Vascular CaMKII: heart and brain in your arteries

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Toussaint F, Charbel C, Allen BG, Ledoux J. Vascular CaMKII: heart and brain in your arteries. Am J Physiol Cell Physiol 311: C462–C478, 2016. First published June 15, 2016; doi:10.1152/ajpcell.00341.2015.—First characterized in neuronal tissues, the multifunctional calcium/calmodulin-dependent protein kinase II (CaMKII) is a key signaling component in several mammalian biological systems. Its unique capacity to integrate various Ca2+ signals into different specific outcomes is a precious asset to excitable and nonexcitable cells. Numerous studies have reported roles and mechanisms involving CaMKII in brain and heart tissues. However, corresponding functions in vascular cell types (endothelium and vascular smooth muscle cells) remained largely unexplored until recently. Investigation of the intracellular Ca2+ dynamics, their impact on vascular cell function, the regulatory processes involved and more recently the spatially restricted oscillatory Ca2+ signals and microdomains triggered significant interest towards proteins like CaMKII. Heteromultimerization of CaMKII isoforms (four isoforms and several splice variants) expands this kinase’s peculiar capacity to decipher Ca2+ signals and initiate specific signaling processes, and thus controlling cellular functions. The physiological functions that rely on CaMKII are unsurprisingly diverse, ranging from regulating contractile state and cellular proliferation to Ca2+ homeostasis and cellular permeability. This review will focus on emerging evidence of CaMKII as an essential component of the vascular system, with a focus on the kinase isoform/splice variants and cellular system studied.

CaMKII; calcium signaling; endothelium; vascular smooth muscle

SEMINAL WORK FROM BENNETT ET AL. (13) described a unique protein isolated from brain extracts, calcium/calmodulin-dependent protein kinase II (CaMKII) (13). The following years were marked by intensive research on this protein in neurons, driven by the high abundance and significant role of the kinase. Indeed, CaMKII is crucial for long-term memory processes through its capacity to integrate intracellular calcium (Ca2+) oscillations (for review see refs. 91, 119, 180). A tissue-specific distribution of the four mammalian CaMKII isoforms (α, β, δ, and γ) was initially established, with CaMKIIα and β being restricted to brain while δ and γ are expressed in peripheral tissues (104, 168). Additional work led to the identification of CaMKII as an important regulator of cardiac gene expression (168) and intracellular Ca2+ dynamics (183), further evidenced by its involvement in cardiac arrhythmia and hypertrophy (for review see refs. 110, 149, 162). More recently, a growing body of evidence in the vascular smooth muscle cell (VSMC) literature suggests a crucial involvement of CaMKII in the regulation of vascular functions. Indeed, VSMC migration, proliferation, and hypertrophy (28, 41, 87, 89, 143) as well as VSMC-dependent regulation of vascular tone were shown to be controlled by CaMKII (76, 135). CaMKII functions in endothelial cells (ECs) remained, however, largely unexplored despite significant potential involvement of the kinase. Recent work shows that CaMKII regulates crucial endothelial functions, including vascular permeability and nitric oxide (NO) production (18, 32, 68, 78, 117). In light of the rapidly changing and sometimes divergent nature of the literature on vascular CaMKII, this review will highlight exciting findings and future perspectives for the multifunctional kinase in vascular smooth muscle and endothelial cells.

Glossary

AIP Autocamtide-2-related inhibitory peptide
AMPAR α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPK 5’-AMP-activated protein kinase
ANGII Angiotensin II
AA Arachidonic acid
BAEC Bovine arterial endothelial cell
BKCa Large conductance Ca2+-activated K+ channels
BPAEC Bovine pulmonary arterial endothelial cell
BREC Bovine retinal endothelial cell
Ca2+/CaM Calcium/calmodulin complex

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<table>
<thead>
<tr>
<th><strong>CaM</strong></th>
<th>Calmodulin</th>
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<tbody>
<tr>
<td><strong>CaMKII</strong></td>
<td>Calcium/calmodulin-dependent protein kinase II</td>
</tr>
<tr>
<td><strong>Ca,1.2</strong></td>
<td>Voltage-gated L-type calcium channel</td>
</tr>
<tr>
<td><strong>CCE</strong></td>
<td>Capacitative calcium entry</td>
</tr>
<tr>
<td><strong>Cdk2</strong></td>
<td>Cyclin-dependent kinase 2</td>
</tr>
<tr>
<td><strong>Clca</strong></td>
<td>Ca	extsuperscript{2+}-activated chloride channels</td>
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<tr>
<td><strong>CPEAC</strong></td>
<td>Calf pulmonary arterial endothelial cell</td>
</tr>
<tr>
<td><strong>CREB</strong></td>
<td>cAMP response element-binding protein</td>
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<tr>
<td><strong>Cyt c</strong></td>
<td>Cytochrome c</td>
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<tr>
<td><strong>EC</strong></td>
<td>Endothelial cell</td>
</tr>
<tr>
<td><strong>EEL</strong></td>
<td>External elastic laminae</td>
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<tr>
<td><strong>HDMEC</strong></td>
<td>Human dermal microvascular endothelial cell</td>
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<tr>
<td><strong>ERK</strong></td>
<td>Extracellular signal-regulated kinase</td>
</tr>
<tr>
<td><strong>FBS</strong></td>
<td>Fetal bovine serum</td>
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<tr>
<td><strong>H2O2</strong></td>
<td>Hydrogen peroxide</td>
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<tr>
<td><strong>HDAC</strong></td>
<td>Histone deacetylase</td>
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<td><strong>HUVEC</strong></td>
<td>Human umbilical vascular endothelial cell</td>
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<tr>
<td><strong>HETEs</strong></td>
<td>Hydroxyeicosatetraenoic acids</td>
</tr>
<tr>
<td><strong>iNOS/NOS2</strong></td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td><strong>JAK2</strong></td>
<td>Janus kinase 2</td>
</tr>
<tr>
<td><strong>JNK</strong></td>
<td>c-Jun NH	extsuperscript{2}-terminal kinase</td>
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<tr>
<td><strong>K</strong></td>
<td>Potassium</td>
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<tr>
<td><strong>KCa2.3.x</strong></td>
<td>Small conductance Ca	extsuperscript{2+}-activated potassium channels</td>
</tr>
<tr>
<td><strong>Kf</strong></td>
<td>Inward rectifying K	extsuperscript{+} channel</td>
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<td><strong>KN-62</strong></td>
<td>4-[(2S)-2-[[5-isouquinolinsulfonyl]methylamino]-3-oxo-3-(4-phenyl-1-piperazinyl)propyl]phenyl isouquinolinesulfonic acid ester</td>
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<td><strong>KN-93</strong></td>
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<td><strong>Kv</strong></td>
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<td>Myosin light chain</td>
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<td>Lipopolysaccharides</td>
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<td><strong>LTP</strong></td>
<td>Long-term potentiation</td>
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<td><strong>MAPK</strong></td>
<td>Mitogen-activated protein kinase</td>
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<tr>
<td><strong>MEP</strong></td>
<td>Myoendothelial projection</td>
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<tr>
<td><strong>MEK</strong></td>
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<td>Methionine</td>
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<td>Myosin light chain kinase</td>
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<tr>
<td><strong>MMP</strong></td>
<td>Matrix metalloproteinase</td>
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<tr>
<td><strong>Na,1.5</strong></td>
<td>Sodium voltage-gated channel</td>
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<tr>
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<td>Norepinephrine</td>
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<tr>
<td><strong>NMDAR</strong></td>
<td>N-methyl-D-aspartate receptor</td>
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<tr>
<td><strong>nNOS/NOS1</strong></td>
<td>Neuronal nitric oxide synthase</td>
</tr>
<tr>
<td><strong>NO</strong></td>
<td>Nitric oxide</td>
</tr>
<tr>
<td><strong>PAEC</strong></td>
<td>Pulmonary arterial endothelial cell</td>
</tr>
<tr>
<td><strong>PAH</strong></td>
<td>Pulmonary arterial hypertension</td>
</tr>
<tr>
<td><strong>PAR1</strong></td>
<td>Protease-activated receptor 1</td>
</tr>
<tr>
<td><strong>PDGF</strong></td>
<td>Platelet-derived growth factor</td>
</tr>
<tr>
<td><strong>P13K</strong></td>
<td>Phosphoinositide 3-kinase</td>
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<td><strong>PKA</strong></td>
<td>Protein kinase A</td>
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<tr>
<td><strong>PKC</strong></td>
<td>Protein kinase C</td>
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<tr>
<td><strong>ROCK</strong></td>
<td>Rho-associated protein kinase</td>
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<tr>
<td><strong>ROS</strong></td>
<td>Reactive oxygen species</td>
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<tr>
<td><strong>RyR</strong></td>
<td>Ryanodine receptor</td>
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<tr>
<td><strong>Ser</strong></td>
<td>Serine</td>
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<tr>
<td><strong>SERCA</strong></td>
<td>Sarco/endoplasmic reticulum Ca	extsuperscript{2+}-ATPase pump</td>
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<tr>
<td><strong>SMC</strong></td>
<td>Smooth muscle cell</td>
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<tr>
<td><strong>SR</strong></td>
<td>Sarcoplasmic reticulum</td>
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<td><strong>Thr</strong></td>
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<td><strong>TNF-α</strong></td>
<td>Tumor necrosis factor-α</td>
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<tr>
<td><strong>VEGF</strong></td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td><strong>VSAC</strong></td>
<td>Vascular smooth muscle cell</td>
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The Blueprint

