Medical Hypotheses 81 (2013) 216–218

Contents lists available at SciVerse ScienceDirect

Medical Hypotheses

journal homepage: www.elsevier.com/locate/mehy

EPAC–STX interaction may play a role in neurodevelopment/neurogenesis

Ali Razmi [a,b,*], Samane Jahanabadi [a,c], Mousa Sahebgharani [a], Mohammad-Reza Zarrindast [a]

[a] Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
[b] Pharmacology and Applied Medicine Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
[c] Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

ARTICLE INFO

Article history:
Received 15 January 2013
Accepted 26 April 2013

ABSTRACT

During embryonic life a group of cells become proliferated, migrated and differentiated to develop central nervous system. Migration has been suggested to be due to accumulation of polysialic acid (PSA), a negatively-charged glycoside, on the outer cell membrane. The same event happens to PSA in a tumor mass as well. Polysialylation is the product of polysialyl transfrase isozymes; STX (ST8SIA2), the embryonic active isoform, and PST (ST8SIA4), expressed in adults CNS. Additionally, cAMP concludes to activation of PKA and EPAC resulting to the initiation of gene expressions which are highly required during development. EPAC, the latter known target of cAMP in mammalian nervous system, has proliferative properties in the developing CNS. We propose for the proper action of EPAC, namely CNS development, the presence of STX and its elevation after EPAC activation is mandatory. This hypothesis is put forward after observing, in a preliminary experiment, a relationship between EPAC activation and STX mRNA expression levels in rat hippocampus. The interaction between EPAC and STX may be suggested to be through EPAC-induced gene expression of the latter. From the above assumptions one may suggest the use of EPAC activators as neurogenesis inducers and its inhibitors as tumor modulators.

Background

cAMP pathway

Cyclic adenosine monophosphate (cAMP) is the second messenger of the receptors which are coupled to Gs proteins. It relays signal from cell membrane to the next response transducer agents after its production by adenylyl cyclase. Cyclic nucleotide-gated and hyperpolarization-activated channels, cyclic nucleotide-activated ion channels, protein kinase A (PKA) and exchange protein directly activated by cAMP (EPAC) are the known targets of the cAMP [1]. Among these, PKA and EPAC activation initiate the phosphorylation cascade including ERK (extracellular signal-regulated kinase), Rap (Ras-related protein), Rho [2,3] and phospholipase C [4]. The phosphorylations could conclude to activation of nucleus targets following by gene expression and new protein synthesis.

NCAM and PSA-NCAM

NCAMs (Neural Cell Adhesion Molecules) of the CAM's immunoglobulin superfamily, mediate neural development by their homophilic and heterophilic interactions. They involve in neurite outgrowth, cell migration, differentiation, synaptogenesis and survival procedures [5–8]. In spite of their zipper role in adhesion of neurites, NCAM interactions could initiate intracellular signals [9]. NCAMs are expressed in growth cones and construct cell–cell contacts. However, the controlling process of their gene expressions are largely unknown [10].

NCAM is affected by several posttranslational modifications such as polysialylation. Polysialic acid (PSA), an α2,8-linked polymer of sialic acid, was discovered for the first time by Jukka Finne in a developing brain [11]. Negative charges which surround PSA polymers absorb more water molecules near the outer cell membrane which leads to repulsion and increasing distances between cells during cell migration in the developing CNS [12]. Polysialylation is the product of two isozymes; ST8SIA2 (STX), predominantly expressed during embryonic life, and ST8SIA4 (PST), responsible for polysialilation of NCAM in adults rat brain [13].

CNS development

Involvement of cAMP

cAMP has a dominant role in axon formation during neuronal development. The decline of cAMP activity is the characteristic of the later stages of vertebrate embryogenesis which keeps going down during postnatal and adult neural growth [14]. Since the increase of cAMP levels is accompanied with axon outgrowth and guidance in neural regeneration following injury [15], the same

* Corresponding author. Address: Pharmacology & Applied Medicine Department of Medicinal Plants Research Center, Institute of Medicinal Plants, Academic Center for Education, Culture and Research (ACECR), Kavosh Boulevard, Supa Boulevard, P.O. Box 31375-1369, Kordan, Karaj, Iran. Tel.: +98 912 3945632; fax: +98 26 34764021.
E-mail address: arazmi@razi.tums.ac.ir (A. Razmi).

