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Review

New paradigms in cAMP signalling

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ABSTRACT

Signalling through adenosine 3′5′ monophosphate (cAMP) is known to be important in virtually every cell. The mapping of the human genome over the past two decades has revealed an unexpected complexity of cAMP signalling, which is shared from insects to mammals. A more recent technical advance is the ability to monitor intracellular cAMP levels at subcellular spatial resolution within the time-domains of fast biochemical reactions. Thus, new light has been shed on old paradigms, some of which turn out to be multiple new ones. The novel aspects of cAMP signalling are highlighted here: (1) agonist induced plasticity – showing how the repertory of cAMP signalling genes supports homeostatic adaptation; (2) sustained cAMP signalling after endocytosis; (3) pre-assembled receptor-Gs–adenylyl cyclase complexes. Finally, a hypothetical model of propagating neuronal cAMP signals travelling form dendrites to the cell body is presented.

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1. Brief synopsis of the main components of intracellular cAMP signalling

The molecular toolbox of cAMP signalling has been reviewed extensively (Houslay and Milligan, 1997; Antoni, 2000; Conti and Beavo, 2007; Houslay et al., 2007), hence only a cursory treatise necessary for understanding the subsequent sections is provided.

1.1. Adenylyl cyclases (ACs) and cyclic nucleotide phosphodiesterases (PDEs)

There are 10 mammalian AC genes each of which encodes a different protein. Transmembrane domain ACs (tmACs) are differentially controlled by heterotrimeric G proteins, intracellular Ca^{2+} and protein phosphorylation (Taussig and Gilman, 1995; Antoni, 2000; Willoughby and Cooper, 2007). In addition, the soluble enzyme AC10 is uniquely controlled by bicarbonate ions (Kamenetsky et al., 2006). More recent results also show selective targeting of tmAC isoforms to membrane microdomains such as

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lipid rafts (Ostrom and Insel, 2004; Crossthwaite et al., 2005) and differential association with scaffolding proteins (Dodge et al., 2001), particularly A-kinase anchoring proteins (AKAPs), all contributing to the formation of intracellular cAMP compartments (Lynch et al., 2006; Dessauer, 2009). Several signalling pathways converge on ACs, which act as molecular signal integrators. The AC isoforms have distinct tissue distributions indicating non-redundant physiological roles (Sadana and Dessauer, 2009). At the single cell level, expression of multiple AC-s isotypes is the rule rather than the exception. The situation with PDEs is similar to that of ACs, an even greater genetic variability is evident for these enzymes displaying unique regulation of cAMP hydrolysis, subcellular targeting and tissue topography (Houslay and Milligan, 1997; Conti and Beavo, 2007).

1.2. Downstream targets of cAMP

The classical downstream target of cAMP is the protein kinase A (PKA) family of proteins which has been extensively characterized (Taylor et al., 2004). The important advance in the field is the discovery of the multiplicity of AKAPs, that have emerged as scaffolding proteins for a bewildering variety of signallosomes (Logue and Scott, 2010). Relatively novel targets of cAMP include ion channels directly influenced by cAMP. These come in two flavours – the hyperpolarization-activated and cyclic nucleotide-modulated channels (HCN) and channels directly modulated by cyclic nucleotides (CNG channels). Both of these channel types increase the electrical activity of cells in response to cAMP. Various types of PDE are modulated by cAMP usually via the N-terminal domains (Keravis and Lugnier, 2010). Last, but not least, exchange proteins activated by cAMP (EPACs) are the most recently recognized family of proteins the functional activity of which is regulated by cAMP–EPACs modulate the activity of the small GTPase Rap1 and have important functions regulating cell shape and secretory granule dynamics (Gloerich and Bos, 2010). Importantly, the cAMP sensitivity of this panoply of targets spans a 30-fold dynamic range. The PKA isoforms are the most responsive and are saturated at 1 μM, cyclic nucleotide gated channels are close to maximally active at 10 μM whilst EPAC may require up to 40 μM cAMP to be fully active.

