O₂ rather than a direct effect of H₂S alone. (51)

As can be appreciated, substantial variation and redundancy exists among mediators of endothelium-dependent dilation across species and vascular beds. Beyond their ability to modulate vascular tone, these released substances have broader paracrine effects, modulating other physiological processes including inflammation, proliferation, fibrosis, apoptosis, and thrombosis. (1) To achieve such broad effects, these mediators are capable of moving beyond the vascular wall to influence surrounding parenchymal cells. It is likely that the diversity in mediator compounds coordinates in paracrine fashion, both tissue perfusion and tissue function. The nature of compensatory dilator substances may contribute to such diversity in physiology and pathology. (52)

The K⁺ ion itself is considered an EDHF. Edwards et al in 1999 concluded that K⁺ transferred from endothelial to smooth muscle cells was in fact an EDHF.(53) The authors went on to show that gap junction inhibitors (carbenoxolone and gap 27) eliminated ACh-induced smooth muscle hyperpolarization in carotid arteries indicating that gap junctions play a role in the radial propagation of electronic signals between the endothelium and medial layer of the vascular wall.(54) This work was expanded upon by Dora and colleagues who demonstrated that activation of SK_{Ca} channels in endothelial cells in response to ACh results in hyperpolarization of smooth muscle cells even in the presence of the NOS inhibitor, L-N^G-Nitroarginine methyl ester (L-NAME).(55) Further, this group showed that carbenoxolone inhibits endothelial-dependent smooth muscle hyperpolarization and subsequent relaxation in mouse mesenteric arteries.(56)

Non-Endothelial-Derived Paracrine Communication

Parenchymal Cell Influences

Since Furchgott's findings in 1980, the intimal layer of the vessel wall has been viewed as a paracrine tissue, capable of forming and releasing various factors to influence the medial layer and thus overall tone (see above section). During this period of discovery the outermost layer, the adventitial layer, comprised of fibroblasts, collagen, nerve endings, and the vasa vasorum, was primarily considered structural support for the blood vessel.(57) This view was changed by innovative studies demonstrating *in vivo* transfer and expression of recombinant eNOS in the adventitia of canine cerebral arteries.(58) Later studies would show improved vessel relaxation in both normal and atherosclerotic cerebral arteries indicating that adventitial cells can possibly compensate for loss of endothelium-derived NO.(59, 60) What has emerged from these and other studies is that adventitial fibroblasts are able to regulate vascular function through release of vasoactive mediators and may compensate for decreased endothelial delivery of NO during times of stress or disease.(61) On the contrary, the adventitia can also impair NO-mediated vasorelaxation. This was demonstrated by Pagano and colleagues who showed that NADPH oxidase activity in the rabbit aorta adventitial layer was capable of generating superoxide, ($O_2^{\cdot -}$) thus decreasing NO bioavailability.(62) Interestingly, eNOS gene transfer to the adventitial layer of rabbit carotid arteries decreases the contractile response to phenylephrine and may serve as a novel therapeutic strategy for cardiovascular disease where NO bioavailability is diminished.(63)

Vasoactive compounds also arise from plasma cells which have a critical role in the inflammatory process and can trigger significant dilation throughout the vasculature. (64) For instance histamine and serotonin are both considered vasoactive amines that cause profound dilation once released from mast cells and/or platelets. Substance P, a vasoactive peptide, is released from secretory vesicles found in sensory neurons and is also capable of vasodilation as well as triggering mast cell degranulation. (64)

Perivascular Fat

Perivascular adipose tissue (PVAT) was considered primarily a structural feature of arteries until Soltis and Cassis showed that the presence of PVAT decreased noradrenaline-induced constriction in rat aortic rings.(65) However interest in this novel paracrine peri-vascular region did not gain traction until ten years later when perivascular inflammation was observed following angioplasty in porcine coronary arteries.(66) Broad support exists for the idea that PVAT contributes to endothelial dysfunction and reduced vascular tone in subjects with cardiovascular disease.(67-69) Similar to the endothelium, multiple PVAT-derived relaxing factors (PVRF) have emerged as being responsible for vasodilation. In 2007 Fesus et al demonstrated using an adiponectin knock-out ($Apn^{-/-}$) rat model that adiponectin, a protein secreted by adipocytes ("adipokine") is a vasodilator of rat aorta and mesenteric arteries.(70) The cholesterol-lowering lipophilic HMG-coA reductase inhibitor Atorvastatin activates release of H_2S from PVAT causing subsequent relaxation in denuded rat aortic vessels.(71) Angiotensin 1-7 (Ang 1-7) was identified immunohistochemically in rat aortic PVAT where it stimulates an endothelium-dependent vasodilation inhibitable by A779, an antagonist of the Ang1-7 endothelial cell receptor (Mas receptor).(72) Using rat aortic rings,

