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ABSTRACT

The well-known second messenger cyclic adenosine monophosphate (cAMP) regulates the morphology and physiology of neurons and thus higher cognitive brain functions. The discovery of exchange protein activated by cAMP (Epac) as a guanine nucleotide exchange factor for Rap GTPases has shed light on protein kinase A (PKA)-independent functions of cAMP signaling in neural tissues. Studies of cAMP-Epac-mediated signaling in neurons under normal and disease conditions also revealed its diverse contributions to neurodevelopment, synaptic remodeling, and neurotransmitter release, as well as learning, memory, and emotion. In this mini-review, the various roles of Epac isoforms, including Epac1 and Epac2, highly expressed in neural tissues are summarized, and controversies or issues are highlighted that need to be resolved to uncover the critical functions of Epac in neural tissues and the potential for a new therapeutic target of mental disorders.

1. Introduction

Epac (exchange protein activated by 3′-5′-cyclic adenosine monophosphate [cAMP]) has been investigated functionally and pathophysiologically as a potential therapeutic target of various diseases since it was identified in the late 90s as a novel cAMP target protein by two independent groups (1, 2). Two variants of Epac (Epac1 and Epac2) act as cAMP guanine-nucleotide exchange factors (GEFs) to activate the small GTPases Rap, Rap1, and Rap2 in a protein kinase A (PKA)-independent manner (1, 2), and Epac-activated Rap controls a variety of biological processes (3). The Epac1 and Epac2 proteins are encoded by two different genes: *RAPGEF3* (chromosome 12:47,734,363–47,771,040 for human; chromosome 15:97,744,770-97,767,972 for mouse) and *RAPGEF4* (chromosome 2:172,735,274-173,052,893 for human; Chromosome 2: 71,981,240-72,257,474 for mouse), respectively, which both give rise to multiple transcripts. Among potential transcripts produced from *Rapgef3*, transcript variant 1 of Epac1 encoding the longest isoform, Epac1A with 923 amino acids has been well studies relatively to other transcripts or splicing variants, but differences in functional significance and tissue expression distribution of Epac1 isoforms still remain unclear (4). Contrast to Epac1, transcriptional variants from alternative promoters and splicing variants encoding Epac2 isoforms, Epac2A1, Epac2A2, Epac2B, and Epac2C and tissue distribution of Epac2 protein have been studied well in mice (5) and it is well conserved across human (Table 1). Whereas Epac1 is ubiquitously expressed in nearly every tissue, Epac2 has been detected only in the brain, adrenal glands, pancreas, and liver (5). In the brain, Epac2 expression in neurons is more abundant than but works with Epac1 to regulate neural function (2). Interestingly, Epac2 isoforms Epac2A1, Epac2A2, Epac2B, and Epac2C exhibit distinctive patterns of expression (5). Epac1 and Epac2 proteins, meanwhile, have considerable similarities in structure and they have the amino-terminal regulatory region and carboxyl-

terminal catalytic region in common. The regulatory region is composed of a cyclic nucleotide-binding (CNB) domain for functional binding of cAMP and Epac activation, and a Dishevelled, Egl-10, Pleckstrin (DEP) domain. On the other hand, the catalytic region comprises a RAS exchange motif (REM) domain, a RAS-association (RA) domain, and a guanine nucleotide exchange factor for Ras-like small GTPases (RasGEF) domain, also known as CDC25 homology domain (Figure 1). The DEP domain is involved in plasma membrane localization of Epac1 and subcellular localization of Epac2. The REM region stabilizes the RasGEF domain without direct interaction with the small GTPases. The RA domain helps perinuclear localization of Epac1 and regulate plasma membrane localization of Epac2 (4, 6). Structurally, Epac1 consists of identical structural subdomains with those found in Epac2B although their exon sequences and transcripts are different (Figure 1).

