Molecular Cloning of a cDNA Encoding the "61-kDa" Calmodulin-stimulated Cyclic Nucleotide Phosphodiesterase

TISSUE-SPECIFIC EXPRESSION OF STRUCTURALLY RELATED ISOFORMS*

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We have isolated a 2287-bp cDNA encoding the 61-kDa calmodulin-stimulated cyclic nucleotide phosphodiesterase (CaM PDE) from a bovine brain library. A large open reading frame within the cDNA encodes a 530-residue polypeptide which is identical to the sequence of the purified protein previously determined by direct amino acid sequencing. Moreover, COS cells transfected with the cDNA express a cAMP and cGMP hydrolytic activity that is stimulated by calcium and calmodulin, confirming that the cDNA represents a mRNA species encoding a CaM PDE isozyme.

RNase protection analyses indicate that either 61-kDa CaM PDE mRNA or structurally related transcripts encoding different CaM PDE isoforms are expressed in a tissue-specific manner. Total RNA isolated from brain (cerebral cortex, basal ganglia, hippocampus, cerebellum, and medulla/spinal cord), heart, aorta, liver, kidney outer medulla, kidney papilla, trachea, and lung completely protected a 410-base antisense riboprobe corresponding to sequence encoding a portion of the catalytic domain. Little or no protection was detected using adrenal cortex, adrenal medulla, liver, kidney cortex, spleen, or T-lymphocyte total RNA. Only brain RNA completely protected a 240-base antisense riboprobe corresponding to the 61-kDa CaM PDE amino terminus encompassing a putative calmodulin-binding domain. However, heart, aorta, liver, kidney