cAMP signalling in the vasculature: the role of Epac (exchange protein directly activated by cAMP)

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Abstract

The second messenger cAMP plays a central role in mediating vascular smooth muscle relaxation in response to vasoactive transmitters and in strengthening endothelial cell–cell junctions that regulate the movement of solutes, cells and macromolecules between the blood and the surrounding tissue. The vasculature expresses three cAMP effector proteins: PKA (protein kinase A), CNG (cyclic-nucleotide-gated) ion channels, and the most recently discovered Epacs (exchange proteins directly activated by cAMP). Epacs are a family of GEFs (guanine-nucleotide-exchange factors) for the small Ras-related GTPases Rap1 and Rap2, and are being increasingly implicated as important mediators of cAMP signalling, both in their own right and in parallel with the prototypical cAMP target PKA. In the present paper, we review what is currently known about the role of Epac within blood vessels, particularly with regard to the regulation of vascular tone, endothelial barrier function and inflammation.

Introduction

The second messenger cAMP controls a range of vascular processes, including endothelial barrier function [1], VSMC (vascular smooth muscle cell) proliferation [2] and vasodilatation [3]. cAMP is formed within vascular cells following the stimulation of G_sPCRs (G_s-protein-coupled receptors) by vasoactive transmitters such as adenosine and adrenaline and the activation of the enzyme adenylate cyclase. The physiological effects of cAMP can then be mediated by three different effectors, namely PKA (protein kinase A), CNG (cyclic-nucleotide-gated) ion channels, and the most recently discovered Epacs (exchange proteins directly activated by cAMP).

Over the years, PKA has been shown to have numerous important functions in the vasculature, including promotion of VEC (vascular endothelial cell) barrier stability [1] and mediation of vasodilatation [4,5]. Similarly, CNG ion channels are expressed in VECs and VSMCs [6], and appear to contribute to the regulation of vascular tone [7,8]. Since their discovery in 1998, several studies have shown that Epac can act in parallel with, or independently of PKA to mediate cAMP-induced effects in a variety of cellular contexts [9,10], including the pancreas [9], kidneys [11], the immune system, neurons and lungs [12], and the brain and heart [13]. In the present review, we examine what is currently known about the role of Epac in the vasculature, focusing on its function in VSMCs and VECs, with emphasis on how Epac regulates vasodilatation, inflammation and VEC barrier function.

Expression, structure and activation of Epacs

Epacs are GEFs (guanine-nucleotide-exchange factors) for the small Ras-like GTPases Rap1 [14,15] and Rap2 [16]. Two Epac isoforms exist, termed Epac1 and Epac2. Epac1 (Epac), also known as cAMP GEF-I (RAPGEF3 in humans), is ubiquitously expressed, but is particularly abundant in the heart and kidneys [14,15]. By contrast, expression of Epac2, also known as cAMP GEF-II (RAPGEF4 in humans), is largely confined to the brain, kidney and pancreas [14]. Alternative splicing and differential gene promoter use gives rise to three variants of Epac2, named Epac2A, Epac2B and the liver-specific variant Epac2C [17,18]. A homologue of Epac, termed Repac (related to Epac) (RAPGEF5 in humans), also exists [16].

Epac1 is strongly expressed in VSMCs [19–21]. However, whereas expression of Epac2 protein has been reported in human airway SMCs (smooth muscle cells) [22], within VSMCs Epac2 has only been reported at the mRNA level in rat mesenteric arterial SMCs, where it is 200-fold less abundant than *Epac1* mRNA [23]. In the endothelium, Epac1 protein is expressed in HUVECs (human umbilical vein endothelial cells) [24–27], whereas Epac2 is not expressed

Key words: cAMP, endothelial barrier function, exchange protein directly activated by cAMP (Epac), guanine-nucleotide-exchange factor (GEF), protein kinase A (PKA), vascular smooth muscle, vasculature, vasodilatation.