The unique capacity of CaMKII to integrate various Ca	extsuperscript{2+} signals into specific outcomes involves a complex series of autoregulatory processes attributable to the serine/threonine kinase’s particular structure. Of the four different isoforms of CaMKII characterized in mammals, CaMKIIα and β were first identified in brain and, until recently, were considered as brain specific while CaMKIIδ and γ were found in a broader range of tissues (168). A common organization of functional domains (e.g., N-terminal catalytic domain, central regulatory domain, C-terminal association domain) is shared amongst the four isoforms (90, 168). The association domain, which is unique to CaMKII within the CaMK kinase family, permits a multimeric conformation (or association) of 6 to 12 subunits and is the core of CaMKII’s ability to decode different types of Ca	extsuperscript{2+} oscillations (Fig. 1A; for review, see ref. 163). CaMKII oligomerization is not restricted to identical isoforms (74). Heteromultimerization is actually a key feature of the enzyme, allowing a sophisticated detection of Ca	extsuperscript{2+} signals and the associated response, seen as a capacity to finely tune Ca	extsuperscript{2+}-based intracellular signaling. However, to date the only heteromultimeric complexes to be detected in vivo comprise CaMKIIα/β: initially observed in neurons (22), the presence of CaMKIIα/β complexes was recently demonstrated in native endothelium (169). The inclusion of different isoforms and/or splice variants can convey unique properties to the CaMKII oligomer. For example, CaMKIIB binds F-actin, an ability not shared by other CaMKII isoforms, which can be useful to other subunits through heteromultimerization (145). Similarly, including splice variants can result in distinct subcellular distributions, as evidenced by the nuclear distribution of CaMKIIα compared with the sarcoplasmic reticulum restricted localization of CaMKIIδ (109). Therefore, CaMKII heteromultimerization expands the already broad range of interlinked signaling pathways into an amazing web of possibilities.

Initiation of CaMKII activation, common to all CaMKII isoforms, requires an increase in intracellular Ca	extsuperscript{2+} levels. In resting conditions, the catalytic activity of the enzyme is inhibited by the binding of the autoinhibitory sequence of the regulatory domain to the catalytic domain. As the first step...
The activation of phosphorylated CaMKII subunits will persist and already activated subunit (bound to Ca\(^{2+}\)) which becomes active in a Ca\(^{2+}\) phosphorylates an adjacent CaM-bound subunit (Fig. 1A). 

The activated (CaM-bound) subunit phosphorylates a subunit of CaMKII within the oligomeric complex and bind to a subunit of CaMKII within the oligomeric complex ("Ca\(^{2+}/CaM-dependent activation"). This disrupts the association between the regulatory autoinhibitory sequence and the catalytic domain, thus relieving inhibition and allowing phosphorylation of serine/threonine residues (reviewed in ref. 70). Such activity, being Ca\(^{2+}\)/CaM dependent, would closely parallel the subsequent decline in intracellular Ca\(^{2+}\) concentration and the resulting dissociation of the Ca\(^{2+}/CaM\) complex from the CaMKII subunit. The unique feature of CaMKII resides largely in the ability of activated subunits within the oligomer to undergo trans-autophosphorylation (138). Activated CaMKII subunits can phosphorylate threonine 286 residue (Thr286 for CaMKII\(\alpha\); Thr287 for \(\beta/\delta/\gamma\)) of an adjacent and already activated subunit (bound to Ca\(^{2+}/CaM\)) (20, 141). The activation of phosphorylated CaMKII subunits will persist even upon Ca\(^{2+}/CaM\) dissociation, a state called "Ca\(^{2+}/CaM independent" or "autonomous" activation (Fig. 1B)(94, 142). Autophosphorylation at Thr286 also decreases (1,000-fold) the rate of Ca\(^{2+}/CaM\) dissociation ("CaM-trapping") from CaMKII subunits, allowing the enzyme to remain active longer. In addition, a subsequent elevation in intracellular Ca\(^{2+}\) level will increase both the extent and duration of activation of a partly active CaMKII multimer (i.e., more active subunits) (130). This heteromultimeric structure combined with the ability of trans-autophosphorylation to augment and prolong activation allows CaMKII to integrate divergent forms of Ca\(^{2+}\) signaling (in frequency and amplitude) by converting a wide range of inputs into specific outcomes. The extent and duration of CaMKII activation is also influenced by the rate of dephosphorylation by protein serine/threonine phosphatases such as PP1 (17, 157), PP2A (64, 157), and PP2C (48).

Although less studied, autophosphorylation at threonine 305/306 (Thr305/306) also impacts on CaMKII activity; however, Thr305/306 phosphorylation is preceded by Thr286 phosphorylation (56, 125). In addition, autophosphorylation at Thr253 has been reported from in vivo studies (38) and is thought to be responsible for the targeting of CaMKII to neuronal postsynaptic densities (108). Modulation of prolonged CaMKII activity is not limited to autophosphorylation processes, as the enzyme is also sensitive to cellular redox state (27, 43), a potentially very important feature for an enzyme expressed in vascular cells and thus exposed to various degrees of oxidative stress. Anderson’s group showed that increased levels of reactive oxygen species (ROS) is associated with the oxidation of methionine residues (Met281/282) of CaMKII\(\beta\), which may be functionally analogous to autophosphorylation at Thr286/287 (43). Indeed, Met281/282 residues lie within the CaMKII regulatory domain and their oxidation prevents the regulatory domain from associating with the catalytic domain, thus maintaining CaMKII in an active conformation. This paired methionine motif is conserved in CaMKII\(\alpha\), \(\delta\), and \(\gamma\) isoforms, while the first methionine is replaced by a cysteine in CaMKII\(\alpha\). As with methionine, cysteine residues are susceptible to oxidation. However, both ROS- and autophosphorylation-dependent mechanisms require binding of Ca\(^{2+}/CaM\) to the regulatory domain for initial activation of a subunit (67, 122).

The Origins

The literature contains many reviews discussing the expression, subcellular distribution mechanisms of activation, function, and regulation of neuronal and cardiac CaMKII (21, 42, 54, 56, 61, 65, 152, 153). Accordingly, this section includes only a brief overview of CaMKII in these tissues as an introduction to its biological and cellular functions. CaMKII was initially characterized in brain tissue as a Ca\(^{2+}\)-dependent kinase controlling presynaptic neurotransmitter release, neuronal excitability, and postsynaptic regulation of learning and memory through long-term potentiation (LTP) (21, 57, 65, 70, 152). Indeed, upon synaptic stimulation, CaMKII converts transient Ca\(^{2+}\) signals into LTP, a sustained response (57, 92). NMDAR (51, 84, 158) and AMPAR receptors, two glutamate-gated ion channels, are the main targets for learning and memory.
CaMKII regulation of LTP. CaMKII phosphorylation of the AMPAR GluR1 subunit increases single-channel conduction (10, 34, 100, 154, 165), and is essential to LTP since transgenic mice lacking the corresponding phosphorylation site are characterized by a deficiency in LTP (10, 100, 195). CaMKII also regulates the trafficking and assembly of proteins such as GluA2 subunits within AMPAR, thus modulating Ca\(^{2+}\) permeation of the channels (97). Furthermore, the importance of CaMKII in learning and memory has been confirmed by reports of altered associated cognitive functions in mice deficient in CaMKIIe (147) or expressing CaMKIIe-Thr286Ala, which cannot undergo phosphorylation at position 286 (52).

