Cyclic AMP Sensor EPAC Proteins and Their Role in Cardiovascular Function and Disease

Frank Lezoualc’h, Loubina Fazal, Marion Laudette, Caroline Conte

Abstract: cAMP is a universal second messenger that plays central roles in cardiovascular regulation influencing gene expression, cell morphology, and function. A crucial step toward a better understanding of cAMP signaling came 18 years ago with the discovery of the exchange protein directly activated by cAMP (EPAC). The 2 EPAC isoforms, EPAC1 and EPAC2, are guanine-nucleotide exchange factors for the Ras-like GTPases, Rap1 and Rap2, which activate independently of the classical effector of cAMP, protein kinase A. With the development of EPAC pharmacological modulators, many reports in the literature have demonstrated the critical role of EPAC in the regulation of various cAMP-dependent cardiovascular functions, such as calcium handling and vascular tone. EPAC proteins are coupled to a multitude of effectors into distinct subcellular compartments because of their multidomain architecture. These novel cAMP sensors are not only at the crossroads of different physiological processes but also may represent attractive therapeutic targets for the treatment of several cardiovascular disorders, including cardiac arrhythmia and heart failure. (Circ Res. 2016;118:881-897. DOI: 10.1161/CIRCRESAHA.115.306529.)

Key Words: cardiovascular disease ■ cyclic nucleotide ■ heart failure ■ remodeling ■ signaling pathways

Cyclic adenosine 3′,5′-monophosphate (cAMP) is one of the most studied signaling molecules that plays a critical role in cellular responses to extracellular stimuli in the cardiovascular system. It controls a wide range of biological effects, including cell proliferation, differentiation, and apoptosis.1 cAMP is produced from ATP by transmembrane adenyl cyclase activation on Gs-coupled G protein–coupled receptors.2 In addition, soluble adenyl cyclase is a second intracellular source of cAMP and can be activated by divalent cations in various subcellular compartments.3 The intracellular level of cAMP depends not only on its production by adenyl cyclase but also its degradation by a large family of cAMP phosphodiesterases (PDEs), which catalyze the hydrolysis of cAMP into 5′-AMP.4 PDEs are key actors in limiting the spread of cAMP and seem critical for the formation of dynamic microdomains that confer specific response to various hormones.4 Besides specialized membrane structures that may also limit cAMP diffusion, A-kinase anchoring proteins function to tether cAMP effectors and PDE enzymes into defined cellular compartments and, therefore, maintain localized pools of cAMP to control the cellular actions of the second messenger.5

Until recently, the intracellular effects of cAMP were attributed to the activation of protein kinase A (PKA) and cyclic nucleotide–gated ion channels. In 1998, a family of novel cAMP effector proteins named exchange proteins directly activated by cAMP (EPACs) was discovered.6,7 The EPAC protein family is composed of EPAC1 and EPAC2, which act as guanine-nucleotide exchange factors (GEFs) for the small G proteins, Rap1 and Rap2, and function in a PKA-independent manner.6,7 On binding to cAMP, EPAC promotes the exchange of GDP for GTP, hereby inducing the activation of the small G protein Rap.8 In contrast, Rap-GTPase–activating proteins enhance the intrinsic GTP hydrolysis activity of Rap leading to GTPase inactivation.9 The cycling of Rap between its inactive and active states provides a mechanism to regulate the binding to effector proteins.

The discovery of EPAC proteins has broken the dogma surrounding cAMP and PKA, and uncovered new perspectives for the understanding of cAMP signaling in the cardiovascular system. The functions of these cAMP-sensitive GEFs in various subcellular compartments and cellular contexts are currently being unraveled, but given the availability of EPAC-specific ligands and engineered mouse models, a large body of evidence indicates that EPAC proteins are involved in multiple biological actions of cAMP, such as cardiac hypertrophy and vascular inflammation. In this review, after a description of EPAC protein structures and mechanism of activation, we discuss recent advances in the discovery of novel pharmacological modulators of EPAC (Figure 1). We provide an overview of the roles of EPAC proteins, their signalosome and compartmentation in cardiovascular function and diseases. We also discuss the therapeutic potential of EPAC ligands in the treatment of cardiovascular diseases.

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Correspondence to Frank Lezoualc’h, PhD, INSERM UMR-1048, Institut des Maladies Métaboliques et Cardiovasculaires, Université Toulouse III-Paul Sabatier, 1 Ave Jean Poulhès, BP 84225, 31432 Toulouse Cedex 4, France, E-mail Frank.Lezoualch@insERM.fr
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EPAC Proteins

EPAC Genes and Tissue Distribution

EPAC proteins were discovered by 2 independent groups in 1998.6,7 On the basis of their initial finding that cAMP-induced activation of Rap1 was insensitive to inhibition of PKA, de Rooij et al6 identified a protein named EPAC (corresponding to EPAC1 isoform) in the database as a genomic sequence encoding domains with homology to cAMP-binding sites and GEFs for Ras-like proteins. Independently, the same EPAC1 protein and another EPAC isoform named EPAC2 were found in a differential display screen for novel cAMP-binding domain-bearing proteins enriched in the striatum.7 A third member of the family known as REPAC (for related to EPAC), has been identified; however, this protein lacks the cAMP-dependent regulatory sequences present in EPAC1 and EPAC2 (Figure 2).10,11 Both the EPAC proteins are cAMP regulated Rap-GEFs that favor GDP/GTP exchange and thus the activation of the small GTPases, Rap1 and Rap2.6,7 EPAC1 and EPAC2 are, respectively, encoded by Rapgef3 and Rapgef4 genes, which display similar genetic organization. Rapgef3 transcription leads to the synthesis of one 4-kb long mRNA, whereas alternative promoter usage and differential splicing of Rapgef4 generate 3 different EPAC2 isoforms (named EPAC2A, EPAC2B, and EPAC2C).12,13

EPAC1 and EPAC2 exhibit distinct expression profiles that may vary depending on developmental stages and pathophysiological situations. EPAC1 is nearly ubiquitously expressed with high levels of expression in the heart, blood vessels, uterus, kidney, and central nervous system.6,7 In contrast, the epigenetical regulation of Rapgef4 alternative promoters leads to a restricted expression pattern of EPAC2 isoforms.13 EPAC2B, which is similar to EPAC1 in domain structure, is detected in the adrenal gland and the endocrine pancreas, whereas EPAC2C mRNA was reported only in the liver.12,14

Table 1. Pharmacological and functional properties of exchange protein directly activated by cAMP (EPAC) agonists and antagonists. BRET indicates bioluminescence resonance energy transfer; CNBD, cyclic nucleotide-binding domain; and GEF, guanine-nucleotide exchange factor.