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http://dx.doi.org/10.1016/j.mehy.2013.04.047

phenomenon may also be involved in CNS developement. Thus, in embryonic life neural growth study, investigations of the cAMP pathway and the involved proteins are mandatory.

Proliferation

PKA and EPAC as downstream effectors of cAMP exert complementary effects in controlling cAMP-stimulated cell proliferation. Thus, the extent of proliferation can differ depending on PKA or EPAC activity. The effects of EPAC activity on proliferation is confirmed by several experiments. For instance, cAMP is shown to be involved in the rat thyroid cell proliferation with EPAC and PKA synergistically acting in nucleotide-mediated mitogenesis [16]. On the other hand, the anti-proliferative property of EPAC by cAMP is still controversial. There are studies which suggest that the increased PKA activity is responsible for anti-mitogenic effect of elevated cAMP. Other reports indicate the involvement of PKA-independent mechanisms, including EPAC over-expression, for anti-proliferative effects of cAMP [16].

Axon outgrowth

Myelination is the result of cAMP reduction in the mature neurons. Returning cAMP levels to embryonic stage causes axon regeneration of the mature neurons [15]. This function of cAMP is mediated by PKA in two phases: initial phase by inhibition of Rho [17] and the later one involvement of transcription factor (CREB) activation [18]. Rho GTPase mediates axon regeneration inhibitory properties of myelin proteins and chondroitin sulphate proteoglycan [19]. Furthermore, applying PKA specific siRNA leads to deficit in axon outgrowth [20].

Axon guidance

cAMP level changes modulate attraction and repulsion of the growth cone leading to axonal guidance in a developing CNS. Exposure to cues like acetylcholine, neurotrophin-3, brain derived neurotrophic factor (BDNF) and netrin-1 induces increased intracellular activity of cAMP followed by cone attraction, while repulsion is a consequence of low activity [21]. For example, stimulation of netrin enhances PKA activation and subsequently influences axon guidance through localization of the related receptors [22]. PKA and EPAC, the downstream effectors of cAMP, seem to mediate different signaling mechanisms on growth cone in embryonic and adult neurons. Thus, the EPAC or PKA activity can verify whether the response to a guidance cue will be attractive or repellent. Whilst high levels of EPAC results to attraction in the embryonic life, activation of PKA favors repulsion in adult neurons [23].

Hypothesis

We propose a hypothesis that differentiates the regulatory and proliferatory properties of the cAMP pathway in the CNS. The regulatory roles could be attributed to PKA, whilst proliferative actions are EPAC-mediated. Therefore, modulation of cAMP pathway by manipulation of related enzymes might have beneficial effects in clinical settings directly related to embryonic or adult neurogenesis.

Empirical data

The idea of the present hypothesis is originated from our previous experiment regarding the hippocampal cAMP pathway functions on rat's passive learning and memory. In exploring the probable role of cAMP signaling on the expression of NCAM and polysialylating enzymes, qRT-PCR data revealed a relationship between EPAC and St8sia2.

Briefly, 8-*p*CPT (8-(4-chlorophenylthio)-2′-*O*-methyladenosine-3′,5′-cyclic monophosphate), an EPAC activator, and 8-Br-cAMP, a selective PKA activator, were injected into hippocampus bilaterally through a guidance cannula for three consecutive days. After behavioral test, the rats' hippocampi were collected for evaluation of the proteins changes. Total RNA were extracted and cDNA was synthesized through Maniatis protocol [24]. Quantitative real-time PCR experiments were done using QuantiFast SYBR Green PCR Kit, rat's St8sia2, St8sia4 and Gapdh primers (Qiagen, Germany). Products specificity was confirmed using agarose gel electrophoresis and melting curve analysis. Changes in expressions were calculated using $2^{-\Delta\Delta C_T}$ method [25] and were presented as fold changes in expression using REST 2008 software.

St8sia4 (PST) mRNA expression levels were decreased in 8-Br-cAMP - treated and were not changed in 8-*p*CPT treated hippocampi (Fig. 1a). The unexpected results were obtained in expression levels of STX mRNA of the tissues which were received 8-*p*CPT; as +4 and −2-fold changes in EPAC and PKA activated hippocampi, respectively (Fig. 1b).

Evaluation of the hypothesis

There are a lot of proteins involved in developmental processes of embryonic life of which some will continue to play a regulatory role through the adult life as well. Regarding neuronal development in embryonic life, some proteins play a dominant role while others will have an increasing pattern in the later stage.

