The International Journal of Biochemistry & Cell Biology 56 (2014) 66–73

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Cyclic AMP and the regeneration of retinal ganglion cell axons☆

Mats Hellström[1], Alan R. Harvey*

School of Anatomy, Physiology and Human Biology, The University of Western Australia, Crawley, WA 6009, Australia

ARTICLE INFO

Article history:
Received 20 February 2014
Received in revised form 10 April 2014
Accepted 22 April 2014
Available online 4 May 2014

Keywords:
Retinal ganglion cell
Regeneration
Cyclic AMP
Protein kinase A
Exchange protein activated by cAMP

ABSTRACT

In this paper we present a brief review of studies that have reported therapeutic benefits of elevated cAMP on plasticity and regeneration after injury to the central nervous system (CNS). We also provide new data on the cellular mechanisms by which elevation of cyclic adenosine monophosphate (cAMP) promotes cytokine driven regeneration of adult CNS axons, using the visual system as the experimental model. cAMP is a second messenger for many intracellular signalling pathways. Elevation of cAMP in the eye by intravitreal injection of the cell permeant analogue (8-(4-chlorophenylthio)-adenosine-3′,5′-cyclic monophosphate; CPT-cAMP), when added to recombinant ciliary neurotrophic factor (rCNTF), significantly enhances rCNTF-induced regeneration of adult rat retinal ganglion cell (RGC) axons into peripheral nerve (PN) grafted onto transected optic nerve. This effect is mediated to some extent by protein kinase A (PKA) signalling, but CPT-cAMP also acts via PI3K/Akt signalling to reduce suppressor of cytokine signalling protein 3 (SOCS3) activity in RGCs. Another target for cAMP is the exchange protein activated by cAMP (Epac), which can also mediate cAMP-induced axonal growth. Here we describe some novel results and discuss to what extent the pro-regenerative effects of CPT-cAMP on adult RGCs are mediated via Epac as well as via PKA-dependent pathways. We used the established PN–optic nerve graft model and quantified the survival and regenerative growth of adult rat RGCs after intravitreal injection of rCNTF in combination with a selective activator of PKA and/or a specific activator of Epac. Viable RGCs were identified by βIII-tubulin immunohistochemistry and regenerating RGCs retrogradely labelled and quantified after an injection of fluorogold into the distal end of the PN grafts, 4 weeks post-transplantation. The specific agonists of either PKA or Epac were both effective in enhancing the effects of rCNTF on RGC axonal regeneration, but interestingly, injections that combined rCNTF with both agonists were significantly less effective. The results are discussed in relation to previous CPT-cAMP studies on RGCs, and we also consider the need to modulate cAMP levels in order to obtain the most functionally effective regenerative response after CNS trauma.

This article is part of a directed issue entitled: Regenerative Medicine: the challenge of translation.

1. Introduction

Cyclic adenosine monophosphate (cAMP) has long been known to act as a second messenger for a large number of receptors and plays a key role in transduction and cell signalling in many pathways and biological systems. In the mammalian central nervous system (CNS), cAMP plays an important role in cell survival and the stimulation and guidance of axonal growth during CNS development. However, during later stages of embryogenesis, there is a fall in cAMP levels that is associated with altered axon growth and responsiveness to guidance cues. For example, endogenous cAMP levels are high in post natal day (P) 1 dorsal root ganglion (DRG) neurons which have the ability to grow neurites on MAG and myelin substrates. By P3–P4 however, a decrease in cAMP levels in these sensory neurons is linked to regeneration failure (Cai et al., 2001). A decrease in endogenous cAMP levels is also seen in RGCs as they mature (Cai et al., 2001; Argaw et al., 2008). Down-regulation of cAMP reduces the capacity of neurons to respond to neurotrophic factors (Meyer-Franke et al., 1998; Shen et al., 1999) and contributes to the onset of sensitivity to inhibitory molecules in the maturing CNS environment (Lehmann et al., 1999; Cai et al., 2002).

☆ This article is part of a directed issue entitled: Regenerative Medicine: the challenge of translation.

