Chapter 13
Phosphodiesterase 1: A Unique Drug Target for Degenerative Diseases and Cognitive Dysfunction

Lawrence P. Wennogle, Helen Hoxie, Youyi Peng, and Joseph P. Hendrick

Abstract The focus of this chapter is on the cyclic nucleotide phosphodiesterase 1 (PDE1) family. PDE1 is one member of the 11 PDE families (PDE 1–11). It is the only phosphodiesterase family that is calcium/calmodulin activated. As a result, whereas other families of PDEs 2–11 play a dominant role controlling basal levels of cyclic nucleotides, PDE1 is involved when intra-cellular calcium levels are elevated and, thus, has an "on demand" or activity-dependent involvement in the control of cyclic nucleotides in excitatory cells including neurons, cardiomyocytes and smooth muscle. As a Class 1 phosphodiesterase, PDE1 hydrolyzes the 3′ bond of 3′-5′-cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Here, we review evidence for this family of enzymes as drug targets for development of therapies aimed to address disorders of the central nervous system (CNS) and of degenerative diseases. The chapter includes sections on the potential for cognitive enhancement in mental disorders, as well as a review of PDE1 enzyme structure, enzymology, tissue distribution, genomics, inhibitors, pharmacology, clinical trials, and therapeutic indications. Information is taken from public databases. A number of excellent reviews of the phosphodiesterase family have been written as well as reviews of the PDE1 family. References cited here are not comprehensive, rather pointing to major reviews and key publications.

Keywords Phosphodiesterase 1 • PDE1A • PDE1B • PDE1C • Cyclic nucleotide • Calcium-Calmodulin Stimulation • Phosphorylation • GM-CSF • Gene knockout • Smooth muscle cells • CNS Disease • Cognitive dysfunction • Parkinson's disease • Heart failure • Schizophrenia • Vinpocetine • ITI-214 • Dopamine D1 Receptor • Neuroprotective • NOR Model

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13.1 Introduction and Focus

The focus of this chapter is on the cyclic nucleotide phosphodiesterase 1 (PDE1) family. PDE1 is one member of the 11 PDE families (PDE 1–11). It is the only phosphodiesterase family that is calcium/calmodulin activated. As a result, whereas other families of PDEs 2–11 play a dominant role to control basal levels of cyclic nucleotides, PDE1 is involved when intra-cellular calcium levels are elevated and, thus, has an "on demand" or activity-dependent involvement in the control of cyclic nucleotides in excitatory cells including neurons, cardiomyocytes and smooth muscle. As a Class 1 phosphodiesterase, PDE1 hydrolyzes the 3′ bond of 3′-5′-cyclic nucleotides, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). Here, we review evidence for this family of enzymes as drug targets for development of therapies aimed to address disorders of the central nervous system (CNS) and of degenerative diseases. The chapter includes sections on the potential for cognitive enhancement in mental disorders, as well as a review of PDE1 enzyme structure, enzymology, tissue distribution, genomics, inhibitors, pharmacology, clinical trials, and therapeutic indications. Information is taken from public databases. A number of excellent reviews of the phosphodiesterase family have been written (Bender and Beavo 2006a) as well as reviews of the PDE1 family (Goraya and Cooper 2005). References cited here are not comprehensive, rather pointing to major reviews and key publications.

13.2 Structure

The PDE1 family of enzymes includes three genes, PDE1A, PDE1B and PDE1C, with the following human gene names:

PDE1A: NCBI Gene NP_001245241.1;
PDE1B: NCBI Gene NP_000915.1;
PDE1C: NCBI Gene NP_001177987.1.

PDE1 enzymes are globular, mainly cytosolic proteins. As is the rule in the PDE superfamily, PDE1 enzymes exist as dimers of identical subunit enzymes. The human PDE1B enzyme is depicted in Fig. 13.1 as a stick diagram showing a 536 amino acid protein with an N-terminal regulatory domain (aa 1–197) containing two calmodulin binding sites, a catalytic domain (aa 198–496) containing an H-loop, a dimerization domain, two helix and a M-loop, and a C-terminal extension. The role of the C-terminal is not well understood. Sites of phosphorylation of PDE1 on the N-terminal have been proposed (Kakkar et al. 1999), and may influence calmodulin binding affinity (Sharma et al. 2006), but in general the role of phosphorylation of PDE1 is poorly understood (Beltman et al. 1993). Details of the N-terminal domain including calmodulin binding domains have been proposed (Sonnenburg et al. 1995).

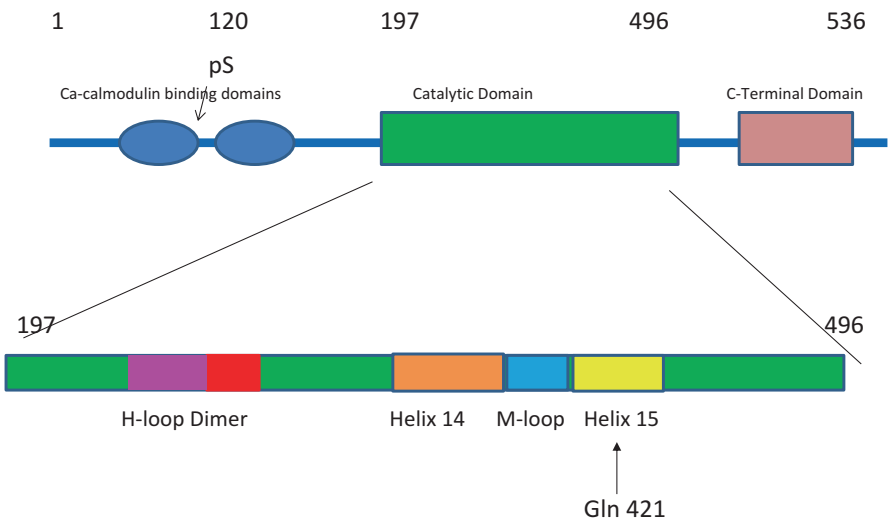


Fig. 13.1 Stick diagram of the secondary structural details of hPDE1B

Several crystal structures of the catalytic core of PDE1 enzymes have been published (Card et al. 2005), including structures with bound inhibitors (Humphrey et al. 2014). An ITI-214 inhibitor-bound crystal structure from our work was recently published (Li et al. 2016). Docking of a PDE1 inhibitor, taken from the patent literature, to a model enzyme catalytic core structure we developed is shown in Fig.13.2. Inhibitors to this class of enzymes are generally competitive for the cyclic nucleotide binding site. Since the publication of the first PDE catalytic domain crystal structure of PDE4, the cyclic nucleotide binding site of this PDE family has been well detailed. This site includes domains of a hydrophobic pocket, a region referred to as a "lid region", a metal binding site and a core pocket. For PDE1 enzymes the active site also includes the Gln421 "switch" (number from hPDE1B isoform) (Zhang et al. 2004). This Gln421amino acid accommodates both cAMP and cGMP, hence referred to as a switch in the case of the PDE1 family, allowing for enzyme activity towards both cyclic nucleotides.

PDE1 isoforms are highly conserved across species (Fig. 13.3). This high degree of amino acid sequence conservation is greater than the sequence conservation seen between human isoforms of PDE1 A, B and C, indicating a fundamental important role of each distinct isoform. Similarly, the sequences of human PDE1A, B, and C are highly homologous (Fig. 13.4). Sequence homology/identity between PDE1 A, B and C can be used to predict potential selectivity of inhibitors. The ~85% homology between these three isoform enzymes at the level of the catalytic domain supports the prediction that inhibitors selective to one PDE1 isoform would be difficult to discover. On the other hand, PDE1 enzymes are quite distinct from other PDE families (2–11) as shown in Fig. 13.5, and PDE1 inhibitors that are highly selective for the PDE1 class over all other classes are now available. This will be discussed

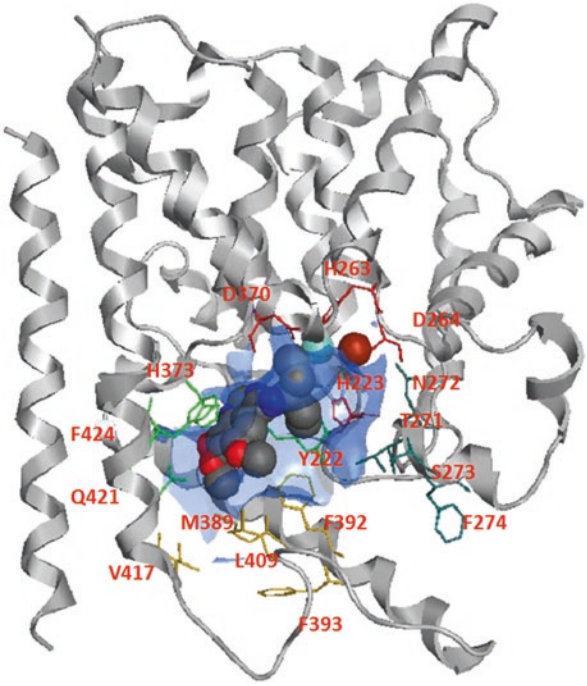
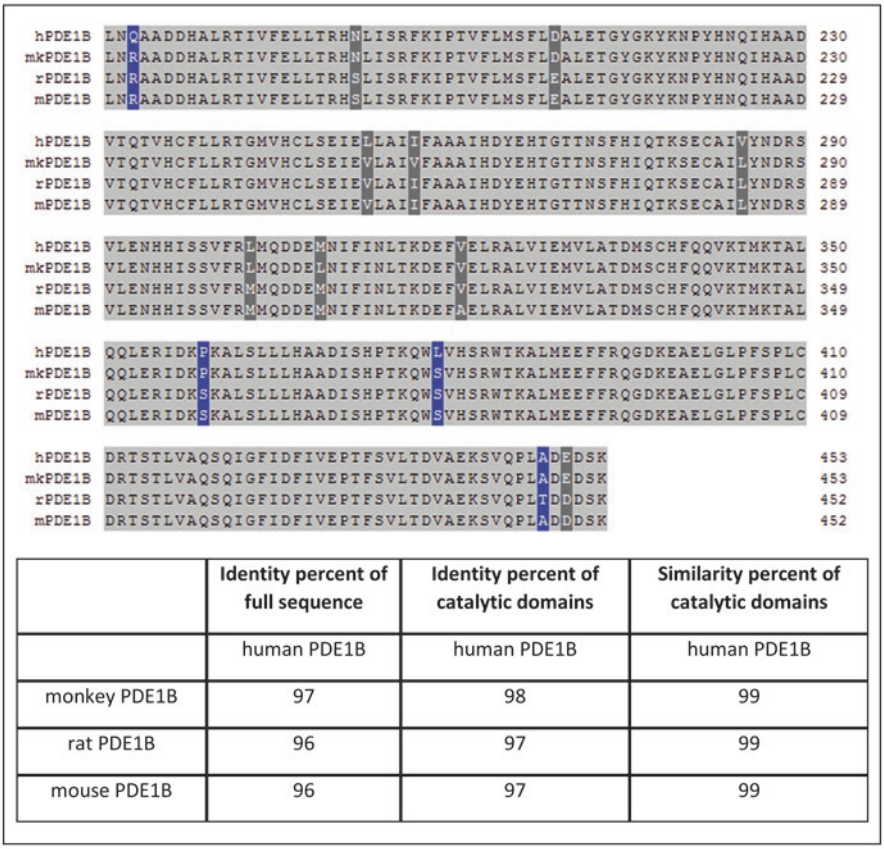