To elucidate the roles of Epac1 and Epac2 in the central nervous system, specific inhibitors and activators have been utilized, such as ESI-05, a selective inhibitor of Epac2 (7), Sp-8-BnT-cAMPS (S0220), a selective Epac2 activator (8), and 8-pCPT-2′-*O*-Me-cAMP (8-CPT [D-007]), which is an activator of Epac1 and Epac2 (8). Although 8-CPT is commonly used *in vitro* and *in vivo* as a general activator of Epac, it was revealed that it is a much more effective activator of Epac1 than Epac2 (5). Thus, the effects of 8-CPT may reflect the role of Epac1 or Epac2 according to the expression level of each isoform in cells or tissues. Additionally, knockout (KO) mouse models have been used to assess the functions of these proteins, such as mice deficient in Epac1 (9, 10), Epac2A (11), or all isoforms of Epac2 (12).

The use of a variety of research tools has revealed the involvement of Epac in a diversity of neuronal functions, including neurotransmitter release (13), neurite growth, and neuronal/glial differentiation (14, 15), and in higher cognitive functions, such as memory, learning, and social interaction (12). This mini-review summarizes recent findings from

research on Epac1 and Epac2, and provides an overview of their diverse roles in the central nervous system. Findings in this emerging field may have implications both for the clinics and for society, and may provide new insights into novel therapeutic approaches for neurological and psychiatric disorders.

2. Roles of Epac at Synapses and in Neurons

2.1. Synaptic remodeling and plasticity

It is well known that synaptic remodeling of spine structures (16) and synaptic plasticity (17) are the two key synaptic mechanisms that underlie the anatomical and physiological bases, respectively, of learning and memory formation. Among the variety of molecular events that mediate these processes, cAMP represents a key intracellular signal that regulates the morphological plasticity of dendritic spines (18) and long-term potentiation (LTP) of synapses (19-21) through cAMP response element binding protein (CREB) or PKA. However, Epac is also involved in synaptic morphology and plasticity.

In cultured mature rat cortical neurons, which express smaller amounts of Epac1 than Epac2, 8-CPT-induced activation of Epac2 results in spine shrinkage, decreased spine motility, and depressed excitatory transmission, with removal of GluA2/3-containing AMPA receptors from synapses. These Epac2-mediated effects were confirmed *in vivo* in cortical neurons of Epac2 KO mice (12, 22). Conversely, point mutations of Epac2 associated with autism (23) induce spine enlargement and stabilization (15). Moreover, studies on the effect of Epac2 on synaptic proteins have revealed that Epac2 balances neuronal excitation and inhibition by stabilizing excitatory synapses and increasing inhibitory synapses (22).

Further electrophysiological studies using Epac2 KO mice or ESI-05 have shown that Epac2 is involved in cAMP-dependent potentiation of hippocampal mossy fiber synapses (13) and LTP at parallel fiber-to-Purkinje cell synapses via activation of GluA3-containing AMPA

receptors (24). Yang et al. demonstrated that KO mice deficient in Epac1 and Epac2 had impaired hippocampal LTP at CA3-CA1 Schaffer collateral synapses but not long-term depression (LTD) as a result of reduced presynaptic glutamate release, whereas no synaptic deficits were detected in the hippocampi of Epac2 KO mice (10). However, deletion of Epac2A results in a failure of NMDA receptor-dependent LTD at the Schaffer collateral-CA1 synapses of hippocampus (11).

Although specific effect of Epac1 or Epac2 was not demonstrated in the following studies, electrophysiological studies using 8-CPT revealed that the Epac-dependent increase of glutamate release at hippocampal excitatory synapses (25) is mediated by phospholipase C (26). Moreover, Epac activation contributes to the maintenance of LTP (27) and pituitary adenylate cyclase-activating polypeptide (PACAP)-induced LTD (28) via extracellular signal-regulated protein kinase (ERK) and p38 mitogen-activated protein kinase (MAPK), respectively, in the hippocampus (Figure 2A). Therefore, effects of Epac on synaptic remodeling and plasticity may be different according to isoforms and these issues remain unresolved yet.

