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EPAC-targeted therapies in cardiovascular system - a review

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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.Epac contains an evolutionally conserved cAMP-binding domain that acts as a molecular switch for sensing intracellular second messenger cAMP levels to control diverse biological functions. Developing the ability to regulate cAMP-mediated signaling through Epac may lead to remarkable new therapies for the treatment of cardiac diseases.

INTRODUCTION

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 cardiovascularsystem. It controls a wide range of biological effects, including cell proliferation, differentiation, and apoptosis. cAMP is produced from ATP by transmembrane adenylyl cyclase on activation of Gs-coupled G proteincoupled receptors. In addition, soluble adenylyl cyclase is a second intracellular source of cAMP and can be activated by divalent cations in various subcellular compartments. The intracellular level of cAMP depends not only on its production by adenylyl 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. Besides specialized membrane structures that may also limit cAMP diffusion, A-kinase anchoring proteins function to tether cAMP effectors and PDEs enzymes into defined cellular compartments and, therefore, maintain localized pools of cAMP to control the cellular actions of the second messenger. Until recently, the intracellular effects of cAMP were attributed to the activation of protein kinase A (PKA) and cyclic nucleotidegated ion channels. In 1998, a family of novel cAMP effector proteins named exchange proteins directly activated by cAMP (EPACs) was discovered. The EPAC protein family is composed of EPAC1 and EPAC2, which act as guanine-nucleotideexchange factors (GEFs) for the small G proteins, Rap1 andRap2, and function in a PKA-independent manner. On bindingto cAMP, EPAC promotes the exchange of GDP for GTP,thereby inducing the activation of the small G protein Rap. Incontrast, Rap-GTPaseactivating proteins enhance the intrinsicGTP hydrolysis activity of Rap leading to GTPase inactivation.The cycling of Rap between its inactive and active states providesa mechanism to regulate the binding to effector proteins.The discovery of EPAC proteins has broken the dogmasurrounding cAMP and PKA, and uncovered new perspectivesfor the understanding of cAMP signaling in the cardiovascularsystem.

cAMP

Eukaryotic cells respond to a wide range of extracellular signals, including hormones, growth factors, and neurotransmitters, by eliciting the generation of intracellular second messengers. Second messengers in turn trigger a myriad of cellular reactions by orchestrating a network of intracellular signaling events. At the cellular level, cAMP plays an important role in virtually every known function such as metabolism, gene

expression, cell division and growth, cell differentiation and apoptosis, as well as secretion and neurotransmission. Synthesis of cAMP in cells is regulated by G protein-coupled receptors (GPCRs), which can either activate or inhibit adenylate cyclase (AC) through the actions of stimulatory (Gs) or inhibitory (Gi) heterotrimeric G proteins. Active ACcatalyses the conversion of ATP into cAMP and pyrophosphate, which catalyse the hydrolysis of cAMP into 5′-AMP. This ensures that the cAMP signal is transient, thereby allowing precise control over the localization, intensity, and duration of the cAMP signal. Elevations in intracellular cAMP lead to the activation of a select range of intracellular effector proteins containing cyclic nucleotide-binding domains (CNBDs), including EPAC enzymes 1 and 2, PKA isoforms , cAMP-responsive ion channels , and Popeye domain-containing proteins .Drugs that target the cAMP system are currently prescribed for a range of medical conditions, including β_2-adrenoceptor agonists such as salbutamol and formoterol, which form the basis of bronchodilators for the treatment of asthma and selective PDE4 inhibitors such as roflumilast , which have shown promise in the treatment of inflammatory diseases such as chronic obstructive pulmonary disorder.

