Structure, Localization, and Regulation of cGMP-inhibited Phosphodiesterase (PDE3)*

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*cyclic AMP and cGMP mediate biological responses initiated by diverse extracellular signals. By catalyzing hydrolysis of the 3'-5'-phosphodiester bond of cyclic nucleotides, cyclic nucleotide phosphodiesterases (PDEs) regulate intracellular concentrations and effects of these second messengers. PDEs include a large group of structurally related enzymes (reviewed in Refs. 1–3). These enzymes belong to at least seven related gene families (PDEs 1–7, Fig. 1), which differ in their primary structures, affinities for cAMP and cGMP, responses to specific effectors, sensitivities to specific inhibitors, and mechanisms of regulation (1–3). Most families are comprised of more than one gene; 14 different PDE genes have been identified. Within different families, tissue-specific mRNAs are generated from the same gene by the use of different transcription initiation sites or by alternative mRNA splicing. Although some aspects of different PDE families will be discussed, this review emphasizes the PDE3 family, including structure-function information and regulation of the adipocyte PDE3, which plays a key role in the antilipolytic action of insulin.

Mammalian PDEs share a common structural organization, with a conserved catalytic core (~270 amino acids) usually located in the C-terminal half (Fig. 1) (4). This region is much more similar within an individual PDE family (~80% amino acid identity) than between different PDE families (~25–40% identity) (1–4). The catalytic core is thought to contain common structural elements important for hydrolysis of the cyclic nucleotide phosphodiester bond, as well as family-specific determinants responsible for differences in substrate affinities and inhibitor sensitivities among the different gene families. It contains a PDE-specific sequence motif, HDWX3HXX2N, and two consensus Zn2+-binding domains, the second of which overlaps the PDE motif (3, 5). PDE3 contains tightly bound Zn2+, which supports catalytic activity (5). The precise role of Zn2+ or other divalent cations in catalytic function of other PDEs has not been defined. Mutagenesis of the first histidine of the PDE sequence motif abolished activity of a recombinant PDE4 expressed in Esherichia coli (6). Histidine- and sulfhydryl-modifying reagents inhibited PDE3 activity (7).

The widely divergent N-terminal portions of PDEs (Fig. 1) contain determinants that confer regulatory properties specific to the different gene families, e.g. calmodulin-binding domains (PDE1); two non-catalytic cyclic nucleotide-binding domains (PDEs 2, 5, and 6); N-terminal membrane-targeting (PDE4) or hydrophobic membrane-association (PDE3) domains; and calmodulin (PDE1),

Distinctive Characteristics of PDE3s

PDE3s, purified to apparent homogeneity from a variety of tissues, can be distinguished from other PDEs by their high affinities for both cAMP and cGMP, with $K_i$ values in the range of 0.1–0.8 $\mu$m and $V_{max}$ for cAMP 4–10 times higher than that for cGMP (1–3, 8).

PDE3 is for several reasons often referred to as the cGMP-inhibited PDE. When different PDEs were first identified, two types (now classified as PDE3 and PDE4) (3) that exhibited a high affinity for cAMP were isolated from various tissues. PDE3, but not PDE4, exhibited a high affinity for both cAMP and cGMP. As might be predicted from their $K_i$ values, cAMP and cGMP were mutually competitive substrates for PDE3. In contrast, cGMP was hydrolyzed poorly by, and did not inhibit, PDE4. Thus, PDE3 was called the cGMP-inhibited PDE to distinguish it from PDE4. In fact, some biological effects of endogenous cGMP may be mediated by inhibition of PDE3, which results in increased cAMP and activation of cAMP-dependent protein kinase (protein kinase A) (9). For example, in rabbit platelets (9), mouse thymocytes (10), and human atrial and frog ventricular myocytes (11), nitrovasodilators (which release nitric oxide and activate guanylyl cyclase) increase cAMP, at least in part, by increasing cGMP, which inhibits PDE3. PDE2 isoforms, which are allosterically activated by cGMP, also serve as a locus for “cross-talk” between cAMP and cGMP signaling systems. PDE2 is highly concentrated in bovine adrenal glomerulosa cells where atrial natriuretic factor inhibits cAMP-stimulated aldosterone biosynthesis, at least in part, by stimulating guanylyl cyclase and increasing cGMP, which activates PDE2 leading to a decrease in cAMP and protein kinase A activity (12).

