Overview of PDEs and Their Regulation

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Abstract—Contraction and relaxation of vascular smooth muscle and cardiac myocytes are key physiological events in the cardiovascular system. These events are regulated by second messengers, cAMP and cGMP, in response to extracellular stimulants. The strength of signal transduction is controlled by intracellular cyclic nucleotide concentrations, which are determined by a balance in production and degradation of cAMP and cGMP. Degradation of cyclic nucleotides is catalyzed by $3',5'$-cyclic nucleotide phosphodiesterases (PDEs), and therefore regulation of PDEs hydrolytic activity is important for modulation of cellular functions. Mammalian PDEs are composed of 21 genes and are categorized into 11 families based on sequence homology, enzymatic properties, and sensitivity to inhibitors. PDE families contain many splice variants that mostly are unique in tissue-expression patterns, gene regulation, enzymatic regulation by phosphorylation and regulatory proteins, subcellular localization, and interaction with association proteins. Each unique variant is closely related to the regulation of a specific cellular signaling. Thus, multiple PDEs function as a particular modulator of each cardiovascular function and regulate physiological homeostasis. (Circ Res. 2007;100:309-327.)

Key Words: cAMP ■ cGMP ■ cyclic nucleotide ■ cell signaling ■ phosphodiesterase inhibitor

Numerous cellular functions are regulated by second messengers, cAMP and cGMP (Figure 1). In the cardiovascular system, blood pressure is regulated by contraction and relaxation of vascular smooth muscle in association with vascular endothelial functions. Beating of cardiac myocytes is accurately controlled to pump blood out of the heart to other parts of the body according to environmental conditions. These events in hemodynamics are ingeniously regulated by extracellular stimulation through alteration of intracellular cyclic nucleotide levels, which are determined by a balance between their production and degradation by $3',5'$-cyclic nucleotide phosphodiesterases (PDEs).1-5 Downstream effector proteins of cAMP and cGMP are cAMP-dependent protein kinase (PKA), cGMP-dependent protein kinase (PKG), cyclic nucleotide-gated ion channels, and cAMP-regulated guanine nucleotide exchange factors (cAMP-GEFs), which are also called exchange proteins directly activated by cAMP (Epacs). PDEs are also downstream effectors of cAMP and cGMP. Recent studies on cyclic nucleotide-mediated signaling have revealed that the signal for each physiological event is independently regulated by compartmentation of certain signaling molecules.6 PDEs are closely related to the regulation of each specific transduction signal, and therefore multiple PDEs play important roles in modulating each cellular function. Our goal here is to give an overview of the expanding molecular characteristics of PDE families principally in humans.
PDE Family

PDEs are classified into classes I, II, and III. Mammalian PDEs, which belong to class I PDEs, have an HD domain (Pfam accession no. PF01966) in the C-terminal half and show high affinity for cAMP and/or cGMP. Protein domains involved in regulation of PDE enzymatic activity and subcellular localization are mainly present in the N-terminal half. Some PDEs have phosphorylation sites targeted by protein kinases and lipid modification sites (Figure 2).

Twenty-one class I PDE genes have been identified in human, rat, and mouse (Figure 3). They are categorized into 11 different families based on structural similarity such as sequence homology, protein domains, and enzymatic properties, including substrate specificity, kinetic properties, and sensitivity to endogenous regulators and inhibitors (Table 1 and the online supplemental Table, available at http://circres.ahajournals.org). Approximately 270 aa in the C-terminal catalytic domain are conserved, with a sequence identity of 35% to 50% among different PDE families. Some PDE families are composed of 2 to 4 subfamily genes showing sequence identity of more than 70% and having identical protein domains organization. Multiple transcriptional products, which are generated from most PDE genes by alternative splicing or transcription from distinct promoters, have actually been identified or predicted in human DNA databases. Thus, the mammalian PDE superfamily is composed of 21 genes and their multiple transcriptional splice variants.

According to the nomenclature used for each PDE isozyme (e.g., HsPDE1A1), the first 2 letters indicate the animal species and the first Arabic number after PDE designates the PDE gene family. This number is followed by a single capital letter indicating a distinct subfamily gene. The last Arabic number indicates a specific splice variant or a specific transcript generated from a unique transcription initiation site (http://depts.washington.edu/pde/pde.html).

The unique characteristics of each PDE gene family are defined by protein domains located in the N terminal to the catalytic unit. As shown in Figure 2, approximately half of PDE gene families (PDE2, PDE5, PDE6, PDE10, and PDE11) have a protein domain termed GAF [Pfam accession no. PF01590] in tandem and are therefore designated GAF-PDE subfamily. The known functions of GAF domains are cGMP binding-mediated allosteric regulation and dimerization of GAF-PDEs. Some GAF domains have also been reported to bind cAMP. Other PDEs (PDE1, PDE3, PDE4, and PDE7–9) have no GAF domain and belong to the non–GAF-PDE subfamily. PDE1 contains a Ca\(^{2+}/\)calmodulin (CaM)-binding site, PDE3 has a transmembrane domain, PDE4 has upstream conserved regions (UCRs), and PDE8 has a response regulator receiver (REC) domain [Response_reg; Pfam accession no. PF00072] and a per–arnt–sim (PAS) domain [Pfam accession no. PF00989]. PDE7 and PDE9 have no specific protein domain in addition to the PDE catalytic domain.

General Characteristics

Fundamental information about the 11 human PDE gene families and PDE inhibitors are summarized in Table 1 and the online supplemental Table.

Phosphodiesterase 1

Three subfamily genes, PDE1A to -C, encode Ca\(^{2+}/\)CaM-dependent cAMP- and cGMP-hydrolyzing PDEs. In humans, PDE1A shows high affinity for cGMP,\(^8\) PDE1B hydrolyzes cGMP with a \(K_m\) value lower and a \(V_{\text{max}}\) value higher than those for cAMP.\(^9\) High affinity for both cAMP and cGMP is observed with PDE1C.\(^8\)

Phosphodiesterase 2

PDE2A hydrolyzes both cGMP and cAMP with similar maximal rates and relatively high \(K_m\) values. PDE2A is allosterically stimulated by cGMP binding to its GAF domain,\(^10\) which enables mutual regulation of both cAMP and cGMP signaling.
**Phosphodiesterase 3**

PDE3A and PDE3B are the subfamily genes of PDE3, which shows high affinity for both cAMP and cGMP. A low $V_{\text{max}}$ value for cGMP compared with that for cAMP lets cGMP function as a competitive inhibitor for cAMP hydrolysis. Therefore, PDE3s are termed cGMP-inhibited cAMP PDEs. The presence of a 44-aa insert in the catalytic domain is a unique characteristic of the PDE3 family. Another special feature is the presence of N-terminal hydrophobic membrane association domains (NHRs).

