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Review

The role of soluble adenylyl cyclase in neurite outgrowth ☆

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ABSTRACT

Axon regeneration in the mature central nervous system is limited by extrinsic inhibitory signals and a postnatal decline in neurons' intrinsic growth capacity. Neuronal levels of the second messenger cAMP are important in regulating both intrinsic growth capacity and neurons' responses to extrinsic factors. Approaches which increase intracellular cAMP in neurons enhance neurite outgrowth and facilitate regeneration after injury. Thus, understanding the factors which affect cAMP in neurons is of potential therapeutic importance. Recently, soluble adenylyl cyclase (sAC, ADCY10), the ubiquitous, non-transmembrane adenylyl cyclase, was found to play a key role in neuronal survival and axon growth. sAC is activated by bicarbonate and cations and may translate physiologic signals from metabolism and electrical activity into a neuron's decision to survive or regenerate. Here we critically review the literature surrounding sAC and cAMP signaling in neurons to further elucidate the potential role of sAC signaling in neurite outgrowth and regeneration. This article is part of a Special Issue entitled: The role of soluble adenylyl cyclase in health and disease.

1. Introduction

Unlike immature neurons that demonstrate a robust capacity to regenerate, adult mammalian central nervous system (CNS) neurons fail to regenerate in response to injury [1,2]. Failed adult regeneration can be attributed to both intrinsic and extrinsic factors. In the adult CNS, injury results in the release or deposition of inhibitory molecules within myelin [3–6] and the glial scar [7–9]. A relative insufficiency of neurotrophic signaling also contributes [10]. Additionally, neurons decrease in their intrinsic growth capacity as a function of maturity, as reflected by a postnatal decline in ability for rapid neurite extension [11]. While simply blocking the expression or presentation of glial-associated inhibitory factors can promote axonal regeneration after injury [6,8], the relative ability of immature neurons to overcome inhibitory cues [12–14] suggests that therapeutic targeting of the factors that influence the intrinsic growth state of the neuron may be effective in regenerative medicine.

Levels of the second messenger adenosine 3′-5′-cyclic monophosphate (cAMP) correlate with intrinsic neurite outgrowth capacity of neurons [15]. The effect of cAMP on neurite outgrowth was first demonstrated in chick dorsal root ganglia cells, in which cAMP treatment increased both length and number of axons [16]. Since then, the role of cAMP in the growth and guidance of axons has been extensively studied. As neurons mature, intracellular cAMP levels decline along with the ability of axons to regenerate after injury [15,17]. In mature neurons, increasing cAMP reverses this effect, and attenuates inhibitory signaling derived from molecules in CNS myelin and the glial scar [18]. Understanding the mechanisms underlying cAMP production may be vital for the development of therapeutic strategies to address regenerative failure in CNS injury. In this review we will discuss soluble adenylyl cyclase (sAC), a member of the adenylyl cyclase (AC) family that has recently been recognized as a potent regulator of neurite outgrowth and neuronal survival [19].

2. cAMP in neurite outgrowth

The induction of neurite outgrowth by cAMP can be divided into 2 phases, the first acting at the growth cone/axonal tip and the second at the cell body and nucleus, inducing transcriptional changes that sustain neurite outgrowth [20,21]. Growth cones are a specialized form of lamellipodia responsible for guiding and exerting tension on the trailing axon [22]. At the growth cone, cAMP-mediated activation of protein kinase A (PKA) causes inactivation of the small G-protein Rho, which results in potentiation of neurite outgrowth [14]. While growth cone dynamics are important for proper axonal guidance and motility, their activities are largely independent from neurite assembly [23]. The ability of cAMP to influence growth cone directionality was first observed in experiments utilizing extracellular, cell-permeable, cAMP gradients, which induced turning and directional attraction of growth cones [24]. When a gradient of cell-permeable cAMP was replaced with a bath application, modulation of responses to other signaling molecules (see below) was observed [25]. In some cases, axons were even found to exhibit opposite reactions to guidance cues

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when cAMP was present versus absent, indicating a potent role of cAMP signaling in the intrinsic regulation of neuronal growth responses [26].