Neuronal excitability is also linked to intracellular Ca\(^{2+}\) levels, and CaMKII has been suggested to modulate neuronal Ca\(^{2+}\) influx. CaMKII phosphorylates voltage-gated L-type calcium channels (Ca,2.1) when expressed heterologously in HEK or neuronal cell lines (72, 99). CaMKII is also a major player in cardiac intracellular Ca\(^{2+}\) homeostasis (21, 30, 45, 148, 164), as confirmed by studies of cardiovascular pathologies (30, 42). CaMKII regulates Ca\(^{2+}\) influx in cardiomyocytes by modulating Ca.1.2 channels and the associated Ca\(^{2+}\) current (16, 194). Furthermore, McCarron et al. (103) showed that inhibition of CaMKII activity prevented the increase in Ca,1.2 current elicited by Ca\(^{2+}\) in smooth muscle cells. In addition to Ca\(^{2+}\) influx, CaMKII also modulates the release of Ca\(^{2+}\) from intracellular stores. Phosphorylation of ryanodine receptors (RyR2) by CaMKII and oxidized-CaMKII was initially reported to stimulate sarcoplasmic reticulum (SR) Ca\(^{2+}\) release (31, 63, 164, 184, 186), although Yang et al. (2007) found that RyR2 phosphorylation by CaMKII reduces Ca\(^{2+}\) release and intracellular Ca\(^{2+}\) dynamics, including Ca\(^{2+}\) sparks and waves (191). Alternatively, CaMKII accelerates cytoplasmic Ca\(^{2+}\) cycling by enhancing SR Ca\(^{2+}\) uptake. Indeed, phosphorylation of proteins involved in refilling SR stores such as the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase pump (SERCA) (188, 189) or phospholamban (PLB) (148, 183) by CaMKII is associated with an increased SR Ca\(^{2+}\) uptake (176, 190). SR Ca\(^{2+}\) regulation appears to be dependent on αKAP, a CaMKII anchoring protein, allowing targeting of the kinase to SR in skeletal myocytes (11). CaMKII-αKAP interaction enhances the capacity of CaMKII to phosphorylate SR proteins such as RyR (55, 177, 186), SERCA (188, 189), and phospholamban (148, 183), and thereby, regulate myocyte Ca\(^{2+}\) homeostasis. Analogous anchoring proteins might very well be involved in directing the subcellular distribution of CaMKII in striated and nonstriated myocytes. Inositol 1,4,5-trisphosphate (IP\(_3\)) receptor type 2 (IP\(_3\)R2)-dependent increases in nucleoplasmic Ca\(^{2+}\) are reduced by CaMKII-mediated phosphorylation at Ser150, which reduces the open probability of the IP\(_3\)-R Ca\(^{2+}\) channel (9, 102). Hence, by directly phosphorylating Ca\(^{2+}\) channels or their accessory proteins, CaMKII is able to regulate both Ca\(^{2+}\) homeostasis and the dynamics of transient Ca\(^{2+}\) signaling events.

Excitable cells are essentially dependent on their membrane potential, and minor changes in their electrophysiological properties can result in significant outcomes. The first evidence that CaMKII may regulate cardiac membrane potential was the demonstration that CaMKII inhibitors autocomtide-2 and KN-63 prevent spontaneous depolarization in sinoatrial node cells (175). CaMKII positively modulates sodium voltage-gated channel (Na\(_a\).1.5), an essential component of cardiac EC coupling, resulting in an increased spontaneous activity (192). Still controversial, the actual CaMKII phosphorylation site in Na\(_a\).1.5 requires further investigation (3, 71). The functional expression of cardiac small conductance Ca\(^{2+}\)-activated potassium channels (K\(_{Ca}\).2.1) is also increased by CaMKII, leading to arrhythmia in ventricular hypertrophy (111). In addition to AMPAR and NMDAR channels, CaMKII also modulates other neuronal ion channels. The open probability of large conductance Ca\(^{2+}\)-activated potassium (K\(^+_\)) channels is increased by CaMKII in neurons. Indeed, van Welie et al. (172) reported a 50% increase in BK\(_{Ca}\) channel open probability when activated CaMKII was included in the patch pipette. Phosphorylation by CaMKII shifted the voltage dependency of BK\(_{Ca}\) towards more negative membrane potentials, resulting in increased channel activity at resting membrane potentials, membrane hyperpolarization, and diminished neuronal excitability (172). Heterologous expression systems have also been used to study the effects of CaMKII activation on ion channels found in neurons and cardiomyocytes, such as the voltage-gated potassium channel (K\(_v\)) largely expressed in cardiomyocytes and dendrites of hippocampal pyramidal neurons. Cotransfection of COS cells with K\(_{4.2}\) and constitutively active CaMKII resulted in increased channel trafficking to the plasma membrane (173). Regulation of K\(_{4.2}\) by CaMKII is not limited to channel trafficking, as electrophysiological studies in rat cardiomyocytes have shown an acceleration of I\(_{Ks,fast}\) (K\(_{4.2}\)-K\(_{4.3}\) complexes) inactivation, potentially occurring through CaMKII-K\(_{4.3}\) interactions (29). Hence, there is accumulating evidence showing direct regulation of membrane potential by CaMKII in excitable cells of cardiac and neuronal origin.

The control of gene expression by CaMKII, another hallmark of the significant role CaMKII plays in regulating key cellular functions, occurs mostly via two main pathways. Through phosphorylation of histone deacetylase (HDAC) in cardiomyocytes and other cell types, CaMKII stimulates the export of HDAC from the nucleus, allowing gene expression (5). This process is involved in CaMKII-induced cardiac hypertrophy (4, 95). Merlen et al. (107) recently showed that nuclear type B endothelin receptors activate CaMKII through IP\(_3\)-R-dependent Ca\(^{2+}\) release in cardiomyocytes and might lead to HDAC phosphorylation. Additionally, the transcription factor “cAMP responsive element binding protein” (CREB) has been identified as a CaMKII target. However, regulation of CREB by CaMKII is complex and involves phosphorylation at two different sites having opposing effects. Although phosphorylation by CaMKII at Ser142 blocks CREB activation, phosphorylation at Ser133 by CaMKII or PKA activates CREB (161). In addition, phosphorylation at Ser142 prevents phosphorylation at Ser133 from activating CREB. The negative effect of Ser142 phosphorylation appears to be dominant, as constitutively active CaMKII only activates CREB when Ser142 was replaced with alanine (161).

Decades of research on CaMKII in cardiac and neuronal tissue have led to significant advancements in our understanding of Ca\(^{2+}\)-dependent regulation of many cellular processes. More recently the vascular field has become interested in the role of CaMKII, as there exist vascular counterparts to several of the cellular functions controlled by neuronal/cardiac CaMKII that play critical roles in cardiovascular homeostasis.
The main physiological function of vascular smooth muscle cells is to develop force through contraction to maintain appropriate levels of vascular tone. Despite the major influence of vascular endothelial cells, vascular smooth muscle function is essentially dependent on intrinsic mechanisms. These mechanisms, such as those regulating myocyte contraction, are sophisticated. Moreover, processes guiding VSMC proliferation and migration are also critical as they have been associated with vascular pathologies such as atherosclerosis or hypertension (reviewed in refs. 26, 137). Similar to neurons and cardiomyocytes, CaMKII appears to modulate several VSMC functions (Table 1). VSMCs show a variety of intracellular Ca\(^{2+}\) signals with distinct kinetics, frequencies, and subcellular origin (44, 121), providing a wide range of potential sources for CaMKII activation with specific intracellular localization.