Fig. 13.2 Structure of human PDE1B and inhibitor PF-04471141 (PDB ID: 4NPV). PDE1B is shown in gray ribbon and PF-044711141 is shown in a space-filled model and colored by atom-type. Amino acids participating in the binding of inhibitor are shown and labeled. Zinc and Magnesium ions are shown in cyan and red balls. The molecular surface of the binding pocket is shown in light blue. The core pocket is in green; the lid region is in dark cyan; the metal binding pocket is in red, and the hydrophobic pocket is in yellow

later. With the most recent class of inhibitors being described below, the agents are sufficiently specific to be suitable for target validation studies.

The possibility has been raised of a calcium-activated protease called calpain to cleave PDE1 enzymes between the calmodulin domains and the catalytic domain (Kakkar et al. 1999). This cleavage, if it occurs, would unleash the enzyme from control by calcium and calmodulin and would be likely to further contribute to the progression of degenerative diseases (Sharma et al. 2006). In Fig. 13.6, the cleavage site for PDE1B is depicted as between amino acid (aa) 126 and 127 of the human enzyme (Sharma et al. 2006).

PDE1 N-terminal calmodulin binding domain structure has not been resolved in high resolution, but structural information is included in Fig. 13.6. A conserved potential phosphorylation site just C-terminal from the second calmodulin domain at Threonine 148 of hPDE1B is present. Calcium-calmodulin activated kinase II (CaMKII) is responsible for phosphorylation of PDE1B, while PKA is able to phosphorylate PDE1A and C (see Table 13.1) (Sharma et al. 2006; Florio et al. 1994; Heredia et al. 2003). Phosphorylation results in a decreased affinity of the enzyme



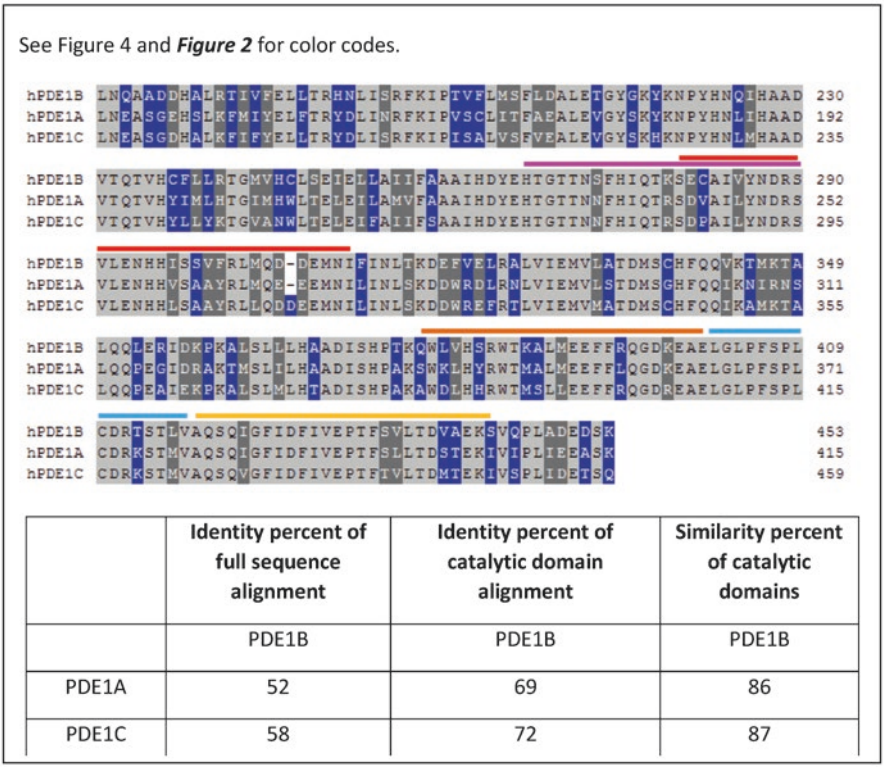
	Identity percent of full sequence	Identity percent of catalytic domains	Similarity percent of catalytic domains
	human PDE1B	human PDE1B	human PDE1B
monkey PDE1B	97	98	99
rat PDE1B	96	97	99
mouse PDE1B	96	97	99

Fig. 13.3 Alignment of the amino acid sequence of the catalytic domain of PDE1B across species. Sequence alignment of the catalytic domains of PDE1Bs from different species. *h* human, *mk* rhesus monkey, *r* rat, *m* mouse. *Blue* non-conserved substitution, *Dark Grey* conserved substitution, and *Light Grey* identical amino acids. Alignment of sequences was performed with the CLUSTAL W multiple sequence alignment program. Definitions for similarity and identity are given in a manuscript by Higgins et al. (1996)

for calmodulin (Florio et al. 1994). In the first calmodulin binding domain there is a basic nine amino acid insert in PDE1C versus 1A and 1B (Fig. 13.6 bottom). The implication of this insertion is not understood.

13.3 Enzymology

As discussed above, the basic architecture of cyclic nucleotide phosphodiesterases includes N-terminal regulatory domains attached to C-terminal catalytic domains. Among the upstream regulatory motifs in different (non-PDE1) PDE families are



	Identity percent of full sequence alignment	Identity percent of catalytic domain alignment	Similarity percent of catalytic domains
	PDE1B	PDE1B	PDE1B
PDE1A	52	69	86
PDE1C	58	72	87

Fig. 13.4 Sequence alignment of the catalytic core of the three human PDE1 isoforms. The color bars above the sequences indicate different motifs as shown in Fig. 13.1. See Fig. 13.3 and Fig. 13.1 for color codes

	1A	1C	2A	3A	4B	4D	5A	6A	7A	8A	9A	10A	11A
PDE1B	69	72	25	36	39	40	24	25	31	32	28	20	28
PDE1B	52	58	20	26	24	25	15	18	21	18	18	12	17

Fig. 13.5 Sequence identity comparison among PDE families. *Top* row is for the sequence identity as a percent of catalytic domain amino acids. *Bottom* row is for the sequence identity as a percent of full length alignments

nucleotide-binding (GAF) domains, ligand binding PAS domains, and UCRs (for Upstream Conserved Region). There are no such domains in the PDE1 family. Instead, unique to the PDE1 family, are Calcium-Calmodulin (Ca^{2+}/CAM) binding motifs (Bender and Beavo 2006a). Regulation in the PDE1 family occurs via tandem upstream calcium-calmodulin binding motifs (Bender and Beavo 2006a). All three PDE1 enzymes catalyze the hydrolysis of both cAMP and cGMP cyclic

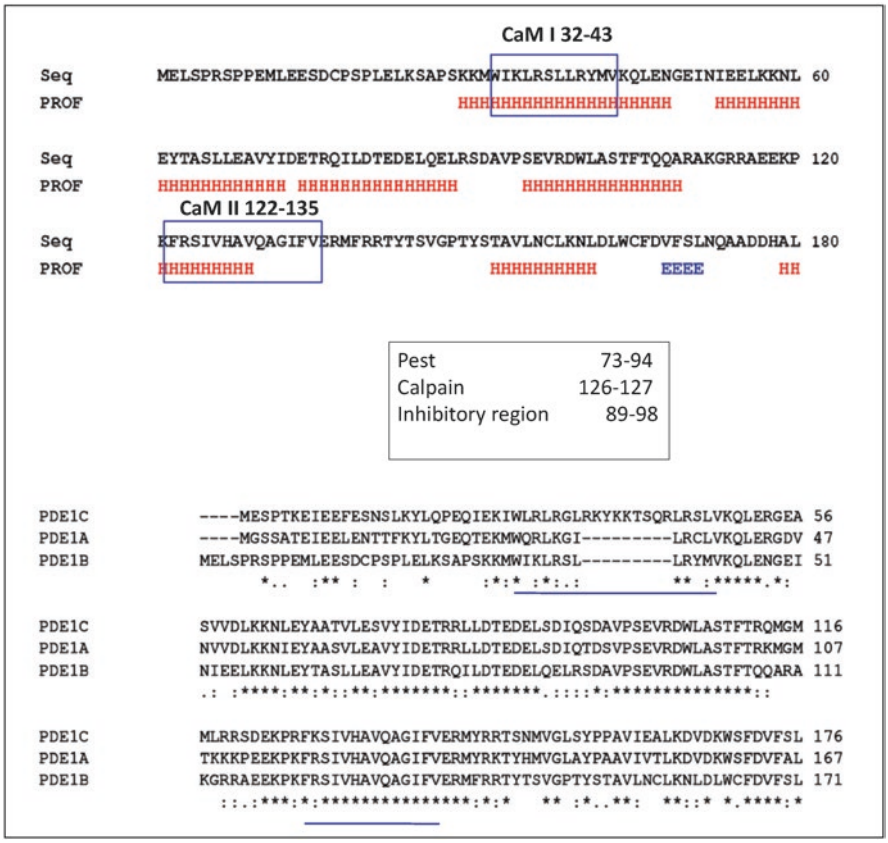


Fig. 13.6 Diagram of the N-terminal regulatory region of hPDE1B. *Top*, diagram of N-terminal region of hPDE1B. Data is derived from information derived by Sonnenburg et al. (1995). *Bottom*, sequence alignment of hPDE1B N-terminal domains. * identical amino acids, : homologous amino acids, . similar amino acid

