Title
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Journal
UCSD Molecule Pages, 1(1)

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Publication Date
2012

UCSD MOLECULE PAGES
doi:10.6072/H0.MP.A001760.01
Volume 1, Issue 1, 2012

Review Article Open Access

Phosphodiesterase 8A, cAMP-specific

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Phosphodiesterase 8A (PDE8A) is a 3′,5′-cyclic-nucleotide phosphodiesterase that specifically catalyses the hydrolysis of cAMP to AMP. PDE8A is one of the two isoenzymes of the PDE8 family, the other being PDE8B. These two highly similar proteins have several common features that distinguish them from other cAMP-specific PDEs: they have very high affinity for the substrate cAMP; they are insensitive to the non-specific PDE inhibitor IBMX. They contain a PAS (Per, Arnt and Sim) and a REC (receiver) domain, both of which are observed in many signal transduction proteins. The possible function(s) of the PAS domain in PDE8 is still unknown. *PDE8A* mRNA is not expressed in all tissues but has been detected in several and is highest in testis, spleen, small intestine, heart, ovary, colon and kidney. Until early 2009, the lack of specific small-molecule inhibitors slowed the study of the physiological relevance of PDE8A; thus to date many aspects of this PDE's functions (that is, whether it is activated or regulated during certain cellular process) remain in large part unknown. So far, functions for PDE8A have been described in Leydig cells, where lack of PDE8A causes increased testosterone production in response to luteinizing hormone; T cells, where it seems to be induced during T cell activation and may control chemotaxis; and cardiocytes, where it seems to regulate calcium handling.

KEYWORDS
mmPDE8A; Pde8a; PDE8A; Phosphodiesterase 8A, cAMP-specific

IDENTIFIERS
Molecule Page ID:A001760, Species:Mouse, NCBI Gene ID: 18584, Protein Accession:AAC40194.1, Gene Symbol:Pde8a

PROTEIN FUNCTION

cAMP and cGMP are intracellular second messengers that mediate the response to a wide variety of hormones and neurotransmitters and modulate many metabolic processes. Their concentration into the cell is finely regulated in time and space by the balance between their synthesis, mediated by adenylate and guanylate cyclases, and their degradation by phosphodiesterases.

Cyclic nucleotide phosphodiesterases (PDEs) are a family of related phospho-hydrolases that selectively catalyse the hydrolysis of the 3′ cyclic phosphate bonds of cAMP and cGMP. To date, 11 families of PDEs have been described, comprising 21 different gene products. Each gene can give rise to several different mRNA products through alternative transcriptional start sites and alternative splicing, which brings the number of estimated functional PDE enzymes to more than 100.

The PDE8 family comprises two distinct but highly similar genes in mammals, PDE8A and PDE8B, and was the first of four families of PDEs to have been identified, initially with the help of bioinformatic analysis of the expressed sequence tag (EST) cDNA databases (Soderling *et al*. 1998; Fisher *et al*. 1998; Gamanuma *et al*. 2003).

PDE8A and PDE8B both have the carboxy-terminal catalytic domain that is common to all PDEs, but they differ from other PDE families in the amino-terminal region, which contains two putative regulatory domains. First, there is a REC domain, homologous to the “receiver” domains of bacterial two-component signaling systems (Galperin *et al*. 2001). This is followed closely by a PAS (Period, Arnt and Sim) domain, which was first described as a regulatory domain present in several proteins involved in the control of circadian rhythms (Dunlap *et al*. 1999; Gilles-Gonzalez and Gonzalez 2004).

PDE8 enzymes are cAMP-specific and have highest affinity for this substrate of all the PDEs (K_M 40-150 nM for cAMP, K_M >100 μM for cGMP) (Fisher *et al*. 1998; Gamanuma *et al*. 2003; Soderling *et al*. 1998). Hence, PDE8 has no appreciable activity towards cGMP.

The first indication of a possible function for PDE8A came from work by Glavas *et al*. (2001), in which a full-length PDE8A protein was identified in human T cells and its expression showed to be upregulated in $CD4^+$ T cells after stimulation. Another study on mouse splenocytes showed that *Pde8A* mRNA levels are increased following cell stimulation with concanavalin A, a lymphocyte mitogen (Dong *et al*. 2006). Moreover, T cell migration in response to chemoattractants was diminished when Pde8A activity was inhibited with the non-selective PDE inhibitor, dipyridamole. Dipyridamole was shown to inhibit PDE8A with a reported IC50 of 4-9 μM (Soderling *et al*. 1998; Fisher *et al*. 1998).