**Phosphodiesterase 4**

Four highly similar subfamily genes, PDE4A to -D, encode cAMP-specific rolipram-sensitive PDEs. The PDE4 family includes a number of splice variants categorized into 3 N-terminal variant groups ("long form," "short form," and "super-short form") based on the presence or absence of N-terminal UCR domains (Figure 2). The long form variants contain UCR1, linker region (LR) 1, UCR2, LR2, and the catalytic domain. The short form and the super-short form variants have LR1-UCR2-LR2 and UCR2 (truncated)-LR2 in the N-terminal region, respectively. UCR1, which includes 1 PKA phosphorylation site, is connected to UCR2 by LR1. UCR2 has a hydrophilic N-terminal region, which intramolecularly interacts with the hydrophobic C-terminal portion of UCR1. UCR1 and UCR2 are involved in PDE4 enzymatic regulation through UCR2 interaction with the catalytic domain and have also been reported to participate in PDE4 regulation.

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**Figure 2.** Schematic representation of the eleven human PDE families. Representative members that constitute the 11 human PDE families are shown here. Each PDE protein is indicated by a thick line. Protein regions are represented by rectangles with patterns. N-terminal variation of PDE1A variants carrying N1, N2, and N3 sequences is shown in a box. Splice variants of PDE5, PDE10, and PDE11 families are also shown in boxes. The 3 isoforms in the PDE4 family are illustrated and boxed. PDE3 enzymes produced by alternative translation initiation are boxed. Reported phosphorylation sites are indicated with arrows. ERK phosphorylation site in PDE4A variants is absent.
The level of cGMP, a second messenger in visual signal transduction, is tightly controlled through regulated cGMP hydrolysis by 3 PDE6 subfamily genes (photoreceptor PDEs). Light-activated transducin stimulates PDE6 activity by removing the inhibitory subunit γ. Membrane hyperpolarization is caused by cGMP elimination, leading to electrical cellular response. 

Phosphodiesterase 5

PDE5A, which has 2 GAF domains (GAF A and GAF B) in the N-terminal half, specifically hydrolyzes cGMP. PDE5A GAF A domain has been reported to be responsible for this enzyme allosteric binding to cGMP, and therefore PDE5A is termed cGMP-binding cGMP-specific PDE. One PKG- and PKA-dependent phosphorylation site in the N-terminal region is related to activation of PDE5A enzyme. cGMP binding to PDE5A GAF A domain promotes phosphorylation, which not only activates the catalytic function but also increases cGMP-binding affinity.

Phosphodiesterase 6

In retinal rod and cone cells, the level of cGMP, a second messenger in visual signal transduction, is tightly controlled through regulated cGMP hydrolysis by 3 PDE6 subfamily genes (photoreceptor PDEs). Light-activated transducin stimulates PDE6 activity by removing the inhibitory subunit γ. Membrane hyperpolarization is caused by cGMP elimination, leading to electrical cellular response. 

Phosphodiesterase 7

Two subfamily genes, PDE7A and PDE7B, encode rolipram-insensitive high-affinity cAMP-specific PDEs (K_m value, approximately 0.2 μmol/L). A PKA pseudosubstrate site is present in the N terminus of PDE7A subfamily.

Dipyridamole nonselectively inhibits PDE7 activity.

Phosphodiesterase 8

PDE8A and PDE8B are the subfamily genes of PDE8. PDE8s are high-affinity cAMP-specific PDEs insensitive to rolipram and 3-isobutyl-1-methylxanthine (IBMX), and contain REC and PAS domains in the N-terminal portion. REC domain functions as a receiver of signals from the sensor component in 2-component signal transduction system in lower organisms. PAS domain is involved in the binding of small ligands and protein–protein interaction. However, regulation of PDE8s via REC or PAS domain is unknown, and obvious endogenous PDE8 activity has not yet been demonstrated in either tissue or cell extracts. Actually, many studies on IBMX-insensitive cAMP PDE activity have been reported. It is true that this activity has, for the most part, not been definitively ascribed to PDE8, but it is very likely attributable to PDE8. Dipyridamole inhibits PDE8 activity but not selectively.

Phosphodiesterase 9

PDE9A specifically hydrolyzes cGMP with high affinity. However, there is no report on the regulation of PDE9A activity or the presence of endogenous PDE9A activity in either tissue or cell extracts. IBMX-insensitive cGMP PDE activity, which has been shown so far, is likely attributable to PDE9.

Phosphodiesterase 10

PDE10A contains 2 N-terminal GAF domains and hydrolyzes both cAMP and cGMP. High affinity for cAMP inhibits cGMP hydrolysis, making this enzyme a cAMP-inhibited dual-substrate PDE. Among newly discovered PDEs, the enzymatic activity of PDE10A is clearly demonstrated in tissue extracts. The enzymatic activity of a chimeric construct of PDE10A GAF domain and cyanobacterial adenyl cyclase is stimulated by cAMP, suggesting a possible allosteric modulation of PDE10A activity by cAMP. Papaverine is known as the most potent inhibitor of PDE10A.

Phosphodiesterase 11

A full-length form, PDE11A, contains 2 GAF domains and a catalytic domain. PDE11A hydrolyses both cAMP and cGMP with similar K_m values. Although a cyanobacterial adenyl cyclase fused with PDE11A GAF domains is activated by cGMP, there is no report on the allosteric regulation of PDE11A enzyme. PDE11A activity has not yet been clearly demonstrated in tissue or cell extracts. Tadalafil has been shown to potently inhibit PDE11A activity still much less potently than it inhibits PDE5A.
PDE Variants and Their Tissue-Expression Patterns

Phosphodiesterase 1

N-terminal and C-terminal variants of PDE1A are divided into 3 groups based on their N-terminal sequences (Figure 2). In humans, PDE1A5 (PDE1A2), PDE1A6 (PDE1A3), and PDE1A9 encode the N-terminal sequence N1, whereas PDE1A1, PDE1A4, and PDE1A8 have the N-terminal sequence N2. The N-terminal sequence N3 is encoded by PDE1A10 to -1A12. These N-terminal sequences differ in Ca2+/CaM-binding domain, and therefore activation by Ca2+/CaM is N-terminal sequence dependent. PDE1As carrying the N2 sequence are activated by Ca2+/CaM, whereas activation of PDE1As with the N1 sequence is not statistically significant. There is no report on the activation of PDE1As with the N3 sequence. In humans, PDE1A5 and PDE1A6 expression is brain specific, whereas PDE1A1 and PDE1A4 expression is ubiquitous but high in the kidney, liver, pancreas, and thyroid gland. PDE1A10 expression is testis specific. In mice, expression of the 61-kDa PDE1 protein (PDE1A) has been reported in the brain.

