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Adenylyl cyclase signalling complexes – Pharmacological challenges and opportunities

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ABSTRACT

Signalling pathways involving the vital second messanger, cAMP, impact on most significant physiological processes. Unsurprisingly therefore, the activation and regulation of cAMP signalling is tightly controlled within the cell by processes including phosphorylation, the scaffolding of protein signalling complexes and subcellular compartmentalisation. This inherent complexity, along with the highly conserved structure of the catalytic sites among the nine membrane-bound adenylyl cyclases, presents significant challenges for efficient inhibition of cAMP signalling. Here, we will describe the biochemistry and cell biology of the family of membrane-bound adenylyl cyclases, their organisation within the cell, and the nature of the cAMP signals that they produce, as a prelude to considering how cAMP signalling might be perturbed. We describe the limitations associated with direct inhibition of adenylyl cyclase activity, and evaluate alternative strategies for more specific targeting of adenylyl cyclase signalling. The inherent complexity in the activation and organisation of adenylyl cyclase activity may actually provide unique opportunities for selectively targeting discrete adenylyl cyclase functions in disease.

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1. Introduction

The concept that hormones or other cellular regulators act from outside the cell to generate an internal second messenger arose with cAMP. The nucleotide is involved in regulating numerous processes, that can range from fast to slow, or from widespread to highly specialised. It occupies a paradigmatic place in cellular signalling in that, rightly or wrongly, the issues surrounding cAMP signalling became paradigms for how second messenger signalling was to be viewed in general. Thus, the principles established in cAMP signalling either translate directly to other systems or at least they have been assessed in other systems. For example, the cAMP field established phosphorylation and kinases as one of the major covalent devices for regulating cellular activity. Phosphorylation cascades, with their cycles of intertwined feedbacks and feed forwards of phosphorylation and dephosphorylation, are now accepted as the central motif of cellular signalling. Of course, mechanisms for terminating signals are just as important in shaping the nature and range of signals. The scaffolding of numerous signalling proteins is key to the actions and regulation of growth factors and both this concept and its application were readily established for cAMP signalling. In this way, recognition of the organisation inherent

Abbreviations: AC, adenylyl cyclase; AKAP, A kinase anchoring protein; BACE, β-secretase; C1, AC catalytic domain 1; C2, AC catalytic domain 2; CaM, calmodulin; GPCR, G protein-coupled receptor; PDE, phosphodiesterase; PKA, protein kinase A; PKC, protein kinase C; PP2A, protein phosphatase 2A; PPi, pyrophosphate; SOCE, store operated calcium entry.

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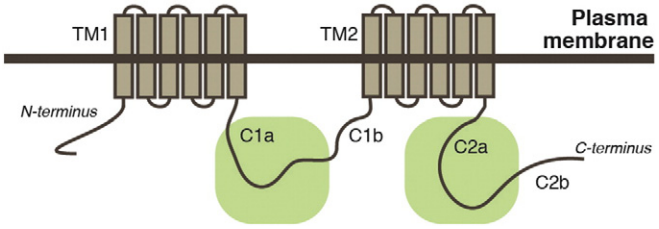

Fig. 1. Structural domains of the membrane-bound ACs. The nine membrane-bound ACs comprise an intracellular N-terminus, two transmembrane regions (TM1 and TM2) of six transmembrane-spanning domains linked by an intracellular region containing the C1a and C1b regions, and an intracellular C-terminus containing the C2a and C2b regions. The highly conserved catalytic domain is formed following the association of the C1a and C2a regions (indicated by green shading). More divergence in length and sequence occurs in the C1b, C2b and N-terminal domains of the ACs.

in cAMP signalling was a reciprocal benefit from exporting the notion of phosphorylation cascades from the cAMP to growth factor fields. Localism – a consequence of organisation in signalling – may have been proposed by the development of calcium dyes and the elegant organisation of cardiomyocyte calcium signalling, but it is today a directly demonstrable cornerstone feature of cAMP signalling.

Notwithstanding the sixty-year study of cAMP, and the range of processes that cAMP regulates, or perhaps because of the complexity now surrounding cAMP signalling, strategies for interrupting cAMP signalling need to go far beyond the simple level of inhibition of the enzyme that produces the signal, adenylyl cyclase (AC). In this regard cAMP may yet again be pointing the way forward to creative strategies for interfering with general signalling processes. In this review we will address the family of ACs, in terms of their physiological roles, their biochemical and cell biological properties, their organisation and association within the cell, and the nature of cAMP signals. We will assess strategies for interfering with cAMP signalling at these various points in an effort to direct us towards the most fruitful way of addressing the roles of this central second messenger.

