Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11079–11083, November 1992
Neurobiology

Molecular cloning of DNA encoding a calmodulin-dependent phosphodiesterase enriched in striatum

(cyclic nucleotides/basal ganglia/dopamine)

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Communicated by Karl H. Beyer, Jr., August 24, 1992 (received for review June 2, 1992)

ABSTRACT **A murine cDNA for the 63-kDa calmodulin-dependent phosphodiesterase (CaM-PDE), PDE1B-1, was isolated by using polymerase chain reaction with degenerate primers followed by the cloning of a full-length cDNA from a whole-brain phage library. The nucleotide sequence of 2986 base pairs contains an open reading frame encoding a protein of 535 amino acids (M_r = 61,231) with a predicted isoelectric point of 5.54. The deduced protein sequence shows ≈60% identity with that of the 61-kDa isoform (PDE1A2), consistent with the proposal that these proteins arise from two separate genes [Novack, J. P., Charbonneau, H., Bentley, J. K., Walsh, K. A. & Beavo, J. A. (1991) *Biochemistry* 30, 7940–7947]. Southern blot analysis suggests high nucleotide-sequence conservation of the *PDE1B1* gene among mammalian and avian species. A single ≈3600-nucleotide mRNA transcript was seen in all brain regions, with striatum containing 4- to 30-fold higher levels than other areas. In nonneural tissues, low amounts of PDE1B1 mRNA were detected in lung, spleen, thymus, and testis; hybridization to several larger mRNA species was also seen in thymus and testis. By using nucleic acid probes for *PDE1B1*, the mechanisms that control its highly selective gene expression can now be studied at the molecular level.**

Cyclic nucleotides have a central role in the signal transduction processes of neurons. By mediating protein phosphorylation cascades, these second messengers are responsible for regulating a diverse array of functions such as gene expression, cellular ultrastructure, and permeability of ion channels. Intracellular levels of cyclic nucleotides are modulated through events that affect their synthesis by adenylate and guanylate cyclase and their degradation by cyclic nucleotide phosphodiesterase (PDE). PDEs comprise an extended family of isozymes that can be divided into five broad categories (1–3). Among these is a subset of PDE isozymes that is regulated by Ca^{2+}/calmodulin (CaM), providing a means for direct interactions between the Ca^{2+}/CaM and cyclic nucleotide second-messenger systems. At least five different CaM-dependent PDE (CaM-PDE) isoforms have been identified and characterized from various tissues including brain, lung, heart, lymphocytes, and testis (4–9).

In neuronal tissue, biochemical and immunological studies have identified two CaM-PDE isoforms that exhibit apparent subunit M_r values of 61 and 63 kDa, and it is known that the holoenzymes are homodimers (5, 10). Although present in all brain regions, CaM-PDE is highly enriched in neuronal populations such as neocortical and hippocampal pyramidal cells and cerebellar Purkinje cells (4). Immunoreactivity is seen throughout the extensive dendritic arborizations of these major output neurons as well as in the somatic cytoplasm. Interestingly, recent electron microscopy studies showed a highly localized distribution of CaM-PDE in the postsynaptic region of asymmetric synapses, while showing little reactivity in other dendritic areas (11). This localization suggests that CaM-PDE subserves an important function in the dendrites of neurons that integrate multiple convergent inputs. During development of the brain, CaM-PDE increases rapidly in periods of active synaptogenesis (12). However, detailed immunological studies have shown complex patterns of CaM-PDE expression during this period, suggesting that the enzyme may be important transiently in the development of certain brain regions rather than simply increasing in relation to increased innervation (12). To date, the neuronal distribution and roles of individual CaM-PDE isoenzymes in the mature and developing central nervous system have not been determined. To understand the biological roles and regulation of CaM-PDE isoforms at a molecular level, we have initiated studies to characterize cDNA clones from mammalian brain and report here the sequence of the 63-kDa isoform of CaM-PDE.*

METHODS

Materials. Restriction enzymes and Klenow fragment of DNA polymerase I were purchased from Promega or United States Biochemical. DNA sequencing was completed by using the Sequenase DNA sequencing kit from United States Biochemical. Components for polymerase chain reaction (PCR) were obtained from Perkin–Elmer/Cetus, and oligonucleotide primers were synthesized with a Cyclone Plus DNA synthesizer (MilliGen/Biosearch, Novato, CA). Hybond nylon membranes for screening phage libraries, and Southern and Northern blots were from Amersham. A Riboclone cDNA synthesis kit that uses avian myeloblastosis virus reverse transcriptase was purchased from Promega. [^{32}P]dATP, [^{32}P]dCTP, and [^{35}S]dATP were purchased from DuPont/NEN. All electrophoresis reagents were obtained from Bio-Rad. A Southern blot of *Eco*RI-digested genomic DNA from several species (Zoo-blot) was purchased from Clontech.