Gao et al demonstrated that anti-contractile effects of PVAT involve the release of NO and H₂O₂ (73). PGI₂ and palmitic acid methyl esters have also emerged as potential PVRFs.(74, 75)

Mechanisms through which PVRFs induce smooth muscle hyperpolarization and relaxation remain unclear. PVAT-derived H₂O₂ may directly activate sGC in smooth muscle cells to initiate relaxation however evidence also suggests that PVRF activates endothelial cells to release NO with downstream K⁺ channel activation. Scavenging of NO, NOS inhibition, high extracellular K⁺, and inhibition of K_{Ca} channels block PVAT-dependent vasodilation. (76) As with EDRFs, the effect of PVRFs on vascular tone vary among species and may contribute to the heterogeneity observed between vascular beds. For example, peripheral vessel PVAT primarily promotes vasorelaxation whereas coronary PVAT impairs endothelial-dependent vasodilation and induces constriction.(77, 78) The nature of the fat depot may account for some of this variation since pericoronary adipose is much more pro-inflammatory than subcutaneous fat stores.(79) Although virtually all arteries are in contact with adipose tissue, the amount of PVAT at each specific artery can vary and may also account for variation. For instance coronary PVAT increases with age and is more abundant in men versus women. (80) As the majority of data has been derived from animal studies, additional studies in human tissue are required to fully understand the effect of PVAT on vascular tone.(81)

Metabolic Vasodilation

The most important and powerful regulator of coronary microvascular tone is cardiac metabolism. Coronary arterioles are designed to provide optimal second-to-second regulation of flow to meet the heart's metabolic demand.(82) This requires close communication between cardiac myocytes and the vasculature. In response to a stressor that increases local oxygen demand, vasoactive metabolites released from the myocardium act directly on coronary arterioles to increase local blood flow. In addition, the mechanism by which myocardial metabolism evokes an increase in local blood flow also involves remote signaling through shear stress. Myocardial metabolite-induced local arteriolar relaxation increases blood flow and dynamically shifts a larger percentage of overall vascular resistance upstream to larger arterioles and small arteries. The local increase in flow is also experienced by the upstream vessels serving the same myocardial area. In response to flow-induced shear stress the endothelium releases NO which promotes dilation. This further reduces overall vascular resistance specifically in the region of myocardium where metabolism is increased. In this way the metabolic vasodilation in the microcirculation recruits proximal vascular segments to further facilitate an increase in flow.

Regional Vessel Communication

Ascending Vasodilation and Gap Junctions

Following the first observation of remote vasodilation in the frog hindlimb by Krogh in the 1922,(83) a wealth of information has surfaced regarding the mechanisms involved in this tightly regulated process by which a local vasodilator stimulus is transmitted up- and down-stream. Elegant experiments performed by Segal and Duling in the late 1980's demonstrated

that microiontophoretic application of ACh in the hamster cheek pouch resulted in local as well as propagated vasodilation. The observed dilations were bidirectional and blocked by inhibitors of gap junctions including hypertonic sucrose solution, CO₂, octanol,(84) and carbenoxolone (54). The ensuing thirty years of experimentation have strengthened the hypothesis that gap junctions, bidirectional channels that connect the cytoplasm between adjacent cells, allow for a rapid means of communicating electrotonic impulses within the arteriole wall.(85)