Abbreviations: AKAP9, A-kinase-anchoring protein 9; α_{2C}-AR, α_{2C}-adrenoceptor; BK_{Ca}, Ca^{2+}-sensitive large-conductance K^+; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; Cdc25-HD, cell division cycle 25 homology domain; C/EBP, CCAAT/enhancer-binding protein; CNB, cAMP–nucleotide binding; CNG, cyclic-nucleotide-gated; eNOS, endothelial nitric oxide synthase; Epac, exchange protein directly activated by cAMP; EPC, endothelial progenitor cell; F-actin, filamentous actin; GEF, guanine-nucleotide-exchange factor; HCASMC, human coronary artery smooth muscle cell; HMVEC, human microvascular endothelial cell; HPAEC, human pulmonary aortic endothelial cell; HUVEC, human umbilical vein endothelial cell; IK_{Ca}, Ca^{2+}-sensitive intermediate-conductance K^+; IL-6, interleukin 6; JAK, Janus kinase; K_{ATP}, ATP-sensitive K^+; MLC, myosin light chain; MLCP, MLC phosphatase; MYPT1, myosin phosphatase targeting subunit 1; 8-pCPT, 8-(4-chlorophenylthio)-2′-*O*-methyladenosine 3′,5′-cyclic monophosphate; 8-pCPT-AM, 8-pCPT acetoxymethyl ester; PDE, phosphodiesterase; PKA, protein kinase A; REM, Ras-exchange motif; Repac, related to Epac; ROCK, Rho-associated protein kinase; RyR, ryanodine receptor; SK_{Ca}, Ca^{2+}-sensitive small-conductance K^+; SMC, smooth muscle cell; SOCS3, suppressor of cytokine signalling 3; STAT, signal transducer and activator of transcription; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α; VEC, vascular endothelial cell; VE-cadherin, vascular endothelial cadherin; VEGF, vascular endothelial growth factor; VGCC, voltage-gated Ca^{2+} channel; VSMC, vascular smooth muscle cell; vWF, von Willebrand factor; WPB, Weibel–Palade body.

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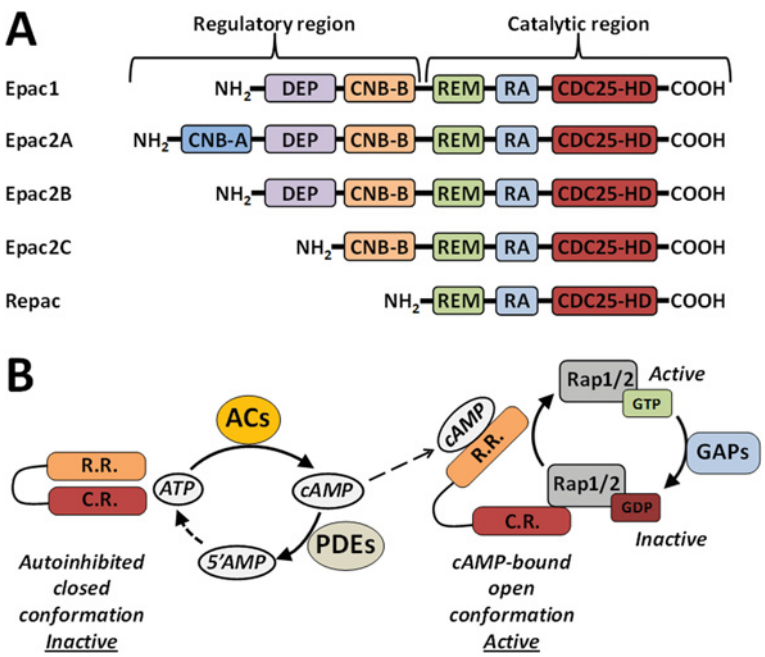
Figure 1 | Structure and activation of Epacs

(**A**) Primary structure of Epac family proteins showing domain organization. Epac consists of a regulatory region and a catalytic region, each of which are subdivided further into functional domains. The organization of these domains is the same in each family member with the exception of Repac, which lacks the typical Epac regulatory region and is a constitutively active homologue of Epac. Epac1, Epac2A, Epac2B and Epac2C each contain a CNB-B domain to which cAMP binds to activate these proteins. Epac2A contains a further CNB domain termed CNB-A, whereas the liver-specific Epac2 variant Epac2C lacks the DEP domain. (**B**) Mechanism of Epac-induced activation of Rap1 and Rap2 (Rap1/2). Activation of cytosolic or membrane-bound adenylate cyclases (ACs) induces conversion of ATP into cAMP, which binds to CNB-B within the regulatory region (R.R.) of Epac, inducing a conformational change that allows the binding of inactive GDP-bound Rap1/2 to the catalytic region (C.R.) of Epac and the removal of GDP. Spontaneous binding of GTP to Rap1/2 activates Rap1/2, allowing it to exert its downstream biological effects. GTPase-activating proteins (GAPs) such as RapGAP increase the intrinsic GTPase activity of Rap1/2, thereby catalysing the hydrolysis of GTP to GDP causing Rap1/2 to return to an inactive state. PDEs hydrolyse cAMP to 5′-AMP, inducing a lowering in global cAMP levels.



in these cells [24,25,28]. Similarly, Epac2 is undetectable in haemopoietic cells [29], although Epac2 expression has been reported in HPAECs (human pulmonary aortic endothelial cells) [30], and inducible expression of *Epac2* and *Repac* mRNA has been described in HMVECs (human microvascular endothelial cells) [31].