Preparation of Cerebellar cDNA from Purkinje and Granule Cell Layers. Cerebellar Purkinje and granule cell layers of C57BL/6 adult male mice (NCI) were isolated by the micropunch dissection procedure described by Palkovits (13) and Palkovits and Brownstein (14). Briefly, the cerebellum with the lower brainstem was frozen on a specimen holder with dry ice, and 300-μm coronal sections were cut on a freezing microtome. Purkinje and granule cell layers of the vermis and paramedian lobes were microdissected with a 300-μm (inside diameter) micropunch needle. RNA was isolated by the method of Cathala *et al.* (15), and poly(A)$^+$ mRNA was selected by using oligo(dT)-cellulose chromatography. Poly(A)$^+$ mRNA was reverse-transcribed by using a *Not*

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Abbreviations: PDE, phosphodiesterase; CaM, calmodulin; CaM-PDE, calmodulin-dependent phosphodiesterase; UTR, untranslated region.

*The nucleotide sequence reported in this paper has been deposited in the GenBank data base (accession no. L01695).

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                -95 GCACG AGGCCGCAGGAACTGCAGCCCTGCTAGCTT GGGCCGAGCAGAAACACACCGGCCTGGCTG GTCCACGCCAGCCGCAGACAGTGGCTGAGC  -1
ATGGAGCTGTCCCCCCGCAGTCCTCCAGAG ATGCTGGAGTCGGATTGCCCGTCACCCCTG GAGCTGAAGTCAGCCCCCAGCAAGAAAATG TGGATTAAGCTACGGTCTCTGCTGCGCTAC 120
M E L S P R S P P E  M L E S D C P S P L  E L K S A P S K K M  W I K L R S L L R Y  40

ATGGTGAAGCAGTTGGAGAATGGGGAGGTT AACATCGAGGAGCTGAAGAAAAACCTGGAA TACACAGCTTCCCTGCTGGAAGCTGTCTAC ATTGATGAGACAAGGCAAATCCTAGACACT 240
M V K Q L E N G E V  N I E E L K K N L E  Y T A S L L E A V Y  I D E T R Q I L D T  80

GAAGATGAGCTTCGGGAACTTCGGTCAGAT GCTGTGCCTTCAGAGGTGCGGGACTGGTTG GCCTCCACCTTCACCCAGCAGACCCGAGCC AAAGGTCGCAGGGCAGAAGAGAAACCCAAG 360
E D E L R E L R S D  A V P S E V R D W L  A S T F T Q Q T R A  K G R R A E E K P K  120

TTCCGAAGCATCGTGCATGCTGTGCAGGCT GGGATCTTCGTGGAGCGGATGTTCCGGAGA ACGTATACCTCTGTGGGCCCCACATACTCT ACTGCAGTCCACAACTGTCTCAAGAACCTG 480
F R S I V H A V Q A  G I F V E R M F R R  T Y T S V G P T Y S  T A V H N C L K N L  160

GACCTCTGGTGCTTTGATGTCTTTTCCTTG AACCGGGCAGCCGATGACCACGCTCTGAGG ACCATTGTTTTTGAGTTGCTGACTCGGCAT AGCCTCATCAGCCGCTTTAAGATTCCCACA 600
D L W C F D V F S L  N R A A D D H A L R  T I V F E L L T R H  S L I S R F K I P T  200

GTGTTTCTGATGAGTTTTCTGGAGGCCTTG GAGACAGGCTATGGGAAATATAAGAATCCT TACCACAACCAGATCCACGCAGCCGACGTG ACCCAGACTGTCCATTGCTTCCTTCTCCGC 720
V F L M S F L E A L  E T G Y G [K Y K N P Y H N] Q I H A A D V  T Q T V H C F L L R  240
                                F1

ACAGGCATGGTGCACTGCCTGTCAGAGATT GAGGTCTTGGCCATCATCTTTGCTGCAGCC ATCCATGACTATGAGCACACAGGCACAACC AACAGCTTCCACATTCAGACCAAGTCAGAA 840
T G M V H C L S E I  E V L A I I F A A A  I H D Y E H T G T T  N S F H I Q T K S E  280

TGTGCCATCCTGTACAATGATCGATCGGTG CTGGAGAATCACCACATCAGCTCTGTCTTT CGAATGATGCAGGATGATGAGATGAACATT TTTATCAATCTCACCAAGGATGAATTTGCA 960
C A I L Y N D R S V  L E N H H I S S V F  R M [N Q D D E M N] I  F I N L T K D E F A  320
                                F2

GAGCTGCGGGCCCTGGTTATCGAGATGGTG TTGGCCACAGACATGTCCTGCCATTTCCAG CAAGTGAAGACTATGAAGACAGCACTGCAG CAGTTGGAAAGGATTGACAAGTCCAAGGCC 1080
E L R A L V I E M V  L A T D M S C H F Q  Q V K T M K T A L Q  Q L E R I D K S K A  360