<table>
<thead>
<tr>
<th>Agonists</th>
<th>EPAC isomers</th>
<th>Binding site</th>
<th>EC50/AC50</th>
<th>Biological effects in cultured cells</th>
<th>Biological effects in vivo</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>8-CPT (8-(4-Chlorophenylthio)-2′-0-methyladenosine-3′,5′- cyclic monophosphosphate)</td>
<td>EPAC1 preferentially</td>
<td>CNBD-B</td>
<td>EC50=5.6 μM (EPAC1 GEF activity)</td>
<td>Used in a multitude of cultured cell types (including cardiovascular cells)</td>
<td>Induces positive inotropic and pro-arrhythmic effects (ex-vivo) in rodents</td>
<td>8, 16, 17, 31, 32, 33, 34, 52, 106, 107, 112</td>
</tr>
<tr>
<td>8-CPT-AM (8-(4-Chlorophenylthio)-2′-0-methyladenosine-3′,5′-cyclic monophosphate, acetoxymethyl ester)</td>
<td>EPAC1 preferentially</td>
<td>CNBD-B</td>
<td>-</td>
<td>Used in a multitude of cultured cell types (including cardiovascular cells)</td>
<td>-</td>
<td>16, 31, 35</td>
</tr>
<tr>
<td>Sp-8-CPT (Sp-8-(4-Chlorophenylthio)-2′-O-methyl-cAMP)</td>
<td>EPAC1 preferentially</td>
<td>CNBD-B</td>
<td>-</td>
<td>Used in a multitude of cultured cell types (including cardiovascular cells)</td>
<td>-</td>
<td>16, 31, 36, 53, 118</td>
</tr>
<tr>
<td>Sp-8-BnT-AMPs (S-220) (S-8-Benzyloxadoline-3′,5′-cyclic monophosphorothioate, Sp- isomer)</td>
<td>EPAC2</td>
<td>CNBD-B</td>
<td>AC50 = 0.1 μM (EPAC2 GEF activity)</td>
<td>Potentiates glucose-induced insulin secretion from primary human pancreatic islets</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>Sulfonylurea drugs (Tolbutamide, glibenclamide)</td>
<td>EPAC2A</td>
<td>CNBDs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>39, 42</td>
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<table>
<thead>
<tr>
<th>Antagonists</th>
<th>EPAC isomers</th>
<th>Binding site</th>
<th>IC50</th>
<th>Biological effects in cultured cells</th>
<th>Biological effects in vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE3F4 (8-Benzyloxadoline-3′, 5′-cyclic monophosphorothioate, Sp isomer)</td>
<td>EPAC1</td>
<td>-</td>
<td>10 μM (EPAC1 GEF activity)</td>
<td>Inhibits EPAC1-induced hypertrophy markers and autophagy in primary cardiomyocytes</td>
<td>-</td>
<td>33, 51-53</td>
</tr>
<tr>
<td>5225554</td>
<td>EPAC1</td>
<td>-</td>
<td>71 μM / 4 μM (EPAC1 BRET sensor)</td>
<td>5376753 inhibits EPAC1-mediated migration of rat cardiac fibroblasts</td>
<td>-</td>
<td>54</td>
</tr>
<tr>
<td>5376753</td>
<td>EPAC1</td>
<td>-</td>
<td>0.43 μM / 0.7 μM (EPAC2 GEF activity)</td>
<td>ESI-05 inhibits EPAC2-induced proarrhythmic Ca2+ in adult rat cardiomyocytes</td>
<td>-</td>
<td>45, 119</td>
</tr>
<tr>
<td>ESI-07 (unlabeled)</td>
<td>EPAC2</td>
<td>-</td>
<td>8.4 μM / 4.0 μM / 5.9 μM (competitive assay of 8-NBD-cAMP with EPAC2)</td>
<td>HUC0197 and HUC0198 block EPAC1 and EPAC2-mediated Akt phosphorylation in H9C2/S7T cells</td>
<td>-</td>
<td>44, 45</td>
</tr>
<tr>
<td>ESI-09 (5-[(2E)-5-(1-carboxy-2-propyl)-1H-indol-3-yl]-2-[1-(chloro phenyl) hydrazino]-3-azaproprionitrile)</td>
<td>EPAC1/EPAC2</td>
<td>CNBD-B</td>
<td>3.2 μM for EPAC1 / 1.4 μM for EPAC2 (GEF activity)</td>
<td>Suppresses pancreatic cancer cell migration and invasion</td>
<td>Protects mice against nicotine-induced injury</td>
<td>46-48</td>
</tr>
</tbody>
</table>
EPAC2A (originally named EPAC2) is broadly expressed, and this EPAC isoform is found in the brain and endocrine tissues, such as pituitary and pancreatic islets. Compelling evidence indicates that EPAC1 and EPAC2 expression levels change throughout development. Both EPAC1 and EPAC2 are detected in mouse embryonic heart, but exhibit different expression profiles. For several organs such as the kidney or heart, the ratio EPAC1/EPAC2 strongly increases in adult in comparison with fetal organs, suggesting that both proteins contribute differentially to organ development and function. Although the underlying molecular mechanisms involved in the regulation of EPAC expression are unknown, various studies indicate that the expression levels of EPAC1 and EPAC2 proteins are altered in pathophysiological situations. With respect to cardiac remodeling, EPAC1 levels and to a lesser extent EPAC2, are upregulated in isoproterenol-induced left ventricular hypertrophy and in pressure overload-induced hypertrophy. On the contrary, EPAC1 expression decreases after myocardial infarction. Of note, a functional hypoxia responsive element in the EPAC1 promoter was recently identified suggesting that EPAC1 may be regulated by hypoxic conditions. In humans, EPAC1 is the main EPAC isoform expressed in the heart and its level is increased in left ventricular samples from patients with heart failure (HF), suggesting a potential role for EPAC1 during late stages of cardiac remodeling. Also only EPAC1 is upregulated in the area of intimal thickening after injury of mouse femoral artery. Therefore, the dysregulation of EPAC expression, and perhaps an imbalance in the expression of EPAC isoforms observed during cardiovascular remodeling suggest a potential implication of these cAMP–GEFs in cardiovascular disease progression (see below).

**EPAC Protein Structure and Mechanism of Activation**

EPAC1 and EPAC2 are multidomain proteins with molecular weights of ≈105 kDa and ≈115 kDa, respectively, and share the same structural organization (Figure 2). Except for REPAC, all EPAC isoforms contain a cyclic nucleotide–binding domain (CNBD-B) that binds cAMP with high affinity. EPAC1 can interact with the microtubule accessory protein light chain 2 (LC2), which increases the affinity of EPAC1 for cAMP. The additional CNB domain (CNBD-A) present in EPAC2A binds cAMP with a lower affinity than the conserved CNB-B and is not required for EPAC2 activation. The regulatory domain of EPAC also contains a Dishevelled, Egl-10, Pleckstrin (DEP) region, which is involved in EPAC subcellular localization. Ezrin/radixin/moesin (ERM) proteins and phosphatidic acid (PA) are involved in the spatial regulation of EPAC1. The catalytic domain of EPAC is composed of a catalytic CDC25 homology domain (CDC25-HD) stabilized by a Ras-exchange motif (REM). The small GTPase Ran and Ran-binding protein 2 (RanBP2) anchor EPAC1 to the nuclear pore. Ras binds to the EPAC2 Ras association (RA) domain to regulate EPAC2 membrane localization and biological function of EPAC2. A putative mitochondrial-targeting sequence (MTS) and a functional nuclear pore localization signal (NLS) are located at the N-terminal part and CDC25-HD of EPAC1, respectively.