Epacs are multidomain proteins that comprise an N-terminal regulatory region and a C-terminal catalytic region (Figure 1A). The domains within the catalytic region of Epac1, Epac2A, Epac2B, Epac2C and Repac each comprise a REM (Ras-exchange motif) domain, a putative RA (Ras-association) domain and a Cdc25-HD (cell division cycle 25 homology domain) (Figure 1A). The regulatory region of Epac1 consists of a DEP (dishevelled/Egl-10/pleckstrin) domain, responsible for plasma membrane targeting of Epac following cAMP binding [32], and a CNB (cAMP–nucleotide binding)-B domain, to which cAMP binds with high affinity to activate the protein.

Upon binding of cAMP to the CNB-B domain (K_d 2.8 μM [33]), Epac undergoes a conformational change from an autoinhibited inactive conformation, wherein the regulatory domain sterically hinders Rap binding to the catalytic domain, to an active conformation capable of binding and activating Rap1 and Rap2 [34] (Figure 1B). The Cdc25-HD domain forms a binding site for Rap1/2 [35], and this interaction is stabilized by the REM domain to allow exchange of GDP for GTP on Rap1/2 to render Rap1/2 active (Figure 1B). The absence of the N-terminal regulatory domain in Repac accounts for its constitutive activity [16], whereas Epac2A contains a second, low-affinity, CNB domain (CNB-A) at its N-terminus (Figure 1A), which is involved in allowing plasma membrane localization of Epac2A [17], but is not required for cAMP-induced activation [36].

Physiological role of Epac in the vasculature

Epac-mediated regulation of VSMC proliferation and migration

In health, VSMCs directly regulate vascular tone and blood pressure and maintain blood vessel integrity, whereas aberrant regulation of VSMC proliferation or contractility

can result in the progression of various vascular diseases [37]. VSMCs are normally quiescent, but proliferate towards the tunica intima in pathologies such as atherosclerosis and restenosis [38–40]. In rat aortic SMCs, PKA and Epac operate synergistically to negatively regulate cell proliferation via a Rap1-independent mechanism [23]. The precise mode by which Epac regulates VSMC proliferation remains to be determined and may be cell-type-dependent. For example, in rat aortic SMCs, activation of Epac and PKA together has been shown to attenuate activation of ERK (extracellular-signal-regulated kinase) 1/2, and JNK (c-Jun N-terminal kinase), and inhibit expression of cell cycle regulators cyclin D1 and Skp2 (S-phase kinase-associated protein 2) [23]. By contrast, reduction in PDGF (platelet-derived growth factor)-stimulated proliferation of HCASMCs (human coronary artery smooth muscle cells) by adenosine seems to occur in a PKA-independent manner via Epac-mediated down-regulation of the early gene nuclear receptor NR4A1 [41].

VSMC migration is essential for normal vascular development, and is also a key feature of pathophysiological states such as atherosclerosis, wound healing and restenosis [42]. In murine femoral arteries, Epac positively regulates VSMC migration during vascular development, as well as during neointimal hyperplasia in response to vascular injury [43]. Importantly, expression of PKA is down-regulated under conditions of neointimal thickening, and selective activation of PKA has been found to reduce rat aortic SMC migration [43], together suggesting that Epac and PKA fulfil opposite roles as positive and negative regulators of VSMC migration respectively. It seems that Epac may regulate VSMC migration by controlling integrin activity, as it has recently been shown to induce integrin $\beta 1$ activation to facilitate VSMC adhesion to fibronectin [44]; however, Epac may also control VSMC migration by regulating secretion of extracellular matrix components, such as down-regulation of expression of the glycosaminoglycan hyaluronan in HCASMCs [45].

Epac-mediated regulation of vascular tone

Following the advent of Epac-selective agonists such as 8-pCPT [8-(4-chlorophenylthio)-2′-*O*-methyladenosine 3′,5′-cyclic monophosphate] [46], which allow Epac-mediated signalling to be dissected from other cAMP-activated pathways, it was demonstrated that Epac could induce relaxation of adrenaline-contracted rat aortae [47]. Further work has suggested that Epac mediates VSMC relaxation by distinct mechanisms, including indirect modulation of K^+ channel activity [19,21], and attenuation of RhoA activity [20] (Figure 2).