From northern blot analysis, PDE8A is particularly abundant in testis, and protein expression was confirmed in spermatozoa (Baxendale and Fraser 2005; Vasta *et al*. 2006). In addition, using a *Pde8a* knockout mouse as model, Vasta and colleagues showed that PDE8A is also present in Leydig cells in testes, where it contributes to controlling the steroidogenic response to luteinizing hormone.

PDE8 inhibition using dipyridamole in a dose-dependent manner increased cAMP levels in bovine cumulus-oocyte complexes and subsequently delayed oocyte nuclear maturation (Sasseville *et al*. 2009). Also, PDE8A modulates excitation-contraction coupling in ventricular myocytes, where its ablation is marked with an increase in calcium transients and calcium spark frequency (Patrucco *et al*. 2010).

It was recently shown that increasing cAMP in effector T cells by inhibiting PDE8 with a new selective small molecule inhibitor (PF-04957325) suppressed the expression of integrins

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Published online: 19 Jan 2012 | doi:10.6072/H0.MP.A001760.01

required for adhesion to endothelial cells (Vang *et al*. 2010). In addition, using Pde8-ablated mice and using the inhibitor PF-04957325 Tsai *et al*. showed that Pde8a and Pde8b both regulate corticosterone production in the mouse adrenal gland.

REGULATION OF ACTIVITY

As with other PDEs, PDE8A activity depends on the presence of divalent cations, and sequence analysis has revealed the presence of conserved metal binding sites in the catalytic domain. *In vitro* experiments showed that maximal PDE8A activity is obtained in the presence of either Mg^{2+} or Mn^{2+} at concentration above 1 mM (Fisher *et al*. 1998). Kinetic studies on recombinant human PDE8A catalytic domain showed that manganese is slightly more efficient than magnesium, and thus might serve as the catalytic metal ion in a physiological setting. However, a biological preference remains to be shown (Wang *et al*. 2008).

Computational analysis of the coding sequence of PDE8A indicated several potential phosphorylation sites (at least three protein kinase A/protein kinase G and tyrosine kinase consensus sequences, plus a site for protein kinase C phosphorylation). Whether PDE8A is phosphorylated or whether this modification regulates the catalytic activity has not been shown (Wang *et al*. 2001).

IBMX, a non-selective inhibitor for most PDE families with IC50 of 2–50 μM, does not effectively inhibit PDE8 activity, unless the concentration of drug is increased to beyond 200 μM (Fisher *et al*. 1998; Soderling *et al*. 1998; Gamanuma *et al*. 2003). The crystal structure of PDE8A's catalytic domain bound to IBMX has been resolved, and data interpretation indicates an important role for Tyr 748 in the unfavorable interaction between IBMX (and presumably other common PDE inhibitors) and the catalytic pocket (Wang *et al*. 2008). Mutation of Tyr 748 to phenylalanine increased the sensitivity of PDE8A to IBMX about tenfold, confirming a pivotal role of this residue in determining PDE8A's selectivity to inhibitors.

The PDE8-selective inhibitor (PF-4957325) has a reported *in vitro* IC50 of 0.7 nM for PDE8A and < 0.3 nM for PDE8B, and is > 2,000-fold more selective over other PDE isoforms (IC50 > 1.5 μM). This inhibitor also shows no activity in an off-target selectivity panel screened at 10 μM (Vang *et al*. 2010, see this reference for a structural depiction).

The presence of protein domains such as the REC and PAS domains predicted by the PDE8A amino acid sequence lead us to expect some form of regulation of its catalytic activity by these domains, as is well established for other PDE families (for example, the GAF domain in PDE2, PDE5 and PDE6 and the Ca^{2+}-calmodulin binding domain in PDE1).