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**Figure 2.** Exchange protein directly activated by cAMP (EPAC) multidomain proteins and their interacting partners. EPAC proteins contain a regulatory region and a catalytic region, which are subdivided into functional domains. Except for REPAC, all EPAC isoforms contain a cyclic nucleotide–binding domain (CNBD-B) that binds cAMP with high affinity. EPAC1 can interact with the microtubule accessory protein light chain 2 (LC2), which increases the affinity of EPAC1 for cAMP. The additional CNB domain (CNBD-A) present in EPAC2A binds cAMP with a lower affinity than the conserved CNB-B and is not required for EPAC2 activation. The regulatory domain of EPAC also contains a Dishevelled, Egl-10, Pleckstrin (DEP) region, which is involved in EPAC subcellular localization. Ezrin/radixin/moesin (ERM) proteins and phosphatidic acid (PA) are involved in the spatial regulation of EPAC1. The catalytic domain of EPAC is composed of a catalytic CDC25 homology domain (CDC25-HD) stabilized by a Ras-exchange motif (REM). The small GTPase Ran and Ran-binding protein 2 (RanBP2) anchor EPAC1 to the nuclear pore. Ras binds to the EPAC2 Ras association (RA) domain to regulate EPAC2 membrane localization and biological function of EPAC2. A putative mitochondrial-targeting sequence (MTS) and a functional nuclear pore localization signal (NLS) are located at the N-terminal part and CDC25-HD of EPAC1, respectively.
(CNBD-B) to the CNBD of PKA and the bacterial transcriptional factor cAMP receptor protein (Figure 2).\textsuperscript{11} Similar to PKA, EPAC is activated by Gs-coupled G protein–coupled receptors and, therefore, responds to physiological relevant cAMP concentrations as revealed in studies using EPAC-based probes.\textsuperscript{21} EPAC2A has an additional low-affinity cAMP-binding domain (CNBD-A), which is unable to induce GEF activity after cAMP binding (Figure 2).\textsuperscript{11,22} Both the CNBD-A and the Disheveled/Egl-10/pleckstrin domain are involved in EPAC subcellular localization. Independently of its cAMP-binding capacity, CNBD-A is implicated in EPAC2 targeting to the plasma membrane of pancreatic cells.\textsuperscript{12} In addition to the temporal control of EPAC1 GEF activity, cAMP can also promote the translocation of cytosolic EPAC1 toward the plasma membrane.\textsuperscript{23} Importantly, a phosphatidic acid–binding motif within the Disheveled/Egl-10/pleckstrin domain is a necessary determinant of plasma membrane association that is required for the proper cellular function of EPAC1.\textsuperscript{24}

The catalytic region consists of a Ras-exchange motif that stabilizes the GEF domain, a Ras association domain, which targets EPAC2 to the plasma membrane through its interaction with Ras.\textsuperscript{25,26} and a CDC25 homology domain (CDC25-HD) that exhibits GEF activity for Rap1 and Rap2 (Figure 2).\textsuperscript{8} The CDC25-HD of EPAC1 contains a functional nuclear pore localization signal.\textsuperscript{27} The linker region located between the regulatory and the catalytic regions contains the hinge and the lid, which are implicated in the communication between both parts of the protein.\textsuperscript{28} In the absence of cAMP, EPAC GEF activity is inhibited because of an intramolecular interaction between its regulatory and catalytic domains, thus preventing the binding of the Rap effector (Figure 3). The unbound CNBD-B that is common to EPAC1 and EPAC2 is sufficient to mediate this inhibitory effect.\textsuperscript{11,29} Binding of cAMP to EPAC induces large conformational changes within the protein and releases the autoinhibitory effect of the N terminus of the protein, leading to Rap activation (Figure 3). Mechanistically, the interaction of the phosphate sugar moiety of cAMP structure with the phosphate-binding cassette of CNBD-B induces a movement of the hinge, which stabilizes the interaction of cAMP with the lid and liberates the catalytic domain. This allows binding of Rap to the GEF, which catalyzes the exchange of GDP to GTP.\textsuperscript{28–30} Interestingly, based on cAMP-dependent conformational change of full-length EPAC, various EPAC-based fluorescence resonance energy transfer biosensors have been developed to measure cAMP dynamics in healthy and diseased cells.\textsuperscript{21}

**Figure 3. Mechanism of exchange protein directly activated by cAMP (EPAC) activation.** cAMP is produced from ATP by adenylyl cyclase (AC) in response to Gs-coupled G protein–coupled receptors (GPCRs) stimulation and activates its classical downstream effector, cAMP-dependent protein kinase A (PKA). Phosphodiesterases (PDE) degrade cAMP and thereby regulate the duration and intensity of cAMP signaling. EPAC function in a PKA-independent manner and represents a novel mechanism for governing signaling specificity within the cAMP cascade. Binding of cAMP to the high-affinity cyclic nucleotide–binding domain (CNBD-B) of EPAC induces marked conformational changes in the protein, which leads to exposure of the catalytic region for binding of Rap GTPase to catalyze the exchange of GDP for GTP. Rap–GTPase–activating proteins (Rap-GAPs) enhance the intrinsic GTP hydrolysis activity of Rap leading to GTPase inactivation.
EPAC and PKA were synthesized based on the observation that the 2′-hydroxyl of cAMP is not required for the binding to EPAC, in contrast to PKA and other direct cAMP effectors.\textsuperscript{32} A screen of cAMP analogs substituted at this critical 2′-position led to the identification of the 8-(4-chloro-phenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate, a specific and efficient activator of EPAC that does not activate PKA.\textsuperscript{32} This compound abbreviated here as 8-CPT (also known as 007 or 8-pCPT-2′-O-Me-cAMP in the literature) exerts roughly a 100-fold selectivity toward EPAC over PKA, and it has become a widely used tool in EPAC functional characterization (Figure 4).\textsuperscript{8,16} 8-CPT is more efficient than cAMP in activating recombinant EPAC1 isoforms in vitro but it is a weaker agonist than cAMP in activating the EPAC2 isoform.\textsuperscript{33} Activation of EPAC1 by 8-CPT is dependent on the unique Gln270 residue in EPAC1. As this amino acid is not conserved in EPAC2, 8-CPT is a poor activator of EPAC2.\textsuperscript{34}

To improve 8-CPT permeability, Vliem et al\textsuperscript{35} synthesized an acetoxymethyl ester form of 8-CPT, which readily crosses the plasma membrane and is hydrolyzed directly by cellular esterases to release the biologically active parent compound (Figures 1 and 4). 8-CPT-acetoxymethyl ester is 100-fold more efficient on EPAC1 activity than its parental molecule in cultured cells, without affecting EPAC specificity.\textsuperscript{33} However, caution should be taken in using such EPAC activator because some studies indicate that in addition to their primary effects, 8-CPT and related analogs such as the nonhydrolyzable Sp isomer of 8-CPT (Sp-8-CPT) can also bind and inhibit PDEs and thus indirectly increase intracellular cAMP and 3′-5′-cGMP levels, and eventually activate PKA, PKG, and cyclic nucleotide–gated channels.\textsuperscript{36} Furthermore, another source of EPAC-independent effects of 8-CPT–modified analogs involves the formation of active intracellular metabolites, which can influence cellular function.\textsuperscript{37,38} Thus, potential off-target effects of 8-CPT and its derivatives limit their use in vivo. An important finding that will certainly help in the identification of the differential biological effects of EPAC isoforms in cardiovascular cells is the recent characterization of a specific EPAC2 activator. On the basis of crystal structures of EPAC2 in complex with several cAMP analogs, Schwede et al\textsuperscript{34} identified a cAMP derivative, which contains a benzyl-thio group (BnT) at 8-position of the Sp-cAMPS and interacts specifically with the K405 residue of EPAC2 (Figures 1 and 4). This EPAC2 agonist named Sp-8-BnT-cAMPS (or S-220) efficiently activates EPAC2, with minor effects on EPAC1 and PKA in a human osteosarcoma U2OS cell line, although PKA activation has been observed in a biochemical kinase assay.\textsuperscript{34} Interestingly, Zhang et al\textsuperscript{39} reported that sulfonylurea drugs, widely used for the treatment of type 2 diabetes mellitus, are selective activators for EPAC2A (Figure 1). The authors showed that sulfonylurea-stimulated insulin secretion was reduced in EPAC2 knockout mice and sulfonylureas such as tolbutamide and glibenclamide were capable of activating an EPAC2A-based fluorescence resonance energy transfer sensor in MIN6 β cells.\textsuperscript{39} Although other groups failed to detect the direct activation of EPAC2A by sulfonylureas using cell-free system,\textsuperscript{40,41} a recent study suggested that sulfonylureas and cAMP cooperatively activated EPAC2A through binding to CNBD-A and CNBD-B, respectively.\textsuperscript{42}