Epac1 can induce relaxation of phenylephrine-contracted rat portal veins via a Rap1-dependent reduction in the activity of the small GTPase RhoA, leading to disinhibition of MLCP (myosin light chain phosphatase) activity and dephosphorylation of MLC (myosin light chain) [20].

We have shown recently that activation of Epac mediates relaxation of phenylephrine-contracted rat mesenteric arteries by promoting the opening of Ca^{2+}-sensitive K^+ channels within both VSMCs and VECs [21]. Application of 8-pCPT-AM (8-pCPT acetoxymethyl ester) increased the frequency of Ca^{2+}-sparks from RyRs (ryanodine receptors) in VSMCs, activating BK_{Ca} (Ca^{2+}-sensitive large-conductance K^+) channels and resulting in membrane hyperpolarization [21] (Figure 2). Importantly, Epac-mediated vasodilatation of rat mesenteric arteries is also partly dependent on an intact endothelium. Direct activation of Epac with 8-pCPT-AM resulted in elevated global $[Ca^{2+}]_i$ (intracellular Ca^{2+} concentration) within mesenteric VECs, as well as the opening of SK_{Ca} (Ca^{2+}-sensitive small-conductance K^+) and IK_{Ca} (Ca^{2+}-sensitive intermediate-conductance K^+) channels [21]. Outward K^+ current through these channels would be expected to cause endothelium hyperpolarization, which can either spread directly to the underlying VSMCs via gap junctions, or the effluxing K^+ may activate Na^+/K^+-ATPases and K_{IR} (inward-rectifier K^+) channels on VSMCs to induce VSMC membrane hyperpolarization. In addition, the eNOS (endothelial nitric oxide synthase)-nitric oxide (NO) pathway also seems to be involved in endothelium-dependent Epac-mediated vasodilatation in this tissue [21]. Epac-induced VSMC hyperpolarization resulting from a combination of these VEC-dependent and VEC-independent pathways will lead to the closure of VGCCs (voltage-gated Ca^{2+} channels), thus lowering global VSMC $[Ca^{2+}]_i$ and inducing VSMC relaxation [21] (Figure 2).

