Available online at www.sciencedirect.com

SCIENCE DIRECT®

Cellular Signalling 17 (2005) 1158 – 1173

CELLULAR SIGNALLING

www.elsevier.com/locate/cellsig

In resting COS1 cells a dominant negative approach shows that specific, anchored PDE4 cAMP phosphodiesterase isoforms gate the activation, by basal cyclic AMP production, of AKAP-tethered protein kinase A type II located in the centrosomal region

Angela McCahill[a,1], Theresa McSorley[a,1,2], Elaine Huston[a], Elaine V. Hill[a], Martin J. Lynch[a], Irene Gall[a], Guy Keryer[b], Birgitte Lygren[c], Kjetil Tasken[c], Gino van Heeke[d], Miles D. Houslay[a,*]

[a]*Molecular Pharmacology Group, Division of Biochemistry and Molecular Biology, IBLS, Wolfson Link Building, University of Glasgow, Glasgow G12 8QQ, Scotland, UK*
[b]*Institut Curie, Section Recherche, Unite Mixte Recherche 144-Centre National de la Recherche Scientifique, 75248 Paris, France*
[c]*The Biotechnology Centre of Oslo, University of Oslo, Blindern, N-0317 Oslo, Norway*
[d]*Respiratory Disease Area, Novartis Institutes for BioMedical Research, Wimblehurst Road, Horsham, RH12 5AB, UK*

Received 1 March 2005; accepted 4 April 2005
Available online 17 May 2005

Abstract

We employ a novel, dominant negative approach to identify a key role for certain tethered cyclic AMP specific phosphodiesterase-4 (PDE4) isoforms in regulating cyclic AMP dependent protein kinase A (PKA) sub-populations in resting COS1 cells. A fraction of PKA is clearly active in resting COS1 cells and this activity increases when cells are treated with the selective PDE4 inhibitor, rolipram. Point mutation of a critical, conserved aspartate residue in the catalytic site of long PDE4A4, PDE4B1, PDE4C2 and PDE4D3 isoforms renders them catalytically inactive. Overexpressed in resting COS1 cells, catalytically inactive forms of PDE4C2 and PDE4D3, but not PDE4A4 and PDE4B1, are constitutively PKA phosphorylated while overexpressed active versions of all these isoforms are not. Inactive and active versions of all these isoforms are PKA phosphorylated in cells where protein kinase A is maximally activated with forskolin and IBMX. By contrast, rolipram challenge of COS1 cells selectively triggers the PKA phosphorylation of recombinant, active PDE4D3 and PDE4C2 but not recombinant, active PDE4A4 and PDE4B1. Purified, recombinant PDE4D3 and PDE4A4 show a similar dose-dependency for in vitro phosphorylation by PKA. Disruption of the tethering of PKA type-II to PKA anchor proteins (AKAPs), achieved using the peptide Ht31, prevents inactive forms of PDE4C2 and PDE4D3 being constitutively PKA phosphorylated in resting cells as does siRNA-mediated knockdown of PKA-RII, but not PKA-RI. PDE4C2 and PDE4D3 co-immunoprecipitate from COS1 cell lysates with 250 kDa and 450 kDa AKAPs that tether PKA type-II and not PKA type-I. PKA type-II co-localises with AKAP450 in the centrosomal region of COS1 cells. The perinuclear distribution of recombinant, inactive PDE4D3, but not inactive PDE4A4, overlaps with AKAP450 and PKA type-II. The distribution of PKA phosphorylated inactive PDE4D3 also overlaps with that of AKAP450 in the centrosomal region of COS1 cells. We propose that a novel role for

Abbreviations: PDE4, cyclic AMP specific phosphodiesterase-4; PKA, cyclic AMP dependent protein kinase A/A-kinase; PKA-RI, PKA type I; PKA-RII, PKA type II; AKAP, A-kinase anchor protein; Rolipram, 4-[3-(cyclopentyloxy)-4-methoxyphenyl]-2-pyrrolidinone.

* Corresponding author. Tel.: +44 141 330 5903; fax: +44 141 330 4365.
E-mail address: M.Houslay@bio.gla.ac.uk (M.D. Houslay).

[1] These two individuals contributed equally to this work and should be considered joint first authors.

[2] Present address: Forschungsinstitut für Molekulare Pharmakologie, Campus Berlin-Buch, Robert-Rössle-Str 10, 13125 Berlin, Germany.

doi:10.1016/j.cellsig.2005.04.003

PDE4D3 and PDE4C2 is to gate the activation of AKAP450-tethered PKA type-II localised in the perinuclear region under conditions of basal cAMP generation in resting cells.