2.2. Neurodevelopment

2.2.1. Neurite and axonal growth

Epac1 and Epac2 are differentially regulated during nervous tissue development. Whereas Epac1 is abundantly expressed in the brain at embryonic and neonatal periods but is almost undetectable in adulthood, Epac2 is lowly expressed at embryonic and neonatal stages and increased in the adult brain (29). These findings suggest that Epac1 has a more influential role than Epac2 in neurite outgrowth during development. In support of this, knockdown of Epac1 expression with short hairpin RNA (shRNA) in cultured hippocampal neurons results in fewer polarized neurons with shorter axons via Rap1B inhibition, and cultured hippocampal neurons from Epac1 KO mice display delayed polarization (9). In pheochromocytoma (PC-12) cells,

Epac1 is involved in panaxydol-induced axonal growth via the Rap1-ERK-CREB pathway (30). However, through a Rap-independent mechanism, cAMP-mediated Epac1-dependent Rit activation induced by PACAP38 results in CREB-dependent neurite outgrowth in PC-6 cells (a subline of PC-12 cells) (31). Interestingly, cAMP- and Rap1-independent functions of Epac1 have a reverse effect on neurite outgrowth. For example, in the Neuro2a neuroblastoma cell line, Epac1 accumulates at the plasma membrane because of the lack of importin β1 and neither binds to cAMP nor activates Rap1, thereby inhibiting neurite outgrowth (32) (Figure 2B).

We cannot rule out the possible involvement of Epac2 in neurite and axonal outgrowth, as both Epac1 and Epac2 are expressed throughout the brain. Indeed, RNA interference-mediated knockdown of Epac2 disrupts the architecture of basal dendrites via inhibition of Ras signaling in mature cortical neurons (33). Furthermore, activation of Epac2 with the specific agonist S-220 enhances the outgrowth of neurites from postnatal rat cortical neurons *in vitro* (34). Additionally, short-interfering RNA (siRNA)-mediated knockdown of Epac1 and Epac2 reduces neurite outgrowth, blocks cAMP-induced neurite growth (29), and eliminates the chemoattraction of rat dorsal root ganglia neuron growth cones to netrin-1 (35). Compatibly with these results, Epac1 and Epac2 activation by 8-CPT is involved in rolipram-induced neurite outgrowth from rat dorsal root ganglia neurons (36) and the conversion of PKA-specific cAMP from a proliferative to an antiproliferative signal in PC-12 cells by promoting neurite outgrowth (37). Nevertheless, 8-CPT-mediated activation of Epac does not affect neurite outgrowth in spiral ganglion neurons (38) (Figure 1A). Further studies, including *in vivo* analyses, are needed to identify the precise roles of Epac proteins in neurite and axonal outgrowth.

2.2.2. Neurogenesis and glial differentiation

Although little is known about the role of Epac in neural differentiation during embryonic

neurogenesis, Epac2 is involved in adult neurogenesis in the ventricular-subventricular zone (V-SVZ) and subgranular zone (SGZ) of dentate gyrus, where it is expressed in GFAP-positive neural stem cells and doublecortin-positive neuroblasts and progenitor cells (39). A study using adult Epac2 KO mice showed that this protein is required for progenitor cell proliferation and neurogenesis in the SGZ (40). Other studies report a role of Epac in the differentiation of glial cells, including astrocytes and oligodendrocytes. PACAP-induced astrocytic differentiation of neural precursor cells is mediated by Epac2A activation via calcium ion influx, leading to increased intracellular concentrations (14). Moreover, inhibition of Epac2 with ESI-05 revealed that Epac2 promotes cAMP-dependent differentiation of cultured rat oligodendrocyte precursor cells by regulating the expression of myelin basic protein (41). In addition, whereas cAMP-dependent proliferation of Schwann cells requires PKA activity, activation of Epac by 8-CPT is antiproliferative and also impacts the cAMP-dependent differentiation and myelin formation in peripheral Schwann cells (42). Thus, although little is known about an exact role of Epac1 in neurogenesis and glial differentiation, Epac, especially Epac2 isoform, appears critical for normal development of neurons and astrocytes, and might provide a new therapeutic target to enhance remyelination in the central and peripheral nervous systems.

2.3. Cell death

Cell death is critical for homeostasis in organisms by eliminating excess and injured cells. Apoptosis and autophagy have been characterized as major types of programmed cell death (43). Although the roles of Epac have been extensively studied in cancer (44), less is known about the role of Epac on cell death in the central nervous system.