There are 2 N-terminal variants in humans, PDE1B1 and PDE1B2. PDE1B1 expression is predominant in the caudate nucleus and putamen of the brain and low in the heart and skeletal muscle. PDE1B2 transcripts are found mostly in the spinal cord. Detailed expression of the 63-kDa PDE1 protein (PDE1B) has been reported in mouse brain.

PDE1C1 and PDE1C3 are human N-terminal variants. Rat PDE1C2 encodes distinct N- and C-terminal sequences. PDE1C4 and PDE1C5, both of which encode the same 654-aa protein, are C-terminal variants of PDE1C1 in mice. Human PDE1C expression is high in the heart and brain. Rat PDE1C transcripts are very few in the heart but are abundant in olfactory epithelium. Mouse PDE1C1 and PDE1C5 transcripts are very few in the heart but are abundant in the cerebellum.

Phosphodiesterase 2

The PDE2A gene encodes 3 N-terminal splice variants containing 2 GAF domains. PDE2A3, which is a human variant, is membrane associated probably because of its unique N-terminal sequence. PDE2A transcripts are rich in the brain and moderate in the heart. Immunoactive PDE2A

<table>
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<th>Gene Family</th>
<th>Gene</th>
<th>Chromosomal Localization</th>
<th>Substrate</th>
<th>K_m (µmol/L)</th>
<th>Relevance</th>
<th>Major Tissue Expression</th>
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K_m values of PDE1–6 are from Bender and Beavo and Francis et al. K_m values from PDE7 to PDE11 are from those of human PDEs.
protein is rich in the neocortex and low in other tissues, and its signals are also localized in capillary and venous endothelial cells and in microvessel endothelial cells but not in arterial endothelial cells of intact tissues. In contrast, cultured endothelial cells exhibit PDE2 activity.

**Phosphodiesterase 3**

Transcripts of PDE3A1 (8.2 kb) and PDE3A2 (6.9 kb) carrying distinct 5' regions are produced by alternative transcription within exon 1 in human cardiovascular system. These 2 transcripts encode PDE3A3, which is generated from exon 4, and PDE3A-94. Only PDE3A-94 has the Akt/protein kinase B (PKB) phosphorylation site, which is critical for enzymatic activation. PKA phosphorylation site is present in PDE3A-136 and PDE3A-118, but not in PDE3A-94. PDE3A1 expression is low in cardiac myocytes, but PDE3A2 expression is high in both cardiac and vascular myocytes. PDE3A3 expression is observed in heart, vascular and placental smooth muscle, corpus cavernosum smooth muscle, and platelets. Rat PDE3A transcripts are abundant in the myocardium, smooth muscle, epithelium, megakaryocytes, and oocytes.

Conversely, only PDE3B1 has been identified in humans. PDE3B transcripts are predominant in human adipose tissue. In rats, PDE3B expression is evident in white and brown adipose tissues and is also found in hepatocytes, renal collecting duct epithelium, and developing spermatocytes. PDE3B transcripts are also abundant in embryonic neuroepithelium including neural retina but not in mature nervous system.

**Phosphodiesterase 4**

PDE4A1 encodes a short form protein. Long forms of PDE4A proteins are encoded by PDE4A4B, PDE4A8, PDE4A10, and PDE4A11. In humans, PDE4A expression is fundamentally ubiquitous and relatively high in the brain with variant-specific tissue distribution pattern. PDE4A4B transcripts are found in T cells, and PDE4A10 expression is high in the heart and small intestine. PDE4A11 transcripts are widely observed in various tissues with high expression in fetal brain but not in adult brain. In rats, PDE4A transcripts are rich in brain neurons and the olfactory system.

The long forms PDE4B1 and PDE4B3 and the short form PDE4B2 are human PDE4B variants. PDE4B4, which encodes a long form protein, is an additional variant in rats. PDE4B4 is widely distributed in various tissues with variant-specific tissue distribution pattern. In immune cells, PDE4B and PDE4D isoforms are predominant compared with PDE4A and PDE4C isoforms. Human PDE4B2 transcripts are abundant in leukocytes, especially neutrophils.

scripts of PDE4B2, which is the major PDE4B isoform in normal B cells, are abundant in naive and memory B cells and low in centroblasts and centrocytes. In rats, PDE4B3 transcripts are present in the brain, heart, lung, and liver, whereas PDE4B4 expression is specific to the liver and brain.

The long forms PDE4C1, PDE4C2, and PDE4C3, which are generated from distinct promoters, have been identified in humans. PDE4C-Δ54 and PDE4C-Δ109 transcription is thought to be driven from a separated common promoter. PKA phosphorylation site is present in PDE4C-136 and PDE4C-118, but not in PDE4C-94. PDE4C1 expression is low in cardiac myocytes, but PDE4C2 expression is high in both cardiac and vascular myocytes. PDE4C3 expression is observed in heart, vascular and placental smooth muscle, corpus cavernosum smooth muscle, and platelets. Rat PDE4C transcripts are abundant in the myocardium, smooth muscle, epithelium, megakaryocytes, and oocytes.

Conversely, only PDE3B1 has been identified in humans. PDE3B transcripts are predominant in human adipose tissue. PDE3B expression is evident in white and brown adipose tissues and is also found in hepatocytes, renal collecting duct epithelium, and developing spermatocytes. PDE3B transcripts are also abundant in embryonic neuroepithelium including neural retina but not in mature nervous system.

**Phosphodiesterase 5**

N-terminal variants PDE5A1 to -5A3, which show similar K_m values (eg, ~6 μmol/L) have been identified in humans (Figure 2). One N-terminal phosphorylation site for PKA and PKG is conserved among all 3 variants. In humans, PDE5A5 transcripts are rich in various tissues, especially in smooth muscle tissues, and are also detected in platelets. PDE5A1 and PDE5A2 transcripts are widely distributed. In contrast, specific expression of PDE5A3 in smooth and/or cardiac muscle has been suggested.

**Phosphodiesterase 6**

Splice variants of PDE6A to -6C have not been reported. PDE6A and PDE6B transcripts are present in rod cells, whereas PDE6C transcripts are in cone cells.