CTATCTCTTCTGCTTCATGCTGCTGACATC AGCCACCCAACTAAGCAGTGGTCAGTCCAC AGCCGCTGGACCAAGGCCCTAATGGAAGAG TTCTTCCGCCAGGGTGACAAGGAGGCAGAG 1200
L S L L L H A A D I  S H P T K Q W S V H  S R W T K A L [M E E F F R] Q G D K E A E  400
                                R1

CTGGGCCTGCCCTTCTCTCCACTCTGTGAT CGCACTTCCACATTGGTGGCCCAGTCCCAG ATAGGTTTCATTGACTTCATTGTGGAGCCA ACCTTCTCTGTGCTGACTGATGTGGCAGAA 1320
L G L P F S P L C D  R T S T L V A Q S Q  I G F I D F I V E P  T F S V L T D V A E  440

AAGAGTGTCCAGCCCTTGGCAGATGATGAT TCCAAGCCTAAAAGTCAGCCCAGCTTCCAG TGGCGCCAGCCTTCTTTAGATGTGGACGTA GGAGACCCCAACCCTGATGTGGTCAGTTTC 1440
K S V Q P L A D D D  S K P K S Q P S F Q  W R Q P S L D V D V  G D P N P D V V S F  480

CGTGCCACCTGGACCAAGTACATTCAAGAG AACAAACAGAAGTGGAAGGAACGGGCAGCA AGTGGCATCACCAACCAGATGTCCATTGAT GAACTGTCCCCCTGTGAGGAAGAAGCCCCA 1560
R A T W T K Y I Q E  N K Q K W K E R A A  S G I T N Q M S I D  E L S P C E E E A P  520

TCCTCCCCTGCAGAAGATGAGCACAACCAG AATGGGAATCTGGATTAGGGAACTGGGGGC TGACCAGGTCCTCACTGAGTCCAAAGTCTT CGATGTCATTAGCACTATCCATCAGGACTG 1680
S S P A E D E H N Q  N G N L D * 535

GTTCCCCCATCTGCTCCAAGGGAGTGTGTT GGTTGTGGGAGAGACAGCTCACCTGAGAGC CAAATGTTGGAGCGGGTGGGGTGTGGGAAG GGCCCCTCCCCACCCACAGGCCCTCAGTGG 1800
TCACTGTGCCCATCCCTTGCCTCTGGACTC CTCCTTTGGCCACTTGGGAGGAGTAAGCTC CCTGCAGGCTTCCTACAGTTTGGAGGGGAG GGTCAGAGATGCCAGCCCCCTGGGCCCTCC 1920
CCATCCTTCTTGCCTCCAAGTTTCTAAGCA ATACATTTTGGGGGTTCCCTCAGTCCCCCA CCCCAGATCTTAGCTGGCAGGTCTGGGTTT CCCTTCTCTTCCCTGAGAAAGGGCCAGAAT 2040
AGGATAGGAAGCTGGCAGTTTTCAAAGCCC TGCATGGGAAGGGCAGGTGTCCCTTCAGGT TCCTTCCTAAGATGAGGTGGGGGACATCCT CTTGATCCCATACTTGCACTTCTTCAGCCC 2160
CATTCCCTTGCAAATTCTCTCTCTCCTGTG CCCAGACAGTGGCTGGGAGAGGAGAGGAGC CATTGGGATTCTGTCTGAGGGAAGTCTTTC CTGGAGTCCTCAAATACTTGGGGATGGTCT 2280
GGGCTTGAGGGGCTTGTACCCTGATCTAGA TGGGTTTTTGACCCTTTGCCTCCTTCCTGT CCCCGGGAACTAGGAAGCCCTCATCCGACC AATGTCTGTAAACGCTCTGAGGTCTTCCTT 2400
CAGGATGTATCTTATGAGCAAAGGAAGGAG AGAGCAGCTAGCCCCAGTACAAGCAGGGGG AAGTATTACCCAGTAATGTTGACACAGAGC AGGTCCTTCCTGCCTCCCTTCTCCCCGAAC 2520
TGGCCCACTAAGAGATTTCCCCCTCCCGGG GCCCCTGGTGGGTGGAAGGCAGATGGGGAG GGGCTCAGGGCGGTAGCAACTTGGAGCACC CAGAGCGAGTGCAGCCAGGTAAAGCCTCTT 2640
CCTGTCCTCAGGCTCCTTGCCCCACGTCAC CCCATTTAAGGCCAAGTCCAGGTGACTGCC TCCTCCTCTCTTGTAAATACATGCATTTGT ACAGTGGGCCCTGTTCTTGTGAAGTCCATC 2760
TCCATGGTCATTAGACCTGCCACTCTGAAC CGCATGTGACTCCCCCATGCTCTTGGTCTC CCAGGCCCCTGCTATAGCCAGAGATCAATA AAGAAGGGAGACCGGCAAAAAAAAAAAAAA 2880
AAAAAAAAAA 2891
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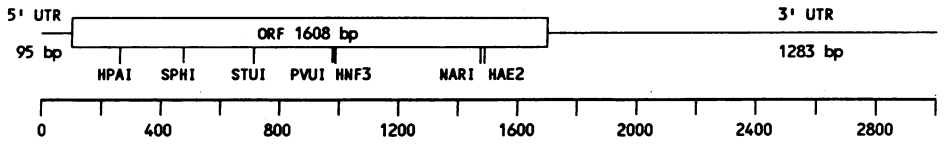