Table 1. *CaMKII* in smooth muscle cells

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Cell Type</th>
<th>Culture Condition</th>
<th>Target</th>
<th>Effect</th>
<th>Function</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>CaMKIIβ2</td>
<td>Rat thoracic aortic SMC</td>
<td>Fresh + NC P3-P10</td>
<td></td>
<td>Increase cell cycle progression</td>
<td>Positively regulates proliferation</td>
<td>(66)</td>
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<td>CaMKII</td>
<td>Rat thoracic aortic SMC</td>
<td>NC P7-P14</td>
<td></td>
<td></td>
<td>Positively regulates proliferation</td>
<td>(126)</td>
</tr>
<tr>
<td>CaMKIIβ2</td>
<td>Rat aortic SMC</td>
<td>NC P3-P10</td>
<td></td>
<td></td>
<td>Positively regulates proliferation</td>
<td>(127)</td>
</tr>
<tr>
<td>CaMKIIδ</td>
<td>Mouse aortic SMC/entire mouse carotid</td>
<td>NC P4-P10</td>
<td>AKT/Mdm2/p53/p21 pathway</td>
<td>Downregulation of p21</td>
<td>Positively regulates proliferation</td>
<td>(89)</td>
</tr>
<tr>
<td>CaMKIIδ</td>
<td>Rat thoracic aortic SMC</td>
<td>NC P4-P8/NC P3-P9</td>
<td>Raf1</td>
<td>Complex with ERK, translocation nucleus</td>
<td>Positively regulates proliferation</td>
<td>(28, 106)</td>
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<tr>
<td>CaMKIIδ</td>
<td>Rat aortic SMC</td>
<td>NC P3-P7</td>
<td>CREB</td>
<td>Inhibits CREB activity</td>
<td>Positively regulates proliferation</td>
<td>(93)</td>
</tr>
<tr>
<td>CaMKIIδ</td>
<td>Mouse aortic SMC/entire mouse carotid</td>
<td>NC P4-P10</td>
<td>MMP9</td>
<td>Increase MMP9 activity</td>
<td>Positively regulates migration</td>
<td>(143)</td>
</tr>
<tr>
<td>CaMKII/CAMKIIδ</td>
<td>Rat thoracic aortic SMC/human aortic VSMC</td>
<td>NC P5-P14/NC P3-P10</td>
<td>ERK</td>
<td>Activated by adhesion, increase ERK phosphorylation</td>
<td>Positively regulates migration</td>
<td>(14, 96)</td>
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<tr>
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<td>Second-order mouse mesenteric SMC</td>
<td>Fresh</td>
<td>Cavβ3 subunit/PLB</td>
<td>Increase Ca(^{2+}) entry, increase SR Ca(^{2+}) load</td>
<td>Positively regulates migration</td>
<td>(128)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Rabbit pulmonary/coronary SMC</td>
<td>Fresh/Fresh</td>
<td></td>
<td>Decrease Cl(^{-}) current</td>
<td></td>
<td>(53, 82)</td>
</tr>
<tr>
<td>CaMKIIγ</td>
<td>Ferret thoracic aorta (± endothelium)</td>
<td>N/A</td>
<td>MLCK/LC20</td>
<td>Increase MLCK/LC20 phosphorylation</td>
<td>Positively regulates SMC contraction</td>
<td>(76)</td>
</tr>
<tr>
<td>CaMKIIγ-G2</td>
<td>Ferret thoracic aorta (± endothelium)/ferret aortic SMC</td>
<td>Fresh</td>
<td>LC20</td>
<td>Increase LC20 phosphorylation, CaMKII plasma membrane recruitment after depolarization</td>
<td>Positively regulates SMC contraction</td>
<td>(101)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Rat thoracic aortic SMC/rat aortic SMC</td>
<td>NC P4-P8/NC P3-P9</td>
<td>NOS2</td>
<td>Resting CaMKII in complex with NOS2/NOS2 trafficking</td>
<td>Production or inhibition of NOS2 activity</td>
<td>(35, 73)</td>
</tr>
<tr>
<td>CaMKIIβ2</td>
<td>Rat aortic SMC</td>
<td>NC P4-P10</td>
<td></td>
<td></td>
<td>Increase VSMC hypertrophy</td>
<td>(87)</td>
</tr>
<tr>
<td>CaMKIIα/ICAM</td>
<td>Rabbit aortic SMC/human aortic SMC</td>
<td>NC P2-P10/NC P4-P10/C (ATTC) P-NS</td>
<td>MEK/PLA(_2)</td>
<td>Increasing MEK/PLA(_2) activity, translocation of MEK/PLA(_2)/CaMKII complex to the nucleus, AA production</td>
<td></td>
<td>(113–115)</td>
</tr>
</tbody>
</table>

NC, noncommercial cell line; C, commercial cell line; N/A, not applicable; P, passage; NS, not specified.

Migration and proliferation. The relationship between CaMKII and VSMC proliferation and migration has been extensively studied and still garners significant interest. Indeed, vasculogenesis and pathological neointimal proliferation are physiologically relevant and enticed research in this field. Interestingly, the expression pattern of CaMKII is linked to myocyte phenotype. Indeed, primary/differentiated VSMCs express primarily CaMKIIγ whereas CaMKIIδ expression is higher in proliferative/cultured VSMCs (66). Platelet-derived growth factor (PDGF) triggers both a rapid activation of CaMKII and CaMKII-dependent rise in intracellular Ca\(^{2+}\) in cultured proliferative VSMCs but not in quiescent myocytes (126). This cytoplasmic increase in Ca\(^{2+}\) and CaMKII activation is important for VSMC migration and proliferation: inhibition of CaMKII with KN-62 (126) or KN-93 (127) almost abolishes PDGF-induced myocyte migration. In a carotid injury model,
medial hyperplasia correlates with an increase in the expression of CaMKIId (≈43%) but not of CaMKIId. Accordingly, neointimal proliferation of VSMCs is significantly lower in CaMKIId−/− mice compared with wild-type mice (89). The involvement of CaMKIId in pathological vascular remodeling can also be extended further, as knockout mice are also protected against structural changes to the external elastic laminae (EEL) following carotid injury (143).

Agonist-induced, CaMKIId-dependent proliferation and migration of VSMCs is not limited to PDGF. Indeed, cultured VSMCs from CaMKIId−/− mice showed similarly reduced migration in the presence of other growth factors (e.g., FBS, TNF-α) but also in the absence of stimulation (143). Accordingly, overexpression of CaMKIId rescued VSMC migration (143). Transgenic expression of an oxidant-resistant form of CaMKIId (methionine-to-valine mutation) did not affect VSMC proliferation in the carotid ligation injury model, suggesting that ROS-dependent activation of CaMKIId is required (199). Despite conflicting reports obtained using cultured cells (66, 106, 127), CaMKIId is still considered as a major regulator of VSMC proliferation and migration.

Cell cycle progression. The modulation of VSMC proliferation by CaMKIId is a consequence of its role in cell cycle control. Indeed, CaMKIId regulates the expression of cyclin-dependent kinase 2 (Cdk2) and cyclin E (89), critical cell cycle activators that promote the transition from G1 to S phase (146). Li et al. (89) showed that the increase in Cdk2 and cyclin E expression in a carotid ligation injury model was prevented by knocking out CaMKIId. Moreover, Cdk2 and cyclin E activity is regulated by the cell cycle inhibitor p21, and p21 expression is higher in carotids from CaMKIId−/− mice that underwent carotid ligation injury than in ligated arteries from wild-type mice. Therefore, the expression pattern of cell cycle regulators appear to be influenced by CaMKIId, as the absence of this kinase correlates with a decrease in VSMC proliferation and cell cycle arrest (89). A detailed signaling pathway encompassing AKT/Mdm2/p53 was further elucidated by Li et al. (89), and CaMKIId was shown to stimulate AKT activation in VSMCs (85). This leads to high levels of Mdm2 phosphorylation at the AKT-specific Ser166 site (198) and the ensuing p53 degradation in WT mice. The loss of p53 significantly decreases p21 expression. In CaMKIId−/− VSMCs, activation of the AKT/Mdm2/p53 pathway is attenuated, p21 expression increases, and proliferation is thus reduced (89).

Crucial to cell cycle control through the sustained expression of proteins such as cyclin D1 (182, 185), the ERK pathway is also targeted by CaMKIId. CaMKIId is required for ERK activation, since its suppression is associated with lower ERK activation (106). A direct interaction between ERK and CaMKIId was demonstrated by coimmunoprecipitation assays that also showed that formation of an ERK-CaMKIId complex was prevented by either U0216, which inhibits ERK activation, or KN-93 (28). The binding of Raf1 with CaMKIId precedes the formation of ERK-CaMKIId complexes (28). Indeed, the interaction of CaMKIId/Raf1 with ERK allows ERK to phosphorylate CaMKIId, leading to nuclear translocation of the macromolecular complex and alterations in transcription.