**Phosphodiesterase 7**

Three variant forms have been reported in human PDE7A subfamily. PDE7A1 and PDE7A2 are N-terminal variants, and PDE7A3 is a C-terminal variant of PDE7A1. PDE7A1 expression is ubiquitous, whereas PDE7A2 transcripts are confined to the heart, skeletal muscle, and kidney. PDE7A3 expression has been demonstrated in the heart, skeletal muscle, spleen, thymus, testis, and peripheral blood leukocytes. In mice, PDE7A expression is highest in the skeletal muscle, followed by the spleen, uterus, heart, brain, and kidney but is insignificant in the testis.

Only PDE7B1 has been identified in humans and mice. In rats, there are 3 N-terminal splice variants, PDE7B1 to -7B3.
Human PDE7B transcripts are observed in the caudate nucleus and putamen of the brain, heart, and several other tissues. In rats, PDE7B expression is particularly high in the testis and neuronal cells of several brain regions and is also detected in the heart, lung, skeletal muscle, and kidney. In mice, PDE7B transcripts are rich in the pancreas and can also be found in the brain, heart, thyroid, and skeletal muscle.

**Phosphodiesterase 8**

In humans, 5 PDE8A splice variants, PDE8A1 to -8A5, have been identified. The longest form, PDE8A1, contains REC and PAS domains. PDE8A transcripts are expressed in various tissues and are abundant in the testis, ovary, small intestine, and colon. In general, PDE8A1 expression is dominant as compared with that of other PDE8A variants. In rats, PDE8A expression is high in the liver and testis.

Five splice variants of PDE8B have also been reported in humans. PDE8B1, the longest form, carries REC and PAS domains. In humans, PDE8B transcripts are predominant in the thyroid gland and are low in most brain areas except the cerebellum. Rat PDE8B transcripts are not confined to the thyroid gland. They are abundant in the brain and are detectable in neuronal cells of several brain regions other than the cerebellum.

**Phosphodiesterase 9A**

Although 21 splice variants of PDE9A have been identified in humans, differences in functional characteristics and subcellular localization among these variants have not yet been reported in detail. PDE9A1 is the longest form of PDE9A variants. Expression of PDE9A transcripts is high in the spleen, small intestine, brain, colon, prostate, kidney, and placenta. Transcripts of PDE9A1 and PDE9A6 (described as PDE9A5 in the literature) are predominant in some immune tissues.

**Phosphodiesterase 10**

Two major N-terminal variants, PDE10A1 and PDE10A2, and several minor variants have been identified in humans. A PKA phosphorylation site in PDE10A2 is the most striking difference between PDE10A2 and PDE10A1. In most human tissues, PDE10A2 expression is higher than that of PDE10A1. PDE10A transcripts are particularly rich in the putamen, caudate nucleus, and testis. PDE10A transcripts are present in neurons of the striatum, caudate nucleus, nucleus accumbens, and olfactory tubercles in rat brain. In mice, PDE10A expression is highest in the testis and brain. Histologically, PDE10A expression is high in the striatal medium spiny neurons (MSNs). However, signal intensity among brain areas in mice, rats, dogs, cynomolgus macaques, and humans is different.

**Phosphodiesterase 11**

Four N-terminal variants are encoded by PDE11A1 to -11A4 (Figure 2). PDE11A4 encodes the longest protein including 2 GAF domains and 2 N-terminal phosphorylation sites for PKA and PKG. PDE11A variants are unique in showing various GAF domain organization.
Phosphodiesterase 4

Expression of certain PDE4 isoforms is altered by rapid or chronic treatment with cAMP-stimulating agents. In human monocyteic U937 cells, steady-state mRNA levels of PDE4A and PDE4B are upregulated by cAMP-stimulating agent β agonist, or rolipram. PDE4B expression is potently stimulated in monocytes by lipopolysaccharide via distinct transduction pathways. Major PDE4 components, PDE4D3 and PDE4D5, are markedly downregulated during differentiation of monocyteic U937 cells to macrophages, whereas PDE4B2 expression is induced. Smoking elevates PDE4A4 and PDE4B2 transcription in peripheral blood monocytes, and significant PDE4A4 upregulation is observed in lung macrophages from smokers with chronic obstructive pulmonary disease compared with control smokers. In rat heart, treatment with 7-oxo-prostacyclin, a stable prostacyclin derivative, causes upregulation of PDE4B3 transcription and downregulation of PDE4D1/2 and PDE4D3 expressions.

Rat PDE4D1/2 transcription is driven by a cAMP-responsive promoter, which is TATA-less and contains a number of GC-rich regions, Sp1, AP1, and AP2 sites and GC-rich regions. Human PDE4D5 transcription starts from a putative promoter containing a number of CCAAT enhancer-binding protein binding sites (C/EBP) and 2 sites of cAMP response element (CRE). Putative promoters for PDE4D6 to -4D8 also contain multiple CRE sites. The promoters for human PDE4A10 and PDE4A11 are TATA-less but include GC-rich islands and Sp1 site.

Phosphodiesterase 5

Both human PDE5A1 and PDE5A2 promoters are TATA-less and contain putative Sp1 sites in the proximal region. Lin et al report PDE5A1 transcription from a further upstream region (318-bp upstream from the initiation ATG codon) and a core sequence for basal promoter activity. The PDE5A1 promoter is stimulated by cAMP or cGMP through Sp1 sites. The PDE5A2 promoter region, which is situated in an intron between the first exons for PDE5A1 and PDE5A2, is highly GC rich and includes potential Sp1 sites, which are important for basal activity and responsiveness to cAMP and cGMP. Lin et al have analyzed PDE5A promoters with COS cells. However, whether these cells are suitable for this type of study is still questionable.

cAMP analogs stimulate transcription of PDE5A, especially PDE5A2 in rat vascular SMCs, whereas cGMP does not. PDE5A upregulation is unlikely to occur during long-term tadalafil treatment accompanying cGMP stimulation in human corpora cavernosa SMCs. No elevation of PDE5A protein, but a slight increase of PDE5A transcripts, has been observed on cGMP stimulation in Tunica albuginea fibroblasts.

Phosphodiesterase 6

The human PDE6A proximal promoter contains 2 indispensable cis elements, a Crx-binding element and an Nrl-response element (NRE). The human PDE6B minimal promoter includes βAp1/NRE and the GC-rich sequence β/Sp4 necessary for in vitro interaction with Nrl and Sp1/Sp4 (especially Sp4) DNA-binding proteins, respectively. The molecular mechanism of PDE6C expression is currently unknown.