FIG. 1. (*Upper*) Nucleotide and deduced amino acid sequence for MBPDE-1, a cDNA for PDE1B-1 (the 63-kDa CaM-PDE isoform) from mouse brain. The nucleotide and deduced amino acid sequences are presented, with positions in the 5' untranslated region (UTR) shown as negative numbers. The single polyadenylylation signal is underlined. Boxes correspond to the region of protein sequence used to make degenerate primers F1, F2, and R1 for PCR. (*Lower*) A map showing the unique restriction endonuclease sites of MBPDE-1. ORF, open reading frame.

I/oligo(dT) primer–adaptor (Promega) according to the manufacturer's instructions.

Isolation and Characterization of cDNA Clones. Degenerate oligonucleotides, corresponding to "sense" or "antisense" primers, were synthesized based on the partial protein sequence of PDE1A2 (the 61-kDa CaM-PDE isoform; ref. 16): F1, 5'-AARTAYAARAAYCCGTAYCAYAA-3'; F2, 5'-ATGCAIGAIGARGARATGYT-3'; and R1, 5'-AIIAA-IAAYTCYTCCAT-3, in which R = A or G and Y = T or C. The relative positions of these oligonucleotides are indicated in Fig. 1. Reaction mixtures (50 μl total) contained 1 ng of "Purkinje/granule cell-enriched" cDNA, 500 ng each of primers F1 and R1, 50 mM KCl, 10 mM Tris·HCl (pH 8.0), 1.5 mM $MgCl_2$, and 0.01% gelatin. The reactions were amplified by using 1 unit of *Taq* polymerase for 40 cycles, with annealing at 42°C for 2 min and extension at 72°C for 2 min.

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PDE1B1  MELSPRSPPEMLESDCPSPLELKSAPSKKMWIKLRSLLRYMVKQLENGEVNIEELKKNLE   60
PDE1A2      GSTAT.TE.LENTTFKY.IGEQTE...QR.KGI..CL.....KLD..VID....I.   56
PDE4A1                                        MPLVDFFCETCSKPWL.GWWDQF.RML  27

PDE1B1  YTASLLEAVYIDETRQILDTEDELRELRSDAVPSEVRDWLASTFTQQTRAKGRRAEEKPK  120
PDE1A2  .A..V..........RL...D...SDIQ..S.............RKMGMMKKKS....R  116
PDE4A1  NRELTHLSEMSRSGN.YSEYISNTFLDKQNE.EIPSPTPRQRA.Q.PPPSVL.QSQPMSQ   87

PDE1B1  FRSIVHAVQAGIFVERMFRRTYTSVGPTYSTAVHNCLKNLDLWCFDVFSLNRAADDHALR  180
PDE1A2  ......V..........Y.KS.HM..LA.PE..IVT..DV.K.S....A..E.SGE.S.K  176
PDE4A1  ITGLKKL.HT.SLNTNYP.F---G.KTDQEDLLAQE.E..SK.GLNI.CYSEY.GGRS.S  144

PDE1B1  TIVFELLTRHSLISRPKIPTVFLMSFLEALETGYGKYKNPYHNQIHAADVTQTVHCFLLR  240
PDE1A2  FMIY..F..YD..N.....VSC.IA.A....V..S........L...........YIM.H  236
PDE1A2  C.MYTIFQERD.LKK.H..VDTM.MYMLT..DH.-HADVA...SL.....L.ST.-V..A  202

PDE1B1  TGMVH-CLSEIEVLAIIFAAAIHDYEHTGTTNSFHIQTKSECAILYNDRSVLENHHISSV  299
PDE1A2  ..IM.-W.T.L.I..MV..............N.....R.DV..............V.AA  295
PDE1A2  .PALDAVFTDL.I..AL.......VD.P.VS.Q.L.N.N..L.LM...E.......LAVG  262

PDE1B1  FRMMQDDEMNIFINLTKDEFAELRALVIEMVLATDMSCH---FQQVKTM--------KTA  348
PDE1A2  Y.L..EE...VL...S..DWRD..N.......S....G.---...I.NI--------RNS  344
PDE4A1  .KLL.EENCD..Q..S.RQRQS..KM..D........K.MTLLADL...VETKKVTSSGV  322

PDE1B1  LQQLERIDKSKALSLLLHAADISHPTKQWSVHSR-WTKALMEEFFRQGDKEAELGLPFSP  407
PDE1A2  ...P.GL..A.TM..I.........A.S.KL.H.-...M.......L.............  403
PDE4A1  .LLDNYS.RIQV.RNMV.C..L.N...PLELY-.Q..DRI.A...Q...R.R.R.MEI..  381