EPAC

Recently, a family of novel cAMP sensor proteins, named Epac (exchange protein directly activated by cAMP) or cAMP-GEF (cAMP-regulated guanine exchange factor), has been identified. These proteins contain a CBD that is homologous to that of PKA R subunits and the prokaryotic transcription regulator, cAMP receptor protein (CRP). Epac proteins bind to cAMP with high affinity and activate the Ras superfamily small GTPases Rap1 and Rap2[1]. Rap1 was initially identified as an antagonist for the transforming function of Ras. Rap1 can be activated in response to a variety of second messengers including cAMP. Although PKA can phosphorylate Rap1 at its C-terminus, PKA phosphorylation is not required for cAMP-dependent activation of Rap1. EPACs are guanine nucleotide exchange factors (GEFs) for the Ras-like GTPases Rap1 and Rap2[2]. There are two mammalian EPAC isoforms, EPAC1 and EPAC. Whereas EPAC1 displays a wide tissue distribution, the expression of EPAC2 is more restricted and appears to be limited to the brain, pancreas, testes, and other secretory cells. The biggest structural difference between EPAC1 and EPAC2 is the presence of an additional CNBD within the N terminus of EPAC2 (CNBD1). CNBD1 exhibits a reduced affinity for cAMP and is unable to induce GEF activity following cAMP binding. Despite this difference, EPAC1 and EPAC2 share structural motifs throughout their regulatory and catalytic domains, with the dishevelledEGLpleckstrin homology domain (DEP), principal CNBD, Ras exchange motif (REM), Ras association domain (RA), and catalytic CDC25 homology domain (CDC25-HD) being heavily conserved between the two isoforms[3]. Regulation of EPAC activity is governed by intermolecular interactions between the regulatory CNBD and catalytic CDC25-HD domains. The 'closed' form of the enzyme is stabilised by a hinge helix and an ionic latch (IL), which lock the CNBD over the CDC25-HD domain; these interactions inhibit GEF activity by limiting substrate access to the CDC25-HD[4]. Binding of cAMP releases salt bridges formed with the IL and unwinds the hinge helix, thereby allowing the CNBD to rotate away, creating an 'open' form where the CDC25-HD is exposed for interaction with GDP-bound Rap1 and Rap2 , this triggers GDP release. 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. In contrast, the epigenetical regulation of Rapgefalternative promoters leads to a restricted expression pattern of EPAC2 isoforms. 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 and subsequent GTP binding and activation, leading to downstream signalling.

EPAC1 and EPAC2 are multidomain proteins with molecular weights of ≈105 kDa and ≈115 kDa, respectively, and share the same structural organization. Both isoforms contain an N-terminal regulatory region and a C-terminal catalytic region. The amino-terminal regulation region consists of a Disheveled/Egl-10/pleckstrin domain followed by an evolutionally conserved cyclic nucleotidebinding domain.cAMP is produced from ATP by