CREB activation is strongly associated with inhibition of VSMC proliferation. Expressing a constitutively active CREB in VSMCs eliminates FBS-induced myocyte proliferation and decreases the expression of growth factors and growth factor receptors such as the PDGF receptor (77). The role of CaMKIId in modulating the cell cycle in VSMCs via CREB was investigated by Singer’s group (93), following up on initial reports of CREB inhibition by CaMKIId-mediated phosphorylation at Ser142 (161). Thrombin- and ionomycin-induced increases in CREB phosphorylation at Ser142 were significantly lower in cells exposed to KN-93 or siRNA targeting CaMKIId (93). In summary, CaMKIId strongly stimulates VSMC proliferation through inhibition of CREB activity.

Interaction with the extracellular matrix. CaMKIId modulation of VSMC migration includes degradation of the extracellular matrix or reduced cellular adhesion. Matrix metalloproteinases (MMPs) are a family of proteases involved in extracellular matrix breakdown or remodeling. Using rat cardiac fibroblasts, Zhang et al. (197) provided the first evidence that CaMKIId regulates MMP expression in the cardiovascular system. A similar relationship exists in smooth muscle (143). Upon stimulation with TNF-α, MMP9 activity, immunoreactivity, and mRNA levels were markedly lower in serum collected from cultured CaMKIId−/− VSMCs relative to wild-type cells (143). These results suggest that CaMKIId regulates MMP9 activity at the level of its transcription or translation; however, the authors suggest that CaMKIId regulates MMP9 posttranscriptionally by increasing the stability of its mRNA. This study established that CaMKIId may significantly influence cell migration through extracellular matrix breakdown.

Cellular interactions with the extracellular matrix, including focal adhesions, are critical for cell migration. Activating integrin signaling triggers Ca2+-transients in VSMCs (139) that are thought to activate Ca2+-dependent proteins involved in cell migration. Integrin-dependent CaMKIId activation was first investigated with PDGF-induced migration of smooth muscle cells (14). In vitro, inhibition of the vitronectin receptor, an integrin found in VSMCs, decreases PGF-induced CaMKIId activation twofold. This led to the suggestion that inhibition of vitronectin receptors suppresses IP3 production and the corresponding changes in intracellular Ca2+ (14). However, Lu et al. (96) reported the activation of CaMKIId by cellular adhesion through an integrin-independent mechanism. Although no evidence of a direct interaction was shown, subsequent phosphorylation of ERK can be blocked by KN-93 or shRNA targeting CaMKIId. This study, along with work from Cipolletta et al. (28), illustrates the complex pathways and reciprocal relationships involving CaMKIId in the control of VSMC proliferation and migration.

Smooth muscle contractility. The primary role as a regulator of vascular tone is conferred to vascular smooth muscle cell by its contractile capacity and is of utmost physiological relevance. In contrast to studies of migration and proliferation, investigation of the involvement of CaMKIId in modulation of vascular tone is mainly limited to native cells, since cultured cells are generally in a proliferative state. Despite a significant body of evidence establishing the regulation of cardiomyocyte contraction by CaMKIId, its role in regulation of VSMC contraction and vascular tone is less well characterized.

Contraction of VSMCs is essentially, but not exclusively, dependent on Ca2+-homeostasis (for review see ref. 181) and relies primarily on Ca2+ entry through CaV1.2 and to a lesser extent on other mechanisms including IP3,R-dependent Ca2+ release from the SR (for review see ref. 62). Investigation of the modulation of Ca2+-homeostasis in VSMCs by CaMKIId, as
described above in neurons or in cardiomyocytes, turned out to be more complex than first expected. Indeed, the most commonly used CaMKII inhibitor, KN-93, appeared to have non-specific effects on smooth muscle Ca\(^{2+}\) and K\(^+\) channels (50, 81, 133), indicating that data obtained using KN-93 in VSMCs requires careful interpretation. Early work by McCarron et al. (103) showed that CaMKII phosphorylates Ca\(_\text{a,1.2}\) in smooth muscle cells. More recently, Grumbach’s group investigated the role of CaMKII in the regulation of Ca\(^{2+}\) homeostasis using TG-SM-CaMKII, a transgenic mouse line with the VSMC-specific expression of a CaMKII inhibitory peptide, CaMKII\(\text{N}\) (128). Prasad et al. (128) reported a reduction of CaMKII activity (−65%), which correlates with an alteration of myocyte electrophysiological properties. Phosphorylation of the Ca\(_{\beta3}\) subunit was lower in VSMCs from TG-SM-CaMKII mice, resulting in a decreased angiotensin II (ANGII)-induced Ca\(^{2+}\) entry. Moreover, SR Ca\(^{2+}\) loading was also diminished by the expression of CaMKII\(\text{N}\), correlating with a 50% reduction of PLB phosphorylation (Thr17) and hence, a greater inhibition of SERCA (128).

Regulation of VSMC membrane potential is an efficient approach to modulate VSMC contraction, owing to the strong voltage-dependency of Ca\(^{2+}\) channel opening. The inward rectifier K\(^+\) channel (K\(_i\)) is a strong modulator of VSMC membrane potential level. Activation by extracellular K\(^+\) leads to VSMC hyperpolarization and vasodilatation (40, 196). Despite the absence of a reported relationship between CaMKII and K\(_{\text{ir}}\), a recent study in primarily cultured bovine pulmonary arterial endothelial cells (PAEC) shows that CaMKII is able to regulate K\(_{\text{ir}}\) current (132). One could then speculate that VSMC K\(_{\text{ir}}\) is also modulated by CaMKII. In addition to potassium currents, the resting membrane potential of native smooth muscle cells is partly regulated by chloride currents and Ca\(^{2+}\)-activated chloride channels (Cl\(_{\text{Ca}}\)) (for review see refs. 23, 80). Leblanc’s group was the first to investigate the role of CaMKII in the modulation of Cl\(_{\text{Ca}}\) channels in VSMCs (53, 82). Inhibition of CaMKII with KN-93 or autocamtide-2-related inhibitory peptide (AIP) resulted in a larger Cl\(_{\text{Ca}}\) current. Moreover, SR Ca\(^{2+}\) loading was also diminished by the expression of CaMKII\(\text{N}\), correlating with a 50% reduction of PLB phosphorylation (Thr17) and hence, a greater inhibition of SERCA (128).

Themes

CaMKII activation alters myofilibrillar function in VSMCs. Decreased CaMKII\(\text{Y}\) expression or pharmacological inhibition of CaMKII activity diminishes contraction in endothelium-denuded ferret aortae in response to high KCl (76). Interestingly, the CaMKII isofrom identified in this study, CaMKII\(\text{Y}\), is different from the isofrom generally considered (CaMKII\(\text{N}\)). Although Kim et al. (76) did not demonstrate a direct association between CaMKII\(\text{Y}\) and myofilibrillar proteins, myosin light-chain kinase (MLCK) activity and subsequent myosin light-chain (LC20) phosphorylation were decreased following inhibition of CaMKII\(\text{Y}\). Rokolya and Singer (135) observed similar effects of CaMKII inhibition on contractile force in porcine carotid arteries. Further investigation of the relationship between CaMKII and the contractile apparatus by Mor-
CaMKII stimulates the production of HETEs, which activates ERK1/2, therein increasing PLA2 activation and AA production (113).

**Pathological hypertrophy.** Vascular disorders such as hypertension have been associated with VSMCs hypertrophy, particularly with the increased cell size (≈35% greater) observed in spontaneous hypertensive rats in comparison to their normotensive counterparts (120). ANGII is a well-described vasoactive endogenous agent with a strong hypertrophic influence (for review see ref. 136). Reports of CaMKII involvement in cardiac hypertrophy led Grumbach’s group to investigate similar roles for vascular CaMKII in response to ANGII. They and others showed that the blood pressure rise induced by ANGII infusion was blunted by inhibition of CaMKII with KN-93 (87). Furthermore, ANGII-induced VSMC hypertrophy was reduced by adenoviral transfection of CaMKIIα. A corresponding exacerbation of VSMC hypertrophy was found in myocytes overexpressing CaMKII (87, 116). Although current literature is limited to ANGII-induced hypertrophy, CaMKII might also be involved in myocyte hypertrophy in response to other stimuli. Furthermore, the mechanisms involved remain unidentified and require further study.