Phosphodiesterase 7

The proximal promoter region for human PDE7A1 and likely for PDE7A3 carries no typical TATA motif but contains a CpG island including 3 potential CRE sites. In Jurkat T cells, overexpression of CRE binding protein (CREB) increases the promoter activity. In human CD4+ T cells, T-cell activation quickly induces PDE7A1 transcription, whereas PDE7A3 induction is slow. PDE7A1 expression is increased in response to intracellular cAMP levels in B lymphocytes. Phorbol myristate acetate stimulates the promoter activity, but the molecular mechanism of this stimulation is still unknown.

Expression of rat PDE7B is stimulated by cAMP stimulants in cultured striatal neurons via cAMP-dependent binding of CRE to CRE site in the putative PDE7B1 promoter region.

Phosphodiesterase 8

Transcription of PDE8A1 is upregulated and reaches a maximum 8 hours after activation of human CD4+ T cells.

Phosphodiesterase 9 to 11

Regulation of the PDE9A, PDE10A, and PDE11A genes is unknown. Transcription of 2 major PDE10A variants, PDE10A1 and PDE10A2, start from the common promoter, which is highly GC rich and has neither a TATA motif nor a CAAT box. The putative promoters for PDE11A1 and PDE11A3 contain a TATA motif. PDE11A4 promoter lacks the TATA motif but includes a GC-rich region, with a CAAT box and Sp1 site.

Enzymatic Regulation of PDEs by Phosphorylation and Association Proteins

Regulators of PDEs such as kinases and association proteins are listed in Table 2 and illustrated in Figure 4.

Ca2+/CaM binding to the N-terminal binding site of PDE1s causes marked stimulation of PDE activity. The binding affinities of PDE1A and PDE1B are reduced by PKA- and CaM kinase II–dependent phosphorylation, respectively. PKA activation has been reported to reduce PDE1C activity in AT20 cells. PDE2A activation by Ca2+/phospholipid-dependent protein kinase has been reported in the liver Golgi–endosomal fraction; however, there is no direct evidence for PDE2A phosphorylation. Phosphorylation of rat PDE2A2 by its associated protein kinase inhibits PDE activity. However, the molecular basis and enzymatic alteration by PDE2 phosphorylation have not yet been investigated in detail.

Activation of PDE3s by PKA- and PKB-mediated phosphorylation has already been discussed (Figure 2). PKA-mediated PDE3A activation functions as negative-feedback regulation in cAMP signaling, and PKB-mediated PDE3B activation in adipocytes greatly contributes to insulin action. However, reported phosphorylation sites of PDE3s are puzzling. In humans, PDE3A is phosphorylated at SHRRTS312 by PKA. Human PDE3A contains a site, KXRXS292, that is similar but not identical to the typical PKB phosphor-
ylation motif RXRXXS. Although PKB phosphorylation of this site is intriguing, detailed analysis of human PDE3A phosphorylation has not been reported. Little is known about PKA- and PKB-mediated phosphorylation of rat PDE3A, which has potential phosphorylation sites.

With regard to PDE3B, human PDE3B contains typical PKB (Ser295) and PKA phosphorylation motifs. However, there is no report on the phosphorylation of these sites. In rat adipocytes, phosphorylation of PDE3B at MFRRP\text{302} by PKA and/or PKB and at QLRRS\text{427} (Ser442 in humans) by PKA has been reported. Mouse PDE3B is phosphorylated by insulin-stimulated PKB at RPRRS\text{273} (Ser295 in humans, Ser279 in rats), MFRRPS\text{296} (Ser318 in humans, Ser302 in rats), and QLRRSS\text{421} (Ser442 in humans). Ser273 in mouse PDE3B is situated in a typical PKB phosphorylation site, but the other 2 sites, “RRXS,” are fundamentally consensus PKA phosphorylation sites. In addition to PKA and PKB phosphorylation, human PDE3A enzyme is phosphorylated by PKC.

PDE4 activity is regulated by phosphorylation in UCR1 by PKA and in the C-terminal region by extracellular signal-regulated kinase (ERK), but the regulation is isoform dependent.\textsuperscript{3,15,16} PKA phosphorylation of the long forms PDE4A4 and PDE4D3 causes their activation probably through disruption of UCR1-UCR2 interaction and UCR2 association with the catalytic region.\textsuperscript{18,19,128,129} ERK phosphorylation of the long form PDE4D3 reduces cAMP-hydrolytic activity (ca. 50% inhibition), which in turn leads to PKA activation. Consequently, PKA phosphorylates PDE4 long forms, which escapes ERK-mediated inhibition. Conversely, ERK phosphorylation stimulates activity of the short forms PDE4D1 and PDE4B2 but does not affect that of the super-short form, suggesting the involvement of UCRs in this regulation.\textsuperscript{16,130} Regulation of PDE4 activity by PKA and ERK phosphorylation depends on PDE4 isoforms and ERK regulatory components in the cells.

The long forms of PDE4 enzymes are activated by phosphatidic acid (acidic) and phosphatidyserine (acidic) but not by phosphatidylcholine (neutral). Alterations in the enzy-

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**TABLE 2. Regulatory Kinases and Association Proteins for PDEs**