Recently, Tsalkova et al\textsuperscript{43} developed a fluorescence-based high-throughput screening assay for identifying EPAC-specific antagonists that directly compete with a fluorescent cAMP

![Figure 4. Chemical structures of exchange protein directly activated by cAMP (EPAC) ligands. Naturally occurring cAMP. 8-CPT preferentially activates EPAC1. 8-CPT-acetoxymethyl ester (AM) has greater membrane permeability than 8-CPT and is cleaved by intracellular esterases to regenerate free biologically active 8-CPT. Sp-8-BnT-cAMPS is an EPAC2-specific agonist. CE3F4 is an EPAC1 inhibitor while EPAC-specific inhibitors (ESI)-05 and ESI-07 inhibit EPAC2 activity.](http://circres.ahajournals.org/)

EPAC Functions and Signaling in the Vasculature

Vascular smooth muscle cells (VSMCs) and vascular endothelial cells (VECs) regulate blood pressure and maintain blood vessel integrity. During stress conditions, VSMCs and VECs undergo a process of phenotypic modulation resulting in the development of cells with proliferative and migratory phenotypes that can contribute to cardiovascular diseases, such as atherosclerosis and restenosis. Because cAMP is a crucial regulator of vascular structural integrity and function, various studies have investigated the role of EPAC in the vasculature.

Regulation of the Vascular Tone

The relaxation of vascular smooth muscle is often mediated through vasodilator-induced elevations of cAMP. Initial evidence for a role of EPAC in the regulation of vasorelaxation came from the observation that the EPAC agonist, 8-CPT and derived compounds directly relaxes several types of vascular smooth muscle preparations, for example cell preparation from rat aorta and pulmonary artery precontracted with endothelin. Consistent with this finding, 8-CPT–induced relaxation is decreased in mouse vessels lacking a direct target of EPAC, Rap1B. Of particular importance, Rap1B knockout mice develop hypertension, in part, via functional changes to VSMC and this indicates an essential role of Rap1 and possibly EPAC in maintaining normal vascular physiology.

Various mechanisms that do not necessarily exclude the participation of PKA are involved in EPAC vasorelaxant effect. EPAC mediates vasorelaxation by indirectly regulating the activation of Ca2+-sensitive and ATP-sensitive K+ channels within the endothelium and on VSMCs. In addition, EPAC-induced arterial smooth muscle relaxation may be a consequence of the activation of endothelial nitric oxide synthase and the subsequent production of nitric oxide, a potent vasodilator molecule in endothelial cells (Figure 5). Of particular importance, activation of EPAC-Rap1 signaling lowers VSMC contractility via the inhibition of the small GTPase RhoA activity, a master regulator of VSMC contraction and the dephosphorylation of myosin light chain (Figure 5). Conversely, in microvascular smooth muscle cells EPAC stimulates RhoA activity to increase the expression of functional α2C-ARs that mediate constriction of small blood vessels. Altogether, these observations indicate that depending on the vascular bed, EPAC may induce vasorelaxation or vasoconstriction. Interestingly, a recent study showed that stimulation of glucagon-like peptide-1 receptor in atrial cardiomyocytes promoted EPAC2 activation, which then mediated the secretion of atrial natriuretic factor. Atrial natriuretic factor release from the atria induced cyclic GMP–mediated smooth muscle relaxation and natriuresis, leading to a reduction of blood pressure. Therefore, the effect of EPAC in the regulation of the vascular tone may be indirect and not only restricted to its biological action in VSMCs and VECs.

Regulation of the VSMC Proliferation and Migration

cAMP is a well-characterized inhibitor of VSMC proliferation implicated in maintaining VSMC quiescence and promoting healing after vessel injury. EPAC can inhibit proliferation in rat and human VSMCs. Similarly, direct activators of EPAC, also inhibit mitogen-induced proliferation of cultured air way smooth muscle cells suggesting that EPAC activation may limit the remodeling observed in diseases of the airways, such as asthma and chronic obstructive pulmonary disease. This effect of EPAC, at least in rat VSMCs, is synergistic with PKA and involves coordinated inhibition of the immediate response gene Early growth response 1 and other factors such as cyclin D1 and S-phase kinase-associated protein-2 (Skp2), that are critical for regulating cell-cycle progression (Figure 5).

Recently, it has been reported that therapeutically relevant concentrations of the prostacyclin analog, beraprost can inhibit PDGF-induced human VSMC migration via activation of the cAMP effector EPAC. The mechanism of this EPAC-mediated inhibition is achieved through the activation
of Rap1 and subsequent inhibition of RhoA, which prevents actin cytoskeletal changes, thereby decreasing cell migration.71 Although this study was performed mainly in vitro and requires further investigation in animal models of vascular remodeling, one could speculate that cAMP-producing agonists such as beraprost might help slow restenosis after angioplasty by inhibiting VSMC migration through EPAC activation. In contrast to the finding of a vasculoprotective effect of EPAC, Yokoyama et al20 has reported that a synthetic EPAC activator used at high concentrations enhanced rat aortic smooth muscle cell migration. The same group recently extended their observation in vivo showing that vascular injury–induced intimal thickening was significantly inhibited in EPAC1-deleted mice.72 The reasons for these discrepancies are unclear but may involve some species differences.

Vascular Endothelial Barrier Function

The endothelium of the vascular system forms a barrier between blood and the extravascular space that controls the extravasation of solutes, macromolecules, and circulating leukocytes. Endothelial barrier (EB) dysfunction results in an increase in vascular permeability, thereby causing edema or inflammatory or metastatic cell infiltration.73 Thus, the selective regulation of vascular permeability is critical for maintaining vascular integrity in homeostasis and disease. Different pools of proteins are assembled on endothelial cells to form membrane domains that create the cohesive structure that accounts for the barrier properties of the vessel wall. Among the barrier-forming adhesive structures, the most important are the adherens junctions (composed mainly of vascular endothelial [VE]-cadherin), gap junctions, and tight junctions.73
It is generally accepted that cAMP under physiological and most pathophysiological conditions stabilizes the EB. EPAC1 is the major EPAC isoform in VECs and activation of EPAC1–Rap1 signaling pathway by 8-CPT enhances EB function in human umbilical vein endothelial cells. On the contrary, the increased permeability induced by inflammatory mediators such as thrombin is prevented by EPAC1 activation. The mechanisms by which EPAC1 mediates VEC barrier function seems to be diverse. For instance, EPAC1-mediated endothelial cell–cell junction stability involves the accumulation of junctional proteins at cell–cell contacts, increased VE-cadherin–dependent cell adhesion, and linearization of cell–cell junctions. Moreover, PDE4D tethers EPAC1 into the VE-cadherin–based complex and the interaction of PDE4D and EPAC1 are critical for coordinating the effects of cAMP on VEC permeability (Figure 5).