PDE3 isoforms are also characterized by their sensitivity to several specific inhibitors and drugs, including cilorast, enoxime, and lixaizone (reviewed in Refs. 13–15), compounds relatively selective for PDE3, with $K_i$ and $IC_{50}$ values at least 10–100-fold lower for PDE3 than for other PDE families. The availability of family-specific PDE inhibitors, especially for PDE3, 4, and 5 (Fig. 1), has facilitated understanding of functions of individual PDEs in regulating specific cyclic nucleotide-mediated processes, e.g. PDE3s in regulation of certain cAMP-modulated processes, including stimulation of myocardial contractility, inhibition of platelet aggregation, relaxation of vascular and airway smooth muscle, and inhibition of proliferation of T-lymphocytes and cultured vascular smooth muscle cells (13–17). The pharmaceutical industry has exhibited considerable interest in developing specific inhibitors of individual PDE families and subfamilies as therapeutic agents to replace widely used but nonselective PDE inhibitors such as theophylline.

That subject is, however, beyond the scope of this review.

A third important general characteristic of PDE3s (discussed in more detail below) involves their phosphorylation and short-term activation in response to insulin as well as to agents that increase cAMP in adipocytes, hepatocytes, and platelets (reviewed in Ref. 18). Other PDEs, in addition to PDE3, are also regulated by phosphorylation (1–3). For example, phosphorylation of PDE1A by Ca2+/calmodulin-dependent protein kinase or PDE1B by protein kinase A reduces affinity of both PDE1 isoforms for calmodulin.

Binding of cGMP to non-catalytic binding sites in the regulatory
domain of PDE5 enhances phosphorylation of PDE5 by cGMP-dependent protein kinase (protein kinase G). Phosphorylation of γ-inhibitory subunits of PDE6 by protein kinase C alters their affinity for the PDE6β catalytic subunits. Effects of phosphorylation on activities of PDEs 1, 5, and 6 in intact cells have not been documented (1–3). A PDE4 isozyme is phosphorylated and activated in response to hormones that increase cAMP in intact cells and by protein kinase A in vitro (1–3). Feedback regulation of both PDE3 and PDE4 activities by cAMP-dependent phosphorylation is likely to be central to intracellular mechanisms for regulating the magnitude and duration of cAMP signals and responses and desensitization to hormone signals (2).

**Figure 1. Structural organization of different PDE families.** Different genes within the same family are designated as A, B, etc. Some family-specific inhibitors are in brackets. The catalytic domain of PDE3 contains an insertion of 44 amino acids (1) that does not align with other PDEs. PDE6 can exist as a αβγ heterotrimer of α catalytic and γ inhibitory subunits; α and β subunits are products of different but closely related genes. This schematic does not emphasize structural diversity found in N- and C-terminal regions of variants in the different gene families, especially PDE1E and PDE4. [P], location of potential phosphorylation sites. Regions involved in calmodulin (CaM-BD) or cGMP binding (cGMP-BD) are indicated. IBMX, isobutylmethylxanthine.

**Figure 2. Model of domain structure and membrane association of PDE3.** A, domain organization of deduced amino acid (aa) identities of PDE3A and PDE3B isoforms. Numbers are percentage of amino acid identity in the indicated domain of the adjacent sequences (PC Gene, FALIGN). B, membrane association of RPDE3B. The deduced sequence of RPDE3B predicts five or six helical, potentially transmembrane, segments in the N-terminal hydrophilic region (PSORT, version 6.3; TM pred [ISREC]). Based on the deduced sequence of RPDE3B, serine-302 within a protein kinase A consensus sequence (RRPS) is phosphorylated in intact rat adipocytes incubated with insulin and/or isoproterenol. The C-terminal catalytic domain is shown with the shaded PDE3 insertion.

