CaM Kinase II-dependent pathophysiological signalling in endothelial cells

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KEYWORDS
CaM Kinase II; Endothelial nitric oxide synthase (eNOS); Hydrogen peroxide; Shear stress; Actin cytoskeleton; Barrier function; Thrombin; Bradykinin

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1. Endothelial isoforms of CaM Kinase II
CaM Kinase II is encoded by four different genes (α, β, δ, and γ) with each isoform exhibiting various splicing variants. The α and β isoforms are most abundant in, and largely restricted to, the neurons, whereas δ and γ isoforms express in most tissues. For cardiomyocytes, δ is the most
prominent isofrom. It has remained unclear which isofrom dominants in the endothelial cells, but δ and γ isofroms have been found in both vascular smooth muscles\(^1\,4\) and endothelial cells.\(^5\) Earlier study by Deli et al.\(^6\) and Kitzbäei et al.\(^7\) also documented an ischaemia-induced activation of α isofrom in cerebral endothelial cells. Various studies from different groups have shown that the endothelial CaM Kinase II is KN93-sensitive (pharmacologic inhibitor). It also shares with the neuronal α isofrom the same conservative kinase domain, which can be competitively inhibited by a peptide targeting at threonine 286.\(^8\,12\)

2. Role of CaM Kinase II in redox-sensitive regulation of eNOS gene expression

Oxidant stress has been shown to contribute to endothelial dysfunction and cardiomypathy.\(^13\,15\) Among biologically relevant reactive oxygen species (ROS), hydrogen peroxide (H\(_2\)O\(_2\)), one of the dismutation/disproportionation products of superoxide anion (O\(_2^*\)\(^-\)), often mediates important signalling events, for example, regulation of endothelial nitric oxide synthase (eNOS) expression and function.\(^8,16\,19\) By producing nitric oxide (NO\(^*\)) to inactivate O\(_2^*\)\(^-\) and its derivatives, eNOS may also contribute to the anti-oxidative activities of endothelial cells. However, the resulting peroxynitrite is still more reactive than NO\(^*\) or O\(_2^*\)\(^-\). Recent studies have shown that it can also become ‘uncoupled’ to produce O\(_2^*\)\(^-\) rather than NO\(^*\). This phenomenon occurs in atherosclerosis or hypertension, representing a mechanism whereby oxidant stress sustains.\(^14,16,17,20\,22\) On the other hand, it also seems to suggest that a potentially compensatory upregulation of eNOS, observed in some hypertensive or diabetic animals,\(^23\,25\) may not be beneficial anymore.

Indeed, H\(_2\)O\(_2\) has been found to upregulate eNOS gene expression\(^8,26\) and mediate angiotensin II-induced uncoupling of eNOS.\(^22\) Interestingly, the critical mediator of H\(_2\)O\(_2\)-dependent upregulation of eNOS mRNA is CaM Kinase II.\(^8\) It turns out that CaM Kinase II can be rapidly phosphorylated upon exposure to exogenous H\(_2\)O\(_2\), resulting in tyrosine kinase activation, and an increase in eNOS gene transcription.\(^8\) These observations demonstrated that CaM Kinase II is indeed redox-sensitive and can unusually lie upstream of a tyrosine kinase janus kinase 2 to result in changes in gene transcription.\(^8\) Although the downstream transcriptional factors involved are yet revealed, cAMP response element binding protein (CREB) is excluded. H\(_2\)O\(_2\) induced a potent activation of CREB and ATF-1, but none of their phosphorylations was affected by inhibition of CaM Kinase II with KN93, or scavenging intracellular calcium with BAPTA/AM (unpublished results). Of note, earlier work by Marsen et al.\(^27\) demonstrated that thrombin induction of endothelin-1 mRNA expression is dependent on CaM Kinase II and its downstream transcriptional factor calcineurin. In human umbilical vein endothelial cells, histamine upregulation of eNOS mRNA and protein expression was also found dependent on CaM Kinase II.\(^28\)

