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CARDIOVASCULAR PHYSIOLOGY

Role of the cAMP-binding protein Epac in cardiovascular physiology and pathophysiology

Mélanie Métrich · Magali Berthouze · Eric Morel · Bertrand Crozatier · Ana Maria Gomez · Frank Lezoualc'h

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Abstract Exchange proteins directly activated by cyclic AMP (Epac) were discovered 10 years ago as new sensors for the second messenger cyclic AMP (cAMP). Epac family, including Epac1 and Epac2, are guanine nucleotide exchange factors for the Ras-like small GTPases Rap1 and Rap2 and function independently of protein kinase A. Given the importance of cAMP in the cardiovascular system, numerous molecular and cellular studies using specific Epac agonists have analyzed the role and the regulation of Epac proteins in cardiovascular physiology and pathophysiology. The specific functions of Epac proteins may depend upon their microcellular environments as well as their expression and localization. This review discusses recent data showing the involvement of Epac in vascular cell migration, endothelial permeability, and inflammation through specific signaling pathways. In addition, we present evidence that Epac regulates the activity of various cellular compartments of the cardiac myocyte and influences calcium handling and excitation–contraction coupling. The potential role of Epac in cardiovascular disorders such as cardiac hypertrophy and remodeling is also discussed.

Keywords Guanine nucleotide exchange factors · cAMP signaling · Calcium · Remodeling

M. Métrich · M. Berthouze · E. Morel · B. Crozatier · F. Lezoualc'h
Inserm, UMR-S 769,
Signalisation et Physiopathologie Cardiaque,
Châtenay-Malabry 92296, France

M. Métrich · M. Berthouze · E. Morel · B. Crozatier · F. Lezoualc'h
Univ Paris-Sud 11, Faculté de Pharmacie, IFR-141,
Châtenay-Malabry 92296, France

A. M. Gomez
Inserm, U637, Physiopathologie Cardiovasculaire,
Montpellier, France

A. M. Gomez
Univ Montpellier 1 & 2, IFR3,
Montpellier, France

F. Lezoualc'h (✉)
Inserm, UMR-S 769, Univ Paris-Sud 11,
Châtenay-Malabry 92296, France
e-mail: Frank.Lezoualch@u-psud.fr

Introduction

Cyclic adenosine 3′,5′-monophosphate (cAMP) is a universal second messenger which is produced from ATP by adenylyl cyclase upon activation of Gs-protein-coupled receptor (GPCR) [6]. cAMP plays central roles in cardiovascular regulation influencing gene expression, cell morphology, and function. In the vasculature, cAMP influences contraction/relaxation of blood vessel smooth muscle cells as well as their movement and the permeability of vascular–endothelial cells (VECs) [4, 29]. In the heart, cAMP regulates many physiological processes such as contractility, relaxation, and automaticity [7]. Besides these physiological roles, various actions of cAMP can be altered in cardiovascular diseases such as hypertrophy and heart failure [65].

Although protein kinase A (PKA) is generally recognized as the primary effector of cAMP signaling [53], other effectors are known to transduce cyclic nucleotide-encoded information. They encompass a class of cyclic nucleotide gated (CNG) cation channels and phosphodiesterases (PDEs) [9, 33, 40, 59]. The role of CNG channels appears to be specific to certain cell types such as pacemaker cells where distinct ion fluxes are regulated. More recently, a family

of proteins directly activated by cAMP was discovered adding another complexity in the cAMP-mediated signaling mechanism [25, 46]. These proteins, named exchange proteins directly activated by cAMP (Epac), are activated by cAMP and function in a PKA-independent manner and therefore represent a novel mechanism for governing signaling specificity within the cAMP cascade. Specifically, they are guanine nucleotide exchange factors (GEFs) for the Ras family of small GTPases, Rap1 and Rap2 [16, 82]. Small G proteins, including Rap, act as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state [17] (Fig. 1). cAMP-dependent activation of Epac promotes the exchange of GDP for GTP, hence switching on the Rap GTPases. The functions of cAMP-regulated GEFs in various cellular contexts are currently being unraveled, but a large body of evidence indicates that Epac is involved in multiple biological actions of cAMP such as insulin secretion and memory formation [15, 38, 70, 82, 103]. In this review, we describe the current understanding of Epac signaling and its roles in cardiovascular physiology and pathophysiology.

Epac protein structure and mechanisms of action

So far, two isoforms of Epac named Epac1 and Epac2 have been identified (Fig. 1). They are encoded by two distinct genes, *RAPGEF3* and *RAPGEF4* [25, 46]. *RAPGEF3* codes for Epac1, whereas *RAPGEF4* generates a long and a short variant named Epac2A and Epac2B, respectively [67]. Epac1 mRNA is expressed ubiquitously with a high expression in the heart, kidneys, ovaries, and thyroid glands, whereas Epac2 mRNA is detectable most notably in the brain, pituitary, and adrenal gland [46, 67]. Based on quantitative RT-PCR and immunoblot blot analysis, Epac1 has been shown to be more abundant than Epac2 in the myocardium [62, 101].