REC domains have an important role in two-component signaling pathways in species such as fungi, slime molds and plants, but their role in mammalian cells is unclear. In the signaling systems, an aspartic acid residue in the REC domain is a target for phosphorylation by a sensory histidine protein kinase, which then induces a conformational change and elicits a downstream response. PDE8A contains a conserved aspartic acid residue in its REC domain that could be the phosphorylation site, but phosphorylation of this site in PDE8 has not been shown (Kobayashi *et al*. 2003).

PAS domains are widespread components of signal transduction proteins, where they serve as universal signal sensors, protein-protein interaction hubs and regulators of effector domains in two-component signaling systems (Möglich *et al*. 2009). PAS domains were originally identified and characterized in several proteins from archaea, eubacteria and unicellular eukaryotes, and are found in all kingdoms of life.

In common with other signal transduction systems, proteins containing PAS domains are modular: PAS sensor (input) domains detect a wide variety of physical and chemical stimuli and regulate the activity of different effector domains in response (output). PAS domains are most frequently found as N-terminal regulators of histidine protein kinase domains, but are also found tethered to kinases, guanylate cyclases, phosphodiesterases, ion channels and chemotaxis proteins. (For more information about PAS domains see this review: Möglich *et al*. 2009). Some authors have suggested regulatory activity by the PAS domain. Wu and Wang reported that a PAS-deleted mutant form of PDE8A showed a 6-fold lower activity than the full-length counterpart in an *in vitro* system (Wu and Wang 2004; see also next section). Thus, the PAS domain seems to enhance the catalytic activity of PDE8A.

A similar conclusion was achieved by comparing the kinetic and structural properties of recombinant human PDE8A forms that contained or lacked the PAS domain (Wang *et al*. 2008). The authors suggest that deleting the PAS domain results in a misfolding of the catalytic domain, which negatively affects its catalytic properties.

INTERACTIONS

Some, but not all, PAS domains bind cofactors such as metabolites, ions, heme and flavin nucleotides (Möglich *et al*. 2009). It is likely that many PAS domains exert their physiological role in the absence of any cofactor. So far, no cofactor has been found to bind the PDE8A PAS domain.

A study by Ping Wu and Peng Wang found that overexpressed recombinant human PDE8A1 (a splice form; see the "Splice Variants" section) can associate with endogenous IκB proteins in human embryonic kidney HEK cells via its PAS domain (Wu and Wang 2004). Functionally, the IκB binding seems to increase PDE8A activity significantly, at least in an *in vitro* system. Whether this interaction occurs under physiological conditions in any tissue or cell that expresses PDE8A remains to be investigated.

PHENOTYPES

In 2006, Vasta *et al*. were the first to describe the generation and characterization of the *Pde8a* knockout mouse. Leydig cells from Pde8a ablated mice show elevated testosterone production compared with wild-type control cells in response to increasing doses of luteinizing hormone (Vasta *et al*. 2006). Cardiomyocytes from these mice also exhibit increased calcium transients and spark frequency than wild-type cells (Patrucco *et al*. 2010). The mechanism by which Pde8a's absence causes either of these phenomena remains unclear. It was also shown that the Pde8 inhibitor PF-4957325 still potentiated pregnenolone production in primary adrenal cells from *Pde8b* knockout animals, while having no effect on *Pde8a/Pde8b* double knockout cells. This indicates that Pde8a has some role in adrenal steroid production, although the extent of this is unknown (Tsai *et al*. 2010).

Phenotypes associated with overexpression of PDE8A are unknown.

MAJOR SITES OF EXPRESSION

Northern blot analysis with a *PDE8A* probe on human mRNA samples from different tissues showed that the messenger transcript is widespread, with higher abundance in testis, ovary, colon and small intestine (Fisher *et al*. 1998). The same analysis conducted on mouse tissues showed high level of mRNA in testis and, progressively decreasing, in liver, kidney, skeletal muscle, heart, eye, ovary, lung and brain (Soderling *et al*. 1998), thus indicating a similar but not totally overlapping expression pattern to human. In rat major expression is found in testis, followed by liver, kidney, heart, thyroid and brain (Kobayashi *et al*. 2003).