2. What are adenylyl cyclases and what to do they do?

2.1. Adenylyl cyclases exert complex physiological effects

Cyclic AMP can impact on most significant physiological processes, from regulatory events to metabolism to growth and differentiation. For instance, cAMP has dramatic effects on the regulation of cardiac contractility by the sympathetic nervous system by affecting ion channels and pumps (reviewed in Boularan & Gales, 2015; Efendiev & Dessauer, 2011); it is centrally involved in the control of glycogenolysis and lipolysis (reviewed in Ravnskjaer, Madiraju, & Montminy, 2016), as well as in the control of hormone release (reviewed in Szaszák, Christian, Rosenthal, & Klussmann, 2008). In addition, cAMP plays critical roles in learning and memory (reviewed in Lee, 2015), and affects cell growth and differentiation (reviewed in Borland, Smith, & Yarwood, 2009; Stork & Schmitt, 2002). However, it is important to consider that in many of these situations, a diverse array of signalling pathways ultimately converge to determine the final outcome of hormonal or regulator control of a process.

2.2. Adenylyl cyclases are diverse

Cyclic AMP is synthesised by enzymes that belong to the nucleotidyl cyclase family; the Class III nucleotidyl cyclases, including all eukaryotic ACs and guanylyl cyclases (the enzymes that synthesise cGMP from GTP), are defined by the sequence homology of their catalytic domains. In mammals, cAMP is produced by 10 AC isoforms that are localised either to the plasma membrane (transmembrane ACs, ACs1–9) or to the cytosol (soluble AC, AC10). Here, we will focus on the nine membrane-bound isoforms of AC (herein referred to as ACs; for a comparison of transmembrane and soluble ACs see Kamenetsky et al., 2006; Steegborn, 2014), which all share the same general architecture (Fig. 1) but different regulatory susceptibilities (Table 1; reviewed in Halls & Cooper, 2011; Sunahara, Dessauer, & Gilman, 1996; Willoughby & Cooper, 2007). ACs can be directly regulated by G proteins following the stimulation of G protein-coupled receptors (GPCRs). Of the 800 or so GPCRs, many act via $G\alpha_s$ to stimulate AC, while other GPCRs inhibit a subset of ACs via $G\alpha_{i/o}$ (Table 1). Indirect regulation of ACs also occurs as a consequence of the activation of distinct signalling pathways. The most prominent of these is calcium, which following binding to calmodulin (CaM) can stimulate AC1 and AC8, and inhibit (independently of CaM) AC5 and AC6. Activation of protein kinases – including calcium acting via CaM kinase, as well as PKC and PKA – also regulates certain of the ACs (Table 1). Many ACs are allosterically regulated via the C1b domain, which is C-terminally adjacent to the catalytic site (Fig. 1); for example, calcium-bound CaM binds to and activates both AC1 and AC8 within this region (Gu & Cooper, 1999; Vorherr et al., 1993), and $G\beta\gamma$ subunits can also positively regulate AC2 via interactions with the C1b domain (Boran, Chen, & Iyengar, 2011).

The nine membrane-inserted isoforms are relatively conserved (around 60%) in their catalytic domains, but quite divergent outside these regions. However, even the most highly conserved catalytic domains of AC5 and AC6 are only 90% identical (Fig. 2). This divergence is potentially enough to allow for significant differences in catalytic

Table 1
Regulation of ACs by G protein, calcium and kinase signalling. Consensus from data reviewed in Cooper and Tabbasum (2014), Halls and Cooper (2011), Sadana and Dessauer (2009). ACs are organised according to sub-group classification; the enzymes are typically categorised as belonging to one of four groups, according to sequence relatedness and initial reports of regulatory properties (Halls & Cooper, 2011). CaM, calmodulin; CaN, calcineurin; PKA, protein kinase A; PKC, protein kinase C; CaMK, calmodulin kinase.

	G proteins			Calcium			Kinases		
Isoform	$G\alpha_s$	$G\alpha_{i/o}$	$G\beta\gamma$	Alone	CaM	CaN	PKA	PKC	CaMK
AC1	Activation	Inhibition	Inhibition		Activation				
AC3	Activation	Inhibition						Activation	Inhibition
AC8	Activation	Inhibition			Activation		Inhibition		
AC2	Activation		Activation					Activation	
AC4	Activation		Activation					Activation	
AC7	Activation							Activation	
AC5	Activation	Inhibition		Inhibition			Inhibition	Activation	
AC6	Activation	Inhibition		Inhibition			Inhibition	Inhibition	
AC9	Activation	Inhibition				Inhibition			