Epac are multi domain proteins characterized by a regulatory region and a catalytic domain. The regulatory region of Epac1 and Epac2B contains two recognized domains, a dishevelled–Egl-10–Pleckstrin (DEP) domain and a high-affinity cAMP-binding domain (cAMP-B). Epac2A shares this organization, but possesses an additional cyclic nucleotide-binding domain, which has low affinity for cAMP and uncertain biological function [24, 79]. The DEP domain of Epac proteins is responsible for membrane association and is required for the translocation of Epac1 to the plasma membrane [24, 72, 75]. The catalytic region of Epac isoforms is constructed from a Ras exchange motif (REM) and a CDC25 homology domain. The crystal structure of the inactive state of the Epac2 protein revealed that the cyclic mononucleotide-binding domain sterically blocks the access of Rap to the catalytic site in the CDC25 homology domain, which is responsible for mediating the exchange activity [79, 80]. Binding of cAMP to Epac induces large conformational changes within the protein and releases the auto-inhibitory effect of the N-terminal region, leading to Rap activation [80]. Epac isoforms can be activated by GPCRs that are positively linked to adenylyl cyclase, including serotonin 5-HT_4 receptors and β-adrenergic receptors (β-AR) [58, 62, 76, 84]. Also of interest, the light chain 1 of microtubule-associated protein 1B (LC1) has been shown to act as a molecular chaperone of Epac1, increasing the binding of cAMP to this GEF and consequently increasing Epac1 signaling in rat pheochromocyta (PC12) cells [14].

Epac proteins are observed at many locations in the cell, including the cytosol, the nucleus as well as the nuclear and plasma membranes [41, 62, 72, 75]. Depending on their cellular localization and molecular partners, Epac proteins activate different downstream effectors [14, 94]. Thus, the distinction between the coupling of Epac to a specific signaling pathway is determined by its localization to micro sub-cellular compartments, explaining the different biological effects of Epac inside a given cell. In this line, muscle-specific A-kinase anchoring protein (mAKAP) plays a key role in compartmentalization of Epac-dependent signaling [20]. It is a scaffold protein that has been shown to localize cAMP-hydrolyzing PDEs, regulatory cAMP-binding subunits of PKA, ERK5, protein phosphatase 2A as well as Epac into specific intracellular compartments, thereby controlling the cellular actions of cAMP [60]. In addition, as for PKA, there is now strong evidence that PDEs contribute to the specificity of Epac signaling in a spatial and temporal manner [41, 60, 77]. For instance, PDE4B activity specifically controls the ability of nuclear Epac1 to drive nuclear export of DNA-dependent protein kinase (DNA-PK), an enzyme that provides a key part of the DNA repair systems, while cytosolic PDE4D regulates PKA-mediated nuclear import of DNA-PK [41]. But PKA and Epac may also be interconnected to regulate cellular processes [49]. In that way, a PDE4 inhibitor roflumilast has been shown to protect cardiac myocytes against NO-induced apoptosis via both cAMP–PKA and Epac-dependent pathways [49]. Therefore, by virtue of the membrane targeting and their ability to interact with other signaling proteins, Epac directs cAMP signaling to specific cellular sites.

Epac-selective cAMP analogs

The major part of available data regarding Epac-dependent functions in the cardiovascular system has been obtained by the use both in vitro and in vivo of Epac activators, which do not discriminate between Epac1 and Epac2. These Epac-

selective cAMP analogs incorporate a 2′-*O*-methyl substitution on the ribose ring of cAMP, a modification that impairs their ability to activate PKA [30, 37, 93]. Among them, 8-pCPT-2′-*O*-Me-cAMP (8-(4-chloro-phenylthio)-2'-*O*-methyladenosine-3',5'-cyclic monophosphate) is the most commonly used Epac agonist and exhibits high affinity for Epac (K_d2.2 μM for Epac1) as well as reduced affinity for PKA (K_d200–300 μM) [30] (Fig. 1). However, like all pharmacological agents, Epac-selective cAMP analogs may be metabolized (i.e., by PDEs) into bioactive products, which may have non-specific effects [31, 73]. Therefore, it is important to mention that the involvement of Epac in a particular function is firmly established only when pharmacological data are supplemented by molecular analyses.

Epac functions in vascular cells

VECs and vascular smooth muscle cells (VSMCs) constitute the bulk of blood vessel cells and cooperate to regulate blood vessel structure and function [78]. VECs and VSMCs of healthy undamaged arteries function primarily to regulate blood vessel lumen diameter by coordinating contractility and relaxation. In contrast, during cardiovascular stresses, VSMCs and VECs undergo a process of phenotypic modulation that results in the development of cells with migratory and proliferative phenotypes, which can contribute to severe vasculopathies, such as atherosclerosis or restenosis after angioplasty [99]. Because cAMP influences many facets of the biology of VSMCs (relaxation–contraction coupling, proliferation, migration, and cellular metabolism) and VECs (proliferation, migration, cellular metabolism, and permeability), it is obviously important to determine how Epac may influence these cellular processes (Fig. 2).