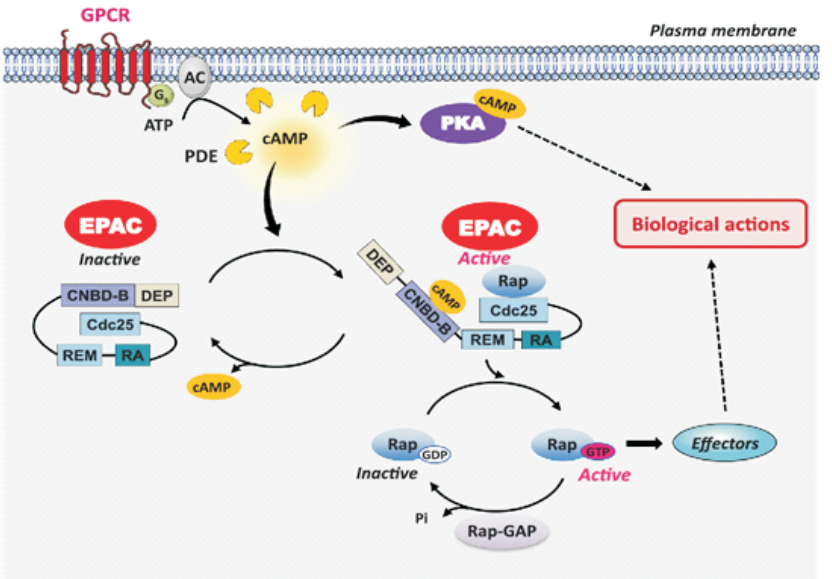

Figure 1: Path way of EPAC activation

adenylyl cyclase (AC) in response to Gαs-coupled G proteincoupled 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 nucleotidebinding 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 GTP. Rap-GTPaseactivating proteins (Rap-GAPs) enhance the intrinsic GTP hydrolysis activity of Rap leading to GTPase inactivation.

PHYSIOLOGICAL ROLES OF EPAC ISOFORMS

I. Insulin secretion

EPAC2 is involved in the potentiation of insulin secretion from pancreatic β cells in response to incretin hormones such as glucagon-like peptide-1 (GLP-1)[6]. The role of EPAC2 in these processes is to promote mobilisation of Ca^{2+} from intracellular Ca^{2+} stores , which in turn triggers Ca^{2+}-induced Ca^{2+} release (CICR)[7] . The ability of EPAC2 to promote Ca^{2+} mobilisation may occur through several mechanisms, including activation of phospholipase C□ (PLC□), interactions with the SERCA Ca^{2+} ATPase in the endoplasmic reticulum, or activation of the type 2 ryanodine receptor. EPAC2-promoted Ca^{2+} release promotes activation of mitochondrial dehydrogenases, leading to an increase in cellular ATP or ADP. This result increase in cytoplasmic ATP promotes closure of ATP-sensitive K^+ channels, leading to membrane depolarisation and an influx of extracellular Ca^{2+} through voltage-gated ion channels[8]. This influx promotes exocytosis and membrane fusion of insulin-containing secretory vesicles.

II. Vascular function

The effects of cAMP on limiting vascular endothelial cell (VEC) inflammation and vascular smooth muscle cell (VSMC) proliferation have been well documented. Sustained IL-6 production appears to drive chronic, low-level vascular inflammation that leads to vascular dysfunction,hypertension, and increased risk of myocardial infarction. An early step in the development of the vascular dysfunction that ultimately leads to the formation of atherosclerotic plaques this may become sufficiently large and occlude vessels and block blood flow. Alternatively, if they are unstable, they may rupture and trigger the formation of thrombi responsible for myocardial infarction or ischaemic stroke. Surgical treatment for atherosclerosis typically involves percutaneous coronary intervention (PCI), a revascularisation procedure involving implantation of a stent into the narrowed coronary artery to physically open the previously narrowed blood vessel lumen and restore blood flow

III. Cellular functions

a. Cell adhesion

One of the first cellular functions attributed to Epac is its ability to enhance cell adhesion. When Epac is ectopically overexpressed in HEK293 cells, it induces flattened cell morphology and increases cell adhesion[9]. A study using a Epac-selective cAMP analog, 8-(4-chlorophenylthio)-2′-Omethyladenosine-3′,5′-cyclic monophosphate, suggests that activation of Epac induces Rap-dependent integrin-mediated cell adhesion to fibronectin in Ovcar3, a human ovarian carcinoma cell line. Subsequent analysis further reveals that cAMP-Epac-Rap1 pathway regulates cell spreading and cell adhesion to laminin-5 through the α3β1 integrin but not the α6β4 integrin[10]. Interaction between Epac1 and light chain 2 (LC2) of the microtubuleassociated protein MAP1A enhances Rap1-dependent cell adhesion to laminin.