**The Last Frontier: Endothelial Cells**

The endothelium is an active and essential component in cardiovascular hemostasis. Intracellular Ca2+, the crux of endothelial signaling and functions, is tightly regulated but the role of CaMKII as an effector or regulator has only recently gathered attention. As in VSMCs, distinct patterns of Ca2+ oscillation have been identified in endothelial cells that differ in their dynamic characteristics as well as source of Ca2+, illustrating the potential for CaMKII in modulating endothelial functions (83, 156, 160). However, conflicting data have been reported, presumably because of differences in the type of endothelial cells used. Indeed, early studies in ECs were largely performed in cultured ECs, notorious for their altered phenotype. Modifications in the protein expression pattern by the culture process and passage number can thus result in signaling pathway adaptation. However, when taken together, findings from various cell lines strengthen the conclusions from cultured ECs studies. Similarly, results obtained using freshly harvested tissue or cells are limited to the respective species unless confirmed in human or across other species. Nonetheless, growing interest in the function of endothelial CaMKII can be noted and is summarized in the following sections as well as in Table 2.

**Endothelial CaMKII expression pattern.** Most studies looking at CaMKII expression in ECs were focusing on the δ and γ isoforms, based on early work suggesting that expression of CaMKIIδ and β is restricted to neuronal tissue. Indeed, Wang et al. (179) probed for CaMKII isoforms in different cultured ECs lines, including human umbilical vascular EC (HUVEC), bovine arterial EC (BAEC), and human dermal microvascular EC (HDMEC). Using a pan-CaMKII antibody, a single band obtained on Western blots (50-kDa) led them to conclude that CaMKIIδ is the endothelial isoform. However, a similar single ≈50-kDa band detected in PAEC was attributed to CaMKIIα (140). Since the predicted molecular weights for CaMKIIα and CaMKIIδ are similar, the use of pan-CaMKII antibodies in immunoblots does not provide sufficient information to discriminate between CaMKIIα and δ. Furthermore, transcripts for CaMKIIγ, γ, and δ variants have been detected in BACE4 cells (7). In contrast, only CaMKIIα was detected by in situ hybridization and Western blot in primary cultured rat brain EC, although CaMKIIδ and γ isoforms were not sought (33). More recently, CaMKIIα, β and δ, but not CaMKIIγ, were found in native endothelial cells from mouse mesenteric arteries (27). Although CaMKIIδ and γ appear to be expressed in smooth muscle cells from the same arteries, CaMKIIα and β were not detected in myocytes. Confocal imaging showed that CaMKIIα and β shared similar intracellular distribution in situ, with clusters found within endothelial-smooth muscle communication structures called myoendothelial projections (MEPs). Both isoforms were also found to be in close proximity and might form heteromultimers (169). The subcellular localization of endothelial CaMKIIδ was distinct, with the enzyme being mainly found at the plasma membrane and in the Golgi (27). Such specific spatial distribution suggests that endothelial CaMKII isoforms might play different roles, being activated by different stimuli and/or having distinct targets and functions. Indeed, CaMKIIα and β were reported to translocate to MEPs upon activation of endothelial Ca2+ signaling (27) while CaMKIIδ did not.

**Cell permeability.** Endothelial permeability is tightly regulated: a leaky endothelial barrier can have deleterious outcomes, but may be required for appropriate physiological functions such as inflammatory processes (for review see ref. 193). The endothelial barrier is passive and relies on tightness of cell-to-cell junctions, which are modulated in inflammation to allow access of immune cells to the tissue. Incubation of bovine pulmonary arterial EC (BPAEC) with a CaMKII inhibitory peptide decreased bradykinin-induced cytoskeletal rearrangement (translocation of cytosolic filament to cell membrane and modification of F-actin). These mechanisms are thought to increase vascular permeability and appear to be modulated by CaMKII (178). Moreover, intercellular communications through gap junctions are dependent on the identity of the connexin subunits forming the hemichannels. Recent in vitro work showed that CaMKII could directly modulate connexin 43, one of the three endothelial connexins (connexins 37, 40 and 43; for review see ref. 32, 69). Bradykinin-induced gap junction association also occurs through a CaMKII-dependent pathway (178). However, additional in vivo investigations are required to better understand role of CaMKII in modulating cell-to-cell communication.

The intracellular signaling regulating transendothelial permeability also appears to involve CaMKII. Previously shown to activate CaMKII (33), thrombin, a major modulator of vascular permeability, was used to elucidate the relationship between CaMKII and EC permeability (19). CaMKII activity in BAECs is increased in the presence of thrombin and endothelial permeability (monitored by albumin clearance and transendothelial electrical resistance measurement) is significantly impaired in the presence of KN-93 (19). Accordingly, CaMKII autophosphorylation at Thr287, used as an index of CaMKII activation, is increased in HUVEC cells exposed to thrombin (179). Therefore, thrombin activation of CaMKII correlates with increased endothelial permeability. From a mechanistic perspective, CaMKII modulation of vascular permeability appears to be linked to the ERK1/2 pathway. As reported in VSMCs, KN-93 significantly impairs ERK activa-
tion in ECs with a concomitant loss of actin stress fibers (18). The CaMKII isoform involved might, however, be different from VSMCs since transfection of HUVEC cells with CaMKIIβ-targeted siRNA did not prevent ERK activation. Interestingly, a critical determinant in thrombin-induced hyperpermeability, the RhoA pathway, is significantly inhibited by CaMKIIβ silencing (179). Since RhoA is an important player for regulation of VSMC contractility (for review see refs. 129, 155, 187), one can speculate that CaMKIIβ may be analogously involved in the control of endothelial permeability through RhoA. The elucidation of the roles other CaMKII isoforms play in the control of endothelial permeability is an interesting avenue and could lead to identification of pharmacological targets for specific signaling pathways.

Migration and proliferation. Both physiological and pathological angiogenesis begins with EC migration and proliferation (124, 144). Plexiform lesions found in patients with pulmonary arterial hypertension (PAH) result from pathological and disorganized cellular proliferation and migration within the arterial lumen (171). Plexiform lesions appear to be enriched in fibronectin, an extracellular matrix component that can act as chemoattractant (105), and thus promote ECs migration. Moreover, thrombin-stimulated migration of pulmonary microvascular ECs (PMVECs) grown on fibronectin can be abolished by inhibition of protease-activated receptor 1 (PAR1). As KN-93 prevents the thrombin/fibronectin-induced EC migration, one or more subtypes of CaMKII are involved (105). These studies suggest that endothelial CaMKII might be an interesting target to limit the formation of plexiform lesions in PAH.

The retina is an established model for the study of angiogenesis, and the role of CaMKII regulating EC migration has been studied in the retinal vasculature. Vascular endothelial growth factor (VEGF) was shown to raise intracellular Ca2+ levels in bovine retinal ECs (BRECs) (8). A twofold increase in AKT activity was also reported in response to VEGF. Although the link between AKT activation and intracellular Ca2+ has not been established in the context of the retinal endothelium, CaMKII is an interesting prospect. Indeed, AKT phosphorylation was significantly diminished by KN-93 in VEGF-treated BRECs (8), similarly to VSMCs. Further evidence of a role of CaMKII in EC migration was provided when KN-62 was shown to reduce BPAEC migration in a wound-healing assay by 30–40% (178).