Of note, Rho GTPases, RhoA and Rac are the main regulators of actin cytoskeleton dynamics and thus of the adherens junctions. They exert opposing effects on EB function. Activation of RhoA increases stress fiber formation and induces contraction via Rho kinase leading to increased gap formation, whereas the activation of Rac1 reorganizes actin cytoskeleton at the cell periphery and thus strengthens the adherens junctions. A growing body of evidence indicates that EPAC1–Rap1 stabilizes EB functions via the regulation of Rac1 activity. Initially discovered in neuronal cells, this connection between EPAC1 and Rac1 involves the Rac-specific GEFs, Tiam1 and Vav2 and mediates protective effects of prostaglandin E2 against vascular barrier dysfunction in human pulmonary artery endothelial cells (Figure 5). Furthermore, activated Rac1 attenuates the effect of the Rho pathway on EB dysfunction via the reduction of RhoA activity, thereby leading to decreased myosin light chain phosphorylation, endothelial cell contraction, and less severe EB dysfunction. EPAC1–Rap1 pathway has also been reported to downregulate RhoA activity through a multimeric protein complex containing the Rap1 effectors, Radil and Rasip1 and the Rho GTPase–activating protein 29 (ArhGAP29), leading to decreased cell contractility and stabilization of VE-cadherin–induced cell–cell adhesion (Figure 5). In addition, the Rap1-binding protein K-Rev1 Interaction Trapped gene 1 (KRIT1) was shown to be involved in EPAC1/Rap1-induced permeability of endothelial cell–cell junctions.

Although EPAC1 activates Rap1, not all EPAC1 effects on vascular EB function can be attributed to the activation of Rap1. Consistent with earlier studies showing the interaction of EPAC1 with microtubule-associated proteins, this cAMP-induced Rap1-independent barrier tightening effect of EPAC1 is also mediated by microtubules and involves A-kinase anchoring proteins. It was demonstrated that A-kinase anchoring proteins 9 interacts with EPAC1 to promote microtubule growth (Figure 5). This pathway induces integrin adhesion at lateral cell borders, and thereby contributes to the EPAC1-induced enhancement of barrier function. Finally, it is interesting to note that in response to elevations in intracellular cAMP the actin-linking protein Ezrin binds to EPAC1 (Figure 5). This molecular complex cooperates to induce cell adhesion and spreading, and may have an important impact on barrier function.

Regulation of Vascular Inflammation

VECs represent a major cellular target for many proinflammatory and anti-inflammatory cytokines. Evidence suggesting an anti-inflammatory action of EPAC1 in VECs came from the work of Sands et al that showed EPAC1 limited the pro-inflammatory interleukin (IL)-6 effects through upregulation of cytokine signaling-3 suppressor gene expression in human umbilical vein endothelial cells. Additional studies demonstrated that EPAC1-induced suppressor of cytokine signaling 3 expression was independent of the classical PKA route of cAMP-mediated transcription but rather involved the mobilization of CCAAT/enhancer–binding protein and c-Jun transcription factors, which directly bound to the suppressor of cytokine signaling 3 promoter (Figure 5). Therefore, the dual functions of EPAC in both downregulating cytokine-mediated inflammation and maintaining the EB integrity suggest a central role for this cAMP–GEF in controlling endothelial responses to inflammatory stimuli. But negative regulation of cytokine signaling by EPAC seems not to be limited to VECs. A recent study reported that EPAC1 mediates the inhibitory effect of the PDE4D inhibitor, roflumilast on vascular cell adhesion molecule 1 expression in tumor necrosis factor-α–activated VSMCs through histone methylation. These data identify PDE4 inhibition and EPAC activation as a promising mechanism to target vascular inflammation occurring in atherosclerosis and restenosis. In addition, EPAC (likely EPAC1) exerts an anti-inflammatory role in cigarette smoke extract stimulated secretion of IL-8. Differences between EPAC isoforms may exist because a recent report by Oldenburger and et al showed that EPAC2, but not EPAC1, enhanced cigarette smoke–induced airway inflammation in genetically modified mice. Further work is necessary to fully elucidate the specific role of EPAC isoforms in the regulation of vascular inflammation.

Role of EPAC in Cardiac Function and Diseases

EPAC Effects on Excitation–Contraction Coupling

Calcium (Ca++) is an essential second messenger in the cardiac physiology because its rhythmic variations activate contraction of the heart beat through the mechanism of excitation–contraction coupling. An action potential depolarizes the sarcolemma activating voltage-sensitive L-type Ca++ channels allowing Ca++ to enter into the cell where it binds to ryanodine receptors (RyR) on the adjacent sarcoplasmic reticulum (SR). This binding induces Ca++ release to the cytosol from the SR. This coordinated process named Ca++–induced Ca++ release provides the Ca++ necessary to activate the contraction of the myofibrils. After contraction, cytoplasmic Ca++ is then rapidly pumped back into the SR through the SR Ca++ ATPase or exported out of the cell (mainly via the Na+/Ca++ exchanger), thereby accounting for relaxation.

The sympathetic nervous system provides the most powerful stimulation of cardiac function, brought about via norepinephrine and epinephrine and their postsynaptic β-ARs. In this setting, norepinephrine released from intracardiac nerve terminals activates β-ARs, which increase intracellular cAMP level to enhance PKA activity in cardiomyocytes,
PKA regulates key excitation–contraction coupling proteins to increase cardiac contraction (inotropy) and relaxation (lusitropy). Indeed, PKA can phosphorylate L-type Ca\(^{2+}\) channels, stimulating Ca\(^{2+}\) current amplitude, which triggers larger SR Ca\(^{2+}\) release to induce myofilament contraction. Phosphorylation of phospholamban by PKA on Ser16 relieves its tonic inhibition on SR Ca\(^{2+}\)-ATPase activity, thus, enhancing the rate of Ca\(^{2+}\) uptake in the SR (lusitropic effect). In addition, the effect of PKA on myofilament protein phosphorylation also modulates contractility by decreasing their Ca\(^{2+}\) sensitivity and thus favoring relaxation.