* Corresponding author at: School of Anatomy, Physiology and Human Biology, M309, The University of Western Australia, 35, Stirling Highway, Crawley, WA 6009, Australia. Tel.: +61 8 6488 3294; fax: +61 8 6488 1051.
E-mail address: alan.harvey@uwa.edu.au (A.R. Harvey).
[1] Current address: Laboratory for Transplantation and Regenerative Medicine, Dept. of Obstetrics and Gynaecology, Sahlgrenska Academy, University of Gothenburg, Sweden.

http://dx.doi.org/10.1016/j.biocel.2014.04.018
1357-2725/

In the absence of therapeutic interventions, cAMP levels are reduced in the brain after traumatic injury (Atkins et al., 2007) and in the retina after ON injury (Cui et al., 2003). Experimental elevation of cAMP promotes a regenerative response by alteration of neurotrophin receptor levels, changing cellular responsiveness to adhesion molecules and growth inhibitory molecules, and/or by activation of transcription factors and altering cellular metabolism (Meyer-Franke et al., 1998; Cai et al., 2002; Li et al., 2003; Gao et al., 2004; Spencer and Filbin, 2004; Lemons and Condic, 2006; Peace and Shewan, 2011; Hannila et al., 2013). Many *in vivo* studies have shown that elevation of cAMP in injured neurons can promote axonal regrowth, thus cAMP and its downstream targets are interesting candidates in studies aimed at stimulating the repair of damaged central pathways and tracts in the adult CNS (e.g. Corredor et al., 2013; Cui et al., 2003; Dell et al., 2013; Liu and Brady, 2004; Lu et al., 2004; Pearse et al., 2004; Qiu et al., 2002; Hannila and Filbin, 2008; Peace and Shewan, 2011; Hannila et al., 2013; Lau et al., 2013; Lu et al., 2012; Luo et al., 2013; Yin et al., 2013).

Intracellular cAMP elevation can be achieved via direct delivery of cAMP analogues (Shen et al., 1999; Cui et al., 2003; Park et al., 2004; Pearse et al., 2004) or by forskolin (Chierzi et al., 2005; Watanabe et al., 2003), or by inhibiting the hydrolysis of cAMP by phosphodiesterase inhibitors (such as IBMX or Rolipram) (Nikulina et al., 2004; Pearse et al., 2004; Chierzi et al., 2005; Costa et al., 2013). cAMP levels can also be raised by activation of the soluble adenylate cyclase, a cytosolic enzyme that converts adenosine triphosphate (ATP) to cAMP (Corredor et al., 2012).

1.1. cAMP in visual system repair

The visual system has been widely used to study mechanisms that suppress or promote axonal regeneration after CNS injury (e.g. Harvey et al., 2006; Benowitz and Yin, 2008; Berry et al., 2008; Fischer and Leibinger, 2012; Morgan-Warren et al., 2013). Using the rodent optic nerve (ON) crush model, elevation of cAMP has been reported to potentiate the regeneration of axotomized retinal ganglion cells (RGCs) (Choi et al., 2003; Monsul et al., 2004; Watanabe et al., 2003), and has been shown to potentiate the regenerative effects of oncomodulin-stimulated RGCs (Kurimoto et al., 2010; de Lima et al., 2012). Furthermore, when a peripheral nerve (PN) graft is transplanted onto the cut optic nerve (PN–ON graft) in adult rats, intravitreal injection of a cell permeant analogue of cAMP (8-(4-chlorophenylthio)-adenosine-3′,5′-cyclic monophosphate; CPT-cAMP) is ineffective by itself, but has a significant additive effect on axonal regeneration when injected in combination with recombinant ciliary neurotrophic factor (rCNTF) (Cui et al., 2003; Park et al., 2004). Compared to intact animals, activity of a downstream mediator of cAMP – protein kinase A (PKA) – was reduced after ON injury and PN transplantation, however injection of CNTF plus CPT-cAMP returned PKA levels close to normal, an effect that was blocked by the PKA pathway inhibitor KT5720 (Park et al., 2004). Overall, the beneficial effect of CPT-cAMP on RGC axonal regeneration was found to be mediated to a large extent by protein kinase A (PKA) signalling (Park et al., 2004; Rodger et al., 2005) although inhibition of mitogen-activated protein kinase (MAPK/ERK) or phosphotidyl inositol-3′-phosphate-kinase (PI3K)/akt also reduced the effects of CPT-cAMP on RGC axonal regeneration (Park et al., 2004).

