137P FROM INTEGRINS TO ENOS: THE ENDOTHELIAL CELL AS A MECHANORECEPTOR

I. Fleming & R. Busse, Institut für Kardiovaskuläre Physiologie, Klinikum der J. W. Goethe-Universität, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

Hemodynamic forces, such as wall shear stress and cyclic stretch, exerted at the endothelial cell surface by flowing blood and pulsatile changes in pressure, are important in ensuring the continuous release of vasoactive endothelial autacoids.

Viewed on a purely structural basis, an endothelial cell is comprised of a membrane stretched over the nucleus, which is supported by a frame composed of micro-tubules, intermediate filaments and actin fibres. These structural components transverse the cell and terminate in characteristic lateral, basal and apical adhesion complexes. The whole structure is stabilised by association with adapter proteins inherent to these contact sites, as well as by attachment to the extracellular matrix at focal adhesion points and to adjacent cells at cell-cell contacts. Thus, even under resting conditions, the entire endothelial cytoskeleton is maintained under tension.

This tensegrity architecture implies that an externally applied mechanical load is superimposed upon pre-existing forces and is redistributed through the entire cell by the cytoskeletal scaffold. Re-orientation of cytoskeletal filaments relative to specific adapter proteins results in the activation of lipid and protein kinases/phosphatases without the need of a specific mechanoreceptor or the autocrine release of an endothelial activating factor. Rather than there being one site of mechanical stress sensing, the signalling pathways activated by the mechanical perturbation of endothelial cells are determined by the sites where there is the greatest alteration in force.

One of the most rapid events to occur following the application of shear stress to endothelial cells is the tyrosine phosphorylation of cell-cell boundary proteins, specifically of adhesion molecules, such as β-integrins, PECAM-1, and cytoskeleton-associated proteins such as paxillin.

The phosphorylation of these proteins (usually by the tyrosine kinase Src) is the signal which recruits signalling molecules to this site to initiate mechanochemical coupling. The phosphatidylinositol 3-kinase, for example, associates with phosphorylated VE-cadherin and PECAM-1 and activates protein kinase B/Akt. Once activated, Akt directly phosphorylates the endothelial NO synthase (eNOS) and enhances NO production without the need for intracellular Ca^{2+} to be increased.

There are additional signalling pathways activated in parallel following the application of shear stress. For example, eNOS is rapidly translocated to the nucleus via an Src- and Akt-independent pathway possibly involving Rac, Hsp90 and a motor protein such as dynein.

138P CAVEOLINS IN SIGNALLING, ONCOGENIC TRANSFORMATION AND MUSCULAR DYSTROPHY

Michael P. Lisanti, Department of Molecular Pharmacology & the Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Signal transduction pathways regulate growth, differentiation, and the development of an organism. In adult animals and humans, signal transduction maintains homeostasis or balance. When homeostatic mechanisms are interrupted, an illness or disease may ensue. Caveolae are plasma membrane specializations and caveolins are structural proteins used by cells to form caveolae (or "little caves" of 50-100 nm in diameter). We and other investigators have discovered that caveolae organelles may be important both in normal signal transduction and in the pathogenesis of a number of human diseases, including cancer and muscular dystrophy.

In my talk, I will update the working definition of caveolae, describe the functional roles of the caveolin gene family, and summarize the evidence that supports a role for caveolae as mediators of a number of cellular signaling processes. For more information, see the reviews listed below.

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139P INTERACTIONS BETWEEN THE NITRIC OXIDE AND HEME OXYGENASE SIGNALLING PATHWAYS IN ENDOTHELIAL AND SMOOTH MUSCLE CELLS IN OXIDATIVE STRESS

Giovanni E. Mann, Centre for Cardiovascular Biology & Medicine, GKT Sch Biomedical Sciences, KCL, Guy's Campus, London SEI

Atherosclerosis is a major contributor to cardiovascular disease (Ross, 1993), and the gaseous monoxides nitric oxide (NO) and carbon monoxide (CO) play important roles as cellular messengers involved in the regulation of vascular smooth muscle tone (Moncada et al., 1991, Maines, 1997). Increased expression of inducible isoforms of nitric oxide synthase (iNOS) and heme oxygenase (HO-1) have been detected in human atherosclerotic lesions (Buttery et al., 1996; Wilcox et al., 1997; Wang et al., 1998). Accumulating evidence in endothelial and smooth muscle cells indicates that NO donors and endogenously generated NO can induce expression of HO-1 in vascular endothelial and smooth muscle cells (Foresti et al., 1997; Durante et al., 1997; Hartsfield et al., 1997; Datta & Lianos, 1999). Furthermore, induction of HO-1 in vascular and other cells types may afford protection against NO and peroxynitrite mediated toxicity (Foresti et al., 1999).