Quantitative real-time PCR analysis conducted to detect the tissue distribution of the two major splice variants of human *PDE8A* (*PDE8A1* and *PDE8A2*) gave the following results (in decreasing order):

PDE8A1: testis, spleen, colon, small intestines, ovary, placenta, lymph nodes, prostate, pancreas, kidney, T helper 1 (Th1) cells, Th2 cells, total leukocytes, lung, brain, liver, thymus, heart, bone marrow and skeletal muscle.

PDE8A2: spleen, testis, ovary, placenta, prostate, colon, pancreas, leukocytes, kidney, brain, Th1 cells, lymph nodes, heart, lung, small intestines, Th2 cells, liver, thymus, bone marrow, skeletal muscle (Wang *et al*. 2001).

Protein expression of PDE8A was reported in the following organisms, tissues and cell lines:

- T cells (human) (Glavas *et al*. 2001)

- Spermatozoa (mouse) (Baxendale *et al*. 2005; Vasta *et al*. 2006)

- Leydig cells (mouse) (Vasta *et al*. 2006)

- Ovary (mouse and bovine) (Sasseville *et al*. 2009)

- Brown adipose tissue (Zapala *et al*. 2005; Kraynik *et al*. 2009)

- Ovary theca cells (human) (Chen *et al*. 2009)

- CCRF-CEM T leukemic cells and Jurkat cells (human origin) (Dong *et al*. 2010)

- Adrenal gland (mouse) (Tsai *et al*. 2010)

Please view the EST and GEO Expression profiles for more information.

Additional reference

Kraynik *et al*. 2009. *FASEB J.* 2009, 23, Meeting Abstract Supplement; 582.5.

SPLICE VARIANTS

The human *PDE8A* gene has several transcript variants that seem to be produced by alternative splicing and alternative start sites (Wang *et al*. 2001). PDE8A2 is a spliced variant of PDE8A1 that lacks the PAS domain, and PDE8A3 is a truncated protein lacking both the PAS and REC domains. PDE8A4 and PDE8A5 are identical truncated proteins, with different untranslated sequences, that are longer than PDE8A3 but are still missing both the PAS and REC domains. Experimental evidence that each transcript variant corresponds to a different protein isoform is still missing.

REGULATION OF CONCENTRATION

Putative binding sites for several transcription factors have been found in the promoter region of the human *PDE8A* gene. These include ATF1, E2F1 and TAF1, which are involved in cAMP-responsive transcriptional regulation (Wang *et al*. 2001). No experimental evidence of a physiological role for promoter regulation has been reported so far.

In $CD4^+$ T cells, mRNA and protein levels for PDE8A1 become maximally upregulated by about 8 hours after CD3 and CD28 stimulation (Glavas *et al*. 2001). A similar upregulation was also reported in mouse splenocytes that have been stimulated with concanavalin A (Dong *et al*. 2006). In contrast to these findings, Wang *et al*. (2001) stated that in human $CD4^+$ T cells co-stimulated via CD3 and CD28, *PDE8A* mRNAs were downregulated, but the experimental data supporting this statement were not reported in the paper.

ANTIBODIES

There is a mouse monoclonal antibody, P4G7, specific for both mouse and human PDE8A. It is targeted to the N-terminal PAS domain (Glavas *et al*. 2001).

Santa Cruz, Fabgennix, Abcam and Scottish Biomedical provide commercial antibodies.

REFERENCES

Baxendale RW, Fraser LR (2005). Mammalian sperm phosphodiesterases and their involvement in receptor-mediated cell signaling important for capacitation. *Mol Reprod Dev*, 71, 4.

Chen C, Wickenheisser J, Ewens KG, Ankener W, Legro RS, Dunaif A, McAllister JM, Spielman RS, Strauss JF (2009). PDE8A genetic variation, polycystic ovary syndrome and androgen levels in women. *Mol Hum Reprod*, 15, 8.

Dong H, Osmanova V, Epstein PM, Brocke S (2006). Phosphodiesterase 8 (PDE8) regulates chemotaxis of activated lymphocytes. *Biochem Biophys Res Commun*, 345, 2.

Dong H, Zitt C, Auriga C, Hatzelmann A, Epstein PM (2010). Inhibition of PDE3, PDE4 and PDE7 potentiates glucocorticoid-induced apoptosis and overcomes glucocorticoid resistance in CEM T leukemic cells. *Biochem Pharmacol*, 79, 3.