PDE1B1  LCDRTSTL-VAQSQIGFIDFIVEP---TFSVLTDVAEKSVQPLADDDSKPKSQPSFQWRQ  463
PDE1A2  ....K..M-.....................-----.L...ST..III..IEE..T.TPSYGAS.R  459
PDE4A1  M..KH-.AS.EK..V....Y..H.LWE.WAD.VHPDAQDILDTLE.NRDWYHSAIR.SPS  440

PDE1B1  PSLDVDVGDPN----PD-------VVSFRATWTKYIQENKQKWKER-AASGITNQMSIDE  511
PDE1A2  SNMKGTTN.GTYS--..YSLASVDLK..KNSLVDI..Q..ER...L-..Q.EPDPHKNSD  516
PDE4A1  .P.EEEP.GLGHPSL..KFQFELTLEEEEEEDSLEVPGLPTTEETFL..EDARA.AVDWS  500

PDE1B1  LSPCEEEAPSSPAEDEHNQNGNLD                                       535
PDE1A2  .VNA..KHAETHS                                                   529
PDE4A1  KVKG-----..ITVVEVAERLKQETASAYG......(85 amino acids)            610
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FIG. 2. Comparison of deduced amino acid sequences from cDNAs encoding PDE1B-1 and PDE4A1 high-affinity cAMP PDE (rat dunce; RD1; ref. 25), with the sequence of PDE1A2 (the 61-kDa CaM-PDE isoform) determined by protein sequencing (16, 24). Positions of identity are indicated by periods. Underlined amino acids represent conservative substitutions between all three sequences. Dashed lines are gaps generated to achieve best sequence alignment. The CaM-binding and conserved catalytic domains defined by Charbonneau *et al.* (16, 24) are enclosed in boxes (residues 27–47 and 197–450 of PDE1B1, respectively).

The resulting DNA fragment was amplified by using primers F2 and R1 in a second round of PCR to ensure that the fragments were for a CaM-PDE. To prepare a hybridization probe, a 242-base-pair (bp) DNA fragment was generated by PCR, purified by gel electrophoresis, and radiolabeled by primer extension with the Klenow fragment of DNA polymerase I and specific primers corresponding to the 5′ ends of each strand. This probe was used to screen a mouse brain Uni-Zap XR cDNA phage library (Stratagene) by plaque hybridization. cDNA inserts were excised *in vivo* as phagemids in *Escherichia coli*, and plasmid DNA was prepared by the alkaline lysis procedure (17). DNA sequencing was completed by the dideoxynucleotide chain-termination method (18).

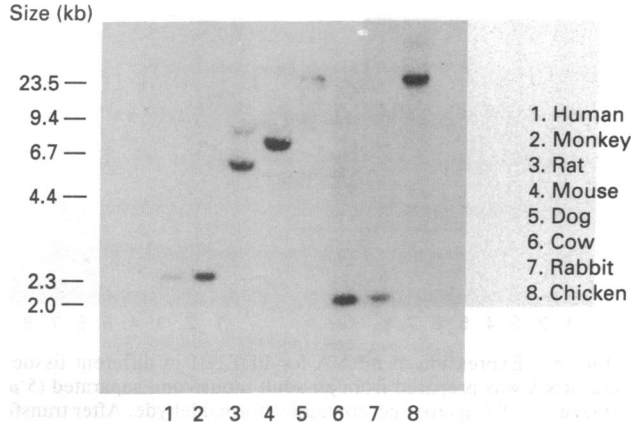

FIG. 3. Southern blot analysis of the *PDE1B1* gene from mammalian and avian species. A Southern blot containing genomic DNA digested with *Eco*RI (Zoo-blot, Clontech) was hybridized with a 241-bp ^{32}P-labeled PDE1B-1 cDNA probe (bp +773 to +1013). DNA samples (8 μg per lane) were from human (lane 1), monkey (lane 2), rat (lane 3), mouse (lane 4), dog (lane 5), cow (lane 6), rabbit (lane 7), and chicken (lane 8).

DNA (Southern) and RNA (Northern) Blot-Hybridization Analysis. RNA from several mouse tissues (C57BL/6) was isolated by the method of Cathala *et al.* (15) and prepared for Northern blots as described (19). Samples of RNA were fractionated in 1% agarose gels containing formaldehyde, stained with ethidium bromide, and transferred to Hybond nylon membranes electrophoretically. Northern and Southern blots were hybridized using radiolabeled probes (specific activities, ≈1–2 × 10^9 dpm/μg) prepared by primer extension of template with an antisense primer or both sense and antisense primers, respectively (20). Final washes for all blots were done at 55°C in 0.015 M NaCl/0.015 M sodium citrate, pH 7/0.1% sodium dodecyl sulfate (0.1× SSC/0.1% SDS).