Epac and VSMC migration

The functional roles of Epac proteins in VSMCs are just beginning to be defined. In contrast with PKA, direct

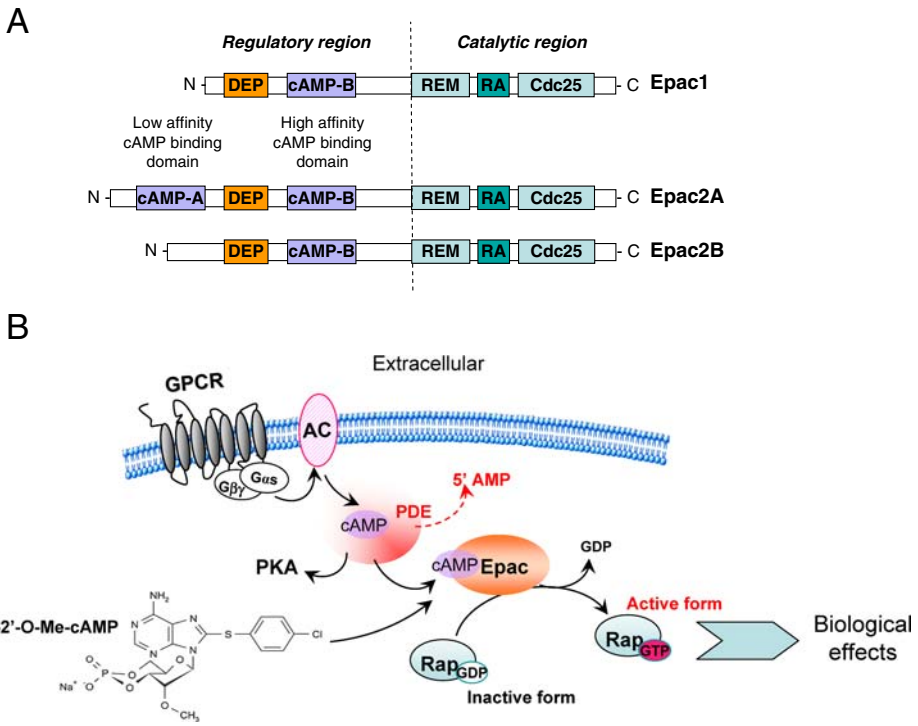

Fig. 1 Structure and mode of action of Epac proteins. **a** Schematic representation of Epac proteins. Epac1 and Epac2 are multidomain proteins encoded by two distinct genes and are characterized by a N-terminal regulatory region that directly binds cAMP with high affinity (*cAMP-B*). Epac2A possesses a second lower affinity cAMP-binding domain whose role is unknown (*cAMP-A*). The catalytic domain of Epac is composed of a CDC25 homology catalytic site stabilized by a REM. Epac proteins also possess a dishevelled, Egl-10, pleckstrin domain (*DEP*) responsible for membrane association. Epac2B is a splice variant of Epac2 and lacks the first cAMP-A-binding domain. Epac2B is expressed in the mouse adrenal gland. **b** cAMP is produced by adenylyl cyclase (*AC*) in response to GPCR stimulation and activates its classical downstream effector, protein kinase A (*PKA*). PDE degrade cAMP and thereby regulate its availability for diffusion. The identification of Epac proteins as novel sensors for cAMP has broken the dogma surrounding cAMP and PKA. Epac proteins are nucleotide exchange factors for the Ras-like small GTPases, Rap1 and Rap2, that function independently of PKA. Upon binding of cAMP, Epac undergoes conformational changes that release the C-terminal catalytic region responsible for Rap activation. 8-pCPT-2′-*O*-Me-cAMP is a synthetic cAMP analog that specifically activates Epac