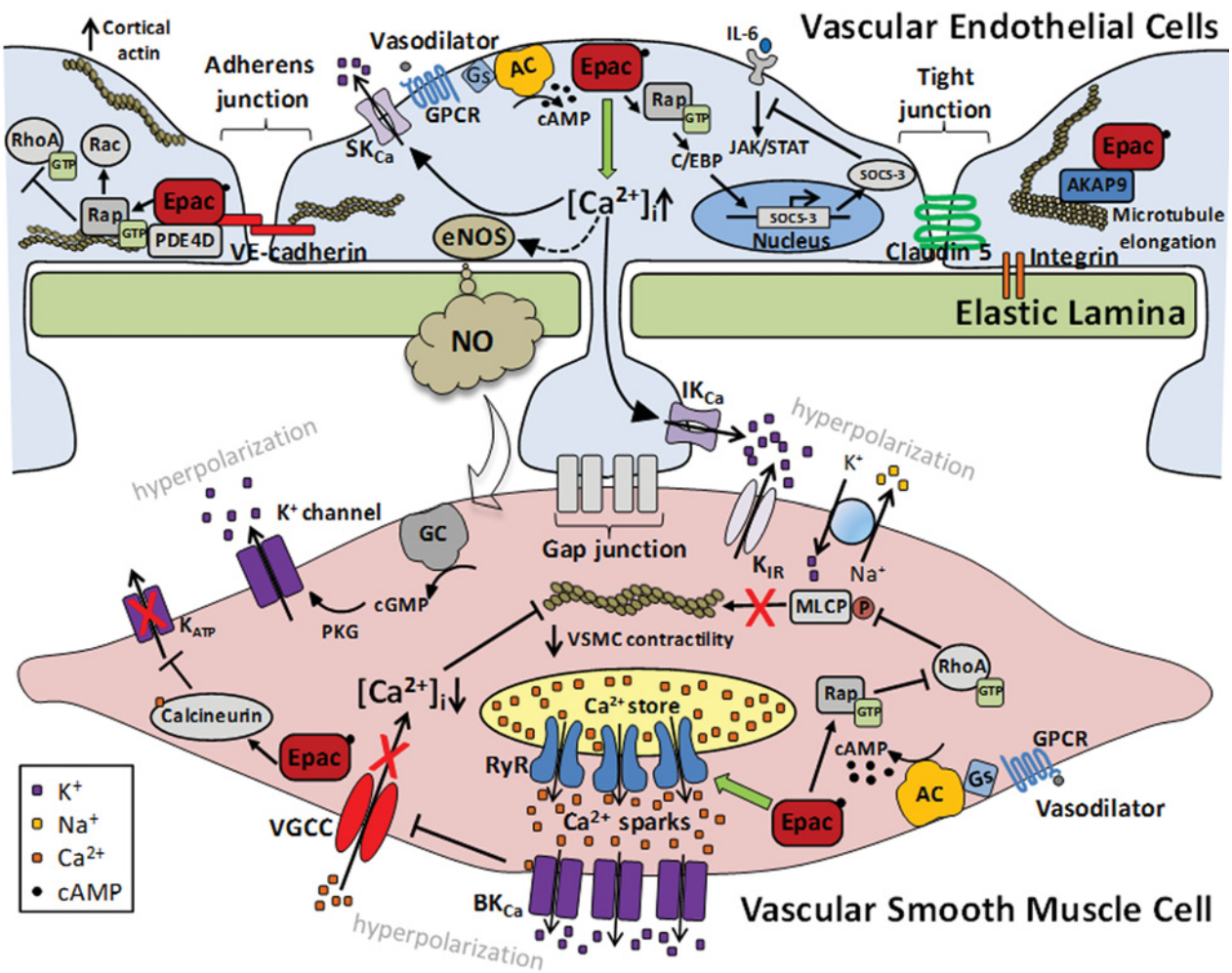
Intriguingly, long-term activation of Epac can promote transcription and mobilization of α_{2C}-ARs (α_{2C}-adrenoceptors) to the plasma membrane of microvascular SMCs [48]. This process involves Rap1A-dependent RhoA activation, leading to activation of ROCK (Rho-associated protein kinase) and ROCK-mediated stabilization of F-actin (filamentous actin) [48]. This is especially interesting given that Epac activation attenuates RhoA activity in other VSMCs [20], and in HUVECs [30], and supports the notion that Epac may have distinct roles within different vascular beds. Given that α_{2C}-ARs are key mediators of vasoconstriction under conditions of cellular stress [49], it is interesting to speculate that Epac may exist in a feedback loop whereby acute Epac activation elicits vasodilatation, whereas chronic activation of Epac may promote vasoconstriction. Indeed, this may also provide physiological relevance to Epac-mediated inhibition of vascular K_{ATP} (ATP-sensitive K^+) channels observed in rat aortae [19].

Epac-mediated regulation of VEC barrier function

The vascular endothelium comprises a continuous monolayer of VECs lining the luminal wall of blood vessels, forming a semi-permeable barrier that controls movement of plasma solutes and regulates circulating cell diapedesis into the extravascular tissue. Endothelial junction integrity is maintained in part by adherens junctions and tight junctions that form cell–cell contacts, aberrations of which can result in disease states such as oedema [50,51]. Cytoskeletal remodelling, characterized by a decrease in actomyosin stress fibres, and an increase in cortical actin at the edge of

Figure 2 | Schematic overview of key Epac functions in the vasculature

See the text for details. In VSMCs, activation of Epac induces RyR-mediated release of Ca^{2+} from intracellular stores (Ca^{2+} sparks), which activate surface BK_{Ca} channels, causing membrane hyperpolarization, closure of VGCCs and a lowering in global [Ca^{2+}], which leads to VSMC relaxation [21]. Epac-mediated vasodilatation is also dependent on a functional endothelium and the activation of SK_{Ca} and IK_{Ca} channels and eNOS [21]. Conversely, in rat aortic SMCs, Epac induces Ca^{2+} release that activates calcineurin and inhibits K_{ATP} channel activity [19]. Activation of Epac/Rap1 inhibits RhoA activity in VSMCs, preventing RhoA-mediated ROCK activation and subsequent ROCK-mediated phosphorylation and inactivation of MLCP, resulting in dephosphorylation of MLC and lowered VSMC contractility [20]. Epac enhances endothelial barrier function by various mechanisms including increased VE-cadherin-mediated adherens junction formation [24,28,57], which may involve Epac targeting via PDE4D [58]. Epac also increases claudin 5-mediated tight junction formation between VECs [54]. Interaction between Epac and the anchoring protein AKAP9 allows Rap1-independent microtubule growth and integrin-mediated adhesion in VECs [55]. Epac mediates rearrangement of cortical actin to the VEC periphery to enhance VEC barrier stability [24,28,30,56,65,66,92]. Epac can also enhance VEC barrier function by modulating Rac activity [54,56,60,64,65]. Epac activates C/EBP transcription factors to induce expression of SOCS3 to block the pro-inflammatory IL-6/JAK/STAT signalling pathway [69,70]. AC, adenylate cyclase; GC, guanylate cyclase; GPCR, G-protein-coupled receptor; Gs, stimulatory G-protein; PKG, protein kinase G.