RESULTS

Cloning and Characterization of a Murine Brain cDNA for PDE1B-1†, the 63-kDa Isoform of CaM-PDE. Previous immunocytochemical studies have demonstrated that CaM-PDE is rich in Purkinje neurons of cerebellum (4, 22); therefore, these and the closely associated granule cell layers were dissected by micropunch from mouse brain, and cDNA was prepared for use in PCR. A 527-bp DNA fragment was amplified from "Purkinje/granule cell-enriched" cDNA by using degenerate primers F1/R1 (Fig. 1). To ensure amplification of CaM-PDE-specific sequences, this fragment was PCR-amplified a second time by using primers F2/R1 to generate a 271-bp fragment. The deduced protein sequence of this fragment was highly homologous to a partial sequence of the 63-kDa bovine CaM-PDE (23), suggesting that the DNA fragment encoded a region of PDE1B-1. A 242-bp probe was generated by PCR amplification of the original F1/R1 fragment and used to screen a mouse brain cDNA phage library.

Of 250,000 plaques screened, 16 positive clones were found, and one possessed an open reading frame of 1608 bp (Fig. 1). This cDNA contained 95 bp of 5′ UTR and 1283 bp of 3′ UTR that included a poly(A) tail preceded by the polyadenylylation signal AATAAA. The deduced amino acid sequence coded for a protein of 535 amino acids with a M_r of 61,231 and pI of 5.54. Comparison of the deduced amino acid sequence of PDE1B1 with that of PDE1A2 (the 61-kDa isoform; ref. 24) showed ≈60% identity and, with conservative substitutions, a similarity of ≈74% (Fig. 2), indicating that these isoforms are derived from two distinct genes (23). Over the 250-amino-acid-conserved catalytic domain defined by Charbonneau *et al.* (16, 24), the identity and similarity between the two PDE sequences increased to ≈71% and ≈86%. Alignment of the conserved catalytic region of PDE4A1, the "high-affinity cAMP-PDE" cloned from rat (RD1; rat Dunce; ref. 25), with that of PDE1B1 and PDE1A2 showed that the PDE4A1 sequence had ≈45% identity and ≈64% similarity with those of both PDE1B1 and PDE1A2 (Fig. 2).

The existence of the gene for PDE1B1 was confirmed by Southern blot analysis of *Eco*RI-digested genomic DNA from several species (Fig. 3). Hybridization of a 241-bp murine probe was seen to DNA from all mammals tested and from chicken, demonstrating a high degree of nucleotide sequence conservation. Based on comparison with other hybridization studies done under identical stringencies and washing conditions (0.1× SSC/0.1% SDS at 55°C), the mouse and chicken

†The classification system proposed by Beavo and Reifsynder (2) was adapted to the standard genetic nomenclature (21) for identification of PDE isozymes as follows: italicized *PDE1B1* (*PDE1B-1* in mouse) for the gene; nonitalicized PDE1B1 for the cDNA, mRNA, and protein in all species including mouse. The 61- and 63-kDa isoforms are classified as PDE1A2 and PDE1B1, respectively.

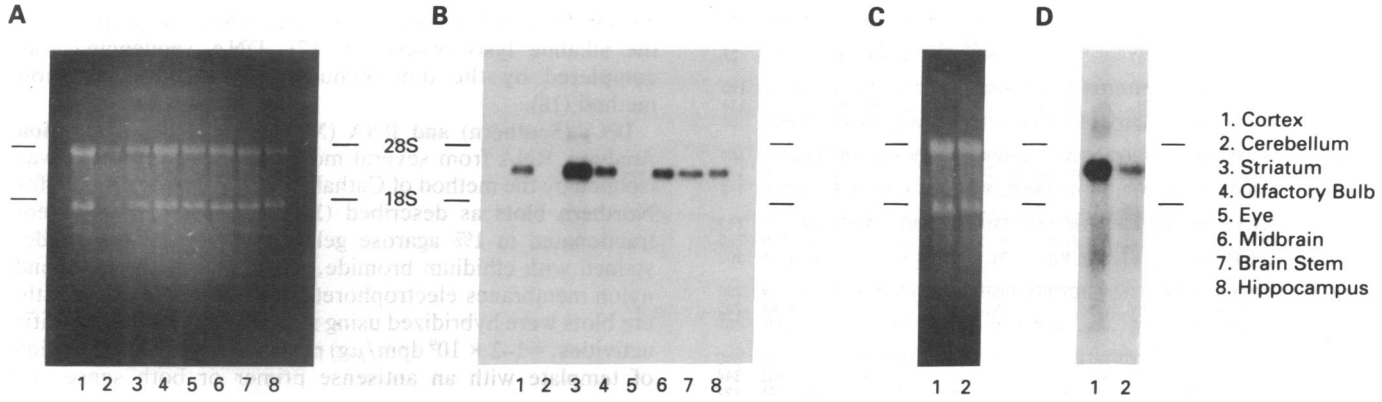

FIG. 4. Expression of mRNA for PDE1B1 in different brain regions. Total RNA was prepared from an adult mouse and separated (1–5 μg per lane) in a 1% agarose gel containing formaldehyde. After transfer to Hybond, the blot was hybridized with a 202-bp ^{32}P-labeled PDE1B-1 antisense cDNA probe (bp +174 to +375). RNA was from cortex (lane 1), cerebellum (lane 2), striatum (lane 3), olfactory bulb (lane 4), eye (lane 5), midbrain (lane 6), brain stem (lane 7), and hippocampus (lane 8). (*A* and *C*) Ethidium bromide-stained RNA gels. (*B* and *D*) Autoradiograms of Northern blots exposed for 2 and 6 days, respectively. *D* was a separate blot containing more total RNA per lane and has been included to detect the presence of PDE1B1 mRNA in cerebellum. Quantification of the hybridization signal is presented in Table 1. Positions of the 18S and 28S rRNAs are indicated by tick marks.