2.3.1. Apoptosis

Whereas cAMP-PKA signaling is neuroprotective, activation of Epac, such as by 8-CPT or

adeno-associated virus-mediated overexpression of Epac1 or Epac2, induces apoptosis of cultured mouse cortical neurons by p38 MAPK-induced upregulation of Bim (known as a Bcl-2 interacting member and Bcl-2-like protein 11) (45) (Figure 2A). Moreover, cortical neurons of Epac1 KO mice are protected from 3-propionic acid-induced apoptosis, and neurons cultured from Epac1 KO mice show increased expression of Bim mRNA and protein (45). Furthermore, by using ESI-05 and a rat model of traumatic brain injury, inhibition of Epac2 was found to reduce the associated neuronal apoptosis (46, 47). Although these findings suggest that Epac1 and Epac2 have a proapoptotic effect in neurons, Epac exhibits antiapoptotic effects in other cells, such as hematopoietic B-CLL cells (48), macrophages (49), and cardiomyocytes (50). Thus, the pro- or antiapoptotic roles of Epac may vary according to cell type and may reflect the differential localization of Epac protein via membrane targeting activities of dishevelled, Egl-10, the pleckstrin domain (51), and Ras association domain (52).

2.3.2. mTOR-independent autophagy

Autophagy is an intracellular lysosomal process for the degradation of endogenous or exogenous materials in the cytoplasm (53). Two signaling pathways are involved in this process: mammalian target of rapamycin (mTOR)-dependent and mTOR-independent pathways (54). Contrary to the role of PKA in mTOR-independent autophagy (55), cAMP-Epac signaling may inhibit this autophagy by activating a series of components of Rap2B-PLC-ε-IP_3 and Ca^{2+}-calpain-$G_s\alpha$ pathways (56) (Figure 2A). In PC-12 cells, 8-CPT delays the clearance of autophagy substrates, and autophagy is induced by inhibiting Epac-activated Rap2B, indicating that Rap2B acts as a downstream regulator of Epac in the mTOR-independent autophagy pathway (56).

3. Roles of Epac in Cognition and Mental Disorders

3.1. Learning and memory

Several *in vivo* studies using KO mice demonstrated that the Epac isoforms impact spatial learning and memory. Mice with double KO of both Epac1 and Epac2 show spatial learning and memory impairments and deficits in reversal learning in the Morris water maze (MWM); however, single KO mice deficient in either Epac 1 or Epac2 KO exhibit normal spatial learning (10). Another group revealed that despite normal performance in the MWM, Epac2 KO mice had a deficit in contextual fear conditioning, with reduced freezing behavior (40). Additionally, inhibition of Epac2 by injection of siRNA resulted in impaired fear memory retrieval in mice (57). Epac2A KO mice similarly show normal spatial learning and memory in MWM and IntelliCage tests but impaired reversal learning, suggesting that Epac2A plays a role in behavioral flexibility (11). Unlike Epac2 KO (40), Epac2A KO mice are insensitive to painful stimuli (11), and so it is not known if this isoform contributes to contextual fear conditioning, but they exhibit normal reward-based reinforcement learning (58).

3.2. Stress and affective disorders

In a postmortem study comparing protein levels of Epac1 and Epac2 in brain tissues from control and suicide groups, Epac2 was significantly higher in the prefrontal cortices and hippocampus of suicide victims with major depression disorder, whereas Epac1 protein expression did not differ from that in the controls (59). Contrary to data from human study, in the hippocampi of mice exposed to acute restraint stress which is used to model depression and anxiety disorders (60), the expression of Epac1 and Epac2 mRNAs was higher in female wild-type mice, and Epac KO mice showed delayed nuclear localization of glucocorticoid receptors along with altered serum corticosterone levels (60). In a study conducted by Zhou and colleagues, Epac2 KO mice displayed anxious and depressive behaviors in normal environments: they spent less time in the center of an open field and exhibited increased

immobility during forced swimming test (40). However, other studies found no evidence that Epac2 deletion alters anxiety (10, 12). Although further study is needed to clarify these discrepancies, the current evidence suggests that Epac1 and Epac2 differentially affect emotional behaviors by regulating cellular responses to stress.