endothelial cells, is also important in promoting VEC barrier stability [52,53].

Several studies have shown that Epac is a key mediator of cAMP-induced endothelial barrier strengthening, both under basal conditions [28,54–56] and in response to oedema-promoting agents such as thrombin, TGFβ (transforming growth factor β) and TNFα (tumour necrosis factor α) [26,28] (Table 1). Since the initial observation that the Epac/Rap1 pathway could negatively regulate activation of the small GTPase RhoA to reverse thrombin-induced permeability [30], several studies have demonstrated that Epac promotes endothelial cell–cell junction stability by a

Table 1 | **Mechanisms of Epac-mediated regulation of endothelial barrier function**

HAEC, human arterial endothelial cell; HDMEC, human dermal microvascular endothelial cell; PAEC, porcine aortic endothelial cell; PAF, platelet-activating factor; RMAEC, rat mesenteric artery endothelial cell; Tiam1, T-cell lymphoma invasion and metastasis 1.

Endothelial cell type	Functional effect of Epac activation on endothelial barrier function	Mechanism of effects	Reference
HPAEC	Decreased thrombin-induced VEC permeability	PKA-independent, Rap1-dependent down-regulation of RhoA activation. Prevention of cortical actin rearrangement and redistribution of VE-cadherin	[30]
HPAEC	Prostaglandin E_2 (PGE_2)- and prostacyclin (PGI_2)-stimulated Epac/Rap1 activation increased VEC barrier stabilization	PGE_2- and PGI_2-induced activation of PKA and Epac resulted in activation of the Rac-specific GEFs Tiam1 and Vav2, leading to Rac activation, increased VEC–VEC adherens junction formation and peripheral actin accumulation	[65]
HPAEC	Decreased thrombin-induced VEC barrier disruption	Mediated by both PKA and Epac/Rap1 pathways, which converge at Rac. Involved Rac-specific GEFs Tiam1 and Vav2, as well as Rac-mediated attenuation of ROCK-mediated MLC phosphorylation by blockade of MLC phosphatase activity	[60]
HUVEC	Decreased basal VEC permeability and lowered thrombin-induced permeability	PKA-independent Rap1-dependent increase in cortical actin at VEC–VEC junctions and enhanced VE-cadherin-mediated adhesion	[28]
HUVEC	Decreased thrombin-induced vascular permeability	Increase in cortical actin and VE-cadherin-dependent VEC–VEC junction formation	[24]
HUVEC	Decreased TGFβ- and TNFα-induced vascular permeability	Rap1-independent induction of microtubule elongation, and an associated increase in cortical actin	[26]
HUVEC	Decreased basal VEC permeability	AKAP9 complexed with Epac inducing Epac1-mediated microtubule polymerization under basal conditions, and promoting integrin-mediated VEC adhesion	[55]
HUVEC	Decreased thrombin-induced VEC permeability	PKA, but not Epac, negatively regulated thrombin induced phosphorylation of MLC and activation of the MLCP regulatory subunit MYPT1, in part by inhibiting the RhoA/ROCK pathway	[91]
HUVEC	Intermedin activated Epac and PKA reducing basal VEC permeability and thrombin-induced hyperpermeability	CGRP (calcitonin gene-related peptide) family member intermedin activated both PKA and Epac, inducing Rac activation and attenuating RhoA signalling, leading to an increase in cortical actin and a decrease in stress fibres	[92]
HUVEC	Increased VEC barrier function, but Epac was not required for basal VEC barrier function	The cAMP-independent Rap GEFs, PDZ-GEF1 and PDZ-GEF2, maintained basal VEC barrier function, but Epac activation increased cortical actin, reduced junction motility and increased barrier function in PDZ-GEF-depleted cells	[66]
PAEC	Protected against hypoxia-reoxygenation-induced VEC barrier disruption and hyperpermeability	Epac-mediated Rac1 activation prevented reoxygenation-induced hyperpermeability, inducing localization of VE-cadherin at VEC–VEC junctions and peripheral actin localization	[56]
HDMEC	Prevented thrombin-induced VEC barrier breakdown	Epac activation increased Rac1 activation, leading to accumulation of VE-cadherin and claudin 5 at VEC–VEC junctions and Rac1-mediated cytoskeletal rearrangement, but did not affect RhoA activity	[93]
HDMEC	Prevented thrombin-induced VEC barrier breakdown	Epac mediated Rac1 activation to attenuate stress fibre formation and enhance endothelial barrier function	[64]
HDMEC	Increased VEC barrier stabilization	Activation of Rac1 leading to VEC barrier enhancement by rearrangement of VE-cadherin and claudin 5 at VEC–VEC junctions	[54]
HAEC	Lowered VEGF-induced VEC permeability	Epac1 tethered to PDE4D and incorporated into VE-cadherin complexes to regulate adherens junction formation	[58]
RMAEC	Lowered PAF-induced VEC permeability	Prevention of PAF-induced redistribution of VE-cadherin	[57]