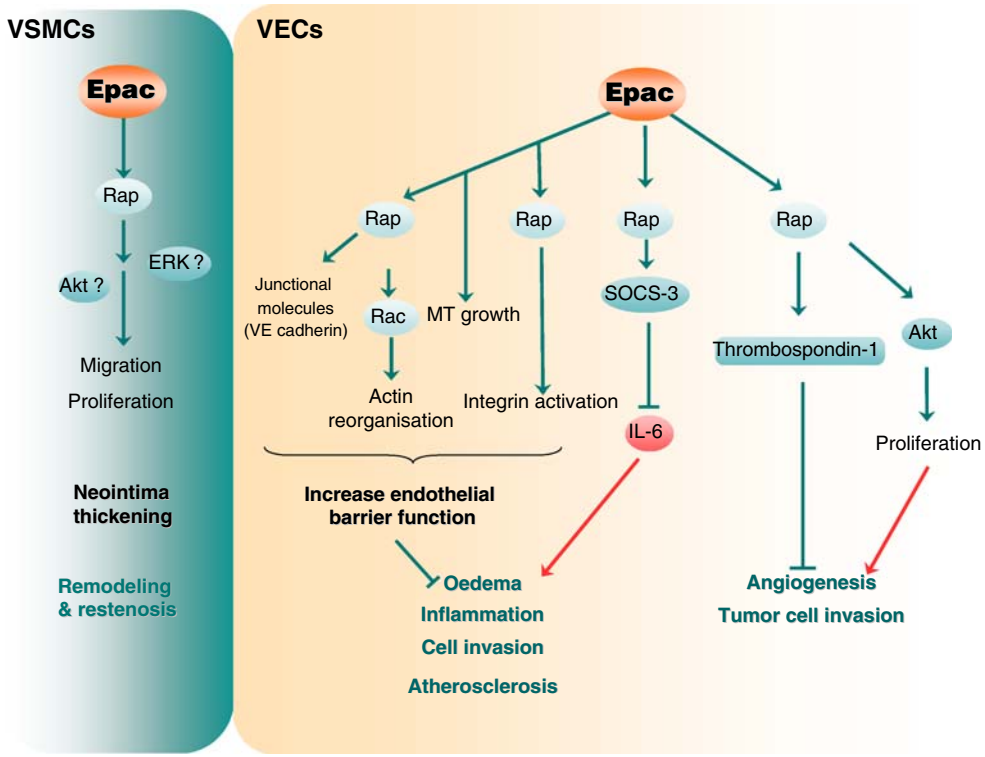

Fig. 2 Role of Epac in vascular smooth cells (*VSMCs*) and vascular–endothelial cells (*VECs*). Epac promotes (probably via Rap1) the migration of various types of VSMCs and facilitates the development of neointima thickening, a process involved in vascular restenosis. It also contributes to the proliferation of airway SMCs induced by β2-adrenergic receptors. In VECs, Epac activation increases endothelial barrier function by increasing junctional molecules such as vascular–endothelial (*VE*) cadherin at cell–cell contacts, by inducing cortical actin rearrangement and enhancing microtubule growth. In addition, Epac mediates activation of integrins involved in adhesion of VECs to the basal membrane. The anti-inflammatory effect of Epac involves the induction of suppressor of cytokine signaling-3 (*SOCS-3*), a negative regulator of IL-6 receptor signaling. Epac/Rap activation induces expression of thrombospondin-1, a key anti-angiogenic molecule. On the other hand, Epac activation stimulates VECS proliferation, a process known to be involved in angiogenesis

activation of Epac promotes the migration of various types of VSMCs and facilitates the development of neointima thickening, a process involved in vascular restenosis [99, 100] (Fig. 2). The pharmacological approach with the Epac-specific agonist, 8-pCPT-2′-*O*-Me-cAMP was verified by siRNA-mediated knockdown of Epac1, which decreased serum-mediated SMC migration [100]. The exact molecular mechanisms that lead to Epac activation and increased migration in VSMCs are unknown. The activation of Rap1, Akt, and/or ERK may be involved, as demonstrated in the regulation of cell adhesion and migration [34, 61, 76, 101]. The role of Epac in the regulation of cell migration is not restricted to VSMCs since several reports have shown that this GEF may influence this cellular process in various cell types including tumor cell migration [3, 51, 57, 101]. Interestingly, Epac contributes to the proliferation of airway SMCs induced by β2-adrenergic receptors indicating that this cAMP-GEF controls different aspects of SMC fates and may also be implicated in the pathological remodeling seen in asthmatic airways [45].

Role of Epac in VECs

Regulation of vascular–endothelial barrier function by Epac

Endothelial barrier function restricts the passage of plasma proteins and circulating cells across endothelial cells, and its dysfunction may result in an increase in vascular permeability, thereby causing edema, inflammatory, or metastatic cell infiltration [91]. Vascular permeability is induced by various pro-inflammatory mediators including thrombin and histamine. Thus, the selective regulation of vascular permeability is critical for maintaining vascular integrity in homeostasis and disease. Several stimuli such as prostaglandins that are linked to cAMP production inhibit vascular permeability of endothelial cell monolayers [34]. Some studies tend to demonstrate that Epac mediates this effect independently of PKA and may represent a new therapeutic target for modulating vascular permeability. Indeed, using pharmacological and molecular approaches in a monolayer of a human

umbilical vein endothelial cell line (HUVEC), it has been reported that increased cAMP levels trigger Epac–Rap1 signaling to reduce vascular permeability by augmentation of junctional molecules such as vascular–endothelial cadherin at cell–cell contacts [22, 34, 48]. Epac1-specific siRNA showed that Rap1 activation by Epac1, but not by Epac2, was responsible for the increase in endothelial adherens junctions [48]. Inversely, Epac signaling antagonizes thrombin-induced cell junction disruption and strongly attenuates the platelet activating factor-induced permeability in HUVECs and intact rat microvessels, respectively [1, 22, 34, 48].

Since the endothelial barrier function is largely dependent on the dynamic of the actin cytoskeleton [12], several groups investigated the involvement of Epac in this process. Epac activation indeed induces reorganization of cortical actin, which supports junctional adhesion molecules thereby contributing to stabilize endothelial barrier function [11, 22, 34, 48]. The effect of Epac on actin remodeling involves the activation of the Ras-like GTPase, Rap, which can then modulate indirectly the activity of the Rho GTPase family such as Rho and Rac. In turn, Rho proteins interact with downstream cytoskeletal and cell adhesion effectors and promote cytoskeletal remodeling [10, 32]. It is suggested that the connection between the Ras and Rho GTPase families probably occurs through the recruitment of specific GEFs of the Rho family (e.g., Tiam, Vav) [10, 11, 22]. Of note, such a cross talk between Rap and RhoGTPases regulated by cAMP and Epac exists in other cellular systems including primary neurons [58, 81, 102]. Finally, besides actin remodeling, Sehrawat and colleagues [85] reported that Epac regulates microtubule dynamics in HUVECs. According to these authors, the effect of Epac activation on microtubule dynamics might have physiological consequences for barrier function as activating Epac could reverse vascular permeability increased by TGFβ and TNF-α, which are cytokines that promote barrier dysfunction by destabilizing microtubule.