b. Cell junctions

Epac1/Rap1 signaling has also shown to contribute to E-cadherin-mediated adhesion. This is consistent with the fact that Rap1 plays an important role in the formation of cell-cell junctions[11]. Stable cell-cell contacts are critical for the barrier function of epithelial and endothelial cells. Endothelial cell junctions are of central importance for regulating vascular permeability. It is well established that cAMP enhances the formation of cell junctions and endothelial barrier function. cAMP decreases basal permeability and reverse vascular leakage induced by inflammatory mediators

c. Differentiation

cAMP has been implicated in regulating differentiation in a variety of cell systems such as, neurite outgrowth in the neuroendocrine model and adipocyte formation from mouse fibroblasts. The role that PKA plays in these processes is controversial and it has been speculated that a PKA-independent cAMP signaling component may be involved. Indeed, several studies have revealed that Epac plays an important role in mediating the effects of pituitary adenylate cyclase-activating polypeptide (PACAP) in inducing neurite outgrowths in PC12 cells and human neuroblastoma SH-SY5Y cells[12]. However, as summarized in a recent Science STKE perspective, the detailed signal transduction pathways that mediate the neurotrophic effects of cAMP are not clear and the involvement of PKA remains contentious.

EPAC IN CARDIAC FUNCTION

➢ Effects on ExcitationContraction Coupling

Calcium (Ca2+) is an essential second messenger in the cardiac physiology because its rhythmic variations activate contraction in each heart beat through the mechanism of excitation contraction coupling. An action potential depolarizes the sarcolemma activating voltage-sensitive L-type Ca2+ channels allowing Ca2+ to enter into the cell where it binds to ryanodine receptors (RyR) on the adjacent sarcoplasmic reticulum (SR). This binding induces Ca2+ release to the cytosol from the SR. This coordinated process named Ca2+-induced Ca2+ release provides the Ca2+ necessary to activate the contraction of the myofibrils [13]. After contraction, cytoplasmic Ca2+ is then rapidly pumped back into the SR through the SR Ca2+ ATPase or exported out of the cell (mainly via the Na+/Ca2+ 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[14]. 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 excitationcontraction coupling proteins to increase cardiac contraction (inotropy) and relaxation (lusitropy)[13]. Indeed, PKA can phosphorylate L-type Ca2+ channels, stimulating Ca2+ current amplitude, which triggers larger SR Ca2+ release to

induce myofilament contraction. Phosphorylation of phospholamban by PKA on Ser16 relieves its tonic inhibition on SR Ca2+ ATPase activity, thus, enhancing the rate of Ca2+ uptake in the SR (lusitropic effect). In addition, the effect of PKA on myofilament protein phosphorylation also modulates contractility by decreasing their Ca2+ sensitivity and thus favoring relaxation.

➢ Cardiac Electric Remodeling

In agreement with the mechanistic findings showing that EPAC activation alters Ca2+ homeostasis, experiments performed on the whole mouse hearts showed that the EPAC activator, 8-CPT induced ventricular arrhythmogenesis[15]. Later, Brette et al[16] 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. 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[17]. EPAC also increases the expression of 2 transient receptor potential canonical channels, transient receptor potential canonical 3 and transient receptor potential canonical 4 channels in isolated rat ventricular cardiomyocytes. Ca2+ influx through these channels may contribute to a proarrhythmic effect of 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 prohypertrophic 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.

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 thenucleoplasm of mature rat cardiomyocytes.[18] This effect of EPAC activation correlates with the perinuclear expression of EPAC, and requires PLC activation (probably via Rap2) and subsequent stimulation of inositol 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. Inositol triphosphate-receptor is a Ca2+ release channel which can, not only generate a local Ca2+ signaling effect at the level of the T-tubularSR junctional complex but also in the perinuclear area of cardiomyocytes, where it has been shown to participate in the excitationtranscription coupling, a process by which local Ca2+ activates gene transcription.

