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Digest

# Exchange proteins directly activated by cAMP (EPACs): Emerging therapeutic targets

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ABSTRACT

Exchange proteins directly activated by cAMP (EPACs) are critical cAMP-dependent signaling pathway mediators. The discovery of EPAC proteins has significantly facilitated understanding on cAMP-dependent signaling pathway and efforts along this line open new avenues for developing novel therapeutics for cancer, diabetes, heart failure, inflammation, infections, neurological disorders and other human diseases. Over the past decade, important progress has been made in the identification of EPAC agonists, antagonists and their biological and pharmacological applications. In this review, we briefly summarize recently reported novel functions of EPACs and the discovery of their small molecule modulators. The challenges and future perspectives are also discussed.



Cyclic adenosine monophosphate (cAMP), also known as 3′,5′-cyclic adenosine monophosphate, is converted from adenosine triphosphate (ATP) by adenylate cyclases (ACs). It is a prototypic second messenger that plays crucial roles in cellular responses to various stimulations, and mediates signaling pathways related to many human diseases including cancer, cardiac and urinary dysfunction, diabetes, immunological diseases and nerve disease.[1–4] Protein kinase A (PKA) and cyclic nucleotide-regulated ion channels were the first discovered cAMP mediators and originally considered as the only ones. In 1998, two independent groups reported another cAMP mediator named exchange proteins directly activated by cAMP (EPACs).[5,6] EPAC is a family of cAMP-binding proteins with guanine nucleotide exchange factors (GEF) activity that directly activate Ras-like small GTPases (Rap1 and Rap2).[5,6] The discovery of EPAC proteins has significantly facilitated the understanding of cAMP-dependent signaling pathway and opens new avenues for developing novel therapeutics targeting cancer, diabetes, heart failure, inflammation, infectious diseases, neurological disorders and other human conditions.[1,7–10]

*Structures and functions of EPAC family proteins:* To date, two members of the EPAC family proteins have been identified, known as EPAC1 (coded by *Rapgf3* gene, in human) and EPAC2 (coded by *Rapgf4* gene, in human).[5,6] EPAC1 (cAMP-GEF-I) is an about 100 kDa molecular weight multi-domain protein that is highly expressed in developing and mature human tissues. The multi-domain protein EPAC2 has three isoforms (EPAC2A, EPAC2B and EPAC2C) with about 115 kDa of molecular weight and is enriched in nervous system and endocrine tissues. EPAC1 and EPAC2 proteins have considerable similarity in the structure and sequence (68% similarity in human).[11] Both EPAC1 and EPAC2 consist of two regions, the N-terminal regulatory region and the C-terminal catalytic region. The regulatory region of EPAC protein includes a disheveled, Egl-10, pleckstrin (DEP) domain and cyclic nucleotide binding domain (CNBD). The C-terminal catalytic regions of EPAC1 and EPAC2 are composed of three basic domains named as cell division cycle 25 homology GEF domain (CDC25-HD), Ras association (RA) domain, and Ras exchange motif (REM) domain.[12,13]

In the absence of cAMP, the activity of EPAC is auto-inhibited. The N-terminal regulatory region and the C-terminal catalytic region of EPAC are held together through intramolecular interactions, thereby preventing Rap binding to the CDC25-HD of EPAC and keeping EPAC inactive (Fig. 1).[14] When cell is stimulated by extracellular signals, ACs are activated through various ligands which bind to G-protein-coupled receptors (GPCRs) and promote the conversion of ATP into cAMP.[15] The binding of cAMP to CNBD allows the regulatory region to rotate about 90° sideways and leaves enough space for Rap binding to CDC25-HD.[15] Consequently, active EPAC catalyzes the exchange of guanosine diphosphate (GDP) to guanosine triphosphate (GTP) and controls Rap-mediated biological functions (Fig. 1). The EPAC signaling pathway plays a critical role in various biological responses including