3.3. Autism spectrum disorders

Screens for genes on human chromosome 2q revealed nonsynonymous variants in *RAPGEF4* (encoding Epac2) in individuals with autistic phenotypes (23). Furthermore, Epac2 KO mice exhibit social interaction impairments in three-chamber tests and ultrasonic vocalization studies, although they show normal working memory and repetitive behaviors (12). Epac2A KO mice also display a stereotypic behavior: making the same decision consecutively during a dynamic foraging task for reward-based reinforcement learning, demonstrating an abnormal decision-making process and perseverative behaviors such as those observed in individuals with autism spectrum disorders (58). These studies also suggest that there are abnormalities of cortical structures, including disrupted cortical columnar organization in the anterior cingulate cortex (12) and a lower density of parvalbumin-expressing GABAergic interneurons in the ventrolateral orbitofrontal cortex (58). However, the role of Epac2 in autistic behavioral phenotypes is still debated. Whereas double deficiency for Epac genes impairs social interactions in mice, this impairment is not seen in single Epac1 or Epac2 KOs (10). Thus, further detailed studies are needed to evaluate the role of Epac in autism.

4. Conclusion

Since the Epac protein was identified in 1998, accumulating data from scientific studies have clarified PKA-independent cAMP functions via Epac in various cell types and tissues. Moreover, specific activators/inhibitors of Epac isoforms and genetic manipulations have

helped uncover the specific roles of Epac1 and Epac2 in a variety of tissues. This mini-review summarizes the specific roles of Epac1 and Epac2 in neural cells and tissues (Figure 2B and 2C). Despite growing evidence suggesting that Epac is an essential protein in the cAMP signaling pathway in neural tissues, a lot of issues remain unsolved. For instance, Epac isoform-specific or Epac localization-dependent anatomical and physiological functions in neurons and higher cognitive functions during different life stages, from embryo to old age, similarly remain shrouded in mystery. Further isoform-specific gene manipulations or drug development specific for Epac2 isoforms will be helpful to elucidate their specific functions. In addition, the roles of Epac1 and/or Epac2 in molecular, physiological, and behavioral phenotypes determined in KO mice need further study. This is also critical for discovering the precise role of Epac proteins in the molecular and pathophysiological mechanisms underlying various diseases, including neurodegenerative and mental disorders.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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FIGURE LEGEND

Figure 1. Schematic representation of domain structure and genomic organization of Epac protein isoforms.

Epac1 and Epac2 consist of the N-terminal regulatory region and the C-terminal catalytic region in common, which are composed of functional multi-domains. The regulatory region contains a cyclic nucleotide-binding (CNB) domain and a dishevelled, Egl-10, Pleckstrin (DEP) domain. The CNB domain of Epac1 and CNB-B domain of Epac2 bind cAMP with a high affinity leading to Epac protein activation. The extra CNB-A domain of Epac2A1 and Epac2A2 bind cAMP with a relatively low affinity compared with the conserved CNB-B domain and is not involved in activation of Epac2. The Dishevelled, Egl-10, Pleckstrin (DEP) domain has a role in the subcellular localization of Epac protein. In the catalytic region, a RAS exchange motif (REM) domain interacting with the guanine nucleotide exchange factor (GEF) region stabilizes a GEF for Ras-like small GTPases (RasGEF) domain which is responsible for biological function of Epac protein. The RAS-association (RA) domain regulates perinuclear localization of Epac1 and plasma membrane localization of Epac2.

Figure 2. Roles of Epac in synaptic plasticity, neurodevelopment, and cell death.