**Tissue-specific Expression, Structure, and Subcellular Location of PDE3 Isoforms**
cDNAs for two PDE3 isoforms (recently classified as PDE3A and PDE3B, respectively (3)) have been cloned from human (H) and rat (R) libraries (19, 20). Deduced sequences of PDE3A (or -B) from different species (rat and human) are more similar than are those of PDE3A and B from the same species (Fig. 2). The human isoforms, HPDE3A and -B, are products of different genes on chromosomes 12 and 11, respectively. Two HPDE3A mRNA species of ~7.6 and ~4.4 kilobases, encoding predicted ~125 and 80-kDa proteins, may be transcribed in a tissue-specific manner, using different initiation sites in the HPDE3A gene (21).

In situ hybridization and Northern blot hybridizations demonstrated overlapping but distinct tissue and cellular distributions of (rat) RPDE3A and RPDE3B mRNAs (20, 22). RPDE3B mRNA was prominent in white and brown adipose cells, hepatocytes, renal collecting duct epithelium, and developing spermatocytes; RPDE3A mRNA was more abundant in heart and vascular smooth muscle (22). The distribution of RPDE3A mRNA in developing rat brain was heterogeneous, whereas RPDE3B mRNA was uniformly present in germinal neuroepithelium and mature neurons (23). PDE3B (not PDE3A) mRNA and enzyme activity (associated with adipocyte particulate fractions) was found in cultured murine 3T3-L1 adipocytes but not undifferentiated 3T3-L1 fibroblasts (24). Human platelet PDE3 is a PDE3A isoform (25). These and other findings suggest that PDE3A and -B likely exhibit cell-specific differences in properties and regulation and may serve cell-specific functions.

Cell-specific expression of different members of the PDE1 family has also been reported (3). In situ hybridization demonstrated that of three different PDE1s in mouse and rat brain, PDE1A mRNA is abundant in cortex and portions of the hippocampus, PDE1B mRNA in the striatal region and dentate gyrus, and PDE1C in olfactory neuronal epithelia (3). Selective expression of different representatives of the same PDE family (or of different families) in different and limited populations of cells (1–3) has important implications not only for regulation of cyclic nucleotide concentrations and their biological effects in specific cells but also in targeting of specific PDEs for therapeutic intervention.