nucleotide sequences for *PDE1B1* are probably >85% identical, consistent with a slow rate of sequence divergence (P. Rathna Giri, R.L.K., and J.W.P., unpublished data).

Expression of PDE1B1 mRNA in Brain and Other Tissues. Hybridization of a 202-bp probe to mRNA from different areas of the brain showed an ≈3600 nucleotide transcript in all regions (Fig. 4 *B* and *D*). Striatum had, by far, the highest level of PDE1B1 mRNA, followed by olfactory bulb, midbrain, cortex, hippocampus, and brain stem; cerebellum and eye showed the lowest level of hybridization. Quantification of the hybridization signal in different regions, after normalization to the amount of total RNA loaded, indicated that striatum contained 4-fold more mRNA than the next highest area (olfactory bulb) and 30-fold more than cerebellum (Table 1). As anticipated, the levels of PDE1B1 message detected in other tissues were small compared to that found in brain, with low amounts of the ≈3600-nucleotide transcript present in lung, spleen, thymus, and testis (Fig. 5). In addition, larger transcripts of ≈5000 and ≈4200 nucleotides were detected in thymus and testis; no hybridization was seen in heart, liver, kidney, or muscle.

Table 1. Comparison of PDE1B1 mRNA levels in different brain regions

Regions in Fig. 4	Total RNA, μg	Blot-hybridization to ^{32}P-labeled probe: Total cpm*	Blot-hybridization to ^{32}P-labeled probe: cpm per μg of RNA	Relative abundance
Fig. 4*B*				
Cortex	3.0	432	144	1.1
Cerebellum	1.0	42	42	0.3
Striatum	1.7	2125	1250	9.1
Olfactory bulb	2.5	708	283	2.1
Eye	2.5	91	36	0.3
Midbrain	2.5	564	225	1.6
Brain stem	2.5	342	137	1.0
Hippocampus	2.5	344	138	1.0
Fig. 4*D*				
Cortex	5.0	663	133	1.0†
Cerebellum	5.0	165	33	0.3†

The data shown in Fig. 4 were evaluated quantitatively by using direct radioimaging (Betagen Betascope 603) of the ^{32}P hybridization signal. The amount of total RNA in each lane was estimated by measurement of OD_{260}, which was in close agreement with ethidium bromide-staining intensities (Fig. 4 *A* and *C*). The data were also quantified by using hybridization probes for 18S rRNA and actin, yielding results that were essentially identical to those above (data provided to reviewers).

*The data reported represent the net cpm collected for 90 min after subtraction of background (x = 81).

†The relative abundance, comparing cortex and cerebellum, in a second experiment (Fig. 4*D*) was essentially identical to the data presented above for Fig. 4*B*.

DISCUSSION

This report describes the use of a microdissection-based strategy to achieve the cloning of a CaM-regulated type I PDE. Over several years, extensive attempts by our laboratory to clone CaM-PDE by using affinity-purified antibodies and degenerate oligonucleotide probes were unsuccessful. Because CaM-PDE is not an abundant enzyme in brain, being ≤0.03% of total brain protein (26), the mRNA is likely to be underrepresented in the majority of libraries. Also, immunocytochemical studies indicated that, although CaM-PDE is expressed robustly in some neurons, this is true only for very select neural populations; thus, whole tissues may not be appropriate as the starting material for cDNA synthesis. Finally, the 3′ UTR of the mRNA may be very long or

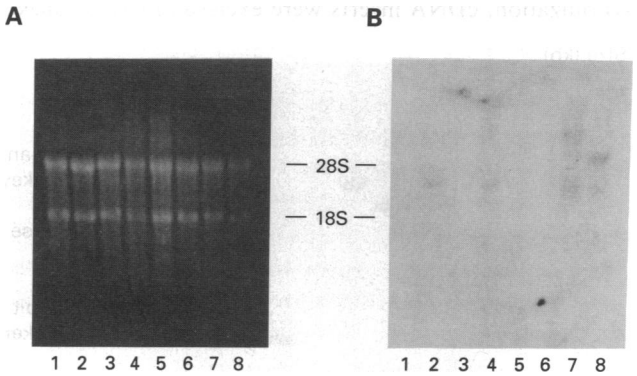

FIG. 5. Expression of mRNA for PDE1B1 in different tissues. Total RNA was prepared from an adult mouse and separated (5 μg per lane) in a 1% agarose gel containing formaldehyde. After transfer to Hybond, the blot was hybridized with a 344-bp ^{32}P-labeled PDE1B-1 antisense cDNA probe (bp +773 to +1116). RNA was from heart (lane 1), lung (lane 2), muscle (lane 3), spleen (lane 4), liver (lane 5), kidney (lane 6), thymus (lane 7), and testis (lane 8). (*A*) Ethidium bromide-stained RNA gel. (*B*) Autoradiogram of the Northern blot after hybridization. Positions of the 18S and 28S rRNAs are indicated by tick marks. Equal loading of RNA was confirmed by hybridizing the same blot with a probe for the 18S rRNA.