nucleotides, though their relative affinities for cAMP and cGMP differs (Bender and Beavo 2006a; Sharma et al. 2006). PDE1A and PDE1B are relatively cGMP-specific, with a higher K_m (weaker affinity) for cAMP than cGMP, while PDE1C hydrolyzes both nucleotides with similar affinity (Table 13.2).

Early purification of the PDE1 isoforms was performed from bovine brain (63 Kd and 60 Kd isoforms—referred to as PDE1B1 and PDE1A2 (Sharma et al. 2006)), heart and lung tissues (Sharma and Kalra 1994). This work revealed enzymes of similar V_{max} activity for cGMP from all tissues. A 63 Kd enzyme from bovine brain was reported to have significantly lower V_{max} for cAMP versus cGMP. Heart enzyme, was activated by significantly lower calcium concentrations in the presence of maximal calmodulin concentration. However, the PDE1 isoform identity of this heart PDE1 was not clearly determined. The issue of the identity of PDE enzyme forms will be discussed further below, under Tissue Distribution.

Table 13.1 Protein—protein interactions of the PDE1 family members as identified in the Pathway Studio Knowledgebase from Elsevier. The protein-protein interactions are annotated by type and contain descriptions that are pulled directly from the public literature by the Knowledgebase software.

Relation	Type	Description	TextRef	# of references	Effect	Source	Mechanism
Calmodulin → CaM-dependent cyclic nucleotide PDE	DirectRegulation	All members of the PDE1 family can be stimulated by Ca(2+) in the presence of calmodulin in vitro. Some PDE1 isozymes have similar kinetic and immunological properties but are differentially regulated by Ca2+ and calmodulin	info:pmid/15988057#abs:2, info:pmid/16786160#abs:4, info:pmid/2561985#abs:1, info:pmid/19741056#abs:3, info:pmid/8569735#abs:4, info:pmid/2543685#abs:2, info:pmid/15599707#abs:8, info:pmid/2286933#abs:8, info:pmid/24222327#abs:4, info:pmid/6088765#abs:3	100	Positive		Direct interaction
Calmodulin → PDE1A	DirectRegulation	Furthermore, all calmodulin isoforms were able to activate bovine calcium/calmodulin-dependent phosphodiesterase. Results obtained with electrophoresis in native conditions indicated that calmodulin is tightly bound to PDE1A. The phosphorylation of calmodulin-dependent phosphodiesterase is blocked by Ca2+ and calmodulin and reversed by the calmodulin-dependent phosphatase	info:pmid/10531384#abs:5, info:pmid/12135876#abs:9, info:pmid/16592937#abs:3, info:pmid/2840101#abs:5, info:pmid/8569735#abs:2, info:pmid/7982300#abs:5, info:pmid/2536706#abs:4, info:pmid/6122610#abs:4, info:pmid/8166665#abs:4, info:pmid/7739760#abs:3	22	Positive		Direct interaction

PKA → PDE1A	ProtModification	The bovine heart calmodulin-dependent phosphodiesterase can be phosphorylated by cAMP-dependent protein kinase, resulting in a decrease in the enzyme's affinity for calmodulin. The cAMP-dependent protein kinase was found to catalyze the phosphorylation of the purified cardiac calmodulin-dependent phosphodiesterase with the incorporation of 1 mol of phosphate/mol of subunit. For example, PDE1A is phosphorylated by cAMP-dependent protein kinase, and thus its affinity for calmodulin is reduced. PDE1A and PDE1C are phosphorylated by cAMP-dependent protein kinase (PKA), whereas PDE1B is phosphorylated by 2+-calmodulin-dependent protein kinase II	info:pmid/8569735#abs:1, info:pmid/7982300#abs:4, info:pmid/1646004#abs:3, info:pmid/10906126#body:369, info:pmid/15763421#body:81, info:pmid/8537356#body:426, info:pmid/15350849#body:10, info:pmid/16102838#body:51, info:pmid/8663227#body:45	9	Unknown		Phosphorylation
PKA → CaM-dependent cyclic nucleotide PDE	ProtModification	Another main difference is that 60 kDa PDE1 isozyme is a substrate of cAMP-dependent protein kinase, whereas, 63 kDa PDE1 isozyme is phosphorylated by calmodulin-dependent protein kinase	info:pmid/22900295#abs:6, info:pmid/12060846#abs:5, info:pmid/9406155#abs:4, info:pmid/9880329#body:255, info:pmid/23459256#cont:72, info:pmid/2986124#title:1, info:pmid/21386978#cont:30	7	Unknown		Phosphorylation

(continued)

Table 13.1 (continued)

Relation	Type	Description	TextRef	# of references	Effect	Source	Mechanism
Calmodulin → PDE1B	DirectRegulation	The activity of one subclass (phosphodiesterase IB) was stimulated severalfold by calmodulin and selectively inhibited by the phosphodiesterase inhibitor TCV-3B, Thus, we may conclude that the basic regulatory properties of the tandem calmodulin-binding domains from Phosphodiesterase1A3 and PDE1B1 were retained in the chimeras, The fully Ca2+−calmodulin-stimulated cGMP hydrolytic activity of PDE1B was comparable with the cGMP hydrolytic activity of PDE5, as measured at 1 μM cGMP, Calcium-bound-calmodulin binds to and activates several molecules in the striatum, including phosphodiesterase 1B, calcineurin, and calcium-calmodulin-dependent protein kinase type II	info:pmid/2158939#abs:2, info:pmid/22484154#body:94, info:pmid/12878685#body:159, info:pmid/22346744#cont:52, info:pmid/10737760#body:119, info:doi:10.1016/j.cellsig.2012.03.019#body:94	6	Positive		Direct interaction

CaM kinase II→ CaM-dependent cyclic nucleotide PDE	ProtModification	The sustained calmodulin-dependent protein kinase II activation depended on synergistic actions of two positive-feedback reactions, calmodulin-dependent protein kinase II autophosphorylation and calmodulin-dependent protein kinase II-mediated inhibition of a CaM-dependent phosphodiesterase, PDE1, Model simulation predicted the following cascade as a candidate mechanism for the calmodulin-dependent protein kinase II contribution to Long-term depression: calmodulin-dependent protein kinase II negatively regulates phosphodiesterase 1, subsequently facilitates the cGMP/protein kinase G signalling pathway and down-regulates protein phosphatase 2A (PP-2A), thus supporting the Long-term depression-inducing positive feedback loop consisting of mutual activation of protein kinase C and mitogen-activated protein kinase	info:pmid/19536203#abs:6, info:pmid/23297306#abs:4, info:pmid/2543685#title:1, info:pmid/15944388#body:201	4	Unknown		Phosphorylation
CaM kinase II→PDE1B	ProtModification	Similarly, phosphorylation of PDE1B by calmodulin kinase II reduces the affinity of PDE1B for calmodulin by 6-fold (Hashimoto et al. JBC 264, 10884-10888, 1989), PDE1A and PDE1C are phosphorylated by cAMP-dependent protein kinase (PKA), whereas PDE1B is phosphorylated by 2+−calmodulin-dependent protein kinase II, cyclic-3',5'-phosphodiesterase1A1 from heart and cyclic-3',5'-phosphodiesterase1A2 from brain are phosphorylated by PKA, whereas PDE1B is phosphorylated by calmodulin-dependent protein kinase II, and all of these phosphorylations can be reversed by calcineurin, a Ca/calmodulin-dependent phosphatase	info:pmid/16968949#body:355, info:pmid/15763421#body:81, info:pmid/9893113#body:209	3	Unknown		Phosphorylation

(continued)

Table 13.1 (continued)

