Soluble Adenylyl Cyclase (sAC) Rescues Neurons from Inhibitory Myelin Cues

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Abstract
Myelin associated proteins are known to pose hurdles to central nervous system (CNS) axon regeneration. The myelin mediated inhibition on axon regeneration can be reversed by brain derived neurotrophic factor (BDNF). BDNF enhances cyclic AMP (cAMP) levels for eliciting its beneficial effects on axon regeneration. A recent article by Martinez et al., revealed that soluble adenylyl cyclase (sAC) is a potential catalytic source for cAMP in BDNF stimulated neurons. Interestingly BDNF stimulated or exogenously administered sAC was able to reverse the inhibitory effects of myelin on axon regeneration suggesting that sAC is a potential therapeutic opportunity in CNS injury.

Keywords: BDNF, cAMP, MAG, sAC, neurite outgrowth, tmAC

Central nervous system (CNS) axon regeneration is cumbersome and incomplete due to resident inhibitory myelin cues (Yiu G, He Z., 2006). Myelin derived proteins such as myelin associated glycoprotein (MAG), Nogo A, ephrin B3 etc. pose tough hurdles for axons to grow along the physical nerve tracts (Yiu G, He Z., 2006). Inhibition of these myelin associated proteins may facilitate regeneration of axons to long-distances, however a complete repopulation of full axonal length is still far from achieving. Enriching nerve regeneration milieu with neurotrophins has been shown to reverse myelin block and facilitate axon regeneration; mediated through multiple mechanisms spanning from transcriptional regulation of growth modulators to modification of critical kinases and phosphatases (Richner et al., 2014). For example, Brain derived neurotrophic factor (BDNF) has been successful in fostering regeneration of both central and peripheral axons (Richner et al., 2014). BDNF actions involve generation of cAMP followed by activation of protein kinase A and CREB (cAMP response element-binding protein) resulting in enhanced axon growth (Cai et al., 1999). In canonical GPCR (G-protein coupled receptor) signaling transmembrane adenylyl cyclase (tmAC) functions as a catalytic source for cAMP. However, as BDNF acts independent of GPCR, the exact source of cAMP downstream of BDNF treatment in neurons was a matter of interest. A recent study by Martinez and colleagues demonstrated that soluble adenylyl cyclase (sAC) is a catalytic source for cAMP in BDNF stimulated neurons (Martinez et al., 2014). The authors showed that sAC dependent cAMP production is sufficient for BDNF to reverse myelin mediated inhibition on axon regeneration.

Unlike tmAC, sAC is not membrane anchored, independent of GPCR and distributed in small micro domains within the cell (Zippin et al., 2003). Martinez and colleagues identified the expression of sAC in isolated cortical and cerebellar neurons; localized throughout the cell body and neurites. Interestingly, selective inhibition of sAC, not tmAC, by two distinct pharmacological agents KH7 and OH-E, completely reversed BDNF stimulated outgrowth of neurons that were grown on myelin-rich environment. The reversal in BDNF efficacy on neurite outgrowth, mediated through sAC inhibition, was accompanied by a reduction in cAMP levels indicating the critical involvement of cAMP in BDNF stimulated, sAC dependent neuronal sprouting. Further, siRNA mediated sAC knockdown in cerebellar neurons inhibited BDNF dependent neurite outgrowth *in vitro*. BDNF

treated sAC C1 neurons in which the first catalytic domain of sAC was deleted, failed to outgrow in myelin rich environment. Interestingly, nourishing neurons with sAC using sAC expressing lentivirus enhanced neurite outgrowth *in vitro* and optic nerve regeneration *in vivo* suggesting a potential role for sAC in augmenting neuron regeneration.

Is sAC the exclusive molecular determinant for cAMP downstream of BDNF? The authors showed that inhibition of sAC could completely reverse BDNF dependent cAMP induction in cerebellar neurons. However, there are multiple other possibilities by which BDNF may raise cAMP levels. For example, BDNF may limit the degradation of cAMP through inhibition of phosphodiesterase (PDE) (Gao et al., 2003). BDNF is a potent calcium stimulator. Increased calcium influx activates tmACs which in turn may induce cAMP levels (Ferguson and Storm, 2004). However, the authors have not investigated these possibilities in their experiments. The authors studied sAC dependent cAMP induction only at shorter intervals of BDNF stimulation (2 minutes). Similarly involvement of sAC in BDNF dependent neuronal growth was assessed at shorter intervals of BDNF stimulation; 15-17h. Even though cAMP has shorter half-life, experiments demonstrating the involvement of sAC on cAMP production at longer intervals of BDNF stimulation may give more insights on whether sAC is the rate limiting factor for BDNF dependent cAMP production and enhanced neurite outgrowth.