Keywords: Cyclic AMP specific phosphodiesterase; PDE4; Cyclic AMP dependent protein kinase A; PKA; AKAP; Rolipram; Centrosome; Dominant negative; Compartmentalisation; Compartmentation

1. Introduction

It is well appreciated that the cyclic nucleotide, cAMP, plays a pivotal role as a second messenger in controlling a wide range of cellular functions. These include processes as diverse as memory, heart and smooth muscle contraction, water and electrolyte homeostasis, immune responses, key aspects of metabolism, gene expression, differentiation and apoptosis [1–3].

Analyses of various intracellular messenger systems, namely Ca^{2+}, tyrosyl kinases and inositol phospholipids, have unequivocally established signal compartmentalisation as crucial to normal cellular functioning [4–8]. Thus the use of optical probes has shown that highly localised gradients of Ca^{2+} can form in cells. Indeed, it has even been shown that Ca^{2+} can be funnelled to distinct points within the cell interior by constraining its dispersal through 'inactivation' by uptake into spatially constrained groupings of mitochondria [9,10]. This generates compartmentalised signalling.

The notion that signalling processes are compartmentalised originated from studies analysing the selective activation of protein kinase A (PKA) RI and RII isoforms in cardiac myocytes, by Brunton et al. [11,12]. Recently, a variety of genetically encoded probes have independently identified intracellular gradients of cAMP occurring in cells, providing additional proof for the concept of compartmentalised cAMP signalling [13–20]. There is now a good appreciation that PKA sub-populations, which are localised at distinct sites in cells, can sense and act on intracellular gradients of cAMP [2,3]. A disparate family of PKA anchor proteins, called AKAPs, provide the means of tethering of PKA regulatory (R) subunits to distinct intracellular sites [2,3]. AKAPs achieve this by interacting with the dimerisation interface of, predominantly, PKA type-II (PKA-RII) [21]. As AKAPs show a unique pattern of intracellular location, they serve to constrain the ability of tethered PKA to be activated by spatially localised intracellular cAMP gradients of appropriate magnitude. Additionally, AKAPs serve as multi-functional scaffold proteins that can recruit specific PKA substrates as well as various regulatory proteins. Thus AKAP-tethered PKA-RII populations are set to 'read' specific, spatially localised intracellular cAMP gradients and act accordingly.

The means through which intracellular cAMP gradients are shaped in cells so as to regulate specific AKAP-tethered PKA sub-populations is only just beginning to be appreciated. Adenylyl cyclase family members, located at the plasma membrane, generate cAMP and thereby provide point sources of cAMP generation [1]. The various adenylyl cyclase isoforms show distinct levels of basal activity in resting cells [22–24]. These enzymes can be activated, however, by specific G_s coupled receptors that are localised to distinct plasma membrane sub-domains and allow spatially distinct point sources of cAMP generation. The only means of degrading cAMP in cells, and thus for establishing and shaping cAMP gradients, is through the action of cAMP phosphodiesterases (PDEs) [25–30]. A large, multi-gene family encodes a plethora of PDEs able to hydrolyse cAMP [1]. Detailed examination has shown that a feature of many PDE isoenzymes is targeting to specific intracellular sites, as exemplified in particular detail for the PDE4 family [28]. This, coupled with observations that selective inhibitors of particular PDE families can exert distinct actions of cell function, indicates that PDEs are set to play a key role in underpinning compartmentalised cAMP signalling.

Currently there is considerable interest in PDE4 cAMP phosphodiesterases [25,27,28]. This is because PDE4 selective inhibitors, which act as anti-inflammatory agents and memory enhancers, are being developed for use in treating asthma, chronic obstructive pulmonary disease (COPD) and depression [31–33]. Furthermore, the PDE4D gene has been linked to common atherogenic stroke [34]. However, a key feature of many PDE4 isoforms is their ability to be targeted to specific intracellular sites [27,28]. This was first shown for the PDE4A1 isoform, whose unique, isoform-specific N-terminal region confers exclusive membrane-association [35–38]. Intracellular targeting of PDE4 isoforms is also achieved through protein–protein interactions, as in the binding of SRC family tyrosyl kinases to PDE4A4/5 [39,40], of RACK1 to PDE4D5 [41,42], of myomegalin to PDE4D3 [43], of the immunophilin, XAP2 to PDE4A5 [44] and of βarrestin to various PDE4 isoforms [45,46]. Thus anchored PDE4 isoforms are eminently poised to shape gradients of cAMP in cells and thence to determine the selective activation of AKAP-anchored PKA.

It is generally understood that the key to appreciating the physiological significance of activation of adenylyl cyclase by a G_s coupled receptor is to determine the ratio of PKA activity in stimulated cells compared to that observed in resting cells. In this regard, adenylyl cyclase isoforms vary considerably in their basal activity and are expressed in a cell-type specific fashion [1,22,23], implying that control-