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COMMENTARY

Short-term regulation of PDE4 activity

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By catalyzing hydrolysis of cAMP and cGMP, cyclic nucleotide phosphodiesterases (PDEs) are critical determinants of the intracellular concentrations, and, consequently, the biological effects of these important intracellular second messengers. Eleven functionally distinct, structurally related and highly regulated gene families comprise the PDE superfamily. Many of the individual PDE gene families contain isoform subfamilies generated from closely related but different genes; more than 20 distinct mammalian PDE genes have been identified. Multiple PDEs can also be derived from a single PDE gene by transcription from different promoters, by alternative mRNA splicing, or by translational regulation, with well over 30 different PDE proteins most likely synthesized in mammalian cells. The different PDE families differ in their primary amino acid sequences, substrate affinities and kinetic characteristics, sensitivities to different effectors and selective inhibitors, responses to regulatory molecules, and cellular functions (Houslay *et al*., 1998; Conti & Jin, 2000; Houslay, 2001; Mehats *et al*., 2002). The combined enormous molecular diversity of ligands and receptors, adenylyl and guanylyl cyclases, PDEs and cyclic nucleotide-regulated effector systems provides for the complex integration, specificity and variety of networks and pathways that are involved in generation, transduction, and modulation of cyclic nucleotide signals and actions, and for establishing unique cyclic nucleotide phenotypes that characterize individual cells.

Intracellular pools of cAMP and cGMP are tightly regulated, and seem to be temporally, spatially, and functionally compartmentalized. Most cells contain representatives of more than one PDE gene family, but in different amounts, proportions, and subcellular locations. By virtue of their distinct intrinsic characteristics, their intracellular targeting to different subcellular addresses/locations, and their interactions with molecular scaffolds, cellular structural elements and different regulatory partners, different PDEs integrate multiple inputs and signalling cascades, and modulate the intracellular diffusion and spatial/functional compartmentalization of – as well as the amplitude, duration, termination and specificity of – cyclic nucleotide signals and actions. PDEs will very likely turn out to be critical determinants in establishing and regulating discrete intracellular cAMP/cGMP signalling modules and microdomains.

Dr Miles Houslay and his co-workers have been responsible for a large body of work relating to elucidation and understanding of the molecular diversity, structure/function, regulation and physiological roles of the PDE4 gene family (Houslay *et al*., 1998; Houslay, 2001). PDE4 proteins are quite specific for cAMP hydrolysis, exhibiting a high affinity for cAMP, with limited capacity for cGMP hydrolysis (Houslay *et al*., 1998; Conti & Jin, 2000; Houslay, 2001. Since many preclinical model systems have strongly suggested that PDE4 isoforms regulate cyclic nucleotide pools that in turn regulate various inflammatory and immune responses as well as clinical depression, PDE4 selective inhibitors, including rolipram, cilomilast (Ariflo®), and roflumilast have been developed as potential therapeutic agents, especially for a variety of inflammatory disorders (Torphy, 1998; Essayan, 2001).

PDE4 isoforms also play an important role in homeostatic regulation of intracellular cAMP, which involves upregulation of PDE4 activity in response to increases in intracellular cAMP (especially after receptor-coupled activation of adenylyl cyclase) (Houslay *et al*., 1998; Conti & Jin, 2000; Houslay, 2001; Mehats *et al*., 2002). This increase in PDE4 activity is part of an adaptive response that results in 'desensitization' of cells to effects of increases in cAMP content and to further stimulation by cAMP-generating agents and signals. Two mechanisms for upregulation of PDE4 have been described. One, referred to as 'long-term' regulation, involves cAMP-induced transcription and increased PDE4 gene expression and protein synthesis. The second, 'short-term' regulation, which involves cAMP-dependent protein kinase (PKA)-catalyzed phosphorylation/activation of PDE4 isoforms, occurs within minutes following receptor-mediated activation of adenylyl cyclase, increases in intracellular cAMP and activation of PKA (Houslay *et al*., 1998; Conti & Jin, 2000; Houslay, 2001; Mehats *et al*., 2002). This type of up-regulation of PDE4 is most likely important in acutely and rapidly regulating the magnitude and duration, as well as termination, of cAMP signals. This 'short-term' reversible activation of PDE4, and the implications of this phenomenon, is the subject of the current report by MacKenzie *et al*. (2002) and this commentary.

All mammalian PDEs exhibit a common structural organization, with a conserved catalytic domain situated in the C-terminal portion of the PDE molecules, preceded and followed by divergent N-terminal regulatory regions and C-terminal domains. This structural organization – i.e., conserved catalytic core and unique N-terminal regulatory regions – is recapitulated within the complex and diverse PDE4 family, which is generated from four genes (PDE 4A-D); each PDE4 gene, in turn, is capable of generating multiple, distinct PDE4 isoforms *via* transcription initiation from different promoter elements or alternative mRNA splicing (Houslay *et al*., 1998; Conti & Jin, 2000; Houslay, 2001). As discussed by MacKenzie *et al*. (2002), PDE4 proteins are classified as 'long' or 'short' isoforms, depending on the presence or absence of two highly conserved domains,

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Upstream Conserved Region 1 (UCR 1; 55 aa) and Upstream Conserved Region 2 (UCR 2; 76 aa), located just N-terminal to the catalytic core (Houslay *et al*., 1998; Conti & Jin, 2000; Houslay, 2001). 'Long' PDE4 isoforms contain UCR1 and UCR2; 'short' PDE4 isoforms lack UCR1, and some also exhibit a truncated UCR2 region. Recent studies by the Houslay group and others suggest that UCR1 and UCR2 interact to form a regulatory module that may influence catalytic activity, sensitivity to drugs, and regulatory effects of phosphorylation (Bolger *et al*., 1993; Lim *et al*., 1999; Beard *et al*., 2000).