Dunlap JC, Loros JJ, Liu Y, Crosthwaite SK (1999). Eukaryotic circadian systems: cycles in common. *Genes Cells*, 4, 1.

Fisher DA, Smith JF, Pillar JS, St Denis SH, Cheng JB (1998). Isolation and characterization of PDE8A, a novel human cAMP-specific phosphodiesterase. *Biochem Biophys Res Commun*, 246, 3.

Galperin MY, Nikolskaya AN, Koonin EV (2001). Novel domains of the prokaryotic two-component signal transduction systems. *FEMS Microbiol Lett*, 203, 1.

Gamanuma M, Yuasa K, Sasaki T, Sakurai N, Kotera J, Omori K (2003). Comparison of enzymatic characterization and gene organization of cyclic nucleotide phosphodiesterase 8 family in humans. *Cell Signal*, 15, 6.

Gilles-Gonzalez MA, Gonzalez G (2004). Signal transduction by heme-containing PAS-domain proteins. *J Appl Physiol*, 96, 2.

Glavas NA, Ostenson C, Schaefer JB, Vasta V, Beavo JA (2001). T cell activation up-regulates cyclic nucleotide phosphodiesterases 8A1 and 7A3. *Proc Natl Acad Sci U S A*, 98, 11.

Kobayashi T, Gamanuma M, Sasaki T, Yamashita Y, Yuasa K, Kotera J, Omori K (2003). Molecular comparison of rat cyclic nucleotide phosphodiesterase 8 family: unique expression of PDE8B in rat brain. *Gene*, 319, null.

Möglich A, Ayers RA, Moffat K (2009). Structure and signaling mechanism of Per-ARNT-Sim domains. *Structure*, 17, 10.

Patrucco E, Albergine MS, Santana LF, Beavo JA (2010). Phosphodiesterase 8A (PDE8A) regulates excitation-contraction coupling in ventricular myocytes. *J Mol Cell Cardiol*, 49, 2.

Sasseville M, Albuz FK, Côté N, Guillemette C, Gilchrist RB, Richard FJ (2009). Characterization of novel phosphodiesterases in the bovine ovarian follicle. *Biol Reprod*, 81, 2.

Soderling SH, Bayuga SJ, Beavo JA (1998). Cloning and characterization of a cAMP-specific cyclic nucleotide phosphodiesterase. *Proc Natl Acad Sci U S A*, 95, 15.

Tsai LC, Shimizu-Albergine M, Beavo JA (2011). The high-affinity cAMP-specific phosphodiesterase 8B controls steroidogenesis in the mouse adrenal gland. *Mol Pharmacol*, 79, 4.

Vang AG, Ben-Sasson SZ, Dong H, Kream B, DeNinno MP, Claffey MM, Housley W, Clark RB, Epstein PM, Brocke S (2010). PDE8 regulates rapid Teff cell adhesion and proliferation independent of ICER. *PLoS One*, 5, 8.

Vasta V, Shimizu-Albergine M, Beavo JA (2006). Modulation of Leydig cell function by cyclic nucleotide phosphodiesterase 8A. *Proc Natl Acad Sci U S A*, 103, 52.

Wang H, Yan Z, Yang S, Cai J, Robinson H, Ke H (2008). Kinetic and structural studies of phosphodiesterase-8A and implication on the inhibitor selectivity. *Biochemistry*, 47, 48.

Wang P, Wu P, Egan RW, Billah MM (2001). Human phosphodiesterase 8A splice variants: cloning, gene organization, and tissue distribution. *Gene*, 280, 1-2.

Wu P, Wang P (2004). Per-Arnt-Sim domain-dependent association of cAMP-phosphodiesterase 8A1 with IkappaB proteins. *Proc Natl Acad Sci U S A*, 101, 51.

Zapala MA, Hovatta I, Ellison JA, Wodicka L, Del Rio JA, Tennant R, Tynan W, Broide RS, Helton R, Stoveken BS, Winrow C, Lockhart DJ, Reilly JF, Young WG, Bloom FE, Lockhart DJ, Barlow C (2005). Adult mouse brain gene expression patterns bear an embryologic imprint. *Proc Natl Acad Sci U S A*, 102, 29.