Epac and inflammation

Uncontrolled and excessive migration of leukocytes from the peripheral blood into tissues is a hallmark of chronic inflammation. Importantly, it has been reported that Epac inhibits endothelial leukocyte transmigration [95]. These data suggest that the regulation of the endothelial barrier function by Epac–Rap may have some important biological consequences under conditions in which an exaggerated inflammatory response leads to vascular disease as observed in atherosclerosis [95]. In contrast, Cullere et al. [22] did not observe any blocking effect on neutrophil migration during endothelial Epac1 stimulation. This discrepancy is currently unclear. Various aspects of transendothelial leukocyte migration such as leukocyte adhesion (primarily through the activation of β1 integrins) and migration can also be influenced by Epac [5, 35, 55, 56].

VECs represent a major cellular target for many pro- and anti-inflammatory cytokines. Activated VECs are capable of producing interleukin (IL)-6, and accumulation of IL-6 has been noted within arterial lesions in several models of atherosclerosis [89]. Epac has been identified as a modulator of IL-6 signaling in HUVECs, making this cAMP sensor as a potential anti-inflammatory protein in VECs [83] (Fig. 2). It is demonstrated that the anti-inflammatory effect of Epac is PKA independent and is associated with the induction of suppressor of cytokine signaling 3 (SOCS-3), a bona fide inhibitor in vivo of gp130, the signal-transducing component of the IL-6 receptor complex (Fig. 2) [83]. Targeting the cAMP-Epac–Rap1–SOCS-3 pathway might therefore prove to be a useful strategy for combating pathologies associated with chronic vascular inflammation [15]. Interestingly, the induction of SOCS-3 expression in response to Epac activation occurs at the transcriptional level and involves the binding of the CCAAT/enhancer-binding proteins (C/EBPs) to the promoter of SOC-3 gene [97]. Although it remains to be demonstrated in VECs, PKCα is a critical requirement for efficient ERK- and C/EBP-dependent SOCS-3 induction by Epac in COS cells [13]. It should be noted that the involvement of Epac in inflammatory processes is not limited to VECs since this cAMP-GEF has been reported to display pro- and anti-inflammatory actions in various cell types including dendritic cells and macrophage cell lines [2, 15, 42, 88, 96].

Others roles of Epac in the vasculature

Obviously, other important functions of Epac in the vascular system need to be explored. For instance, a recent observation points out a role of Epac in the regulation of angiogenesis [28]. Angiogenesis is a tightly regulated process that is important in development as well as pathologic disease states such as tumor growth and metastasis [19]. In their study, Doebele and colleagues [28] show that pharmacologic activation of Epac with the cAMP analog, 8-pCPT-2′-*O*-Me-cAMP, inhibits vascular–endothelial growth factor (VEGF)-induced angiogenesis in vivo in a matrigel mouse model as well as in a severe combined immunodeficient mouse model of human angiogenesis. It is proposed that Epac/Rap activation induces expression of thrombospondin-1, a key anti-angiogenic molecule, which counteracts VEGF signaling via MEK5/ERK5 (Fig. 2) [28]. However, it should be noted that Epac activation stimulates in vitro VECS proliferation, a process known to be involved in angiogenesis [66]. Interestingly, 8-pCPT-2′-*O*-Me-cAMP has also been shown to decrease the in vitro ability of aggressive MUN-2B

melanoma cells to engage in vasculogenic mimicry, a process by which a vessel is formed from tumor cells [54]. It is therefore possible that Epac may be involved in the regulation of tumor progression though various effects on new blood vessel formation.

Epac functions in the myocardium

Cellular contraction is initiated by a transient elevation in intracellular calcium ($[Ca^{2+}]_i$ transients). During an action potential, Ca^{2+} influx induced by activation of voltage-dependent L-type Ca^{2+} channels upon membrane depolarization triggers the release of Ca^{2+} via intracellular Ca^{2+} release channels (ryanodine receptors (RyRs)) of sarcoplasmic reticulum (SR) through a Ca^{2+}-induced Ca^{2+} release (CICR) mechanism [7]. Relaxation follows the decrease in $[Ca^{2+}]_i$ mainly by the Ca^{2+} uptake into the SR through the SR Ca^{2+} ATPase and Ca^{2+} extrusion through the Na^+/Ca^{2+} exchanger (Fig. 3).

cAMP, acting through PKA, influences the activity of several key cardiac proteins involved in excitation–contraction coupling, such as L-type Ca^{2+} channels, phospholamban, RyR, and troponin I [8]. These effects produce PKA-dependent increases in Ca^{2+} current (I_{Ca}), SR Ca^{2+} release and uptake, as well as a desensitization of the myofilaments to Ca^{2+}. The net result is the characteristic positive inotropic (contractile force) and lusitropic (relaxation) effects of β-AR activation in cardiac myocytes. In addition, binding of cAMP to the hyperpolarization-activated cyclic nucleotide-gated channels (HCN) that carry the pacemaker current, I_f, helps to increase heart rate in response to a sympathetic stimulation (chronotropic effect). The discovery of the existence of Epac in cardiac myocytes has raised the possibility of alternative, PKA-independent, cAMP-dependent mechanisms of action involved in Ca^{2+} handling.