<table>
<thead>
<tr>
<th>PDE Isozyme</th>
<th>Association Proteins</th>
<th>Kinases</th>
<th>Alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDE1</td>
<td>Calmodulin</td>
<td>PKA</td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE1A</td>
<td></td>
<td>PKA</td>
<td>Reduction of calmodulin affinity</td>
</tr>
<tr>
<td>PDE1B</td>
<td>CaM kinase II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDE3</td>
<td>PKA</td>
<td></td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE3</td>
<td>Akt/PKB</td>
<td></td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE3B</td>
<td>14-3-3 β protein</td>
<td></td>
<td>Regulation of insulin signaling</td>
</tr>
<tr>
<td>PDE3B</td>
<td>PKC</td>
<td></td>
<td>Enhancement of 14-3-3 protein binding</td>
</tr>
<tr>
<td>PDE4 (long forms)</td>
<td></td>
<td>PKA</td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE4A</td>
<td>AKAP95, AKAP149, AKAP450, myeloid translocation gene (MTG)</td>
<td></td>
<td>Anchoring to organella</td>
</tr>
<tr>
<td>PDE4A5</td>
<td>Immunophilin XAP2</td>
<td>ERK</td>
<td>Inhibition of the PDE activity</td>
</tr>
<tr>
<td>PDE4A5</td>
<td>SH3 domains of c-Abl, Crk, Csk, Lyn, Fyn and v-Src</td>
<td>ERK</td>
<td>Inhibition of the PDE activity</td>
</tr>
<tr>
<td>PDE4B</td>
<td>Disrupted in schizophrenia (DISC1)</td>
<td>ERK</td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE4B,D (C) (short forms)</td>
<td></td>
<td>ERK</td>
<td>Perinuclear localization</td>
</tr>
<tr>
<td>PDE4D (B,C) (long forms)</td>
<td></td>
<td>ERK</td>
<td>Adaptor protein</td>
</tr>
<tr>
<td>PDE4D3</td>
<td>Muscle-selective A-kinase anchoring protein (mAKAP)</td>
<td></td>
<td>Regulation of cardiac dysfunction and arrhythmias</td>
</tr>
<tr>
<td>PDE4D3</td>
<td>Guanine nucleotide exchange factor (Epac1)</td>
<td></td>
<td>Scaffold</td>
</tr>
<tr>
<td>PDE4D3</td>
<td>RyR2/calcium-release-channel complex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDE4D5</td>
<td>WD-repeat protein receptor for activated C-kinase (RACK1)</td>
<td></td>
<td>Regulation of (\beta_2)-adrenoreceptor signaling</td>
</tr>
<tr>
<td>PDE4D3/4D5</td>
<td>(\beta) arrestin</td>
<td>PKA, PKG</td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE5A</td>
<td>PDE6\text{γ}</td>
<td></td>
<td>Inhibition of the PDE activity</td>
</tr>
<tr>
<td>PDE6</td>
<td>PDE6\text{δ}</td>
<td></td>
<td>Anchoring to rod outer segment disc membrane</td>
</tr>
<tr>
<td>PDE7A</td>
<td>Myeloid translocation gene (MTG)</td>
<td>PKA catalytic subunit</td>
<td>Inhibition of PKA activity</td>
</tr>
<tr>
<td>PDE7A1</td>
<td>PKA catalytic subunit</td>
<td></td>
<td>Activation of the PDE activity</td>
</tr>
<tr>
<td>PDE8A1</td>
<td>(\text{IкB}) family proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDE10A2</td>
<td>PKA</td>
<td></td>
<td>Alteration of subcellular localization</td>
</tr>
</tbody>
</table>

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Figure 4. Regulation of enzymatic activity in PDE families. Expressive examples of enzymatic regulation in PDE1 to -5 are schematically illustrated. PDE proteins are shown as in Figure 2. Changes in the shape of each PDE enzyme show alteration of enzymatic activity. Activation and reduction of catalytic activity are indicated by open upward and downward arrows, respectively. ERK phosphorylation site is absent in PDE4A variants.
motic properties of PDE4 isoforms by phosphatidic acid and PKA are similar and exclusive.\textsuperscript{131} Phosphatidic acid is believed to bind to a specific region of UCR1.\textsuperscript{17}

Immunophilin XAP2 specifically binds to PDE4A5, resulting in inhibition of PDE4 activity, elevation of rolipram sensitivity, and reduction of PKA phosphorylation.\textsuperscript{132} The motif EELD in UCR2 of PDE4A5 and the C-terminal tetratricopeptide repeat (TPR) domain of XAP2 are involved in this interaction. DISC1 (Disrupted In Schizophrenia 1 gene), a key factor for psychiatric illnesses, interacts with UCR2 of PDE4B and inhibits PDE4B activity. Increased cAMP dissociates DISC1 from PDE4B via PKA activation and reverses PDE4B activity.\textsuperscript{133} Rat PDE4A5 binds more efficiently to SH3 domains of several tyrosyl kinases, especially Lyn and Fyn, through an SH3-binding site within LR2.\textsuperscript{134} This association with SH3 domains significantly reduces PDE activity and increases sensitivity to rolipram.\textsuperscript{135}

There are several reports on the regulation of PDE5 by phosphorylation. PDE5 activity is increased by PKA/PKG phosphorylation at Ser102 of human PDE5A1 in vitro.\textsuperscript{21} Although copurification of PDE6\textsubscript{γ} (Pγ) with PDE5\textsubscript{γ} has been reported, the physiological significance of this association is unclear, owing to no inhibitory effect of PDE6\textsubscript{γ} on PDE5 activity in vitro.\textsuperscript{137} In contrast, PDE6\textsubscript{αβ} is inhibited by PDE6\textsubscript{γ} (see section General Characteristics, above). Association of PDE8A1 PAS domain with lrB stimulates PDE8A activity but does not affect nuclear factor lrB activation.\textsuperscript{138}

Enzymatic regulation of other PDEs is not well understood. The N-terminal sequences of PDE7A, PDE7B, PDE10A2, and PDE11A4 are phosphorylated by PKA in vitro and/or in vivo. However, there is no evidence of enzymatic regulation of these PDEs by phosphorylation. Phosphorylation of PDE8s carrying putative PKA phosphorylation sites has not yet been investigated. There is no report on PDE9A phosphorylation.

### Subcellular Localization of PDEs, Interaction With Association Proteins, and Compartmentation of Cyclic Nucleotide Signaling

The PKA regulatory subunit binds to the PKA catalytic subunit, and the resultant complex is anchored to particulate fractions. cAMP binding to the regulatory subunit of PKA releases the catalytic subunit for enzymatic activation. A-kinase (PKA) anchoring proteins (AKAPs) play a role in PKA anchoring and function as scaffold molecules for many association proteins.\textsuperscript{6} Localization of each AKAP is among the important determinants of specific cAMP signaling in cells. With regard to PKG, association of G-kinase (PKG) anchoring proteins (GKAPs) with PKGs has been reported. Membrane anchoring of PKG II is mediated by N-terminal myristoylation. Cyclic nucleotides are generated in limited cellular space where their producer enzymes and/or receptors are located; however, target proteins for cyclic nucleotides are widely distributed. Some PDEs are reported to be localized in microdomains and/or to interact with association proteins including scaffold proteins. Regulation of intracellular PDE localization is recognized as among the key mechanisms in compartmentation of cyclic nucleotide signal.

Association proteins for PDEs are listed in Table 2 and illustrated in Figure 4.

### Phosphodiesterase 1

PDE1A protein is predominant in the cytosol of contractile vascular SMCs but is localized in the nucleus of neointimal synthetic vascular SMCs.\textsuperscript{138} Nuclear PDE1A is involved in regulation of cell proliferation and apoptosis of actively growing synthetic vascular SMCs.\textsuperscript{139} However, the molecular mechanism of this altered localization is currently unclear. Diffuse cytoplasmic distribution of PDE1B protein has been shown in chick dorsal root ganglion neurons.