While it is known that PKA-induced phosphorylation of Ser^{54} in UCR1 of the 'long' form of PDE4D3 results in activation of this isoform, no comparable information was previously available concerning phosphorylation/activation of an identical PKA consensus sequence (Arg-Arg-Glu-Ser-Phe) located in UCR1 of PDE4A, B, and C 'long' isoforms. As nicely demonstrated in this report, both *in vitro* and in intact cells, PKA-catalyzed phosphorylation of the single consensus Ser in UCR1 of all four PDE4 'long' forms results in activation of the enzymes. In contrast to PDE4D3, however, the PDE4 'long' forms, when phosphorylated, do not exhibit decreased mobility on SDS–PAGE or altered sensitivity to inhibition by rolipram. As discussed by the authors (and reflective of earlier studies by the Houslay laboratory and others), and since unique PDE4 N-terminal regions are thought to target different PDE4 isoforms to different subcellular locations and molecular scaffolds/regulatory partners, the possibility of different PDE4 'long' forms interacting with different AKAPs (A-Kinase Anchoring Proteins) (Dodge *et al*., 2001) suggests that discrete PKA/PDE4 signalling complexes might contribute to generation and regulation of different intracellular signalling microdomains. This would provide mechanisms for different PDE4 isoforms both regulating and compartmentalizing different cAMP signalling pathways and cellular functions, as well as serving as discrete therapeutic targets in specific disease entities. One can safely assume that Houslay and his collaborators will continue to provide answers and pose interesting questions regarding these important aspects of PDE4 biology.

References

BEARD, M.B., OLSEN, A.E., JONES, R.E., ERDOGAN, S., HOUSLAY, M.D. & BOLGER, G.B. (2000). UCR1 and UCR2 domains unique to the cAMP-specific phosphodiesterase family form a discrete module via electrostatic interactions. *J. Biol. Chem.,* **275,** 10349–10358.

BOLGER, G., MICHAELI, T., MARTINS, T., JOHN, S.T., STEINER, B., RODGERS, L., RIGGS, M., WIGLER, M. & FERGUSON, K. (1993). A family of human phosphodiesterases homologous to the dunce learning and memory gene product of *Drosophila melanogaster* are potential targets for antidepressant drugs. *Mol. Cell. Biol.,* **13,** 6558–6571.

CONTI, M. & JIN, S.-L.J. (2000). The molecular biology of cyclic nucleotide phosphodiesterases. *Prog. Nucl. Acid. Res. Mol. Biol.,* **63,** 1–38.

DODGE, K.L., KHOUANGSATHIENE, S., KAPILOFF, M.S., MOUTON, R., HILL, E.V., HOUSLAY, M.D., LANGEBERG, L.K. & SCOTT, J.D. (2001). mAKAP assembles a protein kinase A/PDE4 phosphodiesterase cAMP signaling module. *EMBO J.,* **20,** 1921–1930.

ESSAYAN, D.M. (2001). Cyclic nucleotide phosphodiesterases. *J. Allergy Clin. Immunol.,* **108,** 671–680.

HOUSLAY, M.D. (2001). PDE4 cAMP-specific phosphodiesterases. *Prog. Nucl. Acid. Res. Mol. Biol.,* **69,** 250–316.

HOUSLAY, M.D., SULLIVAN, M. & BOLGER, G.B. (1998). The multienzyme PDE4 cyclic adenosine monophosphate-specific phosphodiesterase family: intracellular targeting, regulation, and selective inhibition by compounds exerting anti-inflammatory and antidepressant actions. *Adv. Pharmacol.,* **44,** 225–342.

LIM, J., J., PAHLKE, G. & CONTI, M. (1999). Activation of the cAMP-specific phosphodiesterase PDE4D3 by phosphorylation: identification and function of an inhibitory domain. *J. Biol. Chem.,* **274,** 19677–19685.

MACKENZIE, S.J., BAILLIE, G.S., MCPHEE, I., SEAMONS, R., MCSORLEY, T., MILLEN, J., MACKENZIE, C., BEARD, M.B., VAN HEEKE, G. & HOUSLAY, M.D. (2002). Long PDE4 cyclic AMP-specific phosphodiesterases are activated by protein kinase A-mediated phosphorylation of a single serine residue in upstream conserved region 1 (UCR1). *Br. J. Pharmacol.,* in press.

MEHATS, C., ANDERSEN, C.B., FILOPANTI, M., JIN, S.-L.C. & CONTI, M. (2002). Cyclic nucleotide phosphodiesterases and their role in endocrine cell signaling. *Trends Endocrin. Metab.,* **13,** 29–35.

TORPHY, T.J. (1998). Phosphodiesterase isozymes: molecular targets for novel anti-asthma agents. *Am. J. Respir. Crit. Care Med.,* **157,** 351–370.

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