Epac in excitation–contraction coupling

The Epac1 isoform is highly expressed in the heart and shows sarcolemmal and perinuclear linear localization in neonatal and adult rat ventricular myocytes [27, 62]. Initially, Epac activation was found to stimulate CICR in pancreatic β-cells confirming a role of this cAMP-GEF in Ca^{2+} handling [44]. Our subsequent analyses in adult rat cardiomyocytes showed that the activity of the cardiac RyR during diastole, measured as Ca^{2+} sparks, was rapidly increased by Epac activation due to Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation of the RyR, confirming a role of Epac in cardiac Ca^{2+} handling [71]. However, published reports on the amplitude of the $[Ca^{2+}]_i$ transient are not unanimous. In this sense, the $[Ca^{2+}]_i$ transient amplitude has been shown to be reduced by acute Epac activation due to a decrease in the SR Ca^{2+} load subsequent to the enhanced diastolic Ca^{2+} leak through the phosphorylated RyRs in rat cardiomyocytes [21, 71]. Conversely, Oestreich and colleagues [69] showed that acute treatment of single mouse ventricular cardiac myocytes with 8-pCPT-2′-*O*-Me-cAMP increased $[Ca^{2+}]_i$ transient amplitude in field-stimulated cells. This process was dependent on Rap1 and phospholipase C-ε, which was also shown to be a downstream effector of Epac in neuronal cells [47, 82, 84]. The reasons for the discrepancies between the two studies are unclear and may involve species differences and/or methodological approaches such as the frequency of cardiac myocyte electrical stimulation.

Regarding the functional impact of Epac activation in cardiac myocytes, one could think that because contraction is activated by $[Ca^{2+}]_i$ transient, which is decreased by Epac in rat cells, contraction would be weaker. However, this is not the case since we recently found that despite a decrease in the amplitude of $[Ca^{2+}]_i$ transient, Epac shows a positive inotropic effect [21]. Specific activation of Epac or overexpression of a constitutively active form of Epac increases myofilament Ca^{2+} sensitivity in permeabilized rat ventricular cardiac myocytes in a PKA-independent manner. This is correlated with an increase in phosphorylation of sarcomeric proteins such as cardiac myosin-binding protein-C and troponin I [21]. Epac-dependent effects on myofilament proteins involve both protein kinase C (PKC) and CaMKII [21].

Thus, until now there are some collected pieces of evidence showing that Epac exerts a role in cardiac Ca^{2+} handling and excitation–contraction coupling. The involved signaling pathway is beginning to be unraveled: phospholipase C, CaMKII, and PKC have been identified so far as Epac effectors [21, 68, 69, 71]. However, more analyses are still needed to further understand Epac actions on EC coupling in physiology and to elucidate its possible implication in Ca^{2+} handling alterations during cardiac pathologies.

Roles of Epac in cardiac electrophysiology

Very little is known concerning the possible role of Epac on cardiac electrophysiology. To our knowledge, only I_{Ca}, main trigger of CICR, has been analyzed. The results collected so far in rat and in mice cardiomyocytes failed to show any significant effect of 8-pCPT-2′-*O*-Me-cAMP on I_{Ca} [68, 71]. However, other ionic currents in cardiac myocytes must be analyzed before excluding any effect of Epac on cardiac electrical activity. For instance in other cell types, Epac has been shown to affect ionic channels function such as the Ca^{2+}-sensitive potassium currents in cerebellar neurons [87] and the ATP-sensitive potassium current ($I_{K,ATP}$) in chromaffin and in vascular cells [43, 74]. Whether Epac effects in these ionic currents are direct or