variety of mechanisms. These studies suggest that the Epac pathway may act independently from, or in parallel with, PKA to enhance endothelial barrier function (Table 1). These mechanisms include strengthening of VE-cadherin (vascular endothelial cadherin)-mediated VEC–VEC adherens junctions [24,28], prevention of VE-cadherin redistribution [57], and rearrangement of adherens junctions and tight junctions [54]. Epac may also be incorporated into VE-cadherin-based complexes with PDE (phosphodiesterase) 4D to facilitate adherens junction formation [58].

Phosphorylation of MLC is central to the regulation of endothelial cytoskeletal rearrangements [52]. MLC is phosphorylated by MLCK (MLC kinase) in a Ca^{2+}/calmodulin-dependent manner, leading to F-actin bundling and stress fibre formation causing tension within the actin cytoskeleton, which can separate VEC–VEC junctions. This process is reversed when MLC is dephosphorylated by MLCP [52,59]. Epac has been shown to inhibit the activity of the small GTPase RhoA in HPAECs [30,60]. ROCK, the downstream effector of RhoA [61], mediates phosphorylation of MYPT1 (myosin phosphatase targeting subunit 1), the regulatory subunit of MLCP, which inactivates MLCP [62], thus resulting in relaxation of actomyosin stress fibres. This results in relaxation of the actin cytoskeleton and facilitates VEC–VEC interactions, which partly accounts for the endothelial barrier-promoting effects mediated by Epac. The role of the Rho-like small GTPase Rac in regulation of endothelial permeability is currently unclear and its function seems to be context- and stimulus-dependent [63]; however, Epac has been reported to both promote [54,64] and attenuate [65,56,60] activation of Rac1 in VECs to enhance barrier function.

Epac activation increases cortical actin arrangement at the cell periphery of VECs, which acts as a scaffold to stabilize VECs, thus strengthening barrier function [24,28,65]. Epac has also been shown to induce Rap1-independent microtubule polymerization to promote junctional stability in HUVECs [26,55]. Importantly, Rap1 can maintain basal HUVEC barrier function via the cAMP-independent Rap GEFs PDZ-GEF1 and PDZ-GEF2 [66]. Rap1 is an important regulator of cell–cell contacts in a variety of cells, and Rap1 activation can be mediated by several Rap1 GEFs [67], which raises the possibility that other Rap1 GEFs, in addition to Epac and PDZ-GEF1/2, may mediate endothelial barrier stabilization via Rap1. Whereas the mechanisms by which Epac mediates VEC barrier function appear to be diverse, it is noteworthy that a recent comprehensive phosphoproteomics study has shown that the majority of proteins that undergo phosphorylation in response to selective activation of Epac in HUVECs are those involved in facilitating cell–cell junction formation, adhesion and actin reorganization [68]. This further supports the notion that Epac is a central regulator of these processes.

Epac-mediated regulation of inflammation

In addition to Epac-mediated strengthening of endothelial barrier function, Epac can negatively regulate the pro-inflammatory JAK (Janus kinase)/STAT (signal transducer and activator of transcription) signalling pathway to attenuate inflammation. In VECs, Epac activates the C/EBP (CCAAT/enhancer-binding protein) family of transcription factors to regulate expression of SOCS3 (suppressor of cytokine signalling 3), which inhibits pro-inflammatory IL-6 (interleukin 6) receptor signalling [69,70] (Figure 2).