What are the cues being modulated by cAMP and how does cAMP exert these effects? Paracrine factors are critical for the proper guidance and development of neurons. One family of structurally related molecules referred to as neurotrophins has been widely studied and is involved in a variety of neuronal functions [27]. The first discovered neurotrophin was nerve growth factor (NGF) [28]. While NGF was the first discovered, and arguably most heavily studied neurotrophin, it may not be the best surrogate from which to infer a role for cAMP in neurotrophin signaling. Only a select few neuronal subtypes respond to NGF, indicating that the neurotrophic effects for this specific molecule may be unique and/or highly specialized [29]. Other well-characterized neurotrophins include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) [27,30]. Like NGF, other neurotrophins induce survival and neurite growth in various, often specific neuronal subtypes. All neurotrophins bind to a sub-family of receptor tyrosine kinases (RTKs) known as tropomyosin receptor kinase (Trk) receptors [31], in addition to binding with a lower affinity to the tumor necrosis alpha-receptor (TNFR) family member p75 [32]. While all neurotrophins bind p75, they differentially bind to for individual Trk receptors. TrkA is specific for NGF, TrkB binds both BDNF and NT4/5, and TrkC binds NT3 [33]. Activation of Trk receptors is similarly sufficient to promote survival and neurite growth [34,35].

BDNF activation of TrkB, as well as the trafficking of this receptor, is cAMP-dependent [36]. In contrast, NGF-mediated and NT-3-mediated phosphorylation of TrkA and TrkC, respectively, do not require cAMP. This is intriguing as NGF and NT-3 have considerably weaker capacity to promote survival or regeneration in retinal ganglion cells [37], while both neurotrophin ligands of TrkB (BDNF and NT-4/5) demonstrate benefit in both regards [38]. Additionally, 'priming' neurons via pre-treatment with BDNF overcome inhibitory cues in CNS myelin in a cAMP-dependent manner [39,40]. The ability of TrkB ligands to modify regenerative capacity is consistent with the theory that cAMP is important in mediating growth and regenerative capacity of neurons.

Other signaling ligands similarly promote neurite growth, albeit through different signaling pathways. For example, pituitary adenylyl cyclase-activating peptide (PACAP) demonstrates similar neurite outgrowth promotion as NGF in PC12 cells [41] and also promotes neurite outgrowth and survival in primary neurons [42–44]. PACAP exerts these effects via activation of transmembrane ACs (tmACs) which associate with the PACAP receptor, PAC1 [41]. PACAP and NGF both activate the small GTP-binding protein Rap1, which leads to sustained activation of ERK mitogen-activated protein kinase, which is required for neurite outgrowth [41,45–48]. NGF activation of Rap1 has been suggested to be dependent on protein kinase A (PKA) activation, which requires cAMP [45,46], albeit whether NGF-dependent neurite extension is cAMP-dependent is controversial [49]. However, synergistic effects of cAMP and NGF on signaling and neurite outgrowth have been observed [50], and NGF-dependent neurite outgrowth is not supported when NGF is replaced by cAMP [51], which confirms that distinct signaling pathways exist for NGF and cAMP.

The role of cAMP in the nucleus-mediated promotion of neurite outgrowth has been most closely tied to phosphorylation of cAMP response element binding protein (CREB) [52,53]. Increased intracellular cAMP leads to phosphorylation of CREB, which is critical for the transcriptional programs needed for neuritogenesis [54]. Constitutively activated CREB results in robust neurite outgrowth and attenuated responsiveness to CNS molecules which inhibit regeneration [53]. Interestingly, these effects mirror the response of neurons pre-treated with exogenous BDNF and/or cAMP [18,53].