(A) Effects of 8-CPT-induced activation and gene deletion of Epac. The 8-CPT activates both Epac1 and Epac2 in neural tissues. Note the stronger effect of 8-CPT on Epac1 activation (thick arrow) than Epac2 (thin arrow). Activation of Epac protein including Epac1 and Epac2 isoforms contributes to LTP maintenance via extracellular signal-regulated kinase 1/2 (ERK) activation, PACAP-mediated LTD induction via activation of p38 mitogen-activated protein kinases (MAPK), apoptosis by p38 MAPK-induced upregulation of Bim (Bcl-2-interacting mediator known as a Bcl-2 like protein 11), and inhibition of mTOR-independent autophagy activation through Rap2B/PLCε/inositol 1,4,5-trisphosphate (IP3) signaling pathway. However,

effects of 8-CPT-induced Epac activation on neurite outgrowth and synaptic glutamate release are quite different and are not determined yet in neural tissue. (**B)** Role of Epac1 in neurite and axonal growth. Epac1 is involved in axonal growth through Rap1/ERK/cAMP-response element binding protein (CREB) signaling pathway. In Rap-independent manner, Epac1 plays a role in enhancement or inhibition of neurite growth via Rit/CREB activation or accumulation itself near plasma membrane regardless of binding to cAMP, respectively. **(C)** Role Epac2 and Epac2A isoform in neural tissues. Epac2 involves in adult neurogenesis and glial differentiation, apoptotic cell death and induces synaptic spine shrinkage and neurite outgrowth. The asymmetric expression of Ras/Epac2/Rap in dendritic compartments of cortical neurons affects the distribution of phosphorylated BRaf (p-BRaf) in dendrites and then maintenance of basal dendrite complexity in cortical neurons. However, the effect of Epac2 on LTP induction and maintenance is a controversial issue showing different results according to neural tissues. Epac2A isoform is required for LTD induction but not LTP in the hippocampus and astrocyte differentiation during development. LTP, long-term potentiation; LTD, long-term depression.

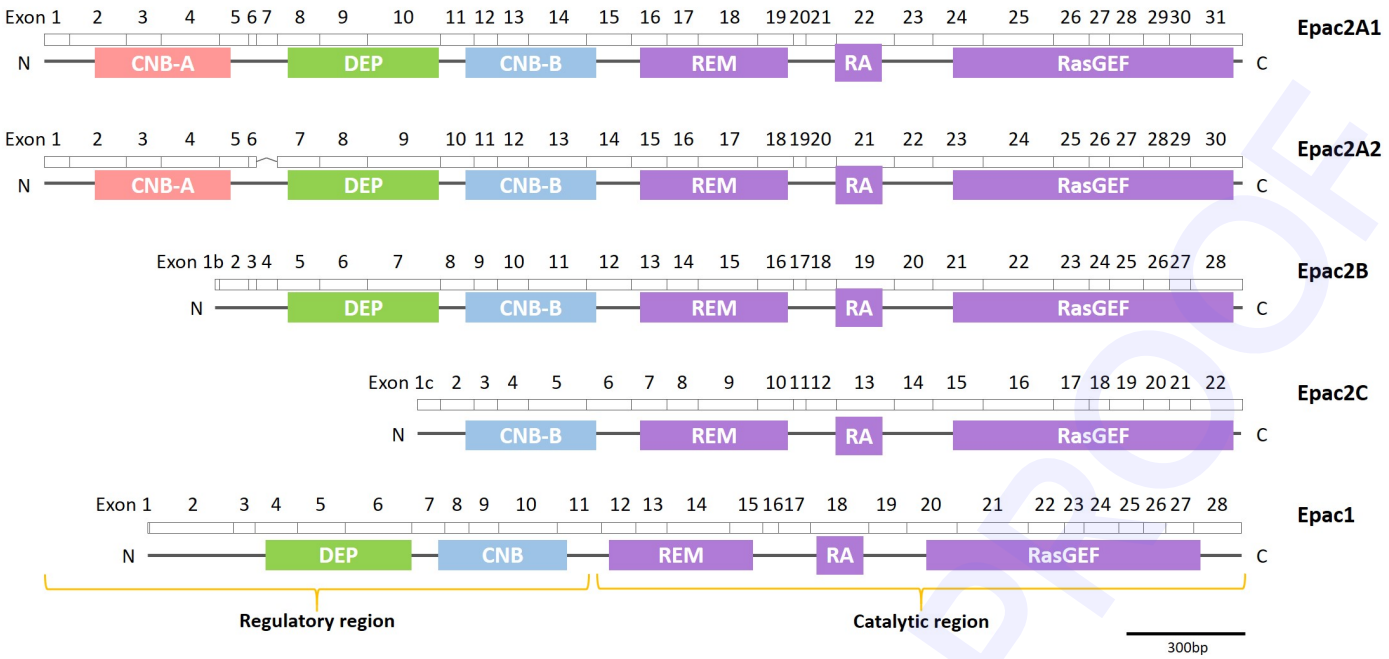

Fig. 1.