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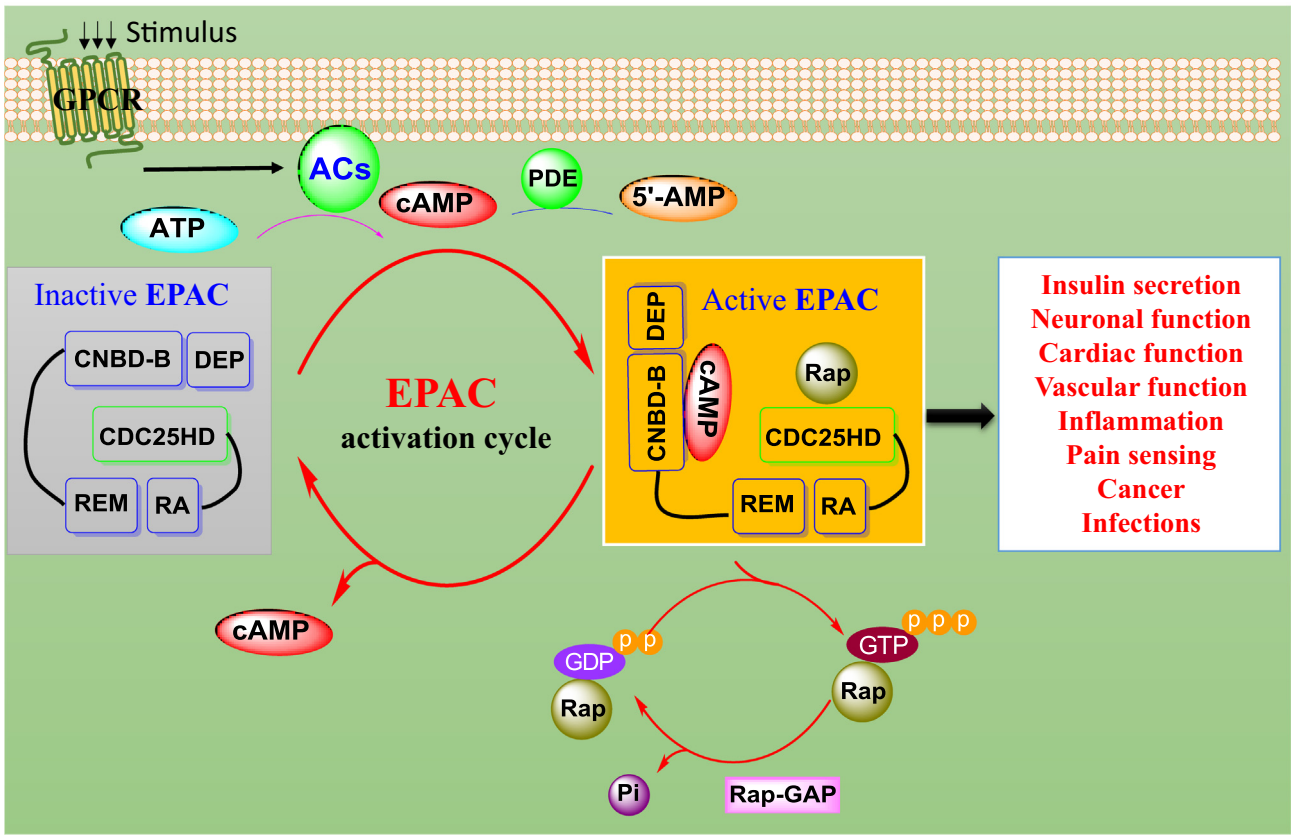


**Fig. 1.** Postulated mechanisms of EPAC activation and associated biological functions. Under the G-protein-coupled receptor (GPCR) stimulation, adenylate cyclases (ACs) convert adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP). The excessive cAMP can be degraded into 5′-AMP by phosphodiesterases (PDEs). The binding of cAMP to inactive EPAC leads to the activation of EPAC, which facilitates the exchange of guanosine diphosphate (GDP) to guanosine triphosphate (GTP) and controls Rap-mediated biological functions. Meanwhile, Rap-GTPase-activating proteins (Rap-Gap) facilitate the intrinsic GTPase activity of Rap to breakdown GTP into GDP and phosphorus inorganic (Pi).