EPAC IN CARDIAC DISEASE

Cyclic AMP (cAMP) is one the most important second messengers in the heart because it regulates many physiological processes, such as cardiac contractility and relaxation. The β-adrenergic receptor (β-AR) belongs to the G protein-coupled receptor (GPCR) superfamily, and is essential for the adaptation of cardiac performance to physiological needs. Upon stimulation of β-AR by noradrenaline (released from cardiac sympathetic nervous endings) and circulating adrenaline, cAMP is produced and activates protein kinase A (PKA), which phosphorylates many of the components involved in the excitation-coupling mechanisms, such as L-type the calcium channel (LTCC), phospholamban (PLB), cardiac myosin binding protein C (cMyBPC), and the ryanodine receptor 2 (RyR2), to modulate their activity[19]. Activation of LTCCs produces an inward Ca2+ current (ICa) that activates RyR2 through the mechanism known as Ca2+-induced Ca2+ release (CICR), which raises cytosolic Ca2+ concentration and activates contraction. Whereas PKA-dependent LTCC and RyR2 phosphorylation results in mobilization of Ca2+ available for contraction, PKA-mediated phosphorylation of phospholamban, a peptide inhibitor of sarcoplasmic reticulum (SR) Ca2+-ATPase promotes increased Ca2+ reuptake in the SR, thereby removing Ca2+ from the cytoplasm and accounting for relaxation. In additionbinding of cAMP to hyperpolarization-activated cyclic nucleotide-gated (HCN) channels that carry the pacemaker current, increases heart rate in response to a sympathetic stimulation (chronotropic effect). From the three β-adrenergic subtypes expressed in the mammalian heart, regulation of cardiac function is ascribed to the β1- and β2-adrenergic receptor subtypes

➢ Epac Signalosome in Pathological Cardiac Remodeling

Given the importance of the β-AR-cAMP pathway in cardiac pathophysiology, several studies aim to investigate the role of Epac proteins in the development of cardiac remodeling and HF. Remodeling pathological disorder comprises multiple attacks of which the best described are the modification of the geometry of the cardiac cavity associated with cardiomyocyte hypertrophy, fibrosis, and alterations of calcium handling and energy metabolism[20]. In the long term, these changes affect cardiac contractility and favor progression of HF, a process predominantly relying on cardiac signaling in response to the β1-AR subtype. Among the two Epac isoforms, Epac1 expression was found to be upregulated in various models of cardiac hypertrophy, such as chronic catecholamine infusion and pressure overload induced by thoracic aortic constriction, as well as in the end stages of human HF[21]. On the contrary, the anti-hypertrophic action of some hormones and microRNA, including the growth hormone-releasing hormone and microRNA-133,

involves Epac1 inhibition. A more direct evidence of Epac1's role in the regulation of cardiac remodeling came from the observation that Epac1 overexpression, or its direct activation with the Epac1 preferential agonist, 8-pCPT-2-O-Me-cAMP (8-CPT), increased various markers of cardiomyocyte hypertrophy, such as protein synthesis and hypertrophic genes in primary ventricular myocytes. It is hypothesized that in the setting of cardiac remodeling, adaptive autophagy antagonizes Epac1-induced cardiac hypertrophy. In vitro studies revealed that the pharmacological inhibition of Epac1 by a tetrahydroquinoline analogue, CE3F4, prevented the induction of cardiomyocyte hypertrophy markers in response to a prolonged β-AR stimulation in rat ventricular myocytes[22]. These findings indicate that Epac1 signaling may provide a novel means for the treatment of pathological cardiac hypertrophy. It is worth mentioning that Epac1 has also been recently identified as a potential mediator of radiation-induced cardiomyocyte hypertrophy, suggesting that this cAMP-sensor is involved in the side effects of anticancer therapy Besides its sarcolemma distribution, Epac1 is also concentrated in the nuclear/perinuclear region of cardiomyocytes, positioned well to regulate nuclear signaling. Specifically, it was shown that Epac1 is scaffolded at the nuclear envelope with phospholipase C (PLC)ε and muscle-specific A-kinase anchoring proteins (AKAPs) to regulate the hypertrophic gene program in primary cardiomyocytes. Interestingly, a detailed analysis of Ca2+ mobilization in different microdomains demonstrated that Epac (probably Epac1) preferentially elevated Ca2+ in the nucleoplasm, correlating with the perinuclear/nuclear localization of Epac1. Additional in vitro studies showed that Epac1, via its downstream effector, the small G protein Rap2, activated PLC to promote the production of inositol 1,4,5-trisphosphate (IP3). Based on this finding, a working hypothesis has been proposed, whereby Epac1 can activate PLC, causing nuclear Ca2+ increase via perinuclear IP3 receptor (IP3-R), which results in the activation of Ca2+-dependent transcription factors involved in cardiac remodeling . Consistently, in cultured cardiomyocytes, it has been reported that Epac activates CaMKII to induce the nuclear export of HDAC4 de-repressing the transcription factor myocyte enhancer factor 2 (MEF2) which activates gene transcription, essential for the hypertrophic program[23]. Collectively, these findings point to Epac1 role in activating the excitationtranscription coupling, the process by which Ca2+ activates gene transcription. Additional Epac hypertrophic signaling have been described and include the GTPase H-Ras, the Ca2+ sensitive protein, calcineurin, and its downstream effector, nuclear factor of activated T cells (NFAT), which are key mediators of cardiac remodeling.