Besides neurotrophin stimulation, neuronal activity is beneficial for both neuronal survival and proper guidance and innervation during development [19,55,56]. In the eye, spontaneous retinal activity is required for proper development, and this spontaneous activity results in increased intracellular calcium and cAMP [57]. Such calcium transients are known to affect in vivo axon extension and pathfinding [58]. Damaged mature neurons have both decreased spontaneous electrical activity and decreased cAMP and calcium influx. The correlation between spontaneous activity of immature neurons and calcium/cAMP levels further supports the importance of cAMP signaling as a therapeutic target for CNS regeneration.

3. How is cAMP produced?

Adenylyl cyclases (ACs) are the family of enzymes that synthesize cAMP. There are 9 mammalian transmembrane ACs (tmACs) that are activated by G-protein coupled receptors (GPCRs). While all tmACs are activated by Gsα, these tmACs are differentially regulated by Gβγ, protein kinase phosphorylation and calcium. In addition to signaling in response to extracellular stimuli at the plasma membrane, tmACs also can continue cAMP production even after clearance from the plasma membrane associated with uptake and trafficking of membrane-derived endosomes [59,60]. In contrast, sAC (ACDY10) is unique among the ACs in lacking transmembrane domains, insensitivity to G-proteins, and bicarbonate-mediated activation, in addition to being activated by calcium. While sAC is most readily detectable in the testis where it has a role in sperm maturation [61], bicarbonate-stimulated cAMP production has been observed in most other tissues and is thought to be associated with intracellular organelles like the mitochondrion and the nucleus [62]. Due to ubiquitous carbonic anhydrases, intracellular pH affects bicarbonate levels that in turn directly modulate sAC activity (Fig. 1) [63]. As mentioned above, sAC is also activated by divalent cations such as calcium [64], via an independent mechanism that can be synergistic with bicarbonate [65].

cAMP and tmAC function have classically been studied using forskolin, a potent pharmacologic activator of all tmACs except AC9 [66,67]. sAC is also insensitive to direct stimulation by forskolin [68]. Elucidating the individual functions of different ACs has been hindered by a lack of useful antibodies for the different ACs, as well as a lack of specific inhibitors [54,69,70]. KH7 is an inhibitor that shows selectivity for sAC and has been used in conjunction with RNA interference (RNAi) to demonstrate sAC-specific functions [71]. Recently, knockout mice for individual ACs, including sAC, have become available that should allow assignment of specific cyclase functions.

4. Soluble adenylyl cyclase

The first reported observation of a "soluble" AC with no transmembrane association was in 1975 [72]. Over 2 decades later the purification and cloning of sAC was reported [73]. The newly discovered sAC cDNA encoded a 187-kDa protein containing 2 catalytic domains (C1 and C2) that demonstrated similarity to AC domains from different cyanobacterial species, suggesting that the sAC catalytic domains evolved as a fusion of 2 distinct bacterial cyclases [73]. The cyanobacterial ACs also demonstrate bicarbonate sensitivity, again supporting an evolutionarily conserved bicarbonate-mediated activity [61,73]. This confirmation of a mammalian, bicarbonate-sensitive AC provided a vital insight into the mechanisms underlying bicarbonate-induced spermatozoa competence and other tmAC-independent, cAMP-mediated cellular events [74–77].

Human sAC is the product of the ADCY10 gene, which is >100 kb and is comprised of >33 exons [64]. While there is a single mammalian sAC gene, there are a number of different sAC mRNAs that have been identified by PCR from various cell types. These isoforms are consistent with ADCY10 mRNA alternative RNA splicing and promoter usage [78,79]. However, validation of the existence of these various sAC isoforms has been difficult due to an inability to reliably detect sAC protein by western blotting in tissues other than the testes. The original purification of sAC from the testis in both rat and human also revealed an abundant, functional 48 kDa protein [80,81]. This smaller sAC may be a cleavage product of the larger 187-kDa protein, although an alternatively-