2. Methodological break-through: molecular biosensors provide direct proof of cAMP compartments and intracellular oscillations

Arguably the most significant recent achievement in furthering our understanding of cAMP signalling has been the introduction of methodologies for monitoring intracellular cAMP levels with spatial and temporal resolutions classical biochemical approaches could not achieve. The evolution of intracellular cAMP sensors from fluorescently tagged PKA subunits delivered into cells by microinjection (Bacskai et al., 1993) to genetically engineered mice expressing EPAC-based cAMP sensor protein (Calebiro et al., 2009) has been reviewed comprehensively (Nikolaev and Lohse, 2006; Willoughby and Cooper, 2008; Calebiro et al., 2010a,b). The chief achievement of these technologies is that unique and localized patterns of cAMP concentrations have been reported that can be connected to specified elements of the cAMP signalling toolbox thus providing important clues for the biological relevance of the genetic diversity of cAMP-signalling proteins. This is not to say that the classical biochemical approaches are no longer useful, as in pharmacological screens the relatively narrow dynamic range of the biosensors restricts their applicability (Hill et al., 2010). However, where practicable, the deployment of sensor-based methods is important for discovery research and for the full validation of the cAMP response measured.

3. A selection of novel paradigms in cAMP signalling

The accounts provided below are by no means a comprehensive treatise of all the novel aspects of cAMP signalling that are beginning to emerge on the back of the biosensor revolution. Notably, cells of the immune system and the role of soluble ACs are not covered, the reader is referred to excellent recent reviews (Gloerich and Bos, 2010; Buck and Levin, 2011). There are also detailed accounts of the multiplicity of cAMP signals involved in the control of insulin release by pancreatic beta cells (Ramos et al., 2008; Leech et al., 2010). The examples highlighted here are as yet not in the mainstream of work in the field, but appear to be highly significant for physiological control. Moreover, these paradigms are amenable to further study, for example, by making use of new biosensor and RNA interference technologies.

3.1. Agonist induced plasticity at the level of AC

The example cited here is the adenohypophyseal corticotrope cell. These cells produce adrenocorticotropin (ACTH) to stimulate adrenocortical steroid production and are thus the main endocrine mediators of the CNS control of the stress-response. Briefly, corticotropes are stimulated by the neuropeptides corticotropin releasing factor-41 (CRF) and arginine vasopressin (AVP) via the neurohaemal interface of the median eminence and are under feedback inhibition by adrenal corticosteroids (Antoni, 1986). With respect to the underlying signalling mechanisms, it is well established that CRF activates adenylyl cyclase (AC) and that this effect is amplified by AVP through activation of protein kinase C (PKC) (Carvallo and Aguilera, 1989; Antoni, 1993). Whilst the exact mechanism of AVP potentiation remains to be established, work over the years clearly points to the involvement of a PKC activated AC such as AC2 or AC7 (Antoni, 1993, 2000). Inhibition by adrenal corticosteroids is largely mediated by the Type 2 nuclear glucocorticoid receptor, and is characterized by distinct time domains (Dallman et al., 1992; Antoni, 1996). The steroid effect that largely determines the size of the stress response in the short and medium term (i.e. from 20 min to 4 h after the induction of stress) is called early delayed inhibition and requires the synthesis of new protein(s) (Dallman et al., 1992; Antoni, 1996). Work in the mouse pituitary cell line AtT20 showed conclusively that the effect of glucocorticoids is mediated by an alteration of the balance of kinase/phosphatase control of STREX-variant, large-conductance (BK) potassium channels (Shipston et al., 1996, 1999). These channels are inhibited by PKA activated by CRF, whilst protein phosphatase 2A recruited by glucocorticoids can override the effect of PKA and thus suppress the stimulation of ACTH release (Tian et al., 1998, 2001).

Significantly, in AtT-20 cells clamping of the CRF-activation of tmAC by Ca^{2+} derived through voltage-operated L-channels appeared to be important for maintaining the efficacy of inhibition by glucocorticoids (Shipston et al., 1994; Antoni et al., 1995). Whilst glucocorticoid feedback inhibition of pituitary ACTH release in non-tumoral rat pituitary corticotrope cells shares the pivotal features of that observed in AtT20 cells, there was no indication for the involvement of BK channels (Lim et al., 1998). Functionally relevant BK- and SK-channels are present in rat adenohypophyseal corticotrope cells, as the ACTH release inhibiting action of atrial natriuretic peptides as well as cyclic GMP analogues is fully opposed upon blockade of these channels (Antoni and Dayanithi, 1990). However, two independent studies concur, that in rat corticotrope cells K^+ channels inhibited by PKA and insensitive to