Under adrenergic stimulation, the Epac1β-arrestin complex is recruited at the β1 -AR, and activates a pro-hypertrophic signaling pathway. Epac1 is also scaffolded at the nuclear envelope with phospholipase C (PLC)ε and muscle-specific A-kinase anchoring proteins (mAKAP) to regulate the hypertrophic gene program. In the nuclear/perinuclear region, PLCε increases nuclear Ca2+ content via the activation of the perinuclear IP3 receptor (IP3-R). Epac1 hypertrophic signaling also involves CaMKII-dependent phosphorylation of RyR2, leading to Ca2+ leak from the sarcoplasmic reticulum and subsequent calcineurin (CaN) activation [24]. The anti-hypertrophic action of the growth hormone-releasing hormone (GHRH) or its agonistic analog, MR-409, involves the protein kinase A (PKA)-dependent inhibition of Epac1 expression. MicroRNA-133 (miR-133) is cardioprotective, and targets several components of β1 -AR signaling. In the context of cardiac ischemia, mitochondrial Epac1 (MitEpac1) is activated by cAMP produced by the soluble adenylyl cyclase (sAC), and increases Ca2+ overload and ROS accumulation to promote mitochondrial permeability transition pore (MPTP) opening and cardiomyocyte apoptosis. α-KG,α-ketoglutarate; β1 -AR, β1 -adrenergic receptor; AC, transmembrane adenylyl cyclase; CaMKIIδ, Ca2+/calmodulin-dependent protein kinase II δ-isoform; cAMP, cyclic adenosine monophosphate; DAG, diacylglycerol; GHRH-R, GHRH receptor; GRP75, chaperone glucose-regulated protein 75; HDAC4, histone deacetylase 4; IDH2, isocitrate dehydrogenase 2; IP3, inositol-1,4,5-trisphosphate; IP3R1, IP3 receptor 1; IP3R1, inositol-1,4,5-triphosphate receptor 1; MEF2, myocyte enhancer factor-2; NFAT, nuclear factor of activated T-cells; PIP2, phosphatidylinositol 4,5-biphosphate; ROS, reactive oxygen species; Ser, serine; TCA, tricarboxylic acid cycle; VDAC1