The inflammatory response involves the binding of circulating leucocytes to VECs via cytokine-induced expression of adhesion molecules on the surface of VECs [71]. In murine brain cerebrovascular endothelial cells, Epac has been reported to mediate PGE_2 (prostaglandin E_2)-induced expression of ICAM-1 (intercellular adhesion molecule 1) [72], which may contribute to leucocyte adhesion, and the development of ischaemia. Furthermore, Epac activation in monocytes can activate $\beta 1$ integrins to facilitate transendothelial cell monocyte extravasion [73], a key event in inflammation [71,74]. Conversely, Epac1 induces activation of $\beta 2$ integrins on EPCs (endothelial progenitor cells) to mediate EPC adhesion to HUVECs [75], which may prove useful as a therapeutic strategy to treat ischaemic stroke. Similarly, Epac-mediated expression of tPA (tissue plasminogen activator) in human brain microvascular ECs [76] may provide a means to counteract ischaemia.

Finally, it is interesting to note that Epac activation can also induce WPB (Weibel–Palade body) exocytosis [77] and secretion of the haemostatic protein vWF (von Willebrand factor) [78] in HUVECs. WPBs secrete various inflammatory cytokines [79], whereas vWF can promote inflammation and ischaemia [80,81]. Collectively, it therefore appears that Epac can mediate both pro- and anti-inflammatory effects.

Epac-mediated regulation of VEC adhesion and migration

Changes in VEC adhesion are necessary to facilitate both VEC migration and normal vascular development, as well as for the progression of inflammation (see above) [82,83]. Epac appears to differentially regulate VEC adhesion in distinct vascular beds. Under conditions where PKA is inhibited, Epac can mediate adhesion of micro-VECs, but not macro-VECs [84]. Interestingly, the differential role of Epac in these cells appears to be due to the actions of PDE3B and PDE4D, which have been shown to complex with Epac to directly regulate Epac activity independently of global cAMP levels [84]. However, the role of Epac in VEC migration is currently unclear, as Epac activation has been reported to both inhibit [31] and not affect [84] VEGF (vascular endothelial growth factor)-induced HMVEC chemotaxis.

Concluding remarks

Over recent years, Epac has emerged as an important mediator of cAMP-regulated processes, most notably as a regulator of VEC permeability, inflammation and VSMC contractility. Although the contribution of Epac within the vasculature *in vivo* has not yet been fully determined, it seems that Epac can work either in parallel with [23,60,65]

or separately from PKA [30,78,84,91]. Epac appears to also have distinct functions within the macrovasculature and microvasculature [54,84]. Key to appreciating the spatiotemporal co-ordination of Epac and PKA signalling will be to better understand the localization of Epac and PKA to sites of cAMP generation and degradation within vascular cells. Indeed, the interaction of Epac with PDEs [76,84] and anchoring proteins such as AKAP9 (A-kinase-anchoring protein 9) [55] has already been shown to be important for facilitating Epac signalling in the vasculature. It is also important to note that Epac can act in a Rap-independent manner in both VSMCs and VECs [23,26]. Future studies should aim to elucidate further the downstream effectors and interacting proteins that facilitate Epac signalling, since clarifying the mechanisms of interaction between Epac and its signalling partners and developing small molecules that modulate these interactions is likely to represent an important means of developing drugs that affect Epac signalling. Given the importance of Epac as a regulator of endothelial barrier function and vasodilatation in animal models, it seems possible that selective cell-specific activation of Epac in the vasculature may allow treatment to counteract oedema and hypertension. Conversely, it has been suggested that administration of an Epac inhibitor may be useful to attenuate VSMC hyperplasia during restenosis [43]. Although three Epac inhibitors have recently been described [85–87], it seems that two of these compounds do not selectively inhibit Epac [88], therefore the development of new selective Epac inhibitors is required.

Finally, it is noteworthy that *Rap1a*$^{-/-}$ and *Rap1b*$^{-/-}$ mice exhibit distinct defects in vascular development [89], but that, whereas *Epac1*/*Epac2* double knockout mice exhibit impaired spatial learning and social interactions [90], to date no abnormal vascular phenotype has been reported. Although this may reflect the possibility that other Rap GEFs are sufficient to maintain Rap1-mediated functions in the absence of Epac during development, it does not explain how Epac-dependent, but Rap1-independent, functions may be maintained. Future studies should aim to address these points to more fully elucidate the roles of this novel cAMP effector *in vivo*.

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