Microsomal heme oxygenases degrade heme to biliverdin and CO, and the cytosolic enzyme biliverdin reductase then catalyzes reduction of biliverdin to bilirubin, both powerful chain-breaking antioxidants (Maines, 1997). Similar to constitutive (eNOS) and inducible (iNOS) isoforms of nitric oxide synthase, two principle isoforms of heme oxygenase have been identified - a constitutive isoform HO-2 (~34 kDa) and an inducible isoform HO-1 (~32 kDa), which is expressed at a low basal level in vascular endothelial and smooth muscle cells and is induced by heavy metals, oxidative stress, inflammatory mediators and oxidized low density lipoproteins (see Siow *et al.*, 1995; 1998; 1999). Although NO and CO modulate intracellular cGMP levels, platelet aggregation and smooth muscle relaxation, CO has a much lower affinity for soluble guanylyl cyclase than NO. Decreased production, bioavailability or sensitivity of target tissues to NO in atherosclerosis may be compensated for by an induction of HO-1, with bilirubin acting as a cellular antioxidant and CO as a potential modulator of vascular tone, as described in the liver (Suematsu *et al.*, 1995).

The potential involvement of stress proteins in protecting vascular cells against oxidant injury in atherogenesis is highlighted by reports that HO-1 gene transfer confers vascular protection (Abraham *et al.*, 1995), induction of HO-1 in LDL receptor knockout mice inhibits the formation of atherosclerotic lesions (Ishikawa *et al.*, 1997) and mice lacking heme oxygenase-2 are more susceptible to oxygen toxicity (Dennery *et al.*, 1998). Anti-atherogenic properties of the vascular heme oxygenase pathway are further highlighted by the report that expression of HO-1 is functionally associated with long term survival of cardiac xenografts (Soares *et al.*, 1998). As endothelium-derived NO synthesis and the sensitivity of target smooth muscle cells may be decreased in atherosclerosis, induction of HO-1 in endothelial and smooth muscle cells could provide an important adaptive mechanism for moderating the severity of ischaemia, thrombosis and atherosclerosis.

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140P GAP JUNCTIONS AND ENDOTHELIAL REGULATION OF VASCULAR TONE

Tudor M. Griffith, Department of Diagnostic Radiology, Wales Heart Research Institute, University of Wales College of Medicine, Cardiff CF14 4XN, UK.

Endothelium-dependent vascular relaxations that are observed in the presence of inhibitors of nitric oxide (NO) and prostanoid synthesis are widely attributed to an endothelium-derived hyperpolarizing factor (EDHF).

Although the existence of such a factor has been demonstrated in cascade bioassay and 'sandwich' preparations constructed from closely apposed arterial strips, in rabbit arteries and veins there is clear evidence that an EDHF transfers preferentially from endothelium to smooth muscle via gap junctions, rather than the extracellular space, following stimulation by agonists such as acetylcholine. These intercellular channels consist of two interlocked connexons, contributed by each coupled cell, which are constructed from six connexin (Cx) protein subunits arranged around a central pore that permits the passage of ions and molecules <1 kDa in size.

In rabbit arteries, EDHF-type relaxations and hyperpolarizations are attenuated in a highly specific fashion by synthetic peptides possessing sequence homology with the Gap 26 and 27 domains of the extracellular loops of the Cx43 connexin subtype. Inhibition of EDHF-mediated responses by such peptides is reversible, whereas inhibition by the established gap junction inhibitor 18-a glycyrrhetinic acid is irreversible. Consistently, in cultured cells expressing Cx43, fluorescence microscopy confirms that peptides do not affect the stability of gap junction plaques (ie clusters of gap junctions in the cell membrane), but that 18-a glycyrrhetinic acid causes plaque disassembly. In rabbit arteries EDHF-mediated responses depend crucially on mobilization of arachidonic acid by a Ca2+-dependent phospholipase A2, but the pathways involved distal to this initial step remain to be elucidated.

Arachidonic acid derivatives produced by the endothelium include cytochrome P450 monooxygenase-derived epoxyeicosatrienoic acids (EETs) and the cannabinoid N-arachidonylethanolamide (anandamide), which have both been proposed as candidate EDHFs. However, in endothelium-intact rabbit arteries, exogeneous EETs and anandamide themselves stimulate EDHF-type relaxations that are attenuated by gap junction inhibitors, thus indicating that they cannot be identical with EDHF. Gap junction-dependent effects may be largest in small vessels, and in the rabbit ear relaxations evoked by acetylcholine are dominated by this mechanism in vessels <100 mm diameter, even in the absence of eNOS inhibition.