Gap junctions permit direct transfer of molecules and ions between adjacent cells allowing for precise control and coordination of regional vascular tone.(86) For instance binding of ACh to the endothelium initiates production of inositol 1,4,5-trisphosphate (IP₃) which subsequently releases Ca⁺² from the endoplasmic reticulum to then stimulate Ca⁺²-activated potassium channels (K_{Ca}) in the plasma membrane. This induces a hyperpolarization that is conducted from endothelial cell to endothelial cells via gap junctions or radially into the medial layer through myoendothelial gap junctions. Vasodilation is ultimately achieved by the closure of voltage-gated Ca⁺² channels.(87) Cell-to-cell contact is accomplished by the physical interaction between hemichannels or connexons, with each connexon containing six connexins (Cx).(88) Interestingly, connexin isoforms exhibit different cellular localization as well as substrate specificity, implying that these connections mediate specific signaling events.(89) Of the twenty known connexin isoforms, five are expressed in the vasculature, including Cx 32, 37, 40, 43, and 45.(90),(91) Using immunohistochemistry techniques, Van Kempen and colleagues have shown that these connexins are differentially expressed throughout the pig, rat, and bovine aortic wall and coronary arteries, with endothelial cell connexons primarily composed of Cx37, while the majority of connexons in smooth muscle cells are comprised of Cx43 (92) which allow for the movement of Ca⁺² between each cell. (93) The complexity of these channels in the vasculature has been confirmed with knock-out studies of specific connexins. Liao and colleagues have shown that deletion of endothelial Cx43 in mice results in hypotension,(94) whereas smooth muscle knockout of Cx43 in the same species initiates accelerated growth of the neointima and adventitia.(95) Interestingly, neointimal formation is reduced in mice following Cx43 deletion in all cell types that express this specific connexin.(96) Substantial evidence indicates that vasoactive compounds modulate gap junctions, and vice versa, both *in vitro* and *in vivo*.(97, 98) Prior studies suggest that NO can either enhance or inhibit gap junction communication based on Cx type and cellular location. In microvascular endothelial cell culture, NO inhibits Cx37 in a non-cGMP-dependent manner(99) that likely involves S-nitrosylation.(100) Studies in human umbilical vein endothelial cells (HUVECs) indicate that NO-induced protein kinase A activation increases formation of Cx40, however inhibition of NOS has no effect on Cx expression. *In vivo* studies support these findings and indicate that NO impairs Cx37-mediated communication.(97) Conversely, connexins can regulate eNOS activity with Cx37 and Cx40 decreasing eNOS activity, with Cx40 being required for proper enzyme expression.(101)

The effect of EETs on gap junctions remains unclear. That EETs increase cAMP levels supports the notion that EETs can enhance gap junction Cx activity via cAMP-dependent phosphorylation.(98) Conversely, H₂O₂ inhibits gap junction-mediated communication in rat epithelial cells through phosphorylation of Cx43. Effects of other vasoactive substances (e.g.

PGI₂, H₂S, adenosine) on gap junction activity are largely unexplored but may play a role in fine vasomotor control.

Myoendothelial Projections

Myoendothelial projections (MEPs), endothelial cell protrusions that extend through the elastic lamina allowing contact with the underlying smooth muscle cell, act as an anatomical hub for gap junctions and intercellular communication across the basement membrane.(102) Under 100nm in diameter, their distribution is heterogeneous throughout the vasculature, with a higher concentration located in smaller caliber arteries as opposed to the larger proximal arteries.(103) To date, only Cx37, Cx40, and Cx43 have been identified within MEPs, with Cx40 shown to be involved in endothelial-derived hyperpolarization (EDH)-mediated vasodilation.(104) Variation in MEP distribution not only occurs within the vascular bed but between microvascular territories, species, and can act as a compensatory pathway for vasodilation with aging or during disease where NO bioavailability is decreased.(105) Also noteworthy, in addition to the traditional endothelium-to-smooth muscle cell signaling, MEPs may contribute to reverse signaling via myoendothelial feedback. In this situation, stimulated elevations in smooth muscle calcium are transmitted to the endothelium via MEPs resulting in endothelial hyperpolarization, which in turn acts as a brake by relaxing smooth muscle.(106)

Vesicular Communication

Microvesicles

In addition to the more classical forms of cell-to-cell communication (e.g. soluble mediators, gap junctions), microvesicles (MV), small particles (1 micron in diameter and lower) released by practically every cell type, have also emerged as a means for remote intercellular communication within the vasculature.(107) The concept of vesicles as tools contributing to cellular crosstalk was introduced decades ago.(108) Originally believed to be the result of cellular necrosis, more recent evidence suggests that vesicle release is a highly regulated active cellular process.(109) Different pathways lead to formation of vesicles of different sizes, and with varying content and membrane composition. Those between 500nm and 1µm in diameter are commonly referred to as ‘microparticles’ (MPs) whereas smaller vesicles, ranging approximately 50nm to 100nm are termed ‘exosomes.’ Collectively, these two populations are referred to as ‘microvesicles.’

