Multiple isozymes of cyclic nucleotide phosphodiesterases (PDEs) are expressed simultaneously in mammalian tissues. To identify and clone these PDEs, a polymerase chain reaction (PCR) strategy was developed using degenerate oligonucleotide primers designed to hybridize with highly conserved PDE DNA domains. Both known and novel PDEs were cloned from rat liver, the mouse K30a-3.3 lymphoma cell line, and a human hypothalamus cDNA library, demonstrating that these PCR primers can be used to amplify the cDNA of multiple PDE isozymes. One unique mouse PDE clone was found to encode a polypeptide identical with the corresponding portion of the bovine brain 63-kDa calmodulin-dependent PDE as reported in the companion article (Bentley, J. K., Kadlecak, A., Shehab, C. H., Seger, D., Sonnenburg, W. K., Charbonneau, H., Novack, J. P., and Beavo, J. A. (1992) J. Biol. Chem. 267, 18676–18682). This mouse clone was used as a probe to screen a rat brain cDNA library for a full-length clone. The conceptual translation of the nucleotide sequence of the resulting rat clone has an open reading frame of 535 amino acids and maintains a high degree of homology with the bovine brain 63-kDa calmodulin-dependent PDE, indicating that this protein is likely to be the rat homolog of the 63-kDa calmodulin-dependent PDE. Expression of the full-length clone in Escherichia coli yielded a cGMP hydrolyzing activity that was stimulated severalfold by calmodulin. Northern blot analysis demonstrated that the mRNA encoding this PDE is highly expressed in rat brain and also in the S49.1 T-lymphocyte cell line. These data demonstrate that the PCR method described is a viable strategy to isolate cDNA clones of known and novel members of different families of PDE isozymes. Molecular cloning of these PDEs will provide valuable tools for investigating the roles of these isozymes in regulation of intracellular concentrations of the cyclic nucleotides.

Cyclic nucleotide phosphodiesterases (PDEs)1 catalyze the hydrolysis of the 3',5' cyclic nucleotides, cAMP and cGMP, to the corresponding nucleoside 5'-monophosphates. These cyclic nucleotides are intracellular second messengers in a variety of endocrine and neural cells. In concert with changes in the rate of cyclic nucleotide synthesis, modulation of cyclic nucleotide degradation by PDEs regulates the intracellular concentration of these second messengers and controls the activity of these cells. Multiple different PDEs are simultaneously expressed and differentially regulated in mammalian tissues and single cell types (1, 2). However, the physiological significance of this complexity in PDE expression is unknown.

The mammalian PDEs are classified by their biochemical properties into five families (1, 3, 4). Calmodulin-dependent PDEs (CaM-PDEs, family I) are stimulated by a calcium-calmodulin complex. The cGMP-stimulated PDEs (cGS-PDEs, family II) are allosterically activated by cGMP. The cGMP-inhibited PDEs (cGI-PDEs, family III) are inhibited by cGMP and cardiac inotropic drugs such as milrinone. The cAMP-specific PDEs (cAMP-PDEs, family IV) have high affinity for cAMP alone and are inhibited by RO 20–1724 and the antidepressant, rolipram. The cGMP-specific PDEs (cGMP-PDEs, family V) have high affinity for cGMP alone. Each family has multiple members, but their relatively low abundance, similar kinetic properties, and extreme sensitivity to proteolysis have complicated their characterization and, even, their enumeration. Recently, molecular techniques have aided these studies but have revealed new levels of complexity. Some members of the CaM-PDE and cGS-PDE families have had full or partial amino acid microsequences determined (5–8). Some members of the cAMP-PDE, cGMP-PDE, and, recently, the cGS-PDE families have had their cDNAs molecularly cloned (9–19). Northern blots have demonstrated the simultaneous presence of mRNA for multiple, nearly identical isozymes of PDEs in many tissues (12). These isozymes are encoded by distinct genes in some cases and alternate splicing from a single gene in other cases (12, 13). Neither amino acid nor nucleic acid sequences have yet been reported for any member of the cGI-PDE family, and no nucleic acid sequences

1 The abbreviations used are: PDE, cyclic nucleotide phosphodiesterase; kb, kilobase; bp, base pairs; CaM, calmodulin; IPTG, isopropyl-ß-D-thiogalactopyranoside; PCR, polymerase chain reaction; cAMP-PDE, cAMP-specific PDE; cGMP-PDE, calmodulin-dependent PDE; cGMP-PDE, cGMP-specific PDE; cGI-PDE, cGMP-inhibited PDE; cGS-PDE, cGMP-stimulated PDE.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) M94537 (rat CaM-PDE), M94538 (mouse CaM-PDE), M94539 (human CaM-PDE), M94540 (rat cGS-PDE), and M94541 (mouse cAMP-PDE).