Interestingly, it has also been shown that CPT-cAMP enhances regeneration of RGC axons by reducing rCNTF mediated activation of suppressor of cytokine signalling protein 3 (SOCS3), an inhibitor of axonal growth (Park et al., 2009; Smith et al., 2009; Hellström et al., 2011a,b). The suppression of SOCS3 by CPT-cAMP appears to require PI3K/Akt signalling (Park et al., 2009), consistent with previous work showing an increase in phospho-Akt levels after intraocular CPT-cAMP injections (Park et al., 2004). Activation of the PI3K/Akt pathway leads to an upregulation of mammalian target of rapamycin (mTOR), now known to facilitate neuroprotection and the regeneration of RGC axons after injury (Park et al., 2008, 2010; Leibinger et al., 2012; Morgan-Warren et al., 2013)

1.2. PKA and Epac are both activated by cAMP

PKA has been reported to be an important mediator of cAMP actions during developmental growth of RGC axons (Argaw et al., 2008), however PKA is now known not to be the only downstream enzyme regulated by cAMP. Another important target for cAMP is the exchange protein activated by cAMP (Epac) (Gloerich and Bos, 2010). As a result of a conformational change induced by cAMP, Epac is able to activate the GTPases, repressor-activator protein 1 (Rap1) and Rap2 (de Rooij et al., 1998; Kawasaki et al., 1998; Gloerich and Bos, 2010), which in turn signal via various effector proteins, including via the extracellular signal-regulated kinase (ERK)/p38-mitogen activated protein kinase (MAPK) pathway (Bos, 2003; Ster et al., 2007). Rap activation is important in cell–cell interactions in many tissues. In the normal CNS, Epac modulates postsynaptic excitability (Ster et al., 2009; Woolfrey et al., 2009; Gloerich and Bos, 2010) and may also play a role in CNS repair after injury (Murray and Shewan, 2008; Peace and Shewan, 2011). Further studies in the nervous system suggest that Epac and PKA can play opposing roles in regulating attractive and repulsive axon guidance respectively (Murray et al., 2009). Both Epac isoforms (Epac1 and Epac2) are expressed in RGCs (Whitaker and Cooper, 2010). Interestingly, the expression of Epac1 is higher early in development and declines over time (Shewan et al., 2002; Murray et al., 2008), at a time when neurons lose their growth potential. On the other hand, Epac2 is initially expressed at low levels but is up-regulated postnatally (Murray et al., 2008).

Given our observation of enhanced RGC axonal regeneration when CPT-cAMP is combined with rCNTF (Cui et al., 2003; Park et al., 2004; 2009; Hellström et al., 2011a,b), and given that both PKA and Epac can mediate cAMP-induced axonal regrowth (Wan et al., 2011; Xu et al., 2012; Murray and Shewan, 2008; Murray et al., 2009), we set out to investigate to what extent the pro-regenerative effects of CPT-cAMP on adult RGCs are mediated via Epac as well as via PKA-dependent pathways. We used the established PN–ON graft model (Vidal-Sanz et al., 1987; Cui et al., 2003; Hu et al., 2007; Hellström et al., 2011a,b) to analyse the survival and regenerative responses of axotomized adult rat RGCs after intravitreal injection of rCNTF, delivered in combination with a selective activator of PKA and/or a selective activator of Epac. Better information about which pharmacological agents are most effective in promoting CNS regeneration in particular circumstances has potential translational value in a clinical setting.

2. Methods

Twenty Wistar rats (8–10 weeks old; Animal Resources Centre, WA) were anaesthetized with a 1:1 mixture (1.5 mL/kg) of ketamine (100 mg/mL) and xylazine (20 mg/mL) and intraorbital ON axotomy was followed by attachment of a 1.5 cm length of autologous PN graft, as previously described (Cui et al., 2003; Hu et al., 2007; Hellström et al., 2011a,b). Eye ointment containing atropine sulphate (10 mg/g, Troy Ilium) was applied to protect the cornea during surgery. The grafts consisted of a segment of tibial nerve, a branch of the sciatic nerve, and were sutured onto the cut ON 1.5 mm behind the optic disc using 10/0 Ethilon suture (Johnson & Johnson, North Ryde, NSW, Australia) without compromising retinal blood supply. Rats also received a subcutaneous injection of buprenorphine (0.02 mg/kg, Temgesic; Reckitt & Colman, Hull, UK) and an intramuscular injection of Benacillin (0.3 mg/kg, Troy Ilium;