In 2005, Morel et al\(^{109}\) showed that the EPAC-specific agonist 8-CPT produces bursts of Ca\(^{2+}\) transients in rat neonatal ventricular myocytes. Subsequent studies performed in isolated rat adult cardiomyocytes demonstrated that EPAC increases the frequency of spontaneous Ca\(^{2+}\) sparks or spontaneous local Ca\(^{2+}\) transients ([Ca\(^{2+}\)]\(_i\)). A Ca\(^{2+}\) spark arises from the activation of a cluster of RyRs, which induces Ca\(^{2+}\) release from the SR. The increased Ca\(^{2+}\) spark from the SR through Ca\(^{2+}\) sparks induces a decrease in the amount of Ca\(^{2+}\) stored in the SR and thus a decrease in the amplitude of electrically evolved [Ca\(^{2+}\)]\(_i\) transients.\(^{104-106}\) This effect of EPAC activation on diastolic Ca\(^{2+}\) leak is independent of PKA but rather requires Ca\(^{2+}\)/calmodulin kinase type II (CaMKII) activation, which phosphorylates the RyR at a key serine residue (Ser2815 or 2814 depending on species), without significant effects on L-type Ca\(^{2+}\) current or Ca\(^{2+}\) removal via Na+/Ca\(^{2+}\) exchange. This RyR phosphorylation after EPAC activation is fully prevented in the presence of U73122, a specific phospholipase C (PLC) inhibitor, indicating that CaMKII acts downstream of PLC. Importantly, the EPAC-induced CaMKII activation, RyR phosphorylation at Ser2814/15 and lack of L-type Ca\(^{2+}\) current modulation have also been confirmed in mouse cardiomyocytes.\(^{107-110}\) In contrast to rat ventricular myocytes, however, study using mouse cardiomyocytes showed that an acute stimulation of EPAC with 8-CPT induced a significant increase in the amplitude of electrically evoked [Ca\(^{2+}\)]\(_i\) transients. This response was abolished in PLCe-deleted cardiac myocytes but rescued by transduction with PLCe, indicating that EPAC is upstream of PLCe. On the basis of this finding, it was suggested that EPAC via a sequential activation of Rap, PLCe, PKCe, and CaMKII is a critical regulator of Ca\(^{2+}\)-induced Ca\(^{2+}\) release downstream of the β-AR.\(^{108,111}\) At first glance, this effect of EPAC on Ca\(^{2+}\)-induced Ca\(^{2+}\) release could be envisaged in acute stimulation, but could not fit for longer EPAC stimulation because the increase in Ca\(^{2+}\) release without increasing Ca\(^{2+}\) entry would rapidly deplete Ca\(^{2+}\) from the SR.\(^{106,112}\)

Development of EPAC knockout mice has helped to clarify the specific involvement of EPAC proteins in the regulation of excitation–contraction coupling. Using knockout mice of either EPAC1 or EPAC2 and double knockout mice, Pereira et al\(^{109}\) provided evidence that EPAC deletion is not critical to baseline cardiac function and does not alter baseline Ca\(^{2+}\) handling. In contrast to the above-mentioned study suggesting a positive inotropic effect of EPAC activation in mouse cardiomyocytes,\(^{111}\) EPAC knockout mice demonstrated that EPAC does not seem to participate in the inotropic response to acute β-AR activation.\(^{109}\) These contradictory results on the inotropic action of EPAC may depend on experimental conditions. For instance, chronic effects are opposed to EPAC acute effects on excitation–contraction coupling.\(^{112}\) Sustained EPAC activation induces a PKA-independent positive inotropic effect, related to elevation of [Ca\(^{2+}\)]\(_i\) transients, associated to SR Ca\(^{2+}\) load increase and enhancement of Ca\(^{2+}\) entry through L-type Ca\(^{2+}\) current in isolated rat cardiomyocytes.\(^{112}\) Moreover, the EPAC agonist, 8-CPT increases cell shortening and myofilament Ca\(^{2+}\) sensitivity in rat cardiomyocytes. This is correlated with an increase in protein kinase C (PKC) and CaMKII-dependent phosphorylation of 2 key sarcomeric proteins, cardiac Troponin I (cTnI) and cardiac myosin–binding protein-C.\(^{113}\)

Of particular importance, EPAC2 and not EPAC1 was shown to be essential for 8-CPT–induced RyR activation and mediate decreased Ca\(^{2+}\) transients and enhanced Ca\(^{2+}\) leak in a PKA-independent manner in mouse cardiomyocytes (Figure 6).\(^{109}\) This is consistent with the localization of EPAC2 along T tubules in mouse cardiomyocytes, indicating that EPAC signaling is compartmentalized.\(^{114}\) Nevertheless, it is important to keep in mind that species differences may exist in the expression profile and biological actions of EPAC isoforms. Finally, β1-AR blockade prevents EPAC-mediated Ca\(^{2+}\)-signaling alterations indicating that this β1-AR subtype is coupled to EPAC2 in mouse cardiomyocytes.\(^{109}\)

Altogether, most findings agree that EPAC does not play a major role in the inotropic response to acute β-AR stimulation compared with PKA, which is the main cAMP effector in this process. However, it is clear that EPAC activation causes CaMKII-dependent RyR sensitization and induces diastolic SR Ca\(^{2+}\) leak, a mechanism contributing to arrhythmias, pathological cardiac hypertrophy, and HF (Figure 6).\(^{115}\)

**EPAC and Cardiac Electric Remodeling**

In agreement with the mechanistic findings showing that EPAC activation alters Ca\(^{2+}\) homeostasis, experiments performed on the whole mouse hearts showed that the EPAC activator, 8-CPT induced ventricular arrhythmogenesis.\(^{107}\) Later, Brette et al\(^{116}\) reported that pharmacological activation of EPAC induced an action potential lengthening because of an inhibition of the steady-state potassium current. This observation is relevant to cardiac diseases because action potential lengthening is correlated in the genesis of arrhythmia by predisposing cardiac myocytes to early after depolarizations and dispersion of repolarization. Further evidence for a role of EPAC in cardiac electric remodeling came from an elegant study performed in guinea pig ventricular myocytes.\(^{117}\) It was shown that sustained β1-AR stimulation activates EPAC1, which decreases the density of slow delayed rectifier potassium K+ current (IKs). The delayed rectifier K+ current system is crucial for cardiac repolarization and reduced IKs promotes arrhythmogenesis. Mechanistically, EPAC1 effects on IKs occurs at the genomic level because it downregulates mRNA encoding the IKs subunit potassium voltage-gated channel subfamily E member 1 via the phosphatase calcineurin and its downstream effector, the nuclear factor of activated T cells (Figure 6).\(^{118}\) EPAC also increases the expression of 2 transient receptor potential canonical channels, transient receptor
potential canonical 3 and transient receptor potential can-
onical 4 channels in isolated rat ventricular cardiomyo-
cytes. Ca\(^{2+}\) influx through these channels may contribute to a proarrhythmic effect of EPAC. Finally, Okumura et al showed that EPAC1 deletion in mice decreased the incidence of atrial fibrillation after pacing probably, through inhibition of hyperphosphorylation of RyR on Serine 2814/2815 and phospholamban on serine-16. However, the authors did not test the involvement of EPAC2 in this process. In another study, EPAC2 activation, instead of EPAC1, was reported to contribute to \(\beta\)-AR–induced ventricular arrhythmia. The reason for the discrepancy may be because of the different cell types used in the 2 studies. Indeed, atrial cardiomyocytes either completely lack or possess only a rudimentary T-tubule network compared with ventricular myocytes in smaller laboratory species, such as the mouse. Because EPAC2 is mostly expressed in T tubules of mouse cardiomyocytes, it is possible that EPAC1 deletion prevents atrial fibrillation. In addition, EPAC isoform expression may vary in function of the cell type. The expression profile of EPAC1 and EPAC2 has not been yet investigated in atrial mouse cardiomyocytes and could be different to ventricular myocytes where both isoforms have been detected. Further in vivo experiments using selective pharmacological modulators of EPAC isoforms should allow for better discrimination of the role of EPAC1 and EPAC2 in cardiac arrhythmias. Of note, EPAC signaling could also concern arrhythmia through other mechanisms than alteration of Ca\(^{2+}\) handling. In particular, some studies reported that EPAC regulates the expression and localization of the main ventricular gap junction protein connexin 43 in a Rap1- and PKC\(\varepsilon\)-dependent manner, thereby influencing the gating function of Gap junctions. Considering the importance of connexin 43 in the regulation of electric cell-to-cell coupling and the pathogenesis of ventricular arrhythmias, we can speculate that EPAC contributes to electric remodeling via alterations of gap junction formation.