Relation	Type	Description	TextRef	# of references	Effect	Source	Mechanism
CAMK → CaM-dependent cyclic nucleotide PDE	ProtModification	Another main difference is that 60 kDa PDE1 isozyme is a substrate of cAMP-dependent protein kinase, whereas, 63 kDa PDE1 isozyme is phosphorylated by calmodulin-dependent protein kinase. Cardiomyocyte contains both a CaMK-regulated isoform of adenylyl cyclase (type III) (23) and a CaM-dependent isoform of cyclic nucleotide phosphodiesterase	info:pmid/22900295#abs:6, info:pmid/10935538#body:113, info:pmid/9138729#body:7	3	Unknown		Phosphorylation
PDE1B → PPP1R1B	ProtModification	Phosphodiesterase 1B and activation of calcineurin that dephosphorylates phosphoThr34 DARPP-32. The activity of DARPP-32, in turn, is regulated by PKA and PKG phosphorylation and, recently, it has been shown that PDE1B is also involved in the regulation of DARPP-32 activity	info:pmid/22346744#cont:805, info:pmid/12967715#body:210, info:pmid/17027094#body:100	3	Unknown		Dephosphorylation
CaM kinase II → PDE1A	ProtModification	The brain 63- and 61-kDa calmodulin-dependent phosphodiesterase isoenzymes (phosphodiesterase1) are phosphorylated in vitro by calmodulin-dependent kinase II and PKA, respectively	info:pmid/8663227#body:45, info:pmid/10799557#body:238	2	Unknown		Phosphorylation
PDE1B → YWHAZ	Binding		info:pmid/16615898, info:pmid/16959763	2		PINA	
PDE1B → PDE1A	ProtModification	Furthermore, the phosphorylation of PDE1A1 (59 kDa) and PDE1A2 (61 kDa) by PKA and of PDE1B1 (63 kDa) by CaM Kinase II decreases their sensitivity to calmodulin activation	info:pmid/16102838#body:51, info:pmid/9102399#body:68	2	Unknown		Phosphorylation
APOA1 → PDE1A	Binding		info:pmid/11991719	1		PINA	
APP → PDE1A	Binding			1		PINA	

ARRB2 → PDE1C	Binding	This may, however, change as a recent study has demonstrated that ß-arrestin2 can co-localize with PDE1C in the olfactory epithelia of rodents (Menco B. J Neuro cytology, 34: 11–36, 2009)	info:pmid/24292836#cont:322	1			
Calcineurin → PDE1C	ProtModification	The resulting Ca 2+ influx would activate calcineurin, which would in turn dephosphorylate and reactivate PDE1C, causing a rapid termination of the cAMP signal	info:pmid/15763421#body:89	1			Dephosphorylation
CALM1 → PDE1A	Binding		info:pmid/12135876	1		PINA	
CALM2 → PDE1A	Binding		info:pmid/12135876	1		PINA	
Calponin → PDE1A	Binding	Using primary vascular smooth muscle cells, we show that cytoplasmic and nuclear PDE1A were associated with a contractile marker (SM-calponin) and a growth marker (Ki-67), respectively	info:pmid/16514069#abs:5	1			
CaM-dependent cyclic nucleotide PDE → ENPP1	Binding	Similarly, unlike NPP1, cell-associated nucleotide pyrophosphatase/ Phosphodiesterase1-2-2 and nucleotide pyrophosphatase/Phosphodiesterase1-2-1 did not display any nucleotide phosphodiesterase activity	info:pmid/15096095#body:151	1			
CaM-dependent cyclic nucleotide PDE → GPHA2	Binding	Moreover, we have provided enticing genetic evidence that Pde1 interacts with Gpa2 in C. albicans but not in S. cerevisiae	info:pmid/20558315#body:182	1			
CaM-dependent cyclic nucleotide PDE → GPR1	Binding	Furthermore we have preliminary evidence (D. Wilson, PhD thesis) that PDE1 also interacts genetically with Gpr1, suggesting that inhibitors of Pde1 when combined with those against Gpa2 and/or Gpr1 could prove effective for combinatorial treatment of C. albicans infections	info:pmid/20558315#body:179	1			

(continued)

Table 13.1 (continued)

Relation	Type	Description	TextRef	# of references	Effect	Source	Mechanism
CaM-dependent cyclic nucleotide PDE→LIG3	Binding	A recent study showed DNA ligase III directly interacts with tyrosyl phosphodiesterase 1 in the single-stranded break repair complex, which is important for maintaining the genomic integrity of developing neurons	info:pmid/16648486#body:59	1			
CaM-dependent cyclic nucleotide PDE→Microtubule	Binding	In bovine brain, PDE1 appears to be associated with neurofilaments and microtubules	info:pmid/15763421#body:98	1			
CaM-dependent cyclic nucleotide PDE→ Neurofilament	Binding	In bovine brain, PDE1 appears to be associated with neurofilaments and microtubules	info:pmid/15763421#body:98	1			
CaM-dependent cyclic nucleotide PDE→PARP1	Binding	Once damage has been recognized, PARP binds to the DNA and recruits X-ray repair complementation group 1(XRCC1) and tyrosol DNA phosphodiesterase 1 (TDP1) to remove the damaged region of DNA, enabling repair proteins to fill-in the missing nucleotides	info:pmid/25774912#cont:36	1			
CAMK→PDE1A	ProtModification	For example, phosphorylation of PDE1A by Ca2+/calmodulin-dependent protein kinase or PDE1B by protein kinase A reduces affinity of both phosphodiesterase1 isoforms for calmodulin	info:pmid/9102399#body:68	1			Phosphorylation
Deaminase→ PDE1A	Binding	Membrane-bound 3'.5'-cyclic nucleotide phosphodiesterase (EC 3.1.4.17) is closely associated physically with nucleotidase and deaminase, thus forming an enzyme cluster of unique catalytic behaviour	info:pmid/6293512#abs:1	1			

LRP1 → PDE1C	Binding	Mechanistic studies revealed that PDE1C plays a critical role in regulating the stability of growth factor receptors, such as PDGF-receptor-beta (PDGFR? known to be important in pathological vascular remodeling. PDE1C interacts with LDL-receptor-related-protein-1 (LRP1) and PDGFRß, thus regulating PDGFRß endocytosis and lysosome-dependent degradation in an LRP1-dependent manner. A transmembrane-adenylyl-cyclase (tmAC)-cAMP-PKA cascade modulated by PDE1C is critical in regulating PDGFRß degradation. Conclusions: these findings demonstrated that PDE1C is an important regulator of smooth muscle cell proliferation, migration, and neointimal hyperplasia, in part through modulating endosome/lysosome dependent PDGFRß protein degradation via LRP1	info:pmid/25608528#abs:10	1			
m_Ubb → PDE1A	Binding			1		PINA	
Nucleotidase → PDE1A	Binding	Membrane-bound 3'.5'-cyclic nucleotide phosphodiesterase (EC 3.1.4.17) is closely associated physically with nucleotidase and deaminase, thus forming an enzyme cluster of unique catalytic behaviour	info:pmid/6293512#abs:1	1			
PDE1A → RPS27A	Binding			1		PINA	
PDE1A → UBA52	Binding			1		PINA	
PDE1A → UBC	Binding			1		PINA	
PDE1A → YWHAZ	Binding		info:pmid/16959763	1		PINA	
PDE1B → POT1	Binding			1		PINA	
PDE1B → UBE3A	Binding			1		PINA	

(continued)

Table 13.1 (continued)

Relation	Type	Description	TextRef	# of references	Effect	Source	Mechanism
PDE1C → PDGFRB	Binding	Mechanistic studies revealed that PDE1C plays a critical role in regulating the stability of growth factor receptors, such as PDGF-receptor-beta (PDGFR? known to be important in pathological vascular remodeling. PDE1C interacts with LDL-receptor-related-protein-1 (LRP1) and PDGFRß, thus regulating PDGFRß endocytosis and lysosome-dependent degradation in an LRP1-dependent manner. A transmembrane-adenylyl-cyclase (tmAC)-cAMP-PKA cascade modulated by PDE1C is critical in regulating PDGFRß degradation. Conclusions: these findings demonstrated that PDE1C is an important regulator of smooth muscle cell proliferation, migration, and neointimal hyperplasia, in part through modulating endosome/lysosome dependent PDGFRß protein degradation via LRP1	info:pmid/25608528#abs:10	1			
PDPK1 → CaM-dependent cyclic nucleotide PDE	ProtModification	The pyruvate dehydrogenase kinase isoenzyme 1 (PDK1) phosphorylates the enzyme of the same name (specifically PDE1), which is the major component of the pyruvate dehydrogenase complex	info:pmid/24086109#cont:155	1			Phosphorylation
PKG → CaM-dependent cyclic nucleotide PDE	ProtModification	Several PDEs including PDE1, PDE3, PDE4, PDE5 and PDE11 are phosphorylated and regulated by cAMP/cGMP-dependent protein kinase, and at least PDE4 is also regulated by tyrosine phosphorylation	info:pmid/11738832#body:154	1			Phosphorylation

PINA Protein Interaction Network Analysis platform (http://omics.bjcancer.org/pina/interactome; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3244997/) Cowley et al. 2012

Table 13.2 K_m and V_{max} data for PDE1 isoforms (Sharma and Kalra 1994; Yan et al. 1995)

	K_m (μM)				V_{max} ratio *cAMP/cGMP*	
	cAMP		cGMP			
Isoenzyme	Sharma and Kalra (1994)	Yan et al. (1995)	Sharma and Kalra (1994)	Yan et al. (1995)	Sharma and Kalra (1994)	Yan et al. (1995)
Bovine PDE1A2	40.0	112.7	3.2	5.1	3.0	2.9
Bovine PDE1B1	12.0	24.3	1.2	2.7	0.3	0.9
Rat PDE1C2		1.2 ± 0.1		1.1 ± 0.2		1.2 ± 0.1

In addition to control via binding of calmodulin, PDE1 activity is inhibited by phosphorylation of the enzyme. Phosphorylation of PDE1A by protein kinase A reduces its affinity for Ca^{2+}/CAM; the EC50 for half-maximal stimulation of cAMP hydrolysis goes from 0.51 to 9.1 nM calmodulin (Sharma and Wang 1985). The phosphorylation site for PKA was mapped to Serine 120 of PDE1A1 enzyme, a site between the two calmodulin binding sites (Florio et al. 1994). PDE1C activity is modulated by phosphorylation by PKA (Loughney et al. 1996; Yan et al. 1996).

The N-terminal control elements in PDE1 have an inhibitory effect on enzyme activity. Proteolytic removal of this region with m-calpain made the enzyme calmodulin-independent, with a K_m and V_{max} (as measured with cAMP) very close to the fully stimulated full-length enzyme (Kakkar et al. 1998). Subsequent molecular cloning and expression has confirmed that the PDE1 catalytic core is constitutively active (Zhang et al. 2004). Specific inhibitors of the PDE1 family enzyme, developed in our laboratories, have equivalent potencies against the Ca^{2+}/CAM-bound holoenzyme and catalytic core.