Table 2. CaMKII in endothelial cells

<table>
<thead>
<tr>
<th>Isom</th>
<th>Cell Type</th>
<th>Culture Condition</th>
<th>Target</th>
<th>Effect</th>
<th>Function</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaMKII</td>
<td>Bovine pulmonary artery EC</td>
<td>NC P3-P5</td>
<td>Iα</td>
<td>Increase Iα current and protein expression</td>
<td>Positively regulates EC migration</td>
<td>(132)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Bovine pulmonary artery EC</td>
<td>NC P4-P10</td>
<td>Filamin/F-actin/gap junction</td>
<td>Filamin translocation/F-actin rearrangement/gap junction formation</td>
<td>(178)</td>
<td></td>
</tr>
<tr>
<td>CaMKII</td>
<td>Bovine pulmonary artery EC</td>
<td>C (ATTC) P19-P24</td>
<td>ERK</td>
<td>ERK activation</td>
<td>Increase vascular permeability</td>
<td>(19)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Human umbilical vein EC</td>
<td>C (Cascade biologies) P3-P12</td>
<td>RhoA</td>
<td>RhoA activation leading to ROCK activation</td>
<td>Hyperpermeability</td>
<td>(179)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Rat pulmonary microvascular EC</td>
<td>NC P-NS/NC P5-P9/NC P4-P10</td>
<td>AKT</td>
<td>AKT phosphorylation following VEGF stimulation</td>
<td>Positively regulates EC migration</td>
<td>(8)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Calf pulmonary artery EC</td>
<td>C (ATTC) P15-P21</td>
<td>IP3/R1 mechanism</td>
<td>Decreasing Ca2+ store release/Favor Ca2+ entry</td>
<td>Positively regulates EC migration</td>
<td>(1, 2)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Mouse mesentery artery</td>
<td>N/A</td>
<td>IP3/R1</td>
<td>Decreases IP3/R1 Ca2+ release</td>
<td>(169)</td>
<td></td>
</tr>
<tr>
<td>CaMKII</td>
<td>Macaque choroid-retinal EC</td>
<td>C (CAS) P3-P4</td>
<td>Fas/JNK</td>
<td>Increase mitochondrial membrane potential/increase cytochrome C release</td>
<td>Positively regulates EC apoptosis</td>
<td>(88)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Porcine aortic EC</td>
<td>NS P29-P30</td>
<td>NOS3</td>
<td>Increase NOS3 activity</td>
<td>Positively regulates NO production</td>
<td>(140)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Human umbilical vein EC</td>
<td>C (Cell system) P-NS/NC P3-P5</td>
<td>NOS3 mRNA</td>
<td>Increase NOS3 mRNA level</td>
<td>Positively regulates NO production</td>
<td>(24, 25, 86)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Rat aorta/human umbilical vein EC</td>
<td>NC P1/NC/N/NC P6-P10</td>
<td>NOS3</td>
<td>Increase NOS3 activity (PSe1177)</td>
<td>Positively regulates NO production</td>
<td>(46, 78, 123)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Native mouse mesenteric EC</td>
<td>N/A</td>
<td>NOS3</td>
<td>Increasing NO production</td>
<td>Positively regulates NO production</td>
<td>(27)</td>
</tr>
<tr>
<td>CaMKII</td>
<td>Human umbilical vein EC</td>
<td>NC P1/NC P4-P10</td>
<td>NOS3</td>
<td>Regulates NOS3 localization</td>
<td>Positively regulates NO production</td>
<td>(46, 178)</td>
</tr>
</tbody>
</table>

NC, noncommercial cell line; C, commercial cell line; N/A, not applicable; P, passage; NS, not specified.
Calcium dynamics. Essential to virtually every endothelial function, numerous facets of endothelial Ca\textsuperscript{2+} dynamics have been scrutinized. Indeed, Ca\textsuperscript{2+} ions are an essential cofactor to basically every endothelial function (37, 112, 166). CaMKII is linked to Ca\textsuperscript{2+} homeostasis in cardiomyocytes and VSMCs but also in vascular endothelium. For example, the role of CaMKII in the ATP-dependent increase in intracellular Ca\textsuperscript{2+} was fully described in calf pulmonary arterial ECs (CPAECs) (1, 2). ATP is known to stimulate both the release of Ca\textsuperscript{2+} from intracellular Ca\textsuperscript{2+} stores and capacitative Ca\textsuperscript{2+} entry (CCE; also known as store-operated Ca\textsuperscript{2+} entry; SOCE) in ECs. Inhibition of CaMKII altered the morphology of ATP-induced Ca\textsuperscript{2+} transients by decreasing the sustained phase without affecting the Ca\textsuperscript{2+} peak amplitude. However, exposure of CPAECs to KN-93 alone (in the absence of ATP) triggered a similar Ca\textsuperscript{2+} transient, even in the absence of extracellular Ca\textsuperscript{2+}. The KN-93-sensitive Ca\textsuperscript{2+} rise thus originates from intracellular stores. Although nonspecific effects of KN-93 were not ruled out, it was proposed that inhibition of CaMKII could evoke a Ca\textsuperscript{2+} transient through Ip/R in the endoplasmic reticulum. Accordingly, inhibition of Ip/R receptors led to a 91% decrease in KN-93-induced Ca\textsuperscript{2+} transients (1). Moreover, endothelial CaMKII is involved in a regulatory feedback loop where the kinase can be activated by local increases in Ca\textsuperscript{2+} arising from intracellular stores (27, 169) in native endothelial cells from mesenteric resistance arteries. Upon activation, CaMKII appears to modulate intracellular Ca\textsuperscript{2+} store content through inhibition of Ip/R activity. Recent advances on local endothelial Ca\textsuperscript{2+} signaling (6, 83, 156, 160) and subcellular compartmentalization of intracellular pathways offer a renewed perspective on the physiological specificity of otherwise broad pathways. Indeed, Ca\textsuperscript{2+} signals within MEP called Ca\textsuperscript{2+} pulsars activate and recruit CaMKII\alpha and \beta to these endothelial cellular projections where the kinase can regulate the activity of other proteins (27, 169). Noteworthy, CaMKII activity in resting endothelium (in the absence of agonist) is sufficient to modulate intracellular Ca\textsuperscript{2+}, since inhibition of CaMKII with KN-93 stimulated Ca\textsuperscript{2+} pulsars in mesenteric arteries. Further studies showed that CaMKII controls local Ca\textsuperscript{2+} dynamics and ER Ca\textsuperscript{2+} levels in native endothelium through inhibition of Ip/R (169). In addition to modulation of Ca\textsuperscript{2+} stores, work on cultured CPAECs showed that CaMKII stimulates CCE (1) in a similar fashion to what has been reported in Xenopus oocytes and skeletal muscle cells (98, 174). Modulation of CCE by CaMKII has also been reported in VSMCs, highlighting the ubiquitous role of CaMKII in regulating Ca\textsuperscript{2+} signals (134). In summary, the reciprocal relationship between CaMKII activation and intracellular Ca\textsuperscript{2+} dynamics is definitely convoluted: the high level of sophistication in CaMKII signaling that is becoming apparent allows for the fine-tuning of critical cellular pathways and functions.

Pathological levels of cytoplasmic Ca\textsuperscript{2+}, as provoked by hyperglycemia, are deleterious to endothelial cells and can trigger apoptosis. Exposure to high glucose levels has been used to explore the involvement of CaMKII in EC apoptosis (15, 170). Incubation of RF/6A cells (a cell line derived from macaque choroid-retinal ECs) in hyperglycemic milieu for 96 hours significantly increased apoptosis. Moreover, intracellular Ca\textsuperscript{2+} release and CaMKII activation were stimulated by increasing glucose levels from 5.5 mM (normal glucose) to 30 mM. Interestingly, increased CaMKII activity did not coincide with changes in enzyme expression. The impact of CaMKII activity on EC apoptosis was demonstrated as a significant reduction of hyperglycemia-induced apoptosis when cells were treated with KN-93 (88). The mechanism involves the modulation of mitochondrial membrane potential and cytochrome c release by CaMKII and, possibly, the fus/JNK cascade (88) similar to macrophages (167).

Nitric oxide regulation. Nitric oxide, the most potent endogenous endothelium-derived vasodilator, is produced primarily by endothelial nitric oxide synthase (eNOS: NOS3) in ECs. Ca\textsuperscript{2+} ions, in complex with CaM, are essential cofactors for NOS3 activation. Therefore, CaMKII through regulation of endothelial intracellular Ca\textsuperscript{2+} signaling could indirectly modulate NOS3 activity and NO production. In addition to activation by Ca\textsuperscript{2+}, posttranslational processes including phosphorylation have been shown to elevate NOS3 activity (for review see refs. 39, 131). Indeed, acute application of the Ca\textsuperscript{2+} ionophore A23187 triggered NO production and release in PAECs in a CaMKII-dependent fashion (140).