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have been reported for members of the CaM-PDE families.

Three isozymes of the family 1 CaM-PDEs have been isolated from the brain (20). The predominant brain isozyme (61 kDa), which is also expressed in small amounts in many other tissues, has been shown to be an alternate splice product of the gene which encodes the predominant, 59-kDa heart isozyme (6, 21). A unique gene encodes a 63-kDa CaM-PDE that has been detected only in brain and phytomemagglutinin-stimulated, but not quiescent, T-lymphocytes (20, 22). A 75-kDa brain isozyme has not been fully characterized (20).

To enable investigation of the role of multiple PDEs in regulation of intracellular cyclic nucleotide concentrations, we attempted to characterize the full range of PDEs present in cells and tissues by a PCR-based molecular cloning technique. Comparison of the known or deduced amino acid sequences for seven PDEs from four mammalian PDE families plus the Drosophila dunce PDE (11) and the high affinity Saccharomyces PDE (23) reveals a central conserved domain (5, 12) of approximately 250 amino acids that is likely to contain the catalytic site (7, 24). Within this domain there are several smaller regions (up to 7 amino acids) of stringently conserved sequence. We designed degenerate PCR primers that hybridize with the nucleic acid coding sequences for these regions and used these primers to identify and clone PDEs from several mammalian tissues. One novel rat cDNA obtained by this approach encodes a CaM-dependent PDE that is expressed in brain and in a T-lymphocyte cell line. Evidence is presented that this cDNA encodes the rat 63-kDa CaM-PDE isozyme. Bentley et al. (25) in the companion article report the isolation of a homologous cDNA encoding the bovine brain 63-kDa CaM-PDE.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—The S49.1 mouse lymphoma cell line was obtained from the American Type Culture Collection (ATCC No. TIB 28). The S49.1 subline, K30a-3.3, which has increased cGI-PDE activity relative to the S49.1 subline, K30a-3.3, which has increased cGI-PDE activity compared with the wild type (26), was a generous gift of H. R. Kaslow (University of Southern California, Los Angeles). The cells were grown in suspension in Dulbecco's modified Eagle's medium-H (750 mg/ml Tris (pH 8.3), 50 mM KCl, 2 mM MgCl2, 0.14 mM each ultrapure dNTP (Pharmacia LKB Biotechnology Inc.), 2 mM dithiothreitol, 100 μg/ml acetylated bovine serum albumin, and primers PDE-5' and PDE-3', at final concentrations of 4 and 10 μM, respectively. The mixture was heated to 94 °C for 3 min before addition of five units of AmpliTaq DNA Polymerase (Perkin-Elmer Cetus) and cycling 40 times at 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min. Human hypothalamic PDE cDNAs were PCR-amplified directly from a λgt11 cDNA library.

After digestion with SauI and EcoRI and electrophoresis in 3% NuSieve Agarose (FMC Bioproducts, Rockland, ME), the PCR fragments of approximately 350 bp were isolated and ligated into SauI- and EcoRI-digested M13 mp18 phage DNA (GIBCO). One-hundred clones were screened by performing dyeoxy-GTP chain termination sequencing reactions (30) and grouping the clones according to the pattern of resulting "G-ladder" bands on a denaturing sequencing gel. From each group a limited number of clones was selected for complete sequencing.

**RNA Analysis—**Poly(A)+ RNA prepared from tissues or cultured cells was glyoxylated and fractionated by electrophoresis through a 10% agarose gel and transferred to a Biotrans nylon membrane (ICN, Costa Mesa, CA) (31). DNA probes were generated by PCR using the primers from within the PDE sequence plus flanking vector primers. DNA from the degenerate PDE primers, PDE-5', and PDE-3', and an M13 mp18 template containing a cloned PDE cDNA fragment. Probes were labeled by random priming (Boehringer Mannheim) in the presence of [32P]dCTP, and hybridization was performed in 50% formamide at 42 °C with washing in 0.1 x SSC at 65 °C.

