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Review

Regulation of cAMP by phosphodiesterases in erythrocytes

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Abstract:
The erythrocyte, a cell responsible for carrying and delivering oxygen in the body, has often been regarded as simply a vehicle for the circulation of hemoglobin. However, it has become evident that this cell also participates in the regulation of vascular caliber in the microcirculation *via* release of the potent vasodilator, adenosine triphosphate (ATP). The regulated release of ATP from erythrocytes occurs *via* a defined signaling pathway and requires increases in cyclic 3',5'- adenosine monophosphate (cAMP). It is well recognized that cAMP is a critical second messenger in diverse signaling pathways. In all cells increases in cAMP are localized and regulated by the activity of phosphodiesterases (PDEs). In erythrocytes activation of either β adrenergic receptors (β_2AR) or the prostacyclin receptor (IPR) results in increases in cAMP and ATP release. Receptor-mediated increases in cAMP are tightly regulated by distinct PDEs associated with each signaling pathway as shown by the finding that selective inhibitors of the PDEs localized to each pathway potentiate both increases in cAMP and ATP release. Here we review the profile of PDEs identified in erythrocytes, their association with specific signaling pathways and their role in the regulation of ATP release from these cells. Understanding the contribution of PDEs to the control of ATP release from erythrocytes identifies this cell as a potential target for the development of drugs for the treatment of vascular disease.

Key words:
erythrocyte, isoproterenol, iloprost, phosphodiesterases, cyclic nucleotides, adenosine triphosphate

Abbreviations: AC – adenylyl cyclase, AKAP – A kinase anchoring protein, ATP – adenosine triphosphate, cAMP – cyclic adenosine monophosphate, cGMP – cyclic guanosine monophosphate, FRET – fluorescence resonance energy transfer, IPR – prostacyclin receptor, PDE – phosphodiesterase, PVD – peripheral vascular disease, UCR – upstream conserved regions

Introduction

Within all mammalian cells, activation of specific G protein-coupled receptors can stimulate the activity of adenylyl cyclase (AC), resulting in the production of cyclic adenosine monophosphate (cAMP). With a diffusion constant of 270–780 $\mu m^2/s$ [4, 12, 78], cAMP could potentially diffuse throughout the cell, activating any of several effector proteins expressed in that cell. However, indiscriminate effector protein activation does not occur, and activation of individual receptors produces highly selective cellular responses. This selectivity suggests that there is compartmentalization of cAMP signaling within cells. Multiple proteins aid in the compartmentalization of cAMP signaling, including ligand receptors, their G proteins, ACs as well as regulatory and scaffold proteins. However,

this compartmentalization is suggested to be most heavily dependent on phosphodiesterase (PDE) activity [5, 78]. PDEs control the diffusion of cAMP by rapidly degrading this cyclic nucleotide within cells and therefore regulate its biological actions.

Eleven PDE families have currently been identified, some of which contain multiple isoforms [9]. PDE families and their individual isoforms are differentiated by their ability to hydrolyze either cAMP, cyclic guanosine monophosphate (cGMP), or both cyclic nucleotides as well as by their cellular and subcellular location, mode of regulation and sensitivity to inhibitors [9]. Although all cells contain PDE activity, the isoforms present vary according to cell type [9]. This diversity in PDE expression allows these enzymes to regulate discrete signal transduction pathways, and ultimately specific physiological processes.

Several studies indicate a role for PDEs in regulation of discrete cell processes. For example, the hypothesis that compartmentalization of cAMP generates specificity of G_s-receptor actions, with PDEs playing a major role, was tested in adult rat ventricular myocytes by characterizing the PDEs involved in regulation of cAMP signals and L-type Ca^{2+} current upon stimulation of different G_s-coupled receptors [50]. These studies demonstrated that receptor-PDE coupling has functional implications downstream of cAMP and identified functional coupling of specific PDE families to specific ligand-activated G_s-coupled receptors as a major mechanism which enables cardiac cells to generate heterogeneous cAMP signals to different hormones [50]. In addition, these studies also suggest that alteration of PDE activity abolishes the compartmentalization of the cAMP signal allowing effector proteins to be activated throughout the cell, eliciting aberrant responses [50]. In support of this hypothesis, fluorescence resonance energy transfer (FRET) analysis in both HEK293 cells and cardiac myocytes allowed for visualization of cAMP compartmentalization and demonstrated that this was dependent on PDE activity [39, 70]. In mouse embryonic fibroblasts, specific isoforms of the PDE4 gene family were functionally coupled to the β_2 adrenergic receptor and ablation of PDE4 in mice altered the response to β_2 adrenergic receptor activation in these cells [11]. Similarly, in cardiac myocytes, disruption of cAMP pools *via* ablation of distinct PDE4 isoforms disrupts the tight regulation of β adrenergic signaling [76]. In the studies mentioned above, stimulation of β1, β2 or prostacyclin receptors (IPR), which are all G_s coupled receptors, increase cAMP with distinct patterns of protein phosphorylation resulting in different downstream effects. In each of these studies, it was concluded that the results were partially due to either the functional coupling or the localization of individual PDE families to each receptor, resulting in the heterogeneity of ligand specific cAMP-mediated effects.

Subcellular localization of PDEs

The subcellular localization of PDEs is recognized to be a key mechanism for compartmentalization of cyclic nucleotide signaling. The amino terminal sequence of the PDE determines the localization of the protein within the cell. PDEs may be found either in the cytosol, associated with the plasma membrane, or organelles of the cell [36, 43]. Another determinant of localization is whether or not the PDE is bound to or interacts with scaffold proteins, which localize PDEs to microdomains within a cell [17, 29, 77]. Clearly, the subcellular location of PDEs is critical for coupling these enzymes to specific signal transduction pathways, which permit specific PDEs to regulate local increases in cAMP produced by activation of ligand specific receptors. This mechanism can target locally produced cAMP to selective effector proteins, resulting in a specific response. Therefore, the fact that cAMP is synthesized in response to receptor-mediated activation of AC in erythrocytes [7, 42, 59, 60] suggests that PDEs within these cells regulate the compartmentalization of cAMP signaling allowing for specific cell responses.

PDE activity in erythrocytes

Although cAMP is considered a ubiquitous second messenger in cells, the presence of AC activity and cAMP production in the erythrocyte was once controversial. It was suggested that mature erythrocytes possess little to no AC activity and therefore could not synthesize cAMP [8, 21, 32, 57]. However, Nakagawa et al. demonstrated that stabilization of AC during isolation of erythrocyte membranes is critical in order