### Phosphodiesterase 2

Rat PDE2A protein anchors to the membrane with the hydrophobic N-terminal portion\textsuperscript{140} and is localized at lipid rafts in neurons.\textsuperscript{141} In cultured neonatal rat ventriculocytes, PDE2A protein is confined to membrane compartment with the sarcomeric Z line. Cyclic nucleotide signaling molecules such as β-adrenoceptor, adenylyl cyclase, and nitric oxide synthase, are also localized with PDE2A in the lipid rafts, suggesting coupling of these molecules in cardiac signal transduction.\textsuperscript{142}

### Phosphodiesterase 3

NHR modules greatly contribute to intracellular localization of PDE3 proteins. PDE3A-136 containing 2 NHRs is exclusively membrane bound. PDE3A-118 carrying NHR2 and PDE3A-94 containing no NHR module are found in both membrane-bound and cytosolic fractions of cardiac and vascular myocytes.\textsuperscript{51} PDE3A and PDE3B proteins containing 2 NHRs are localized in the endoplasmic reticulum.\textsuperscript{143} PDE3B protein is associated with caveolae and lipid raft in plasma membrane fractions of adipocytes, suggesting functional importance of this association in insulin signaling.\textsuperscript{144} A scaffold molecule, 14-3-3β protein, interacts with rat PDE3B protein phosphorylated via phosphatidylinositol 3-kinase/PKB pathway in adipocytes.\textsuperscript{127} 14-3-3 protein also binds to phosphorylated human PDE3A at Ser428 by PKC.\textsuperscript{123}

### Phosphodiesterase 4

PDE4 isoforms show unique intracellular distribution patterns characterized by interaction with several anchoring or targeting proteins such as immunophilin XAP2, DISC1, SH3 domain–containing proteins, β-arrestin, receptor for activated C-kinase (RACK1), and AKAPs.

β-Arrestin downregulates β-adrenoceptor signaling by inhibiting further interaction with guanine nucleotide binding proteins (G proteins) (Figure 5). Adrenergic stimulation causes time-dependent recruitment of β-arrestins and PDE4D isoforms to β-adrenoceptor at the cell membrane level.\textsuperscript{145} β-Arrestin 1 and 2 bind to PDE4 isoforms of all 4 subfamilies through interaction with the C-terminal catalytic domain. PDE4D5 has a distinct β-arrestin–binding site in the N-terminal region.\textsuperscript{146} PKA colocalized with AKAP79\textsuperscript{147} is first activated by cAMP and then phosphorylates the receptor, which causes G\textsubscript{i}-to-G\textsubscript{i} switch and ERK1/2 activation.\textsuperscript{148} G protein–coupled receptor kinase recruited to the membrane phosphorylates β\textsubscript{2}-adrenoceptor, and then β-arrestin/PDE4
complex is recruited to the receptor. This PDE4 recruitment contributes to local cAMP degradation, reduction of PKA activity, and modulation of β-adrenoceptor function, thus constituting a negative-feedback regulation. Moreover, the recruitment may also regulate ERK signaling.\textsuperscript{148,149} Regulation of PDE4 by this mechanism is largely dependent on the expression profile of PDE4 isoforms and coexisting PDE4-scaffolding proteins in the cell.

Ligation of T-cell Receptor (TCR) with CD28 costimulation causes T-cell activation by cAMP reduction through recruitment of β-arrestin/PDE4 complex to lipid raft in microdomain. Regulation of compartmentalized cAMP signaling by β-arrestin/PDE4 complex is considered to be crucial to T-cell regulation.\textsuperscript{150}

RACK1 specifically and directly binds to the N-terminal β-arrestin-binding site of PDE4D5.\textsuperscript{147} RACK1-PDE4D5 complex is present in cytosolic fractions. Because the binding of RACK1 and β-arrestin to PDE4D5 is mutually exclusive, RACK1 might direct cytosolic localization of PDE4D5 by competing with β-arrestin. RACK1 interaction does not alter PDE4D activity but increases its sensitivity to rolipram.

AKAPs are the most important molecules in compartmentation of cAMP/PDE4 signaling.\textsuperscript{6} AKAP149 (mitochondria), AKAP95 (perinucleus), and muscle AKAP (mAKAP) (perinucleus)\textsuperscript{151} bind to PDE4A, PDE4A, and PDE4D3, respectively (Table 2). Nuclear membrane anchoring of mAKAP directs associating PDE4D3 to localize around the nucleus. PKA phosphorylation of PDE4D3 at Ser13 enhances the direct binding PDE4/PII regulatory subunit in the Golgi–centrosome region of β-TC3 insulinoma cells.\textsuperscript{160} N-terminal PKA pseudosubstrate sites in human PDE7A1 bind to PKA catalytic subunits and inhibit kinase activity.\textsuperscript{26,160} Myeloid translocation gene (MTG) protein also binds to and colocalizes with PDE7A in the Golgi of HuT78 cells.\textsuperscript{161} PDE7B localization is unknown. Recombinant human PDE7B1 protein with an N-terminal FLAG-tag expressed in COS-7 cells is cytosolic.\textsuperscript{71}

**Phosphodiesterase 7**

PDE7A2 with an N-terminal hydrophobic region is localized to particulate fractions.\textsuperscript{26} PDE7A1 colocalizes with the RII PKA regulatory subunit in the Golgi–centrosome region of β-TC3 insulinoma cells.\textsuperscript{160} N-terminal PKA pseudosubstrate sites in human PDE7A1 bind to PKA catalytic subunits and inhibit kinase activity.\textsuperscript{26,160} Myeloid translocation gene (MTG) protein also binds to and colocalizes with PDE7A in the Golgi of HuT78 cells.\textsuperscript{161} PDE7B localization is unknown. Recombinant human PDE7B1 protein with an N-terminal FLAG-tag expressed in COS-7 cells is cytosolic.\textsuperscript{71}

**Phosphodiesterase 8**

A consensus sequence for myristoylation/palmitoylation motif, MGCAP, is present in human PDE8A1 and PDE8B1. However, subcellular localization of PDE8 proteins is unknown. N-terminal Xpress-tagged human PDE8A1 and PDE8B1 proteins are found in both cytosolic and particulate fractions of COS-7 cells.\textsuperscript{27}

**Phosphodiesterase 9**

PDE9A1, PDE9A16, and PDE9A17 have a pat7 nuclear localization signal, PLRDRRV. Nuclear localization of recombinant PDE9A1 is determined by the pat7 motif.\textsuperscript{80}

**Phosphodiesterase 10**

Recombinant proteins of PDE10A1 and PDE10A3 are cytosolic.\textsuperscript{162} By contrast, endogenous PDE10A2 protein from rat striatum as well as recombinant PDE10A2, which is found in the Golgi apparatus of transfected PC12H cells, is dominant in membrane fractions. By PKA phosphorylation, PDE10A2 alters subcellular localization from Golgi to cytosol.\textsuperscript{162} cAMP signaling in Golgi and cytosol of neurons is hypothesized to be controlled through alteration of PDE10A subcellular localization by PKA.