**Isolation of Full-length PDE Clone**—Two λgt11 libraries were screened. The Crb series of clones were isolated from a commercial library (Clontech RL1002R, Palo Alto, CA) of oligo(lT)-random primed cDNA from entire adult Sprague-Dawley rat brain. The Arb series were isolated from a library (generous gift of J. Arriza, Duke University) constructed of oligo(dT)-primed cDNA from the entire brain of 6-week-old Sprague-Dawley rats. These libraries were screened using a PCR-generated PDE cDNA probe. Clones obtained were plaque purified and subcloned into the EcoRI site of the plasmid pBS+ (Stratagene, La Jolla, CA). These double-stranded plasmids were completely sequenced in both directions using a ladder of degenerate PDE primers from within the PDE sequence plus flanking vector primers.

**Bacterial Expression of PDE Clone**—PDE cDNA from Arb 5, a λgt11 clone, was subcloned into the bacterial expression vector pET3b (32). The vector was digested with NdeI and BamHI, dephosphorylated, and ligated into the BamHI site of the plasmid pBS+ (Stratagene, La Jolla, CA). These double-stranded plasmids were completely sequenced in both directions using a ladder of degenerate PDE primers from within the PDE sequence plus flanking vector primers.
added to the 3' terminus of the open reading frame of the PDE cDNA encoded in Arb 5. This PCR fragment was ligated into the prepared vector. MAX Efficiency DH5αF’IQ competent Escherichia coli (GIBCO) were transformed and selected by growth in ampicillin-containing medium. Plasmid isolates with the insert of the appropriate size plus to vector without a cDNA insert, were used to transform E. coli BL21(DE3)pLysS, the expression host. Twenty-mI cultures were grown to mid-log phase, and isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 0.4 mM to initiate transcription of the cDNA insert. 2.5 h later, aliquots were harvested by centrifugation according to the method of Thompson and Appleman (33) using 1 µM [3H]cGMP and the extract from 4.5 × 10⁵ cells. Assays performed in the presence of calmodulin contained 500 ng/ml calmodulin (Sigma) and 2 mM CaCl₂.

RESULTS

Design and Use of Degenerate PCR Primers to Amplify PDE cDNAs—To isolate PDE cDNAs, PCR was performed using template cDNA from several mammalian sources with the degenerate PCR primers, PDE-5' and PDE-3'. These primers were designed to hybridize to two highly conserved regions (approximately 350 bp apart) in PDE cDNAs. PCR products of 350 bp length were recovered, subcloned into M13 mp18, screened by examination of G-ladder band patterns, and then each distinct clone type was fully sequenced. Using rat liver template, five different clones were retrieved. Four of these are identical to the cDNA of the four rat cAMP-PDEs (ratPDE1, ratPDE2, ratPDE3, and ratPDE4) that were previously cloned from rat testes (12). The fifth clone likely encodes a novel rat cGSPDE as its deduced amino acid sequence is 99% identical to the corresponding portion of a bovine cGSPDE (5). Characterization of this cDNA will be the subject of a subsequent publication.

From K30a-3.3 mouse lymphoma cell cDNA, two distinct clones were isolated. One is likely to encode the mouse homolog of the rat cAMP-PDE, ratPDE3, as it has 94% nucleotide sequence identity (and 99% deduced amino acid identity) with the corresponding portion of the cDNA for ratPDE3 (12) (data not shown). The other K30a-3.3 mouse clone, named K-17, was found to encode an amino acid sequence 70% identical with the corresponding amino acid sequence of the catalytic domain of the bovine 61-kDa CaM-PDE of Charbonneau et al. (7). The amino acid sequence of this portion of the 63-kDa CaM-PDE of Bentley et al. (25) is identical to that of the mouse CaM. An asterisk indicates an amino acid identical to that of the mouse CaM. Lines above and below the sequences indicate the region of the PCR primers used to clone the PDEs and were excluded from numerical comparisons of the sequences presented in the text.