### EPAC and Cardiac Hypertrophy

Disease-related stresses such as hypertension and myocardial infarction can promote pathological cardiac hypertrophy which...
often leads to HF, a major cause of morbidity and mortality, often associated with sudden cardiac death. Although acute stimulation of β-AR is beneficial for the heart, chronic activation of this pathway triggers pathological cardiac remodeling, which may ultimately lead to HF. All forms of chronic HF in humans are associated with an elevated level of catecholamines that is inversely correlated with survival. Aberrant cAMP signaling through dysregulation of cAMP compartmentalization contributes to cardiac remodeling and HF. Thus, current studies aim at determining the involvement of the cAMP-binding protein EPAC in the process of cardiac pathological remodeling under chronic β-AR stimulation.

As indicated above, there is a potential role of EPAC in pathological cardiac hypertrophy because EPAC myocardial expression (mainly EPAC1) is increased in different animal models of cardiomyopathy and in left ventricle samples from failing heart patients. More recently, microRNA-133, a potent regulator of cardiac hypertrophy was shown to directly target EPAC mRNA and several other components of the cAMP signaling cascade, including the β1-AR and the catalytic subunit β of PKA. In vitro data demonstrated the involvement of EPAC in cardiomyocyte growth. Direct activation of endogenous EPAC with 8-CPT or overexpression of EPAC1, the major EPAC isoform in rat cardiac myocytes, increased various markers of hypertrophy, such as cell-surface area, protein synthesis, and atrial natriuretic factor expression. This hypertrophic effect of EPAC1 was confirmed in isolated adult rat cardiomyocytes. Isobutylmethylxanthine, a nonselective PDE inhibitor, exacerbated EPAC1-induced hypertrophy indicating that PDEs are key regulators of EPAC hypertrophic signaling. Of particular interest, a recent study identified EPAC1 as a potential mediator of radiation-induced hypertrophy in primary cardiomyocytes suggesting that cAMP–GEF is involved in regulating the side effects of anticancer treatment.

In 2008, the finding that β-ARs activated EPAC1 to trigger hypertrophy in a PKA-independent manner in isolated adult rat cardiomyocytes led to the hypothesis that, in addition to the classic cAMP/PKA pathway, β-AR stimulation under certain pathophysiological circumstances might switch on the EPAC-signaling pathway. This assumption has been partially confirmed in vivo using full EPAC1 knockout mice. Indeed, deletion of EPAC1 prevented the increase in cardiac mass and the upregulation of genetic markers of hypertrophy induced by sustained β-AR activation. Importantly, EPAC1 knockout mice displayed an improved cardiac contractile function in response to chronic activation of β-AR. Likewise, mice lacking EPAC1 (and not EPAC2) are cardioprotected against other forms of stress such as pressure overload- and aging-induced cardiac dysfunction.

Taking into account that EPAC1 inhibition may prevent phosphorylation of phospholamban and RyR2, one could speculate that an EPAC1 inhibitor might represent a new means of normalizing Ca2+ cycling in the setting of chronic β-AR–induced HF, and this may be an alternative strategy to current β-AR blocker therapy for the treatment of HF. Further investigation is needed to test this hypothesis.

Consistent with the beneficial effect of EPAC1 deletion on cardiac function during stress conditions, cardiac fibrosis was attenuated in EPAC1 knockout mice subjected to either chronic treatment with isoprenaline or transverse aortic constriction. However, the profibrotic effects of EPAC1 (the main EPAC isoform expressed in cardiac fibroblast) are in opposition to a previous in vitro study showing that EPAC1 overexpression prevented the effects of the profibrotic agents, transforming growth factor β1, and angiotensin II on collagen synthesis in primary rat cardiac fibroblasts. This apparent discrepancy may be because of the differences between in vitro and in vivo experimental conditions.

**EPAC Hypertrophic Signaling**

Initial studies performed in primary cardiomyocytes revealed that EPAC hypotrophic signaling was complex and involved various effector proteins such as the small GTPases Rap2B, PLC, Rac, H-Ras, the Ca2+-sensitive proteins calcineurin and CaMKII, and their downstream prohypertrophic transcription factors such as nuclear factor of activated T cells and myocyte enhancer factor 2. Pharmacological inhibition of either calcineurin or CaMKII was sufficient to block EPAC-induced hypertrophy. This indicates that CaMKII and calcineurin pathways converge on common downstream target genes in the hypertrophic-signaling pathway initiated by EPAC. On the basis of the previous finding of Schmidt et al, which also revealed cross-talk between EPAC and PLCε in neuroblastoma cells, it was reported that the effect of EPAC on H-Ras activation was indirect and involved Rap2B-dependent stimulation of PLC (most likely PLCε), leading to inositol 1,3,5-triphosphate production and subsequent increase in nuclear Ca2+ (see below; Figure 6). Likewise, cardiac-specific deletion of PLCε isoform inhibits the development of cardiac hypertrophy after transverse aortic constriction, further substantiating that EPAC1–PLCε pathway promotes hypertrophy. This inositol triphosphate–receptor–dependent nuclear Ca2+ elevation together with an SR-Ca2+ leak observed after EPAC stimulation may contribute to activate the 2 key prohypertrophic signaling effectors, calcineurin and CaMKII (Figure 6).

Intriguingly, a recent study revealed a key role of EPAC1 in the induction of autophagy during pathological cardiac hypertrophy. Autophagy is a highly conserved process involved in the degradation and recycling of proteins and organelles and plays important roles in homeostasis and cell survival. Specifically, it was shown that inhibition of autophagy potentiates cardiac myocyte hypertrophy activated by EPAC1. The signaling mechanisms underlying EPAC1-induced autophagy involves a Ca2+/calmodulin-dependent kinase kinase β/AMP-dependent protein kinase pathway. It is hypothesized that in the setting of cardiac remodeling induced by EPAC1 activation, autophagy functions as an adaptive mechanism that antagonizes the effect of EPAC1. This scenario is probably because autophagy has been shown to scavenge dysfunctional organelles and modified proteins to avoid cellular toxicity during cardiac remodeling. However, induction of excessive autophagy can also trigger cell death and then may contribute to heart disease pathogenesis. The effect of EPAC signaling on cell death and apoptosis has been explored in multiple cell types. Interestingly, a recent study showed that EPAC1 deletion in mice protected against myocardial apoptosis induced by sustained activation of β-AR. However, some
anti-apoptotic effects of EPAC have been reported in the literature. For instance, in primary cardiomyocytes EPAC1 participates with PKA in the antiapoptotic effects of exendin-4, a glucagon-like peptide-1 receptor agonist. In neonatal mouse cardiomyocytes, it is suggested that the inability of EPAC1 to trigger apoptosis is because of a lack of expression of the proapoptotic factor, Bim. Therefore, the differential effect of EPAC on cell death may certainly reflect distinct compartmentation of EPAC signaling under different circumstances.

**EPAC Compartmentation**

Although EPAC hypertrophic signaling is far to be unraveled, compelling evidence is now accumulating about the formation of EPAC macromolecular complexes that influence EPAC pro-hypertrophic signaling and cardiomyocyte functions. These molecular events occur in several intracellular compartments such as the SR, the plasma membrane and the nuclear/perinuclear area of cardiomyocytes where EPAC isoforms have been differentially localized. The compartmentation of EPAC in the context of cardiac hypertrophic signaling is discussed in the following section.