refractory to extension by reverse transcriptase, or both. In this regard, the 3′ UTR of the PDE1B-1 cDNA contains several extensive G+C regions, which may be difficult to reverse transcribe. With these potential difficulties in mind, a stepwise strategy was devised to prepare mRNA from a micropunched region enriched in CaM-PDE, amplify partial cDNAs by PCR, and use these DNA fragments to isolate a full-length cDNA from a phage library.

Analysis of the deduced amino acid sequence revealed several potential sites for posttranslational modifications. Four CaM-kinase II phosphorylation consensus sites were present in the PDE1B1 protein sequence, consisting of two Arg-Xaa-Xaa-Ser (Ser-465 and Ser-501) and two Arg-Xaa-Xaa-Thr (Thr-143 and Thr-414) motifs. No consensus sequences for protein kinase C or the cAMP-dependent protein kinase were found. These findings are consistent with phosphorylation studies showing that 2–4 sites could be modified by CaM-kinase II *in vitro* (10, 27), resulting in a decreased affinity for CaM, whereas protein kinase C and cAMP-dependent protein kinase did not phosphorylate this isoform. Whether or not these sites are phosphorylated *in vivo* has not been established. An isoprenylation consensus sequence, Cys-Ala-Ala-Xaa, was present in the deduced protein sequence (Cys-281) of PDE1B1. Interestingly, the cysteine substitution occurs at a site that is conserved among at least eight other PDEs, in which either a leucine or valine is present at this position (28, 29). [Partial cDNA clones for PDE1B1 isolated from human and rat brain also encode cysteines at this position, arguing against this amino acid difference being due to a cloning artifact (J.W.P. and R.L.K., unpublished data).] This observation, in conjunction with a report of a 63-kDa immunoreactive species that was found predominantly in light synaptic membrane fractions (12), may suggest a mechanism whereby this isoform associates with cellular membranes.

Little information exists on the regulation of individual CaM-PDE isoenzymes. In lymphocytes, activation by phytohemagglutinin induced the expression and activity of a CaM-PDE that has immunological and biochemical characteristics similar to the PDE1B1 enzyme (8). Also, a recent study identified CaM-PDE as one of two major PDEs in olfactory mucosa and found that it is selectively expressed in olfactory receptor neurons (30). Biochemical and kinetic characterization demonstrated that the olfactory bulb isoenzyme had a substantially higher affinity for cAMP (K_m = 1–2 μM) than seen for other brain CaM-PDEs (K_m = 12–40 μM), suggesting that it is different than the major forms characterized from brain. Data presented here show that mRNA for PDE1B1 is abundant in olfactory bulb; therefore, it is possible that this high-affinity CaM-PDE isoform in receptor neurons may be a subtype of PDE1B1. Interestingly, another CaM-PDE with high affinity for cAMP has been described in a lymphoblastoid cell line (31).

The most provocative finding of this study is the striking regional selectivity of PDE1B1 mRNA in brain, which shows that striatum contains much more PDE1B1 mRNA than any other brain region. Because immunocytochemical studies have not shown striking immunoreactivity for CaM-PDE in striatum (4), this may suggest that the PDE1B1 isoform or variants thereof are the major CaM-PDE in striatal neurons. Although the physiological significance of the high-level expression of PDE1B1 in striatum is not known, the virtually exclusive postsynaptic localization of CaM-PDE seen in electron microscopic studies (11) may argue for an important role in regulating cyclic nucleotides generated by dopamine-coupled adenylate cyclase. However, regulation of CaM-PDE expression appears to be complex and subject to different types of control. Whereas ablating climbing fiber innervation of Purkinje neurons resulted in the selective loss of CaM-PDE immunoreactivity (22), treatments that block or disrupt dopaminergic innervation showed no overall change in CaM-regulated PDE (32). With the development of molecular probes, experiments to address the gene regulation and role of CaM-PDE isoforms in neurons and other tissues should now be possible.

We recognize the special contribution of Dr. M. Palkovits for his skillful preparation of micropunched cerebellar Purkinje and granule cell layers. We also thank Dr. Polavarapu Rathna Giri for his advice and support. J.W.P. was supported by a Pharmacology Research Associate Trainee (PRAT) fellowship from the National Institute on General Medical Sciences.

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