Information regarding the formation of microvesicles has primarily been derived from experiments involving cultured cells. Over the last decade the list of stimuli identified to induce MV release has expanded greatly and includes shear stress,(110) plasminogen-activator inhibitor 1,(111) and tumor necrosis factor alpha.(112) Once in the extracellular space microvesicles can be broken up within minutes, or, circulate and travel to distant sites. These vesicles can circulate throughout the vasculature, activate transmembrane receptors on neighboring cells in an autocrine or paracrine manner, and bind to and release their contents into other cells.(113) The content is varied and can consist of proteins, lipids, and genetic material. Within the spheroid, proteomic studies performed on MPs from various cell types indicate the presence of proteins involved in cytoskeletal arrangement, adhesion, fusion,

protein folding, and metabolism,(114, 115) suggesting a selective composition. Valadi et al showing that exosomes contain functional mRNA as well as non-coding microRNAs.(116) Circulating microRNA has recently entered the spotlight as a potential biomarker for numerous diseases, however questions have been raised pertaining to the stability of secreted microRNA. Microvesicles may provide a protected microenvironment for microRNA allowing them to travel long distances within the vasculature to target sites.(117)

Despite the fact that microvesicles can interact with other circulating cells, the majority of information exchange occurs at the endothelial cell level, thus these particles can have a tremendous impact on vascular homeostasis and overall tone utilizing the vasculature as a “tube system” for remote communication.(118) In fact a strong correlation exists between impaired FMD in patients with chronic disease and the elevation of MPs. Amabile and colleagues reported increased levels of endothelial-derived MPs in patients with chronic renal disease who also exhibited reduced FMD.(119) Likewise, microvesicles collected from patients during acute myocardial infarction abolished ACh-induced relaxation in aortic rings. (120)

Although many studies have shown that MPs can evoke endothelial dysfunction, data regarding the impact of exosomes on vascular function is lacking. Interestingly, Wu and colleagues demonstrated beneficial effects of exosomal transfer of microRNA in cardiac progenitor cells within ischemic myocardium.(121) Although the majority of studies to date have supported the role of microvesicles having a detrimental effect on the vasculature, emerging data indicates that vesicles are capable of eliciting a positive or negative paracrine functional effect depending on their content or origin. Microvesicles as a novel means of remote vascular cell communication is an exciting new area for investigation.

Mechanistic Cross-Talk and Vasodilation

The various means of intercellular communication described in this review allow for redundancy in mechanisms of vasodilation to ensure adequate delivery of blood in order to match the demand of the organ. This redundancy not only allows for compensatory pathways to emerge during times of stress or disease, but also allows for cross talk between local regulatory factors and regional vascular control, providing precise control over tissue perfusion. For instance increased tissue metabolism triggers dilation locally however blood flow to the region is limited without the concurrent upstream vessel dilation initiated by the release of vasoactive substances in response to an increase in flow. Although the initial dilation may be due to metabolic factors, FMD fine-tunes support of flow to that specific vascular bed. Likewise following an initial local hyperpolarization stimulus, gap junctions allow for the propagation of ions resulting in smooth muscle relaxation, or ascending dilation along the vasculature wall. And finally remote communication throughout the vasculature can be achieved via release and downstream engulfment of microvesicles. Together these mechanisms may create a hierarchy of dilator control working in sync to efficiently provide proper flow to the tissue bed.

Summary and Conclusions

As a result of Furchgott's original discovery of an EDRF, a new scientific domain has emerged describing a highly coordinated chemical-based system of communication within and along the vasculature, extending to adjacent tissues. This review highlights the various mechanisms by which vascular intercellular communication can impact vasomotor responses and tissue perfusion. This complex yet coordinated physiological process relies on multiple modalities including paracrine mediators, gap junctions, and myoendothelial feedback. Novel regulators of vascular tone continue to emerge including remote delivery of vasoactive compounds in discretely secreted vesicular packets. The vasculature not only responds to meet tissue metabolic demands, but also directly influences parenchymal cell processes, establishing blood vessels as dynamic components of an organ's health and functional capacity. It is increasingly important to consider vascular function when assessing or treating any disease process.

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Fig. 1. Paracrine Signaling Resulting in Vasodilation

Endothelial activation may lead to formation of NO, PGI₂, AA, EETs, H₂S, or H₂O₂ which subsequently cause smooth muscle cell relaxation through various signaling pathways or via membrane hyperpolarization. The adventitial layer also contributes through activation of eNOS and generation of NO. Cardiomyocytes generate H₂O₂ during metabolism that results in vessel relaxation. Perivascular fat is capable of producing multiple vasoactive compounds including NO, PGI₂, H₂S, H₂O₂, ang 1-7, adiponectin, and palmitic acid. *These compounds elicit vasodilation via membrane hyperpolarization.

Fig. 2. Mechanisms of Intercellular Signaling Leading to Vasodilation

Cell-to-cell communication can occur via endothelial and non-endothelial-derived (e.g. cardiomyocytes, perivascular fat, adventitial cells) paracrine mediators, gap junctions, myoendothelial projections, as well as via vesicular communication (e.g. microparticles, exosomes, circulating microRNA).