The observation that EPAC1 is activated by β-AR to trigger cardiomyocyte hypertrophy raises the question how EPAC can contribute to the signaling specificity of β-AR subtypes. The β1-AR and β2-AR subtypes, which functionally dominate in the heart, are structurally related and activate the G protein stimulatory for adenylyl cyclase, but both induce different sets of signal transduction mechanisms to fulfill distinct, sometimes opposed, physiological and pathophysiological roles.

For instance, chronic stimulation of β1-AR induces cardiac hypertrophy and interstitial fibrosis, whereas increased β2-AR activity is chronically better tolerated and may even be beneficial. On the basis of the finding that β1-AR and β2-AR play distinct roles in cardiomyocytes, studies investigated whether β1-AR and β2-AR differentially regulate EPAC1. Coimmunoprecipitation and bioluminescence resonance energy transfer experiments showed that β1-AR and β2-AR subtypes differentially interacted with the cAMP sensor EPAC1 to induce distinct signaling pathways. Indeed, although both β-AR subtypes activated the EPAC downstream effector Rap1, only β1-AR was able to regulate EPAC1-induced Rap2 prohypertrophic signaling. Further experiments revealed that the differential interaction of EPAC1 with the scaffolding protein β-arrestin 2 contributes to the specificity of β1-AR and β2-AR signaling. Specifically, EPAC1 recruitment to β-1-AR, via its interaction with β-arrestin2, plays an essential and coordinating role in the specific activation of the β1-AR–Ras cardiac hypertrophic signaling. In line with this observation, β-arrestin has been reported to interact with a CaMKII–EPAC1 complex and this interaction was enhanced on β1-AR stimulation in the heart. However, activation of β-AR2 results in the recruitment of a PDE4D5–β-arrestin2 complex and leaves EPAC1–β-arrestin 2 in the cytosol where it activates Rap1 nonhypertrophic signaling. Taken together, these data provide evidence that β1-AR and β2-AR present distinct spatial relation with EPAC1.

A detailed analysis of Ca2+ mobilization in different subcellular microdomains showed that in addition to its action on SR Ca2+ leak, the EPAC agonist, 8-CPT, elevated Ca2+ in the nucleoplasm of mature rat cardiomyocytes. This effect of EPAC activation correlates with the perinuclear expression of EPAC, and requires PLC activation (probably via Rap2) and subsequent stimulation of inositol 1,4,5 trisphosphate receptor activation. In line with this finding, EPAC1-dependent Rap activation stimulates PLCε but no other PLC isoforms. Also, EPAC1 is scaffolded at the nuclear envelope with PLCε and to muscle-specific A-kinase anchoring proteins in primary cardiomyocytes. Insoluble triphosphate-receptor is a Ca2+ release channel which can, not only generate a local Ca2+ signaling effect at the level of the T-tubular–SR junctional complex but also in the perinuclear area of cardiomyocytes, where it has been shown to participate in the excitation–transcription coupling, a process by which local Ca2+ activates gene transcription. Therefore, this EPAC–PLCε pathway may provide a mechanistic basis for the previous findings that EPAC1 regulates hypertrophic gene expression by activating nuclear CaMKII and subsequent phosphorylation of proteins of the epigenetic machinery, especially histone deacetylases (HDACs) and, in particular, class II HDACs (eg, HDAC4 and 5; Figure 6). CaMKII is known to activate nuclear export of class II HDAC, an effect which de-represses myocyte enhancer factor 2–driven transcription, and contributes to hypertrophic remodeling.

Initially described in neonatal cardiomyocytes and later in adult cardiomyocytes, EPAC1 activates CaMKII that induces HDAC4 and 5 nuclear export, thereby relieving the activity of the transcription factor myocyte enhancer factor 2, which activates gene transcription, essential for the prohypertrophic program (Figure 6). This process is regulated by β1-AR and EPAC2 has been recently excluded from this regulation. This makes sense because EPAC2 is mainly distributed at the T tubule. With regard to epigenetic mechanisms, histone phosphorylation is also thought to be important for cardiac hypertrophy as shown by the recent demonstration that CaMKII also phosphorylates histones directly, which may contribute to changes in hypertrophic gene expression. The possibility that ventricular remodeling can be mediated by cAMP/EPAC1/CaMKII-dependent chromatin modifications deserves further investigations.

**Conclusions**

More than 18 years after their cloning, EPAC1 and EPAC2 are now considered as important effectors of cAMP. Because of the availability of the EPAC agonist, 8-CPT and its derivatives numerous functions of these cAMP sensors have been identified mainly in cultured cells. This includes vascular cell proliferation and migration, EB permeability, and Ca2+ handling. Some of the biological effects of EPAC such as cardiomyocyte hypertrophy and arrhythmia, initially described in vitro have been confirmed in animal models for EPAC proteins. However, some arising from the phenotyping of EPAC transgenic mouse models have generated some contradictory results, which may be partially explained by the genetic background of the animals, the products of the EPAC-deleted gene and the complexity of cAMP signaling. Further pharmacological and genetic studies using EPAC isoform-specific ligands are required to better understand the physiological and pathophysiological functions of EPAC in the cardiovascular system.

Given the multidomain architecture and the growing list of their biological actions, EPAC proteins are coupled to a
multitude of effector proteins into various subcellular compartments. Indeed, the findings reviewed in this article demonstrate the existence of EPAC molecular complexes or signalosomes in distinct subcellular compartments that influence a localized cAMP signaling and a specific cellular function. The interaction of EPAC with cell type–specific protein inside subcellular compartments, which coupled with spatial-temporal dynamics of cAMP, is the key mechanisms that determine EPAC functional effects. This is well illustrated in cardiomyocytes where EPAC isoforms have been shown to influence SR and nuclear Ca\textsuperscript{2+} handling, hypertrophic gene expression and more recently receptor trafficking from the Golgi apparatus.\textsuperscript{146} However, our understanding of EPAC compartmentation is still in its infancy and the function of EPAC and its interactome in other cellular compartments, such as the mitochondria, needs to be explored both in healthy and in diseased cardiovascular cells.

Several recent reports now support the involvement of EPAC in the manifestation of cardiovascular disease suggesting that EPAC may represent attractive therapeutic targets for the treatment of various cardiovascular disorders. For instance, the results discussed above suggest that a pharmacological inhibition of EPAC1 slowing cardiac remodeling may be effective for the treatment of arrhythmia and HF and could act in synergy with the β-blockers. To date, most of the aforementioned EPAC pharmacological antagonists have been successfully tested in vitro in biochemical assays and in cultured cells. Further x-ray crystallography and nuclear magnetic resonance spectroscopy studies combined with chemical optimization are required to improve EPAC ligand specificity and bioavailability before they serve as valuable pharmacological probes to elucidate the physiological and pathophysiological roles of EPAC in the cardiovascular system. In addition, future basic and translational studies are needed to elucidate the possible side effects of EPAC antagonists.

Despite the large amount of information, several questions remain. For example, future studies should aim at determining the underlying molecular mechanisms involved in the regulation of EPAC expression, the epigenetic regulation by EPAC as well as human EPAC genetic variants and their potential link to cardiovascular disease. So, this young effector of cAMP still has a bright future.

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Disclosures

None.

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activate Epac2A.

enhance TREK-1 mRNA and K+ current expression in adrenocortical cells. En


Role of EPAC in the Cardiovascular System


Cyclic AMP Sensor EPAC Proteins and Their Role in Cardiovascular Function and Disease
Frank Lezoualc'h, Loubina Fazal, Marion Laudette and Caroline Conte

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