This observation may explain why the magnitude of EDHF-type relaxations generally increases with decreasing vessel size, and suggests that heterocellular gap junctional communication may be of particular importance in the regulation of microcirculatory perfusion.

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141P HOMOCYSTEINE AND ENDOTHELIAL DYSFUNCTION: THE ROLE OF OXIDATIVE STRESS.

Dr. Derek Lang, Department of Pharmacology, Therapeutics & Toxicology, Wales Heart Research Institute, University of Wales College of Medicine, Heath Park, Cardiff, CF14 4XN.

Elevated plasma homocysteine levels are associated with inborn errors in its metabolism. Patients with these disorders have much higher plasma concentrations of homocysteine than normals (³ 80 mmol/L compared to £ 10 μmol/L) and are prone to premature cardiovascular disease. Severe hyperhomocysteinaemia is rare amongst the general population, whereas mild hyperhomocysteinaemia (15-30 μmol/L) is common.

Since the first presentation of "The Homocysteine Theory of Atherosclerosis" in 1969 by Dr. Kilmer McCully, more than 100 clinical and epidemiological studies investigating the relationship between homocysteine and cardiovascular disease have been published. The results of these studies have indicated that even mildly increased concentrations of homocysteine are associated with increased risk of arterial occlusive diseases.

The mechanism of the vascular injury seen in hyperhomocysteinaemia is not known, though growing evidence suggests that endothelial dysfunction plays a major role. Indeed, many human studies using methionine loading, which acutely elevates plasma homocysteine, induces endothelial dysfunction. Conversely, folate therapy, which lowers plasma homocysteine, has been shown to augment endothelial function.

In addition to hyperhomocysteinaemia, many other diseases which affect the cardiovascular system are also characterised by the development of endothelial dysfunction. It is becoming widely accepted that this endothelial dysfunction is due to an increase in oxidative stress. The reaction of oxygen free radicals such as superoxide anions with endothelium-derived nitric oxide could lead to a decreased bioavailability of nitric oxide. The resultant pathophysiological consequences of such a reaction are significant alterations in vascular function and the possible premature development of atherosclerosis. The question of whether hyperhomocysteinaemia results in overproduction of oxygen free radicals by endothelium remains unanswered, however.

Endothelial dysfunction in response to a homocysteine insult has been well described in vitro. This dysfunction is accompanied by increased superoxide anion production, which when inhibited, restores normal endothelial function. Furthermore, in human studies using methionine loading, the induction of endothelial dysfunction can be prevented by the antioxidant vitamin C. These observations therefore suggest that an elevation in plasma homocysteine can give rise to endothelial dysfunction via a mechanism that involves overproduction of reactive oxygen species.

142P TESTING ENDOTHELIAL FUNCTION USING ULTRASOUND

Jonathan Goodfellow, Wales Heart Research Institute, UWCM, Cardiff

Basic research over the past 20 years has clearly demonstrated the crucial role the vascular endothelium plays both in the maintenance of normal vascular physiology and in the pathophysiology of atherosclerosis, where endothelial dysfunction is a key early feature. Clinical studies have identified risk factors for atherosclerosis and shown a clear relationship between their presence and the development of premature cardiovascular disease.

The recognition that endothelial dysfunction plays an important role in atherogenesis and is associated with all the major risk factors for atherosclerosis has helped shift the focus away from structural/anatomical features of vascular disease to the important functional/dynamic features related to endothelial function.

Clinical research has been helped by the development of non-invasive ultrasound techniques which permit in vivo assessment of large artery endothelial function. The endothelium-dependent stimulus of increasing blood flow through an artery results in flow-dependent dilatation that is measured non-invasively using high-resolution ultrasound, the degree of dilatation serving as an indication of the functional integrity of the endothelium. The endothelium-independent stimulus glyceryl trinitrate acts as a control. This technique has been enthusiastically adopted as a non-invasive measure of endothelial function throughout the world.

Using such techniques, endothelial dysfunction has been demonstrated with each of the major risk factors for atherosclerosis, often before the development of overt vascular disease. The severity of the risk factors has been shown to correlate with the degree of endothelial dysfunction.

It is possible to improve endothelial function with interventions such as exercise, antioxidants, fish oils, cholesterol lowering, and hormone replacement therapy. Recent clinical studies using similar interventions have demonstrated reductions in clinical events, thus raising the possibility that the underlying mechanism is improvement in endothelial function.

Testing endothelial function using ultrasound represents a significant advance and provides a convenient method for serial evaluation of vascular function and may lead to greater insights into the nature of endothelial dysfunction and cardiovascular disease.