CaMKII can modulate the activity of proteins such as NOS3 by various means, including transcription. In HUVECs, the histamine-dependent increase in NOS3 mRNA levels was sensitive to CaMKII inhibition but independent of PKC, JAK2, MAPK, and PI3K, suggesting that the CaMKII stimulates NOS3 expression (86). Exogenous application of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) to BAECs increased CaMKII activation (24). NOS3 mRNA levels were also higher in cells exposed to H\textsubscript{2}O\textsubscript{2}, an effect that was attenuated (\~77%) by CaMKII inhibition. Although the precise mechanism involved in the regulation of NOS3 expression has yet to be determined, a CaMKII-dependent increase in the half-life of NOS3 mRNA has been excluded (24). In contrast to the normal dynamic nature of the endothelium’s physiological environment in vivo, conditions in culture are relatively stagnant, without flow and shear stress. Indeed, blood flow continuously exerts a non-stationary force on vascular walls. The impact of oscillatory shear stress on the NOS3-CaMKII interaction was therefore investigated. As expected, a CaMKII-dependent increase in NOS3 expression and NO production was observed in cells exposed to oscillatory shear stress (25).

Phosphorylation of NOS3 is also a common regulatory process not restricted to CaMKII as consensus sites for other kinases (AKT, AMPK, and PKA) have been reported. Phosphorylation of NOS3 at Ser1177 significantly increases NOS3 activity as does substituting Ser1177 with a “phosphomimetic” acidic amino acid (36). Seminal work on CaMKII-dependent phosphorylation of the NOS family was performed on NOS1 (nNOS) in brain tissue (117). Later, NOS3 phosphorylation by CaMKII was demonstrated in HUVECs (46) and rat aortic endothelial cells (78). NOS3 phosphorylation is also strongly altered by CaMKII inhibition in BAECs (123). Endothelial MEPs appear to be enriched in NOS3 (159), whereas CaMKII was shown to translocate upon activation of local Ca\textsuperscript{2+} signaling (27). Phosphorylated NOS3 (Ser1177) immunoreactivity within MEPs was also decreased upon inhibition of CaMKII (27). Furthermore, Ca\textsuperscript{2+} pulsar-dependent NO production, monitored using the fluorescent NO-sensitive dye DAF-FM, was reduced by CaMKII inhibition, suggesting that CaMKII activation serves to amplify Ca\textsuperscript{2+}-dependent NO production (27). Akt is also able to phosphorylate NOS3 at
Ser1177 (49); hence, as CaMKII can also modulate Akt activation, the effect of CaMKII on NOS3 activity may be either direct or mediated via a CaMKII-AKT-NOS3 pathway.

The subcellular localization of NOS3 may also be regulated by CaMKII. Indeed, while membrane-bound NOS3 is inactive, CaMKII activation results in translocation of NOS3 to the cytoplasm (178). The direct interaction of CaMKII with NOS3 may be responsible for this translocation. In support of this, immunoprecipitation assays show increased association of CaMKII with NOS3 in HUVECs stimulated with bradykinin (46). Hence, CaMKII may function as both regulator and targeting subunit for NOS3.

### Vascular CaMKII: Now and Then

As detailed in this review, CaMKII is involved in a wide range of vascular functions (Fig. 2; nonexhaustive representation of the relationships presented in this review), which might explain the growing interest in this enzyme. In light of the complexity arising from oligomerization and the large number of distinct CaMKII variants, additional roles for CaMKII, possibly of interest in vascular cells, likely remain to be identified. As mentioned above, evidence suggests that CaMKII can modulate proteins independently of its kinase activity, serving as an anchoring, targeting, or structural partner. Research into these aspects of CaMKII signaling and their ability to modulate vascular function will expand our understanding of the physiological roles of the CaMKII family.

Among the various functions controlled by CaMKII, Ca\(^{2+}\) homeostasis is intriguing as it allows the enzyme to regulate its own activation. However, the dogma by which CaMKII is activated by global rise in intracellular Ca\(^{2+}\) might shift according to the recent developments in local Ca\(^{2+}\) signaling, especially in endothelial cells. Indeed, as a consequence of its oligomeric structure, CaMKII has the unique property of being modulated by Ca\(^{2+}\) signal amplitude and frequency. Therefore, slight modification in intracellular Ca\(^{2+}\) dynamics might have a profound effect on CaMKII-dependent events. It seems then reasonable to speculate that Ca\(^{2+}\) microdomains might experience distinct patterns of CaMKII activation and, as a consequence, highly localized changes in CaMKII-dependent functions.

The role of CaMKII has largely been studied using pharmacological inhibitors such as KN-93 or KN-62. However, although KN-93 is a relatively specific inhibitor for CaMKII, it has been shown to alter VSMC Ca\(_{1.2}\) channel activity. This important Ca\(^{2+}\) channel may directly influence CaMKII activation as well as CaMKII-independent pathways (50). Moreover, KN-93 has been shown to inhibit K\(_c\) channels (81, 133) and, more recently, I\(_{Ks}\) current (60). Some in vitro studies have also employed molecular tools, including siRNA or AIP expression, in addition to pharmacological inhibitors. Unfortunately, use of these tools cannot seamlessly be extended to in vivo investigations and their use is often restricted to cell cultures. Alternatively, knockout and transgenic animals could be developed. For example, CaMKII\(\beta\) knockout mice are commercially available. A transgenic mouse expressing a CaMKII peptide inhibitor specifically in VSMCs has also been used, as reported in this review. However, there are currently no mouse models (knockout or transgenic) available where endothelial CaMKII isoforms (systemic or endothelial specific) are targeted.

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Fig. 2. CaMKII signaling targets in vascular cells. Schematic summary of CaMKII’s targets in endothelial cells (left, blue), smooth muscle cells (right, pink) or shared by both vascular cell types (middle). ROCK, Rho-associated protein kinase; NOS3, nitric oxide synthase 3; JNK, c-Jun NH\(_2\)-terminal kinase; Cyt c, cytochrome c; IP\(_R\), inositol 1,4,5-trisphosphate receptor; CCE, capacitative calcium entry; ERK, extracellular signal-regulated kinase; I\(_{Ks}\), chloride current; NOS2, nitric oxide synthase 2; CREB, CAMP response element-binding protein; MLCK, myosin light chain kinase; LC20, myosin light chain; MEK, mitogen-activated protein kinase kinase; PLA2, phospholipase A2; AA, arachidonic acid; PLB, phospholamban; SERCA, sarcoplasmic reticulum Ca\(^{2+}\)-ATPase; MMP9, matrix metalloproteinase 9; Ca\(_{\beta3}\), voltage-dependent calcium channel, subunit-\(\beta3\).
Regulation of CaMKII activity is generally expected to involve phosphorylation and/or oxidation as discussed previously although a vascular counterpart to cardiac oxidized-CaMKII remains to be established. Subcellular targeting and translocation are also relevant properties. The anchoring of CaMKII to different cell membranes has been suggested to be important in the formation of signalosomes that include CaMKII and a Ca\(^2+\) source or CaMKII target protein. Emerging evidence on Ca\(^2+\) microdomains could then be of interest, as targeting specific CaMKII complexes to Ca\(^2+\) microdomains would allow for spatial and temporal localization of CaMKII activation and hence CaMKII-mediated cellular functions. Indeed, endothelial \(K_{Ca}2.x\) and \(K_{Ca}3.1\) channels have a distinct intracellular distribution and might be differentially regulated by heterogeneously distributed CaMKII. Studies in skeletal muscle (11, 12) or cardiomyocytes (150, 151) suggest that membrane targeting requires \(\alpha\)KAP, a unique CaMKII subunit transmitting membrane and CaMKII association domains, but lacking a catalytic domain (12). Thus, heteromultimerization of CaMKII isoforms with this subunit may result in targeting of CaMKII complexes to a specific subcellular region such as the SR (11, 150), nucleus (118) or plasma membrane. However, the possible expression and potential role for an analogous vascular membrane-anchoring CaMKII subunit remains to be determined.

Most of the investigations discussed in this review have been performed in cultured ECs and VSMCs and have significantly improved our understanding of the roles of CaMKII in the cardiovascular system. In situ investigations are still required to confirm the findings from cultured cells as discrepancies may occur, as evidenced by distinct differences in the pattern of CaMKII isoform expression in native versus culture ECs. Moreover, it is now clear that CaMKII, and, possibly, isoform-specific targeting, represents an interesting therapeutic avenue for treatment of vascular disease such as hypertension.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

F.T. prepared figures; F.T., C.C., and J.L. drafted manuscript; F.T., C.C., B.G.A., and J.L. edited and revised manuscript; F.T., C.C., B.G.A., and J.L. approved final version of manuscript.

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Themes

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