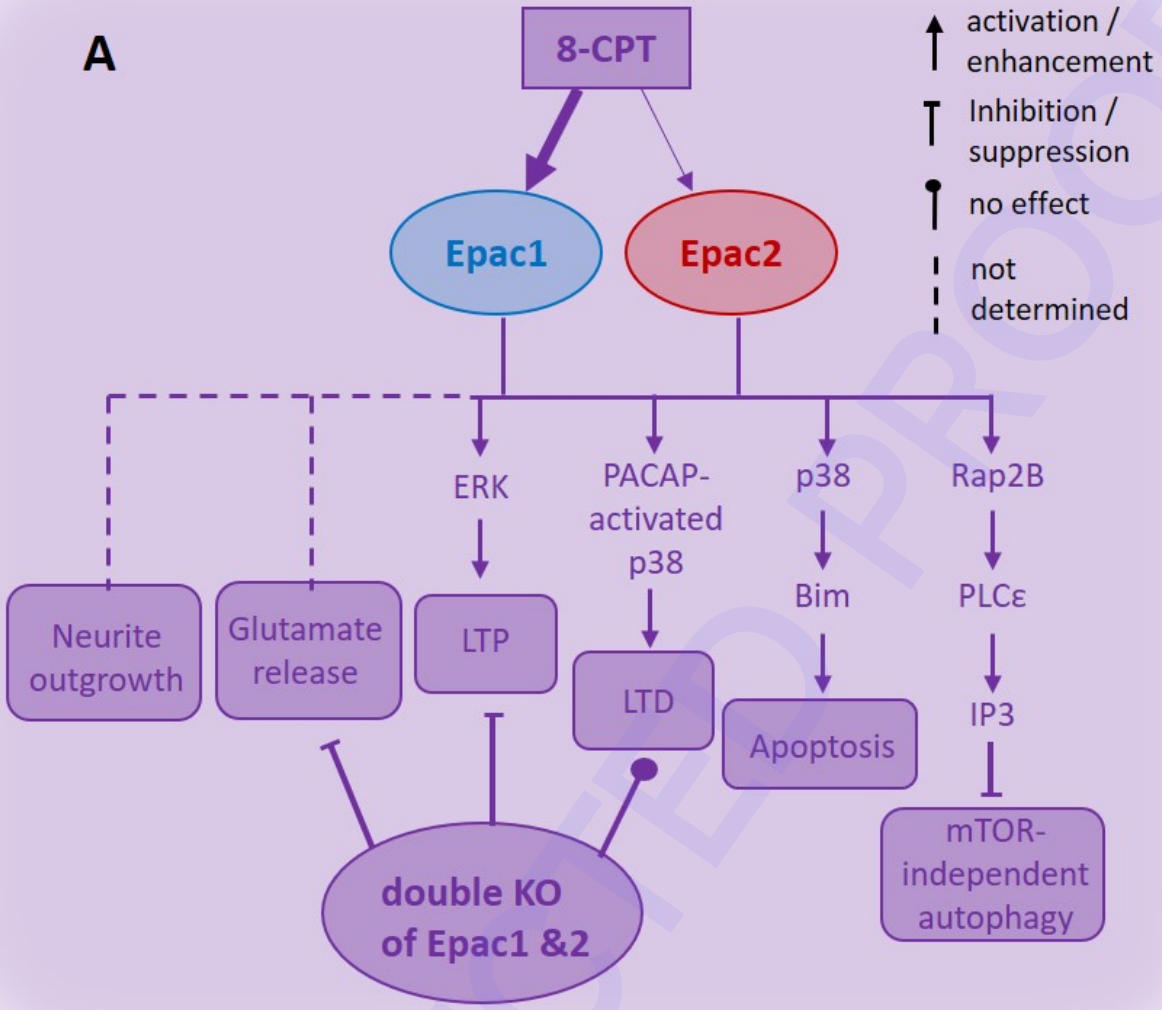

Fig. 2.