Interestingly, control of the PDE1B isoform is modulated by phosphorylation by calmodulin-dependent protein kinase (CamKII) (Kakkar et al. 1996). Given the localization of CamKII in neurons to dendritic spines and post-synaptic densities, this may reflect a calcium-dependent feedback loop that would dampen PDE1B activity in this subcellular region.

13.4 Tissue Distribution of PDE1 Enzymes

The distribution of various PDE1 family members has been studied using a variety of qualitative, semi-quantitative and quantitative methods including: isolation and enzymatic characterization, immunohistochemistry, microarray expression profiling and real-time polymerase chain reaction technology (RT-PCR). The literature on PDE1 tissue distribution is rather complicated as the nomenclature have evolved over time. In addition, other factors should be taken into account to judge the importance of the various PDE1 isoforms in different tissues. First, the relation between

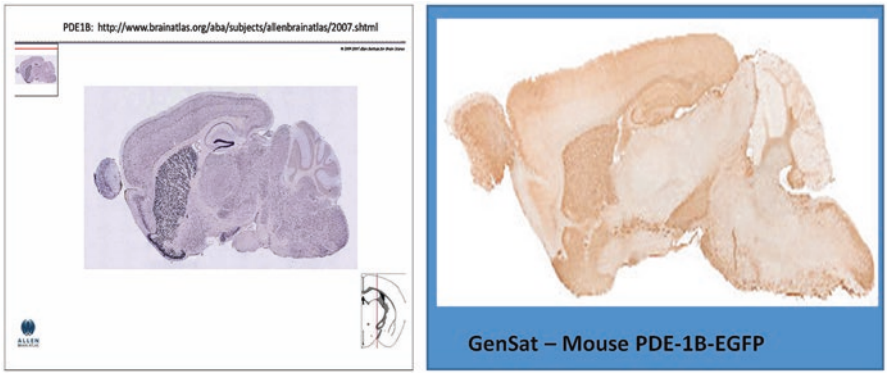


Fig. 13.7 Distribution of PDE1B in mouse brain as revealed by the Allen brain atlas (*left*) and GenSat (*right*) Technologies

mRNA levels quantitated by RT-PCR may not correlate with enzyme levels. Second, tissues contain various cell types. In the heart, cardiomyoctes, endothelial, smooth muscle and, particularly in disease populations, fibroblasts cell types all present different spectrum of PDE1 and other PDE enzymes. Furthermore, cellular compartments undoubtedly exist, as is the case in cardiomyocytes, which may contain higher levels of certain enzymes. Finally, the kinetics of different enzymes and isoforms influence the importance of the various isoforms. PDE1C, with high mRNA levels in the heart, has significantly higher affinity for cAMP versus PDE1A and PDE1B.

Particularly informative information has been collected in human tissues by RT-PCR (Lakics et al. 2010). PDE1B is highly expressed in the brain in the striatum, hippocampus and pre-frontal cortex, where it is highly-co-localized with the dopamine D1 receptor. PDE1B is richly expressed in dopamine-responsive neurons of the caudate putamen and nucleus accumbens. It is also expressed in macrophage cells (Bender and Beavo 2006b). PDE1C is more ubiquitous in the brain, present in the olfactory tubercle, and found abundantly in the cardiomyocyte, lung and heart tissue. PDE1C is a major PDE in the human and rat heart (Sonnenburg et al. 1998) and the major cyclic nucleotide hydrolyzing activity in cardiomyocytes (Vandeput et al. 2007), where PDE3 is also abundantly expressed (Murata et al. 2009). PDE1A is enriched in the brain and, depending on the species (Miller et al. 2011), in the heart. PDE1A is present in activated cardiac fibroblasts (Miller et al. 2011). An interesting variant of PDE1A was described in sperm and kidney by Vasta et al. (2005). Figure 13.7 depicts the localization of PDE-1B in the mouse brain as defined in the Allen Brain Atlas (http://www.brain-map.org/) using in-situ-hybridization, and in the GenSat Atlas (http://www.gensat.org/index.html) using a reporter gene. Enrichment in the striatum of PDE1B implicates a potential role in Parkinson's disease and for use of PDE1 inhibitors in disorders of motor control. The high enrichment in the cardiomyocyte of PDE1C as well a substantial animal model validation reported in the literature, implicates PDE1 inhibitors for heart failure indications (Miller and Yan 2010). There is an interesting literature describing cognitive dysfunction associated with heart failure (Alosco et al. 2014; Feola et al. 2013;

Garcia et al. 2012; Knecht et al. 2012). Additionally, PDE1 enzymes have been found in vascular endothelial cells, smooth muscle cells, fibroblasts and motor neurons.

As mentioned above, the PDE1 enzyme class is known from biochemical isolation studies to be a mainly cytosolic enzyme (Sonnenburg et al. 1998). According to Pathway Studio knowledgebase from Elsevier, some 40 protein-protein interactions of PDE1 isoforms have been documented in the literature (Table 13.1). PDE1A is found in membrane fractions of bovine tracheal smooth muscle and associated with muscarinic M2 acetylcholine receptors in that tissue (Mastromatteo-Alberga et al. 2015). This set of protein interactions is a rather small set, chiefly comprised of calmodulin and relevant kinases, and indicates more work should be done to identify protein-protein interactions of the PDE1 enzyme family.

The subcellular localization of PDE1 enzymes inside neurons and other cells is poorly researched (Beltman et al. 1993; Sonnenburg et al. 1998). The possibility exists of high enrichment of this enzyme in particular microenvironments (Goraya and Cooper 2005). Calcium calmodulin activated kinase II (CAMKII), as an example, is heavily localized with calmodulin in dendritic spines (Lu et al. 2014), reaching high local concentrations, where it plays an important role in synaptic plasticity and memory formation. Calmodulin concentrations in dendritic spines has been estimated to be very high, at around 100 micromolar (Faas et al. 2011). This compares to a half-maximum activation by calmodulin for PDE1 isoforms of approximately 10 nM (Sharma and Kalra 1994). As shown in Table 13.1, a clear interaction is known to occur between calmodulin and PDE1 enzymes, which could serve to concentrate this enzyme family in this microenvironment, contributing to a critical role for PDE1 enzyme in cognitive function.

The relative importance of various PDEs in the heart has been extensively studied (Lee and Kass 2012). This tissue has significant PDE1, 2, 3, 4 and 5 enzymes present and levels vary upon aging and in disease states. Levels of PDE1A and PDE1C vary across species. As mentioned above, in human heart, PDE1C is the predominant PDE1 isoform as measured by RT-PCR. (Lakics et al. 2010) In isolated human cardiomyocytes (as well as Guinea pig, but not rat) PDE1 is the predominant cAMP and cGMP hydrolyzing activity, as reported by Johnson et al. (2012) using a PDE1-selective inhibitor UK90234. Vandeput et al. characterized PDE1C1 subcellular distribution in human myocardium and concluded it to be the major cAMP and cGMP hydrolyzing activity in soluble compartments. PDE3 was found to be the predominant cAMP hydrolyzing activity in microsomal fractions (Vandeput et al. 2007).

13.5 Genomics

As mentioned earlier, catalytic and Ca^{2+}/CaM binding domains of the PDE1 genes are highly conserved between species (Zhang et al. 2000) and across the PDE1 subfamily (Zhao et al. 1997). The National Center for Biotechnology Information

(NCBI) has a substantial and well-organized summary of each of the identified human genes to date (http://www.ncbi.nlm.nih.gov/gene): PDE1A gene has 5 mRNA transcript variants as a result of alternative splicing (Michibata et al. 2001), and they differ mostly in the 5′ and 3′ untranslated regions (UTRs). The sequences encoding the catalytic cores and metal binding sites are entirely conserved. Isoform 1 of PDE1A is encoded by transcript variant 1, represents the longest transcript, and is also considered the "canonical" transcript. Of the species with genome sequences available thus far, 148 organisms have an ortholog of the human PDE1A gene, and slightly fewer organisms have an ortholog for human PDE1B or PDE1C. The human PDE1B gene has 2 transcript variants, with transcript variant 1 encoding isoform 1, the canonical transcript. The human PDE1C gene has 5 transcript variants, and the canonical transcript variant 3 encodes isoform 3. Based on the Genome Reference Consortium's current Human Build 28 patch release 2 (GRCh38.p2), the gene locations for each of the human PDE isoforms are as follows:

PDE1A: Chromosome 2; 182,140,035…182,522,845; 382,811 base pair length
PDE1B: Chromosome 12; 54,549,393…54,579,239; 29,847 base pair length
PDE1C: Chromosome 7; 31,616,777…32,299,404; 682,628 base pair length

Splice variants of the human PDE1A transcript were identified using a cDNA cloning and bioinformatics approach (Michibata et al. 2001). These variants differed in their N-terminal and C-terminal regions. Southern blot analysis of different tissues revealed that certain variants were widely expressed throughout most of the body, while others, such as a variant referred to PDE1A10, were confined to one tissue type (Michibata et al. 2001). Variants all share exons 4–12 of the gene's 17 exons, as exons 6–12 encode the catalytic domain.

A separate, 11.5 kb downstream first exon distinguishes PDE1B2 from PDE1B1 (Bender et al. 2004), and PDE1B2 and PDE1B1 have separate promoters. These promoters are differentially regulated in monocytes versus other cell types. Activation of transcription, rather than post-translational modulation, is primarily responsible for PDE1B2 up-regulation in monocytes. Granulocyte-macrophage colony-stimulating factor (GM-CSF) selectively stimulates transcription of PDE1B2 at a transcriptional start site unique to PDE1B2 (Bender et al. 2004).