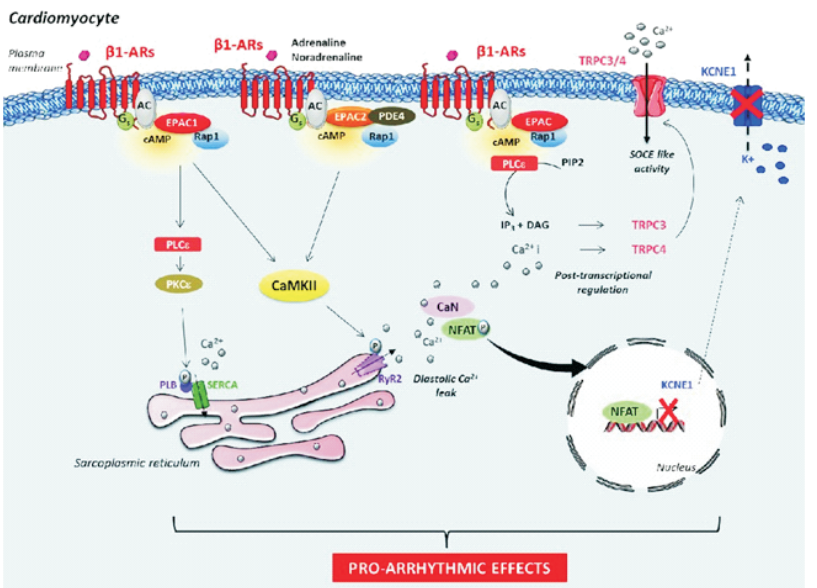

Figure 1: Regulation of arrhythmia by EPAC

➢ Role of Epac in Heart Failure and Arrhythmia

Interestingly, Epac1 KO mice show better cardiac contractility (maintenance of the inotropic reserve) and decreased susceptibility to HF in response to different hypertrophic stress conditions (catecholamine infusion or myocardial pressure overload)[25]. Further evidence for the cardioprotective effect of Epac1 inhibition came from the recent report that Epac1 deficiency attenuates J. Cardiovasc. Dev. Dis. 2018, of 16 type 5 adenylyl cyclase-mediated catecholamine stress-induced cardiac dysfunction[26]. It is interesting to note that Epac1 and Epac2 deleted mice are protected from the incidence of atrial and ventricular arrhythmia, respectively, suggesting a specific role of Epac isoforms in cardiac rhythm disorders. Conversely, direct pharmacological activation of Epac with the cAMP analogue 8-CPT promotes ventricular arrhythmogenesis in isolated perfused mouse hearts[27]. Such arrhythmogenic features were also observed in rat cardiomyocytes, but after sustained Epac activation. Yet, Brette and colleagues reported that 8-CPT induced an action potential lengthening in rat ventricular myocytes, a process involved in the genesis of arrhythmia by predisposing cardiac myocytes to early after depolarizations and dispersion of repolarization

Mechanistically, few studies demonstrated that mainly Epac1 regulated the expression level of proarrhythmic channels, such as the slow delayed-rectifier potassium K+-current (IKs) subunit potassium voltage-gated channel and transient receptor potential canonical 3 and 4 channels that enhance store-operated Ca2+ entry [39,40] (Figure 2). Epac function may also affect susceptibility to arrhythmia via the regulation of gap junction formation[28]. Importantly, in isolated ventricular myocytes, activation of Epac with either 8-CPT or β1-AR induces a spontaneous release of Ca2+ from the SR (a process named Ca2+ sparks) via the CaMKII-dependent phosphorylation of RyR2 on Serine 2814 or 2815 (depending on the species), thereby causing diastolic Ca2+ leak in a PKA-independent manner . Consistent with the localization of Epac2 along T tubules in mouse cardiomyocytes, it has been proposed that the increase of ectopic release of Ca2+ following Epac2 (and not Epac1) activation by β1-AR could be the cause of arrhythmogenic effects in cardiomyocytes. The recent finding that SR Ca2+ leak observed upon PDE4 inhibition involves Epac2 suggests that the interaction of PDE4 and Epac2 are critical for coordinating the pro-arrhythmic effect of cAMP. Adding complexity to the matter, another study showed that Epac1 promoted PLB hyperphosphorylation on Serine 16 via PKCε. This could lead to SR Ca2+ overload with Ca2+ leak and subsequent arrhythmia. Based on the aforementioned studies, the beneficial effect of Epac inhibition seems, therefore, very attractive for the development of novel therapies against HF and arrhythmia. However, few controversies have been reported in the literature. Among them, Yang and colleagues recently reported that pharmacological inhibition of Epac2 with ESI-05 was proarrhythmic in rat[29]. Further pharmacological and genetic studies combining the use of Epac isoform-specific ligands and conditional Epac KO mice are required to better decipher the role of Epac isoforms in cardiac rhythm disorders.