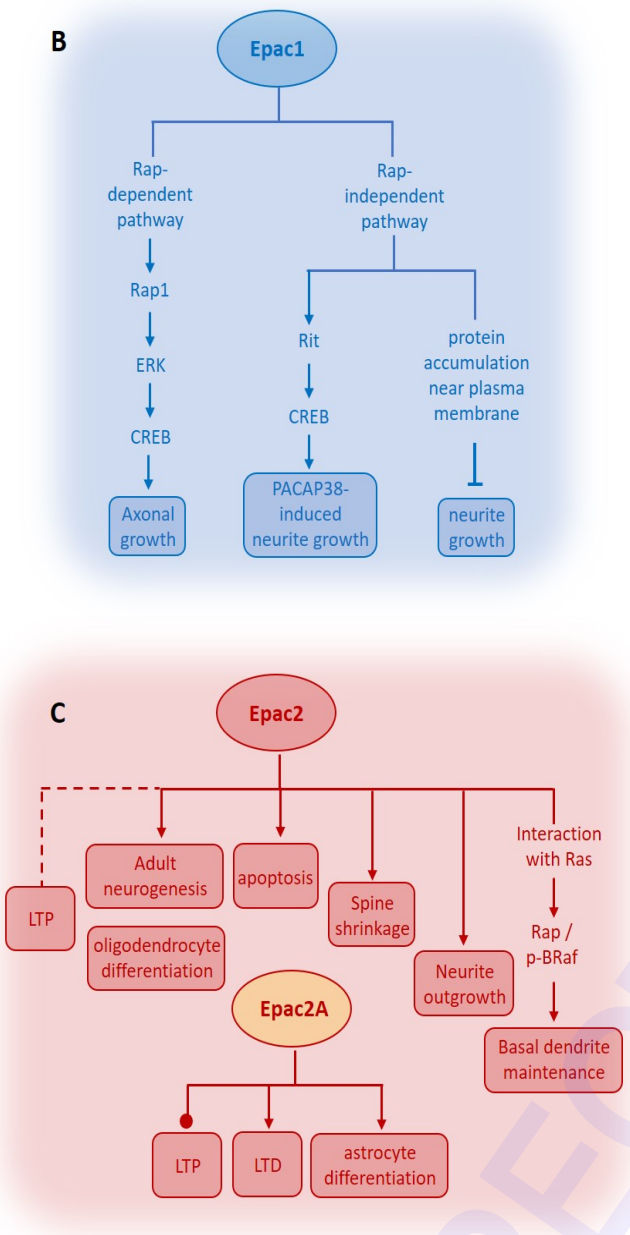

Fig. 3.

Table 1. Summary of Epac2 isoforms

Protein name			Epac2A1	Epac2A2	Epac2B	Epac2C
Transcript name		NCBI	transcript variant 1	transcript variant 2	transcript variant 3	-
		Ensembl	RAPGEF4-202	RAPGEF4-203	RAPGEF4-201	
ID	NCBI	Transcript	NM_001204165.1	NM_019688.2	NM_001204166.1	-
		Protein	NP_001191094.1	NP_062662.1	NP_001191095.1	-
	EBI	Transcript	ENSMUST00000090826.11	ENSMUST00000102698.9	ENSMUST00000028525.5	-
		Protein	ENSMUSP00000088336.5	ENSMUSP00000099759.3	ENSMUSP00000028525.5	-
Exon number			31	30	28	
Protein length (a.a)			1011	993	867	696
Protein expression in tissue[a]		Brain	+	+	-	-
		Adrenal gl.	+	-	+	-
		Pancreas	+	-	+	-
		Kidney	-	-	+	-
		Liver	-	-	-	+
orthologous splicing isoform in human (NCBI ID)			transcript variant 1 (NM_007023.4)	transcript variant 8 (NM_001375866.1)	transcript variant 2 (NM_001100397.2)	

a, citation from reference (5)