The structural organization of PDE3A and -3B proteins is identical. The catalytic domain conserved among all PDEs is in the C-terminal half of the PDE3 molecules (Fig. 1) and is followed by a hydrophilic C-terminal region (Fig. 2). Although the catalytic domains of PDE3A and -3B are very similar, an insert of 44 amino acids in the PDE3-conserved domain, which does not align with sequences in the cognate domains of other PDE families (Fig. 1), differs in PDE3A and -B isoforms (Fig. 2). This insertion, which distinguishes PDE3 catalytic domains from those of other PDEs and may identify subfamilies within the PDE3 family, interrupts the first (of two) putative Zn²⁺-binding domains present in the catalytic domains of all PDEs (5, 19, 20). Whether the 44-amino acid insert in PDE3 is involved in its interaction with substrates and inhibitors remains to be established. The N-terminal portions of PDE3A and -3B are quite divergent (Fig. 2). The N terminus of RPDE3B is enriched in proline residues (20). Both PDE3A and -B contain hydrophobic putative membrane-association domains with several predicted helical transmembrane segments and several downstream consensus sequences (RRXS) for phosphorylation by protein kinase A (Fig. 2). In intact rat adipocytes, serine 302 in a microsomal PDE3B is phosphorylated in response to insulin and agents that increase cAMP (26). A comparison of the properties of full-length and truncated PDE3A and -B recombinants and purified platelet PDE3A after limited proteolysis indicates that the PDE3 catalytic core includes the conserved PDE domain plus some additional N- and C-terminal sequences (Fig. 2), that the N-terminal portions of PDE3A and PDE3B isoforms are not required for PDE3 catalytic activity or sensitivity to specific PDE3 inhibitors (Fig. 2), and that HPDE3A may be more sensitive to inhibition by cGMP than RPDE3B (19, 25, 27, 28). Multiple structural determinants in cGMP interact with PDE3, since different modifications of the guanine ring altered the IC₅₀ values of a series of cGMP analogs for inhibition of cAMP hydrolysis (29).
PDE3s are found in both particulate and cytosolic fractions of cells (8). The open reading frames of PDE3A and B cDNAs predict proteins of ~122–125 kDa (Fig. 2), consistent with monomeric sizes of 130–135 kDa for PDE3 isoforms identified in adiocyte microsomal and cardiac sarcoplasmic reticulum fractions (30, 31). PDE3 isoforms of ~110 kDa and less have been purified from cytosolic fractions of human platelets, bovine ventricular myocardium, and bovine aortic smooth muscle (25, 32–34). The N-terminal hydrophobic regions, which contain several predicted transmembrane segments, are likely to be important in membrane association of PDE3s, since PDE3 and PDE3A recombinants (both full-length and truncated forms, which contain portions of the hydrophobic region) were found predominantly in particulate fractions of S99 cells, whereas recombinants containing the C-terminal catalytic core but lacking the hydrophobic sequences were predominantly cytosolic (21, 27). Whether cytosolic PDE3 isoforms are generated by proteolytic removal of the membrane-association region or result from the use of alternative transcription initiation sites or alternative mRNA splicing is unknown (21, 25, 27, 31–34).

Regulation of Adipocyte PDE3B by Insulin and Agents That Increase cAMP

In adipose tissue, activation of a microsomal PDE3B is a major mechanism by which insulin antagonizes catecholamine-induced release of free fatty acids, a quantitatively important energy source in mammals (18, 35–37). Selective PDE inhibitors such as cilostamide, OPC-3811, and CI-930 blocked the anti-lipolytic action of insulin (38–40). Of a series of CAMP analogs, all of which activated protein kinase A and stimulated lipolysis, insulin effectively inhibited the lipolytic effects of only those analogs that were substrates of protein kinase A (26, 30, 37, 43). In PDE3A, insulin antagonizes cAMP-related modulation of protein kinase A activity and inhibits lipolysis of insulin-stimulated adipocytes (26, 30, 37, 43). In PDE3B, a single serine located in a protein kinase A consensus sequence (-MFRIFPS302LPCISREQ-) is phosphorylated in response to insulin, isoproterenol, or the combination of both (26, 44). cAMP-PDE3 signaling pathways upstream of PDE3B and that protein kinase A is involved in sensitization of the insulin-signaling pathway and activation of PDE3B (Fig. 3). cAMP-enhancing agents have been reported to modulate responses to insulin and other growth factors (44–46). In adipocytes, insulin-stimulated tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) results in translocation of IRS-1 to PDE3 and that protein kinase A is involved in sensitization of the insulin-signaling pathway and activation of PDE3B (42).2 Mitogen-activated protein kinases and pp70S6 kinases are apparently not involved in this cross-talk or in the activation of PDE3B by insulin.

As depicted in Fig. 2B, Ser-302 is just C-terminal to the putative membrane-association domain of PDE3B. This model is consistent with earlier reports of purification of a truncated ~70-kDa PDE3 that was released from rat hepatic membrane fractions by limited proteolysis (48). Phosphorylation of Ser-302 might relieve inhibitory constraints or alter conformation of the catalytic domain, since limited proteolysis of rat adipocyte microsomal fractions from control increased PDE activity to that level, as that in PDE3B phosphorylation/activation of PDE3B, and that protein kinase A is involved in sensitization of the insulin-signaling pathway and activation of PDE3B (42). Mitogen-activated protein kinases and pp70S6 kinases are apparently not involved in this cross-talk or in the activation of PDE3B by insulin.