Martinez *et al*,. and multiple other reports have shown that sAC is sufficient to promote CNS axon growth (Corredor et al., 2012; Martinez et al., 2014). sAC is sufficient to promote the growth of PNS (peripheral nervous system) axons as well(Wu et al., 2006). This raises the question whether supplementation of sAC is a better approach than BDNF administration in enhancing nerve regeneration? sAC and BDNF mediate their effects mainly through cAMP. sAC may directly increase the catalysis of ATP to cAMP and raise endogenous cAMP levels. In contrast BDNF may have multi-cascade approach: activation of sAC, calcium influx and inhibition of PDE for raising cAMP levels (Gao et al., 2003; Martinez et al., 2014). A cAMP independent mechanism also been been attributed to BDNF mediated enhanced neurite outgrowth (Hultman et al., 2014). However aberrant cAMP levels may be detrimental; carcinogenic, induce apoptosis and cause memory dysfunction (Dumaz et al.,2006; paspalas et al., 2013; Ladilov and Appukuttan., 2014). Therefore, even if BDNF is likely a better approach than sAC in raising cAMP levels, it may not be always beneficial to neurons. In contrast, sAC supplementation may deliver optimal levels of cAMP and thus may be a better promising opportunity. A comparative study on the potential of BDNF and sAC in modulating cAMP levels and axon growth in long-term nerve regeneration models would bring additional insights on this argument.

Whether sAC is indispensable for neuron growth and survival is inconclusive from the literature available. sAC has been shown to be essential for RGC (retinal ganglion cells) growth and survival (Corredor et al., 2012) . However, Martinez etal,. showed that the basal neurite outgrowth is unaltered in sAC inhibited neurons. Interestingly sAC C1 knockout mouse shows no defect in basal axonal growth(Moore et al., 2008). Hence, it may be likely that sAC is involved, but dispensable for enhancing axonal growth. The essential Involvement of sAC facilitates netrin mediated growth cone extension is also controversial (Moore et al., 2008; Wu et al., 2011). In their present article, Martinez et al,. demonstrated that sAC is essential for reversing myelin block by BDNF (Martinez et al., 2014) . Additionally the authors showed beyond doubt that sAC supplementation facilitates axonal elongation. Overall Martinez and colleagues reinforce the emerging concept that sAC is a critical endogenous regulator for cAMP in BDNF stimulated and regenerating neurons.

sAC is distributed in micro domains within a cell (Zippin et al., 2003). cAMP being non-diffusive and functionally active in the immediate vicinity around its source, extensive studies on sAC in the context of nerve regeneration would definitely expose the critical roles of these neuronal micro domains in neuron regeneration.

How sAC is regulated in injured neurons; does sAC complement tmAC, is sAC superior to tmAC or is sAC essential for injured neurons to survive and grow, is yet to be uncovered. Also quantification of sAC expression in neurons at pre and post injury intervals may reveal the kinetics of endogenous modulation of sAC and its effect on cAMP synthesis and intrinsic growth capacity of injured neurons. The potential of a combined treatment of neurotrophin or electrical stimulation and sAC in facilitating neuronal growth would be worth a study. It would be also interesting to explore the behavior of sAC in Schwann Cells in injured nerves, which will help in profiling sAC as a therapeutic option for peripheral nerve regeneration.

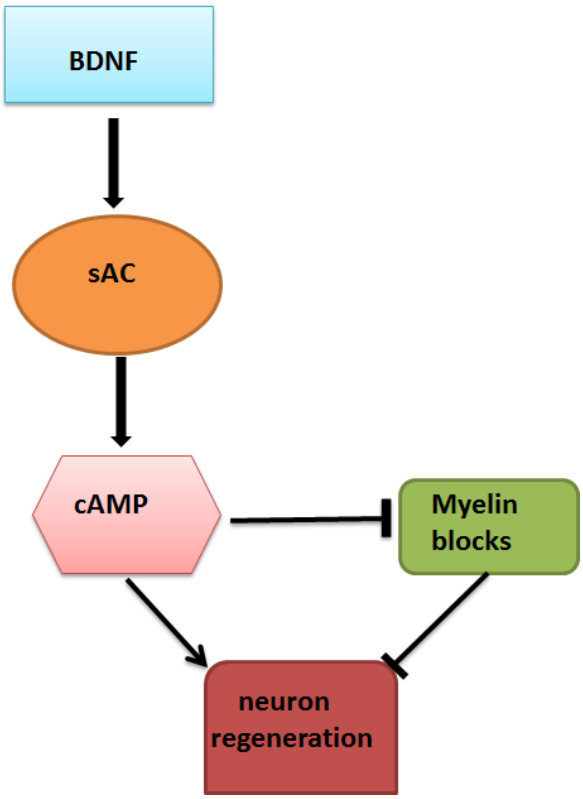

Figure.1. sAC is involved in BDNF dependent neuron regeneration. sAC is activated by BDNF which in turn induce cAMP production. These cAMPs promote neuron regeneration by relieving myelin inhibitory cues.

Conflict of interest

The authors declare no conflict of interest

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