EPAC expression is under constant regulation during CNS development. Microarray data reveal that Pka (Prkaca and Prkacb) expression has an ascending profile in the CNS of the developing mouse [26] while EPAC1, the active isomer, expression shows

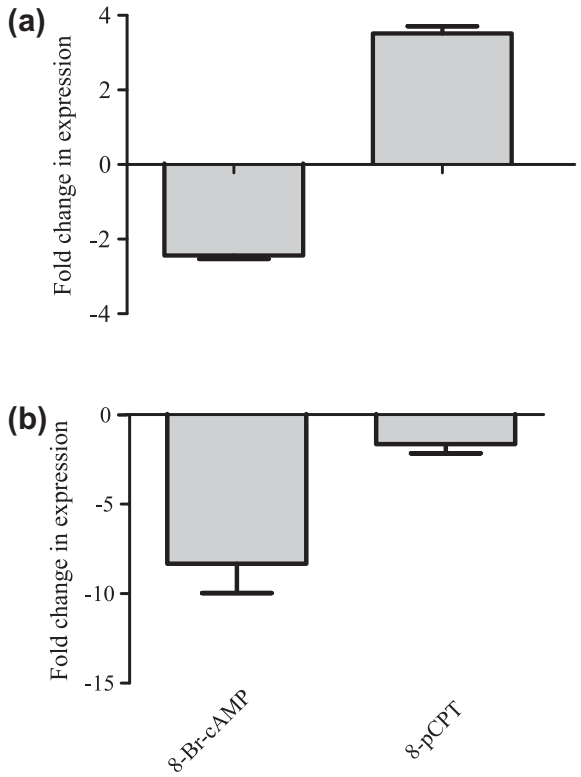

Fig. 1. St8sia2 (a) and St8sia4, (b) mRNA expression of 8-Br-cAMP (1.25 μg), and 8-*p*CPT-2′-*O*-ME-cAMP (5 μg) in the 3 days treated hippocampi. Y axis demonstrates fold changes in mRNA expression levels relative to control group. The mRNA levels in hippocampus of all groups are normalized with mRNA levels of Gapdh. Data are presented as Mean ± STD ($n = 4$, $P < 0.05$ considered as significant difference).

descending trend during development and postnatal rat brain, spinal cord and dorsal root ganglion [27].

Intracellular signal transduction is dependent to kinases as key regulators of a developing brain and cAMP is known as a major propagator of the kinase activation. Moreover, there are couples of anti-tumor substances which act through inhibition of these enzymes like multi-kinase inhibitors and some new compounds [28,29]. Due to over-expression of EPAC in human pancreatic ductal adenocarcinoma cells, applying selective inhibitor of EPAC resulted to antitumor properties without involvement in regulatory kinase [30]. On the other hand, stimulating roles of PSA in tumor cell growth, differentiation [31] and invasion [32] have been reported. PST expresses in normal and tumor tissue, in contrary to STX (the embryonic isoform) which only expresses in proliferative tumor cells [33].

As mentioned in the section of experimental procedure, following the EPAC activation, we have observed an increase in St8sia2 mRNA expression while, following PKA activation a decrease in St8sia2 expression was observed.

If one extrapolates the present data of the hippocampus to entire nervous system, it can be concluded that expression of STX in a developing CNS is dependent on EPAC activation. However, the present data does not rule out the involvement of other cAMP related proteins and other signaling pathways affecting on expression of proteins, a subject which needs further experiments.

Relation between STX/PST expression and EPAC/PKA activation has not been investigated yet in neuronal cells. Both STX and EPAC show proliferatory properties with the same time pattern of expression in the embryonic life. Based on the above observations, EPAC inhibitors can be suggested as a new anti-tumor therapy tool.

It is possible to induce neural sprouting by controlling EPAC function on NCAM modifications [34]. Therefore, administration of EPAC enhancers can stimulate neurogenesis in a clinical approach.

The mechanism of how cAMP levels could modify the growth cone response to axonal guidance is still not fully understood. The involvement of EPAC may be regarded as key regulator of this pathway.

Conflict of interest statement

None.

Acknowledgement

Experiments of the present article were supported by grant No. 10507 from Tehran University of Medical Sciences. We thank Dr. Bijan Djahanguiri for his kind help and advice.

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