In addition to exogenously applied H\(_2\)O\(_2\), oscillatory shear stress (OSS) activates endothelial CaM Kinase II via an increase in intracellular production of H\(_2\)O\(_2\).\(^11\) OSS occurs at bifurcations and branching points of the vascular tree, where atherosclerotic lesions are more frequent and severe.\(^29\,32\) On the other hand, unidirectional laminar shear stress, which occurs in the plain area of the vasculature representing unidirectional, tangential force evenly applied to the endothelial surface, is protective against atherosclerosis.\(^33\) Interestingly, both shear forces upregulate eNOS gene expression but via distinctive signalling mechanisms.\(^8,11,34\) CaM Kinase II is activated by oscillatory shear but inhibited by laminar shear. Inhibition of CaM Kinase II with KN93 blunted eNOS mRNA upregulation by oscillatory shear, so did scavenging intracellular H\(_2\)O\(_2\) by cell-permeable catalase.\(^11\) Different from laminar shear, oscillatory shear induces sustained increase in ROS production, which could potentially induce eNOS uncoupling. Thus, an upregulation of eNOS probably just turns the enzyme into a more efficient ‘peroxynitrite’ generator (peroxynitrite is formed by NO\(^*\) reaction with O\(_2^*\)\(^-\)), when eNOS is partially uncoupled. Also these data would suggest that similar to a potential causal role of CaM Kinase II in cardiac hypertrophy and post myocardial infarction remodeling,\(^35\,40\) CaM Kinase II activation may contribute to chronic endothelial dysfunction by mediating eNOS regulations in response to pathological agonists.

3. Role of CaM Kinase II in phosphorylation-dependent activation of eNOS and calcium-dependent vasorelaxation

In addition to chronic changes in gene expression, CaM Kinase II also mediates rapid activation of eNOS and calcium agonists-induced vasorelaxation. Bradykinin induced eNOS phosphorylation at serine 1179 was found inhibitable by KN93, which is accompanied by an attenuation of eNOS activity.\(^41,42\) Phosphorylation of eNOS often sensitizes eNOS to lower concentrations of calcium.\(^43,44\) Consistent with activation of eNOS, vasorelaxation induced by acetylcholine and calcium agonist A23187 was also attenuated by KN93.\(^42\) KN93, however, had no effects on vasorelaxation induced by NO\(^*\) donors.\(^44\) Thus, it seems that calcium dependent, physiological vasorelaxation is at least in part mediated by CaM Kinase II-dependent rapid enzymatic activation of eNOS. Interestingly, in human umbilical endothelial cells, thrombin activation of NO\(^*\) production was also found dependent on CaM Kinase II.\(^45\) However, whether CaM Kinase II is rapidly inactivated after initial increase in NO\(^*\) production, or cross-talks with other eNOS-activating protein kinase such as AKT/PI3K, remains to be elucidated. It is also unclear that how CaM Kinase II activation by different agonists diverges to differentially mediate patho- or physiologi
cal responses of the endothelium.

4. CaM Kinase II and actin cytoskeleton regulation

Interestingly, translocation of filamin, an actin-binding protein, is dependent on CaM Kinase II activation in endothelial cells\(^46\) with direct phosphorylation of filamin by CaM Kinase II.\(^47\) H\(_2\)O\(_2\) induction of actin stress fiber formation is dependent on p38 MAPK phosphorylation of the actin-binding protein heat shock protein 27 (HSP27).\(^48\) CaM Kinase II not only lies upstream of p38 MAPK/HSP27, but also precedes ERK1/2 activation, with both parallel pathways contributing to formation of actin stress fibers in response to H\(_2\)O\(_2\).\(^12\)
Recent innovative studies indicate the ‘house-keeping’ actin to exhibit important regulatory roles including cytoskeletal rearrangement, control of cell shape and movement, and regulation of gene expression. By determining subcellular localizations of transcriptional factors, or regulating chromatinremodelling complexes, actin can modulate gene transcription. Moreover, via modulation of its actin binding proteins, actin can influence mRNA stability, for example, that of eNOS. Interestingly, eNOS mRNA stability is increased in proliferative endothelial cells comparing with confluent cells, contributing to higher protein abundance. This response is consequent to a reduced binding of an actin-containing complex to the 3′-untranslated region of eNOS.