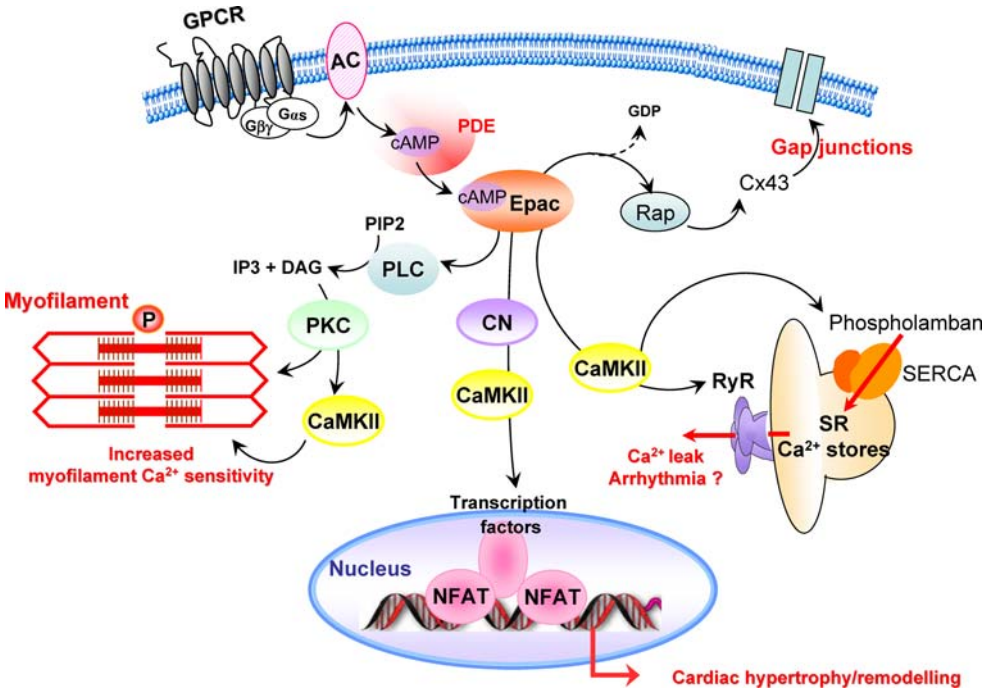

Fig. 3 Epac regulates the activity of various cellular compartments of the cardiomyocyte. Upon binding to cAMP, Epac stimulates PLC, which hydrolyzes PIP2 to produce DAG and IP3 leading to PKC and CaMKII activation. Both kinases are involved in the regulation of contractile protein phosphorylation and myofilament Ca^{2+} sensitivity. Epac-Rap1 signaling cAMP induces Cx43 accumulation in cell–cell contacts and enhances gating function. Epac contributes to the hypertrophic effect of β-adrenergic stimulation through the activation of the small GTPase Ras, Ca^{2+}-sensitive proteins (CaMKII and calcineurin) and downstream transcription factors such as NFAT. This cAMP-binding guanine nucleotide exchange factor also stimulates RyR2 phosphorylation via CaMKII, and subsequent Ca^{2+} leak may contribute to CaMKII and calcineurin activation and arrhythmia. Epac has been reported to phosphorylate phospholamban in a CaMKII-dependent manner. *AC* adenylyl cyclase, *CaMKII* Ca^{2+}/calmodulin-dependent protein kinase II, *CN* calcineurin, *DAG* diacylglycerol, *IP3* inositol triphosphate, *NFAT* nuclear factor of activated T cell, *PLB* phospholamban, *PIP2* phosphatidylinositol bisphosphate, *PLC* phospholipase C, *RyR* ryanodine receptors, *SERCA* SR Ca^{2+} ATPase, *SR* sarcoplasmic reticulum

mediated by intracellular Ca^{2+} mobilization remains unclear. Among the Epac modulated ionic currents cited above, the $I_{K,ATP}$ has an important role in the heart. $I_{K,ATP}$ is normally inactivated by the intracellular ATP concentration present in cardiac cell. In pathological situations such as ischemia, ATP levels fall and $I_{K,ATP}$ is activated, promoting an outward potassium current that hyperpolarizes the cell. Membrane hyperpolarization lowers cell excitability and thus protects the cardiomyocyte in these situations. As indicated below Epac has been shown to decrease $I_{K,ATP}$ in non-cardiac cells [43, 74]. Therefore, one could hypothesize that, if Epac also decreases $I_{K,ATP}$ in cardiac myocytes, this could have deleterious actions and eventually increase excitability and promote arrhythmias. In this sense, Epac has been shown to favor arrhythmias in mice hearts [39]. However, this effect seems to be independent of ionic currents activated during the action potential (AP) since Epac did not alter AP duration and refractory periods. Proarrhythmic Epac effects would be instead dependent on Ca^{2+} handling alterations through CaMKII [39].

Epac and intercellular communication

In the heart, Epac may also modulate intercellular communication at the level of channels called gap junctions (GJ). GJ are formed by two docking connexins constituted by six connexins (Cx). The most predominantly expressed Cx isoform in cardiomyocytes is Cx-43 although Cx-40 and Cx-45 are also present [98]. Few studies have evaluated a possible role of Epac on connexins and GJ in the heart. Prolonged (24 h) treatment of cultured cells with the cAMP analog db-cAMP was shown to increase the expression of Cx-43 and Cx-45 but the molecular mechanisms appeared as disparate [23]. These mechanisms were more recently evaluated by Somekawa et al. [86] who demonstrated that, after 12-h treatment with a cAMP analog and/or specific activators or inhibitors of PKA or Epac, GJ neoformation was both PKA and Epac-dependent with independent mechanisms. This was associated with an increased intercellular communication. A possible role of an acute stimulation of Epac on intercellular communication in the

heart has not been studied yet. Preliminary studies from our laboratory indicated that Epac-induced PKCε stimulation increased Cx-43 phosphorylation, a known target of PKC. It is interesting to note that RhoA GTPase activity was recently shown to increase channels permeability of Cx-43 in rat cardiomyocytes with a modulation by the actin cytoskeleton [26]. Since Rap, which is activated by Epac, can modulate indirectly the activity of the Rho GTPase family members, it is likely that Epac may increase intercellular permeability by this means but this needs to be verified.

Epac in cardiac hypertrophy and remodeling

Epac in cardiac hypertrophy

With respect to Ca^{2+} handling, the role of Epac in cardiac myocytes is not limited to the regulation of the contractile machinery. Indeed, recent research has implicated Epac as a new actor in the regulation of cardiac myocyte hypertrophy through the activation of specific Ca^{2+}-dependent signaling pathway, suggesting that this cAMP-GEF regulates distinct pools of calcium involved in contractility and remodeling [62–64].