insulin secretion, neuronal function, cardiovascular function, vascular function, inflammation, cancer, pain, and infections.[1,7–10]

The EPAC signaling pathway is involved in insulin secretion from pancreatic β cells. EPAC2 promotes glucose-stimulated insulin secretion (GSIS) by regulation of intracellular $Ca^{2+}$ concentration.[16–18] To date, three pathways have been revealed for EPAC2-mediated insulin secretion. First, EPAC2/Rap can activate phospholipase Cε (PLCε), protein kinase C (PKC), ryanodine receptor (RyR) and sarco/endoplasmic reticulum $Ca^{2+}$-ATPase (SERCA).[19,20] Second, EPAC2 can directly interact with sulfonylurea receptor 1 (SUR1), leading to ATP-sensitive potassium channel ($K_{ATP}$) closure in response to the increase in the ATP/ADP ratio, thus regulating the intracellular $Ca^{2+}$ level.[21] Third, interaction of EPAC2 with Rim2, Munc 13-1 and Piccolo potentiates rapid $Ca^{2+}$-dependent exocytosis.[22,23] According to a recent study, EPAC1 may also play an important role in GSIS.[24] The EPAC1 knockout mouse model showed the decreased expression of glucose transporter Glut2 and transcription factor PDX1. Collectively, these studies suggest that EPAC represents a potential therapeutic target for diabetes and obesity.

The interaction of EPAC2 with Rim1 has an important role in regulating neurotransmitter release.[25] In addition, a recent EPAC2 knockout mice model study provides *in vivo* evidence that EPAC2 promotes transmitter release by maintaining the readily releasable pool (RRP) at mossy fiber (MF) synapses in the hippocampus.[26] Growing evidence demonstrates that EPAC participates in neurite growth and neuronal differentiation.[27,28] In PC12 and NS-1 cells, EPAC2 is necessary for mediating growth arrest and neurite extension during neuronal differentiation through the mitogen activated protein kinase (MAPK) pathways including p38 and extracellular signal-regulated kinase (ERK).[29]

Studies based on EPAC1 and EPAC2 knockout mouse model have revealed that EPAC proteins exert significant physiological roles in learning, memory and social interactions in brain.[30] Furthermore, EPAC2-deficent mice show reduced dendritic spine motility and density in cortical neurons, and display defects in social interactions and ultrasonic vocalizations.[31] Thus, targeting EPAC signaling pathways may present a novel strategy for the treatment of CNS diseases.

In the heart, EPAC can enhance cardiac contractility by regulating intracellular $Ca^{2+}$ concentration through PLCε, PKC, RyR and $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) signaling pathways.[32,33] In the hypertrophic heart, EPAC (mainly EPAC1) is found to be overexpressed.[34] It suggests that EPAC may play an important role in cardiac hypertrophy.[35] Activation of EPAC can prevent $H_2O_2$-induced production of reactive oxygen radical and inhibit the activation of caspase-3 and apoptosis in cardiomyocytes.[3] Recently, it was reported that the activation of $\beta_1$-adrenergic receptors ($\beta_1$-AR) could lead to EPAC2-dependent sarcoplasmic reticulum (SR) $Ca^{2+}$ leak and arrhythmia through phosphorylation of RyR2 by CaMKIIδ or PKA.[36] Of note, the distributions between EPAC1 and EPAC2 in mice myocytes are significantly different. EPAC1 is limited in nuclear signaling while EPAC2 is found to gather around the T tubules, indicating that EPAC2 is involved in the arrhythmogenic SR $Ca^{2+}$ leak.[37] EPAC also plays a critical role in the development of cardiac fibrosis.[38] The important involvement of EPAC in cardiovascular functions offers a new direction for the discovery of new treatment of cardiovascular diseases.

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