Common human trans-acting factor AP-1 is reported to be involved in PDE1B transcriptional regulation in monocytes and CHO cells (Spence et al. 1995). Two specific protein kinase C (PKC) isoforms selectively induce production of PDE1B mRNA as an early response to treatment of CHO cells with the tumor-promoting compound phorbol 12-myristate 13-acetate (PMA). This compound is known to signal via activation of PKC and subsequently AP-1 (Spence et al. 1997). Many alterations in PDE1 expression occur at a post-transcriptional stage, for instance, in incidences of traumatic brain injury (TBI) (Oliva et al. 2012).

Transcriptional regulation of each of the PDE1 isoforms has been characterized to some extent. The excitatory effects of cytokine release on PDE1A activity are suppressed in mouse longitudinal smooth muscles. Inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), suggests that this protein complex is involved in upregulation of PDE1A transcription in mouse smooth mus-

cle (Rajagopal et al. 2014). Drug treatment with vasodilators induces PDE1A1 expression, which contributes to nitrate tolerance, as shown in rat aortic VSMSs (Kim et al. 2001). GM-CSF stimulates transcription of PDE1B1 at a start site activated upon monocyte differentiation. Macrophage colony-stimulating factor (M-CSF) stimulation, however, preferentially induces transcriptional upregulation of PDE2 over PDE1B (Bender et al. 2004). Transcription of PDE1C is induced in proliferative human smooth muscle cells (SMCs), and expression is down-regulated upon cellular quiescence (Rybalkin et al. 2003), indicating that PDE1C plays a regulatory role in the cell cycle of SMCs. 7-oxo-prostacyclin treatment was found to increase the transcriptional levels of PDE1C in the rat heart (Kostic et al. 1997).

The roles of PDE1 isoforms in epigenetic mechanisms are unclear. Because PDE1 activity lowers cAMP, it may indirectly inhibit activation of the common transcription factors cAMP/Ca^{2+} response element-binding (CREB) protein and serum response factor (SRF) (Paul et al. 2010). Furthermore, PDE1A is suggested to be an epigenetic regulator of cell cycle growth and proliferation by targeting the epigenetic integrator UHRF1 (Ubiquitin-like, PHD Ring Finger 1). Down-regulation of PDE1A mRNA expression inhibits UHRF-1 and activates the p73 tumor-suppressor protein (Alhosin et al. 2010). RNA-interference knock-down of PDE1A expression in the acute lymphoblastic leukemia Jurkat cell line triggered cell cycle arrest and apoptosis through regulation of these two proteins.

There is little evidence of PDE1 genetic links to CNS disease to date. PDE1 has few SNPs that have been shown conclusively to be linked to disease. However, a recent human genome-wide association study found SNPs in PDE1A that were associated with diastolic blood pressure and carotid intima-media thickness (Nino et al. 2015). Elevated PDE1A and PDE1C mRNA levels were found to be linked with markers of cellular senescence in vascular smooth muscle cells (Nino et al. 2015; Yan 2015). The term senescence refers to a concept of irreparable chromosomal breaks associated with extensive cell passages in culture or age-related vascular disease and mimicked in mouse knockout models lacking nucleotide excision repair genes. A posttranslational regulatory role of PDE1A localization in determining vascular smooth muscle growth has been described (Nagel et al. 2006).

Initially it was proposed that there may be a linkage between PDEs and Major Depressive Disorder. A particular PDE1A variant, rs1549870, was reported to have a significant effect on antidepressant drug response (Wong et al. 2006). Later studies failed to replicate these results (Cabanero et al. 2009; Perlis et al. 2010).

The recent influx of sequencing data regarding the human genome has made it possible to search online databases and compile genetic risk variants within a population. Table 13.3 describes the variants found among 60,706 sequenced human genomes compiled by the Broad Institute's Exome Aggregation Consortium (Lek et al. 2016). The locations of mutations and their consequences are based on the reference genome build GRCh37/hg19. Of note, fewer variations in each of the PDE1 genes have been observed than what would be expected from random mutation rates. PDE1B in particular is predicted to be highly intolerant to loss of function variations (Lek et al. 2016). The generally low frequency of SNPs for this class of enzymes may reflect the vital roles they play. In summary, little information to date

Table 13.3 Highest-frequency variants resulting in amino acid sequence changes or intronic mutations

	PDE1A				PDE1B				PDE1C			
	Variant	Consequence	Allele Freq. (%)	POI	Variant	Consequence	Allele Freq. (%)	POI	Variant	Consequence	Allele Freq. (%)	POI
1	2:183387027 T/C	p.Lys26Arg	0.144	Euro (0.2%)	12:54963142 C/T	p.Ser155Leu[†]	0.287	Euro (0.4%)	7:32209425 A/G	p.Ser94Pro	99.98	
2	2:183106619 AGTTT/A	p.Lys7TyrfsTer7[a]	0.137	Euro (0.4%)	12:54969777 G/T	p.Gly423Gly[b]	0.203	Euro (0.3%)	7:32338337 G/A	p.Ala4Val	80.61	
3	2:183106640 A/C	p.Met1?	0.100	E Asian (3.5%)	12:54967452 G/C	p.Gln341His[b]	0.177	Euro (0.3%)	7:31862756 T/C	p.Ser565Gly	0.809	Euro (1.2%), Latino (0.9%)
4	2:183387015 C/T	p.Arg30His	0.086	Euro (0.1%)	12:54969851 C/T	p.Ala448Val[b]	0.173	Euro (0.3%), S Asian (0.2%)	7:31855625 C/G	p.Val636Leu	0.576	Euro (0.9%)
5	2:183106638 C/T	p.Gly2Ser	0.079	African (1.3%)	12:54955390 C/T	p.Gln8Ter[a,†]	0.098	S Asian (0.2%)	7:31793149 C/T	p.Arg720His	0.573	Euro (Finnish) (2.6%)
6	2:183050553 T/C	p.Ile544Val	0.074	Euro (0.1%), Latino (0.2%)	12:54963045 C/G	p.Ala102Gly	0.075	African (0.4%)	7:31815326 C/T	p.Gly698Ser	0.525	E Asian (6.7%)
7	2:183095749 C/T	p.Arg192His[b]	0.034	Latino (0.3%)	12:54970470 G/A	p.Glu498Lys	0.065		7:31855577 A/C	p.Ser652Ala	0.386	Euro (0.6%)
8	2:183050536 A/C	p.Phe549Leu	0.017		12:54971056 G/A	p.Glu519Lys	0.036		7:31918714 G/C	p.Ala167Gly	0.343	E Asian (1.1%)

	PDE1A				PDE1B				PDE1C			
	Variant	Consequence	Allele Freq. (%)	POI	Variant	Consequence	Allele Freq. (%)	POI	Variant	Consequence	Allele Freq. (%)	POI
9	2:183129104 C/T	p.Val47Ile	0.017	S Asian (0.1%)	12:54963333 G/A	p.Met138Ile[c]	0.033	S Asian (0.2%)	7:31793046 G/C	p.Ile754Met	0.119	African (1.4%)
10	2:183099170 C/T	p.Val152Ile[b]	0.015		12:54943682 C/T	p.Pro9Leu	0.026	African (0.3%)	7:32209545 A/T	p.Trp54Arg	0.052	
11	2:183033002 G/A	p.Ser527Leu	0.014		12:54966485 C/T	p.Thr232Ile[b]	0.021		7:31815334 C/T	p.Arg695His	0.044	
12	2:183050795 A/G	p.Ile463Thr	0.012		12:54962982 C/T	p.Thr81Met	0.017		7:31862774 T/C	p.Ile559Val	0.040	Latino (0.4%)
13	2:183051198 C/T	p.Ser458Asn	0.011		12:54955411 C/A	p.Pro15Thr[†]	0.011		7:31920469 G/A	p.Arg105Trp	0.037	
14	2:183011873 G/A	p.Ser524Phe	0.010		12:54963034 G/A	p.Gly119Glu[c,†]	0.011		7:32338278 A/G	p.Tyr24His	0.035	Latino (0.1%)
15	2:183104889 G/T	p.Pro116Thr	0.010		12:54964027 C/T	p.Thr181Ile[†]	0.010		7:31867930 T/G	p.Thr481Pro	0.035	
1	2:183070619 A/T	c.950 + 48 T > A	31.8		12:54955461 A/G	c.114-5297A > G	70.0		7:31876766 T/C		74.1	
2	2:183053658 C/G	c.1255 + 48G > C	18.8		12:54955324 T/C	c.-45 T > C	66.8		7:32209376 T/C		15.0	
3	2:183066391 C/A	c.1052 + 24G > T	11.5		12:54971347 C/G	c.*17 + 218C > G	50.8		7:32209359 A/G		8.1	
4	2:183066394 C/A	c.1052 + 21G > T	6.8		12:54943775 T/A	c.113 + 6 T > A	1.7		7:32249155 G/GA	c.86-5dupT	7.4	
5	2:183066390 ACAAC/A	c.1052 + 21_1052 + 25delGTTGTinsT	3.2		12:54943620 C/A	c.-13-24C > A	1.7		7:31848763 T/C		5.2	

Table 13.3 (continued)

	PDE1A				PDE1B				PDE1C			
	Variant	Consequence	Allele Freq. (%)	POI	Variant	Consequence	Allele Freq. (%)	POI	Variant	Consequence	Allele Freq. (%)	POI
6	2:183066388 A/C	c.1052 + 24_ 1052 + 27delGTTTinsGTTG	2.4		12:54969936 GGA/G	c.1376 + 53_1376 + 54delGA	1.2		7:32091215 A/T		4.1	
7	2:183066385 A/AC	c.1052 + 30delTinsGT	2.4						7:32249155 GA/G	c.86-5delT	3.8	