Adult myocyte hypertrophy is the compensatory response of the heart to stress and is characterized by non-mitotic growth, addition of new sarcomeres, fetal gene expression, and specific changes in ion channel properties. Maladaptive cardiac hypertrophy can progress to heart failure, a leading cause of morbidity and mortality in industrialized countries [36, 50]. A role of Epac1 in myocardial growth is strongly suggested by its upregulation in various models of cardiac hypertrophy such as chronic isoprenaline infusion and pressure overload induced by thoracic aortic constriction [62, 90]. A more direct evidence of its role in the regulation of cardiac growth came from the observation that Epac1 activation led to morphological changes associated with an increase in cell surface area, protein synthesis, and the expression of cardiac hypertrophic markers such as the atrial natriuretic factor [62, 64]. Consistent with its role in cardiac myocyte hypertrophy, Epac-specific activation triggers signaling pathway involving the Ca^{2+}/calmodulin dependant phosphatase, calcineurin, and the small GTPases, Rac, and Ras in rat cardiac myocytes [52, 62, 64]. Similarly, the hypertrophic effects of Epac are dependent on both calcineurin and CaMKII in adult rat cardiac myocytes [62]. These two well-known calcium-sensitive proteins activate a hypertrophic gene program through the activation of transcriptions factors such as NFAT.

In response to long term β-adrenergic receptor stimulation, Epac induces adult cardiomyocyte hypertrophy in a cAMP-dependent but PKA-independent manner. Knock-down of Epac1 using ShRNA decreases cardiomyocyte hypertrophy induced by β-adrenergic receptor activation in neonatal cardiac myocytes [62]. Altogether, these data combined with the observation that the sympathetic activity is highly increased in heart failure indicate that Epac contributes to the progression of pathological cardiac growth, and its activation may be exacerbated in heart failure, and this contributes to the maladaptive remodeling of the heart [63].These data contrast with the observation that Epac1 inhibits the hypertrophic extracellular signal-regulated kinase 5 (ERK5) pathway by a mechanism involving Rap1 in neonatal cardiomyocytes [27]. As mentioned above, this apparent discrepancy suggests the existence of spatiotemporal dynamics of Epac signaling, which determines its coupling to different effectors and hence functional effect in a given cell type.

Epac in cardiac fibrosis

Recent data pointed to the role of Epac in the regulation of cardiac fibrosis [92, 101]. Cardiac fibroblasts synthesize and secrete components of the extracellular matrix such as fibrillar collagens [18]. In pathological conditions such as those seen in heart failure, excessive deposition of collagen occurs throughout the myocardium (i.e., interstitial fibrosis). Excess fibrosis can lead to impaired diastolic and systolic functions [18]. In their study, Yokoyama and colleagues [101] show that the profibrotic agonist, transforming growth factor β1 (TGFβ1), inhibits Epac1 expression in fibroblasts from multiple tissues. Inversely, overexpression of Epac1 inhibits TGFβ1-induced collagen synthesis in cardiac fibroblast, implying that a decrease in Epac expression is required for profibrotic response. In line with these data, the effect of adenosine agonists to inhibit angiotensin II-stimulated collagen synthesis occurs via a cAMP-Epac pathway in cardiac fibroblasts [92]. The observation that Epac interacts with matrix metalloproteinases [81] suggests that Epac might play a key role in other aspects of tissue remodeling such as the shedding of certain growth factors.

Conclusions and future directions

Besides the well-known cAMP substrate PKA, Epac is now recognized as an incontrovertible factor leading to complex and diversified cAMP signaling pathways. The evidence reviewed here demonstrate that this cAMP-GEF is involved in the control of various cardiovascular functions such as vascular cell adhesion, migration, endothelial barrier permeability, and excitation–contraction coupling. However, some studies are controversial, and it is just the beginning of the functional characterization of Epac, and obviously the list of

its cellular function is growing very fast. Depending of their relative abundance, distribution, partners, and localization, Epac and PKA may act independently, cooperate, or oppose to each other in regulating a specific cellular function. The understanding of Epac and PKA connection represents therefore a real research area of interest but it is also crucial to study the individual contribution of Epac (as an isolated protein or integrated in a macromolecular complex) within the overall cAMP signaling. Further studies will presumably unravel new Epac regulators and effectors as well as specific function for Epac isoforms within the cell. For instance the contribution of Rap proteins in the function of Epac is not always clear as observed in cardiac myocytes. Data are also missing concerning the ability of hormones or neurotransmitters to activate Epac isoforms.

Importantly, Epac interferes with the regulation of cellular mechanisms intimately involved in the manifestation of diseases, such as atherosclerosis, cardiac hypertrophy, and tumor invasion. Thus, Epac proteins are not only at the crossroads of different physiological processes but also may represent attractive therapeutic targets for the treatment of various cardiovascular disorders. An in-depth analysis of the structure, the spatiotemporal regulation, and the mechanism of action of Epac will allow the development of new pharmacological compounds targeting specifically this cAMP sensor. To date, the pharmacology of Epac proteins is poor, and current studies on Epac functional effects clearly suffer from the lack of Epac-selective pharmacological inhibitors. In addition, the development of Epac knock-out mice will help to understand the pathophysiological role of Epac in the cardiovascular system and other tissues.

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