One could speculate that PDE3B is located in microsomal membranes in close proximity to a docking protein for an adipocyte protein kinase A-anchoring protein (50), involved in translocation of protein kinase A (and other signaling molecules) to spatially segregated substrates such as membrane-associated PDE3B. In 3T3-L1 adipocytes such spatial segregation of PDE3B and the lipolytic machinery, resulting in PDE3B regulation of a “pool” of cAMP related specifically to modulation of protein kinase A and HSL, could explain the observed inhibition of the anti-lipolytic effect of insulin by PDE3 inhibitors but not by PDE4 inhibitors (51). In general terms, PDEs may play an important role in the functional or spatial compartmentation of cyclic nucleotide signaling processes. In recent studies on regulation of Ca2+ channels in single isolated frog ventricular myocytes, Jurevics and Fischmeister (52)

2 J. Wijkander et al., unpublished data.
presented evidence to suggest that β-adrenergic receptors, adenyl cyclase, protein kinase A, Ca²⁺ channels, and PDEs (specific PDE isoforms were not identified) were co-localized (52). Their results implicated PDEs as critical regulators in limiting protein kinase A activation to “local” Ca²⁺ channels and preventing diffusion of cAMP to “distant” areas of the same cell. In human and rat pancreatic islet preparations, PDE3, not PDE4, inhibited stimulated insulin secretion, thus, could reflect the presence of PDE3 and PDE4 in different islet cells or different locations in the same cell (53).

Regulation of Other PDEs

Insulin-mediated activation of PDE3 may be an important component in insulin regulation of other cAMP-modulated processes, including growth and differentiation and carbohydrate metabolism. In the frog (Xenopus laevis) oocyte, stimulation of oocyte meiotic maturation by insulin, insulin-like growth factor-I, or Hواصل is associated with activation of oocyte PDE and inhibition of adenyl cyclase, protein kinase A, Ca²⁺, and Ca²⁺-activated protein kinase C (56). Although oocyte PDEs have not been characterized, specific PDE3 inhibitors, but not PDE4 or PDE5 inhibitors, inhibit oocyte maturation (54). It has been suggested (55, 56) that an insulin- and cAMP-stimulated PDE3 in liver (phosphorylated and activated in vitro by protein kinase A (56)) is important in the anabolic effects of insulin (41). In addition to PDE3, an insulin- and cAMP-stimulated PDE3 in liver (phosphorylated by protein kinase A) is associated with activation of oocyte PDE and inhibition of meiotic maturation by insulin, insulin-like growth factor-1, or Hواصل (57). Insulin-mediated activation of PDE3 may be an important component in insulin signal transduction pathways (58). In platelets, specific PDE3 inhibitors prevent aggregation, suggesting that PDE3 is important in platelet function (58). Incubation of intact platelets with insulin or effectors that increase cAMP, as well as treatment in vitro with protein kinase A or a partially purified PDE3 inhibitor, resulted in phosphorylation and activation of platelet PDE3 (32, 59). As was found in adipocytes (30, 43), activation of the platelet PDE3 by insulin correlates with serine phosphorylation (60) although the effects of insulin activation of PDE3 on platelet function are not known. Specific PDE3 inhibitors enhance myocardial contractility and induce vascular and airway smooth muscle relaxation (13–17). Both cardiac (33) and vascular smooth muscle (34) PDE3 isoforms are phosphorylated in vitro by protein kinase A; little is known, however, about the regulation of these enzymes in intact cells.

Many fundamental questions concerning PDE3 isoforms remain to be addressed, including identification of the different PDE proteins in cells and tissues, elucidation of mechanisms for regulation of their gene expression, distribution, and activity, their structure-function relationships, and their role in the action of insulin and agents that increase cAMP and cGMP. Perhaps more fundamental will be understanding mechanisms for regulation of the overall PDE composition of individual cells, functional integration of the different PDEs in establishing and regulating cyclic nucleotide “fingerprints” of individual cells, and the targeting of specific PDEs for therapeutic benefit.

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