POI Population of interest

The EXAC database (http://exact.broadinstitute.org) lists 540 PDE1A variants, 521 PDE1B variants, and 807 PDE1C variants with different frequencies. Listed are the top amino acid changes (*p*) and coding (*c*) changes based upon relative frequencies. Of the 121,412 alleles sequences covered in this database, an allele frequency of 1% would indicate that the variant appeared 1214 times. As with other such sequence databases, the frequencies may be influenced by different consortiums contributing to the database. For example, variant 183387027 is listed at a frequency of 0.144, but in European Finnish populations the frequency is 0.42. In the EXAC database, a plurality of the individuals were from either the Myocardial Infarction Genetics Consortium or the Swedish Schizophrenia and Bipolar Studies Consortium. Reference (Wong et al. 2006), describing the EXACT database can be found at: http://biorxiv.org/content/early/2015/10/30/030338

[a] Loss of function mutation

[b] In the catalytic domain

[c] In a Ca^{2+}/CaM-Binding domain

[†] Not in canonical transcript (ENST00000243052)

p. Indicates change at a protein sequence

c. Indicates change in the coding sequence

p.Ser527Leu Leucine replaces Serine at residue 527

2:183051198 C/T Notation for variant given as chromosome: location/nucleotide variation

Ter Translation stop codon

p.Lys7TyrfsTer7 Tyrosine replaces Lysine, causing a frame shift mutation with stop codon at location 7 in new frame

c.114-5297A>G A to G substitution at nucleotide -5297 from the end of an intron (in the coding DNA positioned between nucleotides 113 and 114)

c.950+48T>A T to A substitution at nucleotide +48 from the start of an intron (in the coding DNA positioned between nucleotides 950 and 951)

*c.*17+218C>G* Nucleotide change 17 base pairs 3′ of the stop codon

c.-45T>C Nucleotide change from T to C at 45 base pairs 5′ of the initiation codon

links PDE1 polymorphism to human disease, based on recent considerable human genome-wide sequencing data. However, this issue is still under-studied. As more information is obtained of this type is developed in disease-specific databases, such as for Parkinson's disease, schizophrenia, and heart failure, it will be interesting to continue to track genetic variations and their potential indications for both disease pathogenesis and drug response.

13.6 Inhibitors in the Public Domain

Until recently, research into PDE1 had lagged behind other PDE families (2–11) in part due the lack of potent and specific inhibitors. The literature on PDE1 was confused by the improper identification of compounds, such as vinpocetine, as selective PDE1 inhibitors. In fact, vinpocetine is a non-selective agent that inhibits other targets with higher affinity than PDE1. In spite of the confusion generated by early studies, there is now a substantial and growing literature implicating selective PDE1 inhibitors as agents for the treatment of cognitive dysfunction, as therapies for neurodegenerative diseases including Parkinson's disease, as well as for disorders of the cardiovascular system. In addition, PDE1B is involved in activation of monocytes to macrophages, which is relevant to inflammatory responses associated with degenerative diseases (Bender and Beavo; Bender et al. 2004; Bender et al. 2005). This literature will be reviewed below. PDE1 is present in activated fibroblasts and contributes to fibrotic diseases as documented in heart failure (Miller and Yan 2010). Recently, Intra-Cellular Therapies, Inc. announced completion of a series of Phase 1 human clinical studies with the clinical candidate ITI-214, a potent and selective PDE1 inhibitor. Manuscripts describing this agent's chemistry (Li et al. 2016) recently was published.

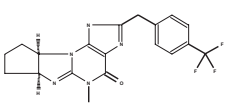
As mentioned, vinpocetine should not be considered a specific PDE1 inhibitor. Curiously, this agent is sold in health food stores as a promoter of memory function. The natural product has been evaluated in six human clinical trials and shown to increase brain vascular blood flow (Patyar et al. 2011). It is not clear which of the target activities of vinpocetine is responsible for this effect (Patyar et al. 2011; Kemeny et al. 2005; Szilagyi et al. 2005; Szatmari and Whitehouse 2003). As shown in Table 13.4 vinpocetine is a very weak PDE1 inhibitor; it interacts with ion channels at nanomolar concentrations. A 2011 review of vinpocetine covers a list of various targets including; voltage-sensitive sodium channels, mitochondrial transition pores, antioxidant properties, and inhibition of the interaction between IκB and IκB kinase (IKK) (Patyar et al. 2011).

Early potent and somewhat selective PDE1 inhibitors were published from efforts at Pfizer and Schering-Plough (Table 13.4). Later efforts at Galapagos and Pfizer demonstrated more potent and more highly selective PDE1 inhibitors. In the 2008 Galapagos patent (WO 2008071650) the authors claimed PDE1C selectivity over PDE1A + B. Our efforts, which started in 2003, led to the discovery of ITI-214 as a potent and highly selective PDE1 enzyme inhibitor with picomolar inhibitory

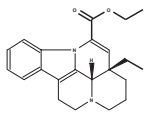
potency and selectivity of 2700-fold versus the first off-target system, PDE4. If one considers a requirement of "greater than 10-fold selectivity" to define a specific inhibitor for one PDE1 isoform (for example PDE1C over PDE1A and PDE1B), we have not seen PDE1 isoform specificity with our proprietary agents, nor when we synthesize and test the literature agents claiming selectivity. As mentioned above, the close PDE1 isoform sequence conservation leads to the prediction that isoform selectivity will be a difficult goal. Importantly, we have seen no downside to hitting all three PDE1 isoforms. Rather, we consider hitting PDE1 A, B and C as beneficial to therapeutic utility with our current PDE1 inhibitors. The rationale for this consideration is that there is no known undesirable effect of this redundancy, and by hitting all three isoforms you can overcome redundancy of isoforms in certain cell types. In human clinical trials, ITI-214 was safe and well tolerated, as described in the clinical trials section below, and reached high plasma drug levels.

Table 13.4 Structures of PDE1 inhibitors

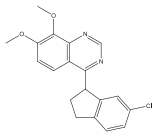
Compilation of Literature PDE1 Inhibitors IC50 (nM)						
	PDE1	PDE4	PDE5	PDE6	Ratio 5/1	Reference
8-MeO-IBMX	1800					
Vinpocetine	58900		>300,000			Ahn et al. 1997
UK-90234	35.4	>1000	1106	8650	31	
SCH51866	70		60		1	
ICOS (IC295?)	490		390000	154000	796	
PF04822163	2-7	7620	>30,000	>29,000		Humphrey et al. 2114
SP31	0.07	700	305		4357	Ahn et al. 1997
SP33	0.6	200	200		333	Ahn et al. 1997
Galapagos 2008	16-83					Unpublished
ITI-214	0.058	158	632	324	10897	Li et al. 2016



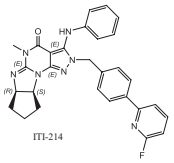
SCH51866



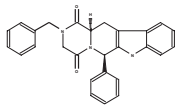
Vinpocetine



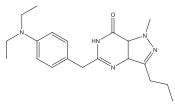
Pfizer PF04822163



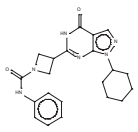
ITI-214



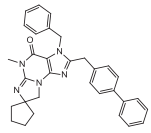
ICOS (IC295)



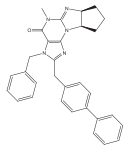
Pfizer UK-90234



Galapagos 2010



Schering Plough 33



Schering Plough 31

The ICOS compound IC295 referred to in the literature was never identified, and so this structure is a representative example from ICOS patent literature

13.7 Pharmacology in Animal Models

Cyclic adenosine monophosphate (cAMP) is the primary intracellular signaling system for the D1 dopamine receptor (Mailman et al. 1986) (as well as for a number of other receptor systems including beta-adrenergic receptors, histamine H2, and various peptide hormone receptors (CGRP, CRF, Melanocortin, and VIP)). The close co-localization in the brain of PDE1B with dopamine D1 receptor has driven efforts to exploit PDE1 inhibitors as cognitive enhancing agents. Cyclic GMP and cAMP are intimately involved in Long-Term potentiation (Kleppisch and Feil 2009), involved in memory consolidation. By amplification of cAMP second messengers, PDE1 inhibitors are likely to be neuroprotective, a subject researched extensively by the Filbin Laboratory (Hannila and Filbin 2008). By amplification of the second messenger signaling systems involving cGMP and cAMP, PDE1 inhibitors have positive effects on memory acquisition, consolidation and retrieval. Cyclic GMP is the primary intracellular signaling system of the atrial natriuretic peptide hormone receptors (Duda et al. 2014; Leitman and Murad 1987), and is also produced by soluble guanylate cyclase via activation of nitric oxide synthase in response to elevated intracellular calcium. In contrast, cAMP is made by adenylate cyclases in response to a number of G-protein coupled plasma membrane receptors. Importantly, the temporal and special concentrations of cyclic nucleotides will vary tremendously, depending on the cell type and stimulus.

PDE1A, B and C knockout mice have been produced and are healthy (Siuciak et al. 2007; Cygnar and Zhao 2009; Ye et al. 2016). PDE1B knockout mice display an interesting "on-demand" phenotype when challenged with a sub-optimal dose of dopamine D1 agonist (Reed et al. 2002; Ehrman et al. 2006).