Role of Mitochondrial Epac in Cardiac Ischemia

Acute myocardial infarction is a leading cause of mortality and morbidity worldwide. Early coronary reperfusion has been established as the best therapeutic strategy to limit infarct size and improve prognosis. However, the process of reperfusion can itself induce cardiomyocyte death, known as myocardial reperfusion injury (I/R), for which there is still no effective therapy[30].Mitochondria have been recognized as playing a central role in both apoptotic and necrotic cell death. Indeed, during I/R injury, cardiomyocyte death is initiated by mitochondrial Ca2+ overload and an excessive production of reactive oxygen species (ROS) which trigger the mitochondrial permeability transition pore (MPTP) opening, resulting in mitochondrial depolarization, swelling, and rupture of the external mitochondrial membrane[31]. This leads to the uncoupling of the respiratory chain, and the efflux of cytochrome c and other proapoptotic factors that may induce apoptosis or necrosis.

Depending on the nature of the stimulus and the cell type used in the study, Epac may play a proapoptotic or antiapoptotic role[32]. For instance, in neonatal rat cardiomyocytes, Epac cooperates with PKA in the antiapoptotic effects of exendin-4, a glucagon-like peptide-1 receptor agonist [33]. Similarly, activation of both PKA and Epac with cAMP analogues confers cardioprotection against I/R injury in isolated rat heart .Interestingly, it is suggested that long-term feeding of an obesogenic high fat diet renders the myocardium less susceptible to I/R induced injury via Epac-dependent signaling. Yet, recent findings using isolated cardiomyocytes from ischemic rat hearts have implied that the cardioprotective effect induced by urocortin-1 involved the Epac2 pathway. On the contrary, in vivo experiments showed that Epac1 genetic ablation in mice protected against myocardial I/R injury with reduced infarct size and cardiomyocyte apoptosis. Consistent with an earlier finding showing the mitochondrial expression of transfected Epac1 in COS-7 cells, Epac1 is expressed in the mitochondrial inner membrane and matrix of cardiomyocytes. A form of Epac1 deleted in its mitochondrial-targeting sequence protects against hypoxia/reoxygenation (a condition mimicking in vivo I/R)-induced cell death, indicating that mitochondrial Epac1 participates in cardiomyocyte death during hypoxic stress[34]. Mechanistic studies demonstrated that during hypoxia/reoxygenation, Epac1 was activated by the type 10 soluble adenylyl cyclase (sAC) to increase mitochondrial Ca2+ uptake and ROS production, thereby promoting mitochondrial death signaling, such as MPTP opening, cytochrome c release, and both caspase-9 and -3 activation. However, these results are not in agreement with another study, which reported that direct activation of sAC with HCO_3^- prevented Ca2+-induced MPTP opening through Epac1, suggesting that Epac1 might protect from cardiomyocyte death[35]. The higher amount of cAMP produced in the model of hypoxia/reoxygenation, and subsequent massive increase in mitochondrial ROS and Ca2+ levels, could potentially account for the observed differences

CONCLUSION

Traditionally, research focused on PKA as one of the main targets of cAMP. However, recent studies indicated that next to classical signaling pathways, there seem to be substantial role for additional cAMP targets, such as Epac, . EPAC in the manifestation of cardiovascular disease suggesting that it 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. 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.

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