5. CaM Kinase II and regulation of microvascular barrier function

CaM Kinase II also appears to be involved in agonist-mediated endothelial cell contraction and barrier dysfunction. Using a well-established model of thrombin-induced endothelial cell barrier dysfunction involving myosin light chain kinase-regulated cytoskeletal rearrangement and contraction, and phosphorylation of the actin- and myosin-binding protein caldesmon, it was demonstrated that similar to thrombin, infection with a constitutively active adenoviral alpha-Cam Kinase II construct induced significant ERK activation, indicating that Cam Kinase II activation lies upstream of ERK1/2. Thrombin-induced ERK1/2-dependent caldesmon phosphorylation (Ser789) was inhibited by KN93, a specific CaM Kinase II inhibitor, or U0126, an inhibitor of MEK1/2 activation. Immunofluorescence microscopy studies revealed phosphocaldesmon colocalization within thrombin-induced actin stress fibers. Pretreatment with either U0126 or KN-93 attenuated thrombin-mediated cytoskeletal rearrangement and evoked declines in transendothelial electrical resistance while reversing thrombin-induced dissociation of myosin from nonenaturing caldesmon immunoprecipitates. These results strongly suggest the involvement of CaM Kinase II and ERK1/2 enzymatic activities in thrombin-mediated caldesmon phosphorylation and both contractile and barrier regulation.

Additionally, in brain capillary endothelial cells, activation of CaM Kinase II δ and γ isoforms was found upstream of voltage-gated potassium channels, resulting in hypoxia-induced cell swelling that likely precedes barrier dysfunction. In this study, antibodies recognizing δ and γ isoforms were used to specifically characterize expression of these endothelial CaM Kinase II isoforms. Interestingly, calcium ion handlings were also recently found regulated by CaM Kinase II in bovine pulmonary artery endothelial cells (marco-). Differential and common roles of CaM Kinase II in macro- and microvascular endothelial cell signaling are summarized schematically in Figure 1.

6. Perspectives of endothelial-specific regulation of CaM Kinase II

In cardiomyocytes, calcineurin, ERK1/2, histone deacetylase, apoptosis signal regulating kinase 1, and NFXB have been found downstream of CaM Kinase II-dependent cardiac hypertrophy. In the endothelial cells, however, the major transcriptional targets or immediate kinase substrates are yet identified. Although eNOS has putative phosphorylation sites for CaM Kinase II, there has been no direct evidence as to whether CaM Kinase II directly phosphorylates eNOS. It remains unclear whether enhanced eNOS phosphorylation upon bradykinin stimulation is a result of CaM Kinase II activation of an intermediate kinase. CaM Kinase II activates ERK1/2 in both endothelial cells and vascular smooth muscle, but it is also unclear whether ERK1/2 can be directly phosphorylated by CaM Kinase II. Whether chronic, endothelium-specific inhibition of CaM Kinase II improves endothelial function or endothelial barrier function in vivo has not been studied. Therefore, much remain to be learned regarding mechanistic insights of CaM Kinase II-mediated endothelial cell signalling.

In summary, despite that endothelial-specific regulations of CaM Kinase II are now only beginning to better understood, data accumulated so far have demonstrated a potentially significant role of CaM Kinase II in endothelial cell pathophysiology. Original research articles focusing on CaM Kinase II signalling in endothelial cells are summarized in Table 1. The critical roles of CaM Kinase II in modulating eNOS expression and function may underlie its possible contribution to atherogenesis where eNOS dysfunction occurs. Activation of CaM Kinase II in microvessels results in barrier dysfunction. On the other hand, transient activation of CaM Kinase II in endothelial cells may have important physiological roles in modulating vascular homeostasis via nitric oxide production and handlings of potassium and calcium ions. Overall the possible connections among these functional roles of CaM Kinase II remain to be fully elucidated.
Table 1  Original articles focusing on CaM Kinase II signalling in endothelial cells

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<tr>
<td><strong>Macrovascular ECs (aortic)</strong></td>
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<tr>
<td>Cai H et al. (2004) J Mol Cell Cardiol, 37:121–125, CaMKII mediation of OSS regulation of eNOS</td>
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<tr>
<td>Li H et al. (2003) Circulation, 107:2348–2354, Role of CaMKII in histamine upregulation of eNOS expression human umbilical endothelial cells</td>
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