There are rather few reports in the literature of the use of potent and selective PDE1 inhibitors in animal models of CNS diseases. This is in large part due to the lack of potent and selective inhibitors available in the public domain. In addition to the work using novel object recognition (NOR) tests in rats, which we have done, we have investigated reversal of catalepsy induced by haloperidol, a potent dopamine D2 receptor antagonist used as an antipsychotic. Haloperidol induces serious extra-pyramidal side effects (EPS) and leads to tardive dyskinesia, a major downside of the potent D2 receptors used to treat schizophrenics. While we have seen that PDE1 inhibitors reverse catalepsy induced by haloperidol when tested in mouse models, PDE10 inhibitors actually exacerbate catalepsy in this assay. This is a major distinction between PDE1 and PDE10. We have generated data indicating wakefulness-promoting properties of PDE1 inhibitors in mouse models, as measured by EEG. Lastly, PDE1 inhibitors are able to potentiate the beneficial effects of sub-maximal L-DOPA when tested in a unilateral 6-hydroxy dopamine lesion model that scores restoration of use of the affected contralateral limb. This set of data has given us optimism that PDE1 inhibitors will potentially treat CNS disorders involving cognitive function, Parkinson's disease and problems in wakefulness. Neurodegenerative diseases could theoretically be treated with PDE1 inhibitors, but this area is under studied.

In contrast, a substantial validation for the use of PDE1 inhibitors to treat heart failure exists in the literature. This work comes mainly from work done by Chen

Yan at Rochester University. It includes use of ICOS PDE1 inhibitors (no structure revealed), PDE1C and PDE1A knock-out mice studies, cellular models, and RNA interference studies (Miller et al. 2011; Miller and Yan 2010; Miller et al. 2009). In addition to effects on cardiomyocyte hypertrophy, this laboratory has documented reversal of fibrosis in heart failure models. Ahn and colleagues working at Schering Plough, published small decreases of blood pressure after treating spontaneously hypertensive rat models with their PDE1 and dual PDE1-PDE5 inhibitors (1997). While more work needs to be done in the areas of heart failure, this work holds promise that PDE1 inhibitors will be effective in heart failure.

13.8 Potential for Cognitive Enhancement in Mental Diseases

Much of the research into cognitive dysfunction in mental disease has focused on the involvement of the pre-frontal cortex (PFC) (Goldman-Rakic 1995; Goldman-Rakic 1994; Goldman-Rakic 1987). In this brain area, well established circuits of pyramidal neurons exist that signal via NMDA glutamate receptors. (Somewhat similar circuits exist in hippocampal regions involved in working memory as described by Tamminga and co-workers (Samudra et al. 2015; Tamminga et al. 2010)). The activity of pre-frontal cortical circuits is dampened by the action of GABA interneurons. The activity of the pyramidal cells is dampened, particularly under stressful conditions, by activation of voltage sensitive potassium channels called hyperpolarization-activated cyclic nucleotide-gated (HCN) and KCNQ channels, both of which are activated by cAMP. These potassium channels, when activated in stressful conditions, can effectively shut down the pyramidal circuits and severely impact memory, particularly in the schizophrenic brain (Arnsten and Jin 2014; Yang et al. 2013).

There is a large literature that indicates hypo-functionality of dopamine D1 receptor in the pre-frontal cortex in patients with schizophrenia (Slifstein et al. 2015; Thompson et al. 2014). As this dopamine receptor is intimately involved in working memory, this hypo-functionality is felt to contribute to the cognitive dysfunction. Importantly, the dopamine D1 receptor plays a pivotal role in many aspects of cognitive function including: speed of processing, attention, vigilance, working memory, reasoning and problem solving (Goldman-Rakic 1996; Goldman-Rakic 1998; Goldman-Rakic 1999). A well supported theory of the etiology of Schizophrenia has proposed cognitive dysfunction as a root cause (Nelson et al. 2009). Moreover, cognitive dysfunction is associated with multiple disorders of the CNS and is well recognized as a component of the cardiovascular disease of heart failure (Moraska et al. 2013). Cognitive dysfunction is compounded by excessive awareness of sensory input, generally suppressed in normal individuals, resulting in overwhelming noise in the PFC.

Over the past decades, substantial efforts have been made to treat schizophrenics with direct-acting dopamine D1 receptors. These efforts have generally failed

(Zhang et al. 2009). These failures are attributed to poor drug bioavailability of first generation agonists such as dihydrexidine (Mottola et al. 1992). In other efforts, the D1 receptor agonists A-86929 (Martin 2011; Giardina and Williams 2001), dinapsoline, dinoxyline, and doxanthrine, were discovered and tested clinically. However, interactions of D1 receptor agonists with D1 receptors in the periphery often lead to side effects resulting in hypotension and tachycardia (Huang et al. 2001). In addition, the failure of these drugs to achieve clinical efficacy may be associated with a diminution of initial positive effects of direct D1 agonists due to receptor desensitization. Dopamine action in this brain area is well known to have an inverted U-shaped activity/[dopamine agonist] relationship. It may be that a rather narrow U-shape response curve contributes to the difficulty of this approach. Apomorphine is a non-selective dopamine agonist with highest potency to D2 receptors, used in the treatment of Parkinson's disease. However, Apomorphine causes emesis, limiting its use.

PDE1 receptor inhibitors act as indirect dopamine D1 receptor agonists and should avoid the problems associated with directly acting D1 receptor agonists. The interest in PDE1 inhibitors revolves around the signal transduction pathway of the D1 receptor (Boyd and Mailman 2012). Dopamine D1 receptors signal via activation of Gs G-proteins to stimulate adenylate cyclase to produce intracellular cAMP (Mailman and Huang 2007). This fact and the co-localization of PDE1B enzyme with the D1 receptor in the pre-frontal cortex indicates PDE1 as a major "turn off" mechanism of the D1 receptor, via hydrolysis of cAMP. Therefore the use of PDE1 inhibitors, by preventing local dampening of cAMP signal transduction, represents an indirectly-acting D1 receptor agonist.

Consistent with these hypotheses, a number of PDE inhibitors have been shown effective in animal models of cognition. These models include the Novel Object Recognition (NOR) model (Reneerkens et al. 2009). The NOR model has a number of advantages as rodents are not perturbed by chemicals or pre-conditioning. This test utilizes the inherent tendency of rodents to explore novel objects. In rats, the recognition of such objects disappears in roughly 4 h. In our standard protocol, a 24 h delay is used and activity at this delay time indicates significant cognitive enhancement. Enhancement of NOR has been demonstrated by a number of PDE inhibitors which include PDE1, PDE2, PDE4, PDE9 and PDE10 (Reneerkens et al. 2009; Bollen and Prickaerts 2012; Reneerkens et al. 2013). Our work has focused on PDE1 inhibitors and we have found a large number of excellent enhancers of NOR, consistent with potency to inhibit PDE1 and to oral bioavailability. PDE inhibitory activity influences multiple aspects of NOR, including cGMP influences on early consolidation and cAMP influences on late consolidation (Bollen et al. 2015).

13.9 Diverse Therapeutic Indications

Based on the animal studies and theoretical arguments, there are a number of potential CNS and non-CNS disorders potentially treated by PDE1 inhibitors. Cognitive dysfunction in Schizophrenia, as discussed above, is well supported by animal

studies. Utility of PDE1 inhibitors as dose-sparing adjuncts to L-DOPA treatment in Parkinson's disease is a second very interesting indication, as is use of PDE1 inhibitors in motor disturbances of a variety of etiologies. More work will need to be done to realize the full potential of these possibilities.

Degenerative disorders of the CNS and the periphery remain attractive potential indications for PDE1 inhibitors. The enormous unmet need for treatments of heart failure justifies continued studies. PDE1 acts particularly in excitatory cellular systems such as neurons and cardiomyocytes where repeated cycles of calcium entry occur during the inherent repetitive cellular excitation. Importantly, the role of PDE1 enzyme during cell excitation is one integrated over the course of a lifetime and so it is particularly relevant to degenerative diseases where excessive intracellular calcium is felt to be responsible, in large part, with the progression of these diseases. Mitochondrial ATP generation is known to be influenced by cAMP (Acin-Perez et al. 2009). Indeed, motor neuron survival and regeneration is clearly benefited by cAMP (Qiu et al. 2002). The important motor neuron survival gene (SMN) has a cAMP-response element (CRE-II), giving further credence to the beneficial role of cAMP to skeletal muscle motor neuron function and survival (Hannila and Filbin 2008; Hannila et al. 2007). These properties of PDE1 indicate that PDE1 inhibitors may additionally have use in motor neuronal survival in spinal muscular atrophy (SMA) and more generally in degenerative disease treatments.

13.10 Clinical Trial Histories

A series of phase 1 human clinical trials with ITI-214, a potent and selective PDE1 inhibitor, were reported in a press release by Intra-Cellular Therapies, Inc. recently (http://ir.intracellulartherapies.com/releasedetail.cfm?ReleaseID=932472). The clinical candidate, ITI-214, was shown safe and well tolerated in normal healthy volunteers over a wide range of doses and in repeat dose studies. High drug levels were found in plasma after oral administration. This study serves to dispel any notion that PDE1 inhibitors will have any obvious liability. To our knowledge, no other potent and selective PDE1 inhibitor has been tested in humans.

In contrast, there are over 1000 clinical trials found in the "ClinicalTrials.gov" database when searched using the keyword "phosphodiesterase". Seventy-seven percent were for cardiovascular indications and 20% for disorders of the CNS. Ten percent of trials were for PDE3 inhibitors, 14% for PDE4 inhibitors, 46% for PDE5 inhibitors and 1% for PDE10.

Clinical trials covering potential cognitive enhancing drugs is a large area, covering a diverse set of targets. Over 1891 trials are listed in clinicaltrials.gov when searched with the term "cognitive dysfunction". Target mechanisms include cholinergic receptors, glutamatergic receptors, phosphodiesterase inhibitors (PDE9, 10, 5 and 4), serotonergic receptors, histaminergic H3 receptor agents and dopamine D1 agonists. However, the lack of new drug therapies to date for cognitive dysfunction indicates this objective may be a difficult one to demonstrate.

13.11 Summary

This review has focused on the potential use of PDE1 inhibitors for various diseases and has attempted to highlight the importance of this target for degenerative disorders and disorders of cognition.

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