**Phosphodiesterase 11**

Subcellular localization of PDE11A is unknown. N-terminal Xpress-tagged recombinant PDE11A proteins are cytosolic in COS-7 cells.\textsuperscript{34,88}

**Phenotypes of Genetically Engineered Animals**

Phenotypes of genetically engineered animals are summarized in Table 2. In this regard, there is no report on PDE1A, PDE1C, PDE2A, PDE4C, PDE5A, PDE6A-C, PDE7B, PDE8A-B, and PDE9A.

**Phosphodiesterase 1**

PDE1B-deficient mice\textsuperscript{163} show increased locomotor activity and deficits in spatial learning.
**Phosphodiesterase 3**

Female PDE3A-deficient mice\(^1\) are infertile because of immature oocytes, demonstrating the involvement of PDE3A in oocyte maturation and fertilization. However, alterations in contraction and relaxation of cardiac and vascular myocytes and platelet aggregation in PDE3A\(^{-/-}\) mice have not yet been reported.

PDE3B transgenic mice (\(\beta\)-cell specific) demonstrate impaired acute insulin response to intravenous glucose loads and reduced insulin secretion in islets in vitro.\(^1\) PDE3B plays a crucial role in regulation of cAMP signaling in \(\beta\)-cell insulin secretion.

**Phosphodiesterase 4**

Knockout mice for PDE4A (PDE4A\(^{-/-}\)), PDE4B (PDE4B\(^{-/-}\)), and PDE4D (PDE4D\(^{-/-}\)) have been reported.\(^1\) Although there are only a few reports on PDE4A\(^{-/-}\) mice, it is believed that no obvious phenotype exists in cardiac or immune system.

Immune response is altered in PDE4B\(^{-/-}\) mice.\(^1\) PDE4B\(^{-/-}\) mice have impaired lipopolysaccharide-stimulated TNF\(\alpha\) production and are resistant to lipopolysaccharide-induced shock. PDE4B has critical function in lipopolysaccharide signaling. In an endotoxin inhalation–induced lung injury model, recruitment of neutrophils is markedly decreased in PDE4D\(^{-/-}\) and PDE4B\(^{-/-}\) mice. CD18 expression and chemotaxis response are decreased in PDE4D\(^{-/-}\) and PDE4B\(^{-/-}\) neutrophils.\(^1\) Neutrophil function is regulated by PDE4B and PDE4D.

PDE4D\(^{-/-}\) but not PDE4B\(^{-/-}\) mice exhibit impaired airway contraction induced by cholinergic stimulation and abolished airway hyperreactivity caused by exposure to allergen, indicating the implication of PDE4D gene in cholinergic airway responsiveness and in development of hyperreactivity.\(^1\)

Progressive cardiomyopathy, accelerated heart failure after myocardial infarction, and cardiac arrhythmias are observed in PDE4D\(^{-/-}\) mice.\(^1\) PDE4D3 is associated with RyR2/calcium-release-channel complex, which is required for heart muscle excitation/contraction. Depletion of PDE4D3 in RyR2 complex enhances PKA phosphorylation of the complex and affects controlled intracellular Ca\(^{2+}\) release, resulting in cardiac dysfunction and arrhythmia.
In the central nervous system, PDE4D has been reported to be linked to cAMP signaling of α1-adrenoceptor in noradrenergic neurons, which may explain the emetic side effect of PDE4 inhibitors.173

**Phosphodiesterase 6**
Mutations in genes generating defective PDE6 enzymes (mainly rod PDE6αβ) cause high-level cGMP accumulation in photoreceptor cells leading to cell death.174

**Phosphodiesterase 7**
T-lymphocyte activation has been proposed to be linked to PDE7A activity.175 However, PDE7A knockout mice (PDE7A−/−) have been reported to show normal in vitro and in vivo T-cell functions, indicating that T-cell activation does not require PDE7A activity.176

**Phosphodiesterase 10**
PDE10A knockout (PDE10A−/−) mice177 show decreased exploratory activity and delayed acquisition of conditioned avoidance behavior. A blunted locomotor response is observed in PDE10A−/− mice following administration of antagonists for the ionotropic N-methyl-D-aspartate receptor that induce locomotor hyperactivity. PDE10A has a particular role in the responsiveness of MSNs to glutamatergic stimulation.

**Phosphodiesterase 11**
PDE11A knockout mice (PDE11A−/−) demonstrate impaired sperm function and spermatogenesis, suggesting involvement of PDE11A activity in spermatogenesis. In humans, a protein corresponding to PDE11A3, which is a testis-specific variant, is undetectable in testicular tissue,87 whereas PDE11A4 protein is present in the prostate, where prostatic fluid involved in sperm activity is produced. The effects of PDE11A disruption on prostatic function are unknown, and therefore the physiological role of PDE11A should not be linked simply to testicular functions.

**Conclusions**
A number of splice variants have been identified in each PDE family, as described here. In regard to certain variants of classical PDEs (PDE1 to -6), molecular characteristics, tissue expression patterns, and expression regulation, all of which are indispensable information to figure out PDE functional roles in cells, have to some extent been investigated. By contrast, less information on newly discovered higher-numbered PDE families (PDE7 to -11) is currently available. Lack of clear evidence for the presence of apparent activity in most of the newly discovered PDEs in tissues and cells is the major obstacle to picture a precise and detailed network of intracellular signaling, where these PDE enzymes are involved in regulation of cellular functions.

Functional roles of PDEs have been studied using PDE family–specific inhibitors to demonstrate pharmacological effects of inhibition of each PDE family. However, PDE inhibitors cannot distinguish isoform-specific and variant-specific functions of PDE. Therefore, isoform-specific, highly selective PDE inhibitors, RNA interference, and genetically engineered animals, such as variant-specific knock-out animals, seem to be potent and plausible tools to solve the specificity issue. Moreover, comparative studies looking at the whole picture of PDEs would be very important to evaluate the involvement of each PDE in specific cellular function and to understand regulation of cyclic nucleotide signaling. Establishment of a standardized platform for PDE research is necessary to interpret further intriguing observations.

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None.

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