From a human hypothalamus cDNA library, three distinct clones were isolated. One, named h337, encodes 95% amino acid (Fig. 2) and has 91% nucleotide sequence identity with the mouse K-17 clone and probably represents the human homolog of the 63-kDa CaM-PDE. A second clone has high nucleotide sequence identity with the rat cGSPDE clone described above and thus is likely to represent a human cGSPDE (data not shown). The third clone isolated was found to have 91% nucleotide sequence identity with a rat cAMP-PDE, ratPDE2 (data not shown) and probably represents the human clone corresponding to this rat PDE.

Distribution of RNA Transcripts of the K-17 PDE in Rat Tissues and S49.1 Cell Line—The tissue distribution of expression of mRNA for the K-17 PDE was investigated by Northern blot analysis. Poly(A)⁺ RNAs from rat brain, heart, liver, kidney, and testis were hybridized with a radiolabeled mouse K-17 probe (Fig. 3). A transcript of approximately 3.0 kb was detected in the rat brain, but no transcript was detected in any of the other tissues. Poly(A)⁺ RNA was also isolated from S49.1 cells, a mouse lymphoma cell line, with T-lymphocyte characteristics (34, 35), and from K30a-3.3 cells, which are derived from the S49.1 line (26). These poly(A)⁺ RNAs hybridized with the K-17 probe producing a strong signal at about 7.0 kb and weaker signals at 4.4 and 12 kb (Fig. 3). In contrast to rat cAMP-PDE transcripts, which have wide tissue distribution (12, 13), and the rat cGSPDE transcripts, which are detected in all rat tissues examined (brain, heart, liver, kidney, and testis, data not shown), K-17 transcripts are relatively restricted in their tissue distribution, here demonstrated to be expressed only in brain and the T-lymphoma cell line.

A Full-length Rat cDNA Clone Encoding a Polypeptide with Sequence Homologous to the Bovine CaM-PDEs—Because K-17 transcripts were found to be abundant in rat brain, the K-17 probe was used to screen two different rat brain cDNA...
libraries constructed in λgt11. Screening of the Arriza library yielded one clone, Arb 5, that includes an insert of 3.0 kb with an open reading frame of 2181 bp commencing with an ATG within a Kozak consensus sequence and preceded by an in-frame stop codon (Fig. 4). Five additional clones, Arb 1–5, each greater than 1.2 kb in length, were subsets of Arb 5. Screening of the Clontech library yielded six clones, each under 900 bp in length. Five of these clones, Crb 1–5, contain overlapping portions of a sequence identical to Arb 5. The sixth clone, Crb 6, has a 3’ portion with sequence identical to Arb 5 but has a completely divergent 5’ sequence. This clone may represent an alternate splice product and will be the subject of a subsequent report. There is 95% nucleotide sequence identity between the mouse K-17 probe and the corresponding portion of the rat Arb 5 clone, and they encode only one amino acid difference (Fig. 2).

The nucleic acid sequence and deduced amino acid sequence of the coding portion of Arb 5 is presented in Fig. 4. Comparison of these rat nucleic acid sequences with those recently reported for the bovine 63-kDa CaM-PDE (25) reveals 97% amino acid and 89% nucleic acid sequence identity, and thus this clone likely encodes a rat 63-kDa CaM-PDE. Comparison of the deduced amino acid sequence of the Arb 5 clone with the amino acid sequence of the bovine 61-kDa CaM-PDE (7) reveals considerable homology but only 57% overall identity.

Expression of the Arb 5 Clone in Bacteria Demonstrates a CaM-stimulated PDE Activity—The DNA fragment consisting of the open reading frame of the Arb 5 clone with a NdeI restriction site at the 5’ terminus and a BamHI restriction site at the 3’ end was produced by PCR using the Arb 5 clone as template. This fragment was subcloned into the expression vector pET3b (32), and two isolates (9 and 16) were transformed into E. coli BL21(DE3)pLysS. This vector/host system has an extremely low level of transcription of the cloned insert prior to induction by IPTG. Host cells containing pET3b with and without the PDE sequence insert were incubated with IPTG for 2.5 h and then lysed. The extract was assayed for PDE activity in the absence and presence of calmodulin and calcium. cGMP hydrolysis was assayed as the host bacteria had an active endogenous cAMP-specific PDE activity that created a high background when cAMP hydrolysis was assayed (data not shown). Extract from host carrying only the pET3b vector without insert had low baseline cGMP PDE hydrolytic activity that did not increase in the presence of calcium and calmodulin (Table I). PDE hydrolytic activity in extract from host expressing pET3b clones 9 and 16 was increased 5-fold over base line in the presence of calcium and calmodulin. This demonstrates that the Arb 5 clone encodes a CaM-PDE. The molecular radius of the expressed protein was estimated by its migration in polyacrylamide gel electrophoresis. Induction of expression of the CaM-PDE by IPTG led to appearance of a 63-kDa protein band detectable both by Coomassie Brilliant Blue staining and by [35S]methionine incorporation that was not present in noninduced host extract (data not shown).

DISCUSSION

In the past several years, the existence of a remarkable heterogeneity of mammalian PDEs has become evident. The role of multiple PDEs in an organism, a tissue, and even a single cell is not yet understood. It has been postulated that there may be differential regulation of transcription and activity, and differential localization of these various PDEs (4). Many PDE isozymes have now been characterized biochemically, but the low abundance, labile activity, and biochemical similarity of many of the isozymes has limited the progress possible by strictly biochemical approaches. We report here a method using degenerate PCR primers to identify some of the different PDE forms expressed at a given time in a tissue of interest.

From rat liver, we have retrieved clones which correspond to each of the four members of the CaM-PDE family previously isolated from rat testis (12, 13). This same preparation of rat liver poly(A)+ RNA in Northern blots revealed only the presence of transcripts of ratPDE3 and ratPDE4 (12, 13). A novel cG5-PDE clone was also isolated from rat liver cDNA. Two PDE clones were isolated from the mouse K30a T-lymphoma cell line. A single cAMP-PDE clone, corresponding to ratPDE2, was found along with a novel clone that encodes a peptide identical to a portion of the bovine 63-kDa CaM-PDE. These clones demonstrate, at least at the mRNA level, that both a cAMP-PDE and a CaM-PDE are expressed simultaneously in these T-lymphocyte-derived cells. There have been conflicting reports of the presence of one or multiple PDE isozymes in lymphoid cells (22, 36), and no CaM-PDE activity has been detected in the K30a cell line (25). Perhaps this enzyme is expressed under these cell culture conditions.

**TABLE I**

| Expression vector | cGMP phosphodiesterase activity* |
|-------------------|----------------------------------|
| pET3b vector alone | - Calmodulin: 0.08  + Calmodulin: 0.02 |
| pET3b/Arb 5 9     | 0.42                             |
| pET3b/Arb 5 16    | 0.43                             |

*Expressed as pmol of cGMP hydrolyzed/min using lysate from 4.5 × 10⁷ cells with 1 μM cGMP, and 500 ng/ml calmodulin and 2 mM CaCl₂, where indicated.

FIG. 4. Nucleotide sequence and predicted amino acid sequence of the rat 63-kDa CaM-PDE clone Arb 5. Stop codons upstream of the first ATG codon and at the end of the coding sequence are marked with asterisks. The nucleotide and amino acid residues are numbered on the right.
conditions, but requires a biochemical modification before it is enzymatically active. From the human hypothalamus, clones corresponding to a cGMP-PDE and the rat cAMP-PDE, rat-PDE2, were amplified. This latter human clone is identical to a portion of the cAMP-PDE clone previously isolated from a human monocyte library (16). The human hypothalamus was also found to have a PDE transcript with sequence very similar to the K-17 sequence suggesting that a 65-kDa CaM-PDE is present in human brain.

All of the clones isolated to date are from three of the five families of PDEs. The cGMP-PDE family members have been found to be present only in photoreceptors, lung, and platelets (38, 39) and thus might not occur in the tissues we have examined. However, members of the cG-PDE family are known to be expressed both in rat liver (40) and in K30a cells (26). Possibly, the cG-PDEs do not share homology with the other PDE families in the regions from which the PCR primers were designed. Alternatively, the cG-PDEs might share these regions of homology, but the PCR primers are not sufficiently degenerate, especially at the critical 3' ends, to allow amplification of the cG-PDEs. The cG-PDEs are extremely low abundance isozymes, and it is also possible that these PCR primers do amplify the cDNA for these PDEs but that more amplified DNA fragments would need to be screened before one for this PDE would be isolated.

The mouse K-17 clone was used as a probe to screen a rat brain library to isolate a corresponding full-length clone, Arb 5. We believe that our Arb 5 clone represents the rat 63-kDa CaM-PDE because: 1) the clone encodes a protein of actual molecular weight 61,325 that has CaM-PDE enzymatic activity when expressed in bacteria, 2) this cDNA is abundant in a mitogenically active mouse lymphocyte cell line and in rat brain, which matches the tissue distribution of the 63-kDa CaM-PDE, and 3) this clone encodes a protein that has 97% amino acid identity (517 out of 535) with the bovine 63-kDa CaM-PDE that is described in the accompanying report (25).

The 63-kDa CaM-PDE has 1 additional amino acid compared with the bovine enzyme (Arg-Arg-Ala at residues 113-115 compared with Pro-Ser). Both species encode their message on a 3-4 kb mRNA.

Several deductions can be made about the biochemical properties of the 63-kDa CaM-PDE based on the predicted amino acid sequence. This protein does share 57% overall amino acid identity with the bovine 61-kDa CaM-PDE with over 70% identity in the putative catalytic domain between the PCR primers, PDE-5' and PDE-3', demonstrating that these proteins are closely related. The rat 63-kDa CaM-PDE has a NH2-terminal domain encompassing amino acids 27-48 which is only 54% identical to the well-characterized calmodulin binding domain of the 61-kDa CaM-PDE (7). However, these rat 63-kDa CaM-PDE amino acid residues are also capable of forming a basic amphipathic a helix, a hallmark of CaM-binding domains, and thus the PDE appears to have a unique CaM-binding domain at this site. The bovine 59- and 61-kDa CaM-PDEs differ only in their CaM-binding domains, and this difference is therefore postulated to confer their differential sensitivity to activation by calcium and calmodulin (21). The distinct CaM-binding domain of the rat 63-kDa CaM-PDE, which is identical to that of the bovine 63-kDa isozyme (25), probably gives these isozymes a unique sensitivity to calcium and calmodulin. The carboxyl termini (residues 437-535 of the rat 63-kDa protein and residues 428-525 of the bovine 61-kDa protein) are completely different, reminiscent of the structure of the members of the cAMP-PDE family that are highly homologous but have divergent sequence at the carboxyl terminus. Possibly, these regions are involved in regulation of PDE activity by phosphorylation. Biochemical evidence suggests that the bovine 63-kDa CaM-PDE is regulated by phosphorylation with CaM-dependent protein kinase II and that the 61-kDa CaM-PDE is regulated by phosphorylation by protein kinase A (20). The deduced amino acid sequence of our clone contains three Arg-X-Ser/ThrCaM-dependent protein kinase II phosphorylation consensus sequences within the carboxyl-terminal 125 amino acids (residues 414, 465, and 501) and a fourth site in the NH2-terminal domain at amino acid residue 143. There are also three casein kinase II consensus phosphorylation sites in the COOH terminus (residues 447, 508, 513, and 522) as well as in the NH2 terminus at residue 80. There is also a possible protein kinase A phosphorylation site at residue 141. However, there is no evidence that casein kinase II phosphorylates this enzyme and there is evidence against protein kinase A regulation of the 63-kDa enzyme (20).

The CaM-PDEs are allosterically activated by binding of a calcium-CaM complex. This complex appears to activate these isozymes by functionally displacing an inhibitory domain removal of the NH2-terminal domain by limited proteolysis yields a constitutively activated, CaM-independent peptide (37). The CaM-PDEs are highly susceptible to proteolysis during purification, and thus most preparations have significant CaM-independent PDE activity. This CaM-independent background activity is the likely explanation for our expression studies that show significant PDE activity before addition of calcium and CaM and only a 2-3-fold activation after their addition.

The PCR-based approach to cloning PDEs that we have described is a general method for cloning PDEs of diverse type. This method should facilitate the currently difficult approach to defining the range of PDE isozymes that are expressed in a single tissue or cell type. With the sensitivity of PCR in amplifying minute quantities of cDNA, it should even be possible to evaluate the PDEs present in a single cell. A general method for cloning mammalian PDEs is also valuable for developing PDE probes across species lines to facilitate the cloning and study of homologous PDEs from different species. Finally, molecular cloning of additional mammalian PDEs will help define the variety of isozymes present within each PDE family and will provide valuable tools for investigating the roles of these isozymes in regulation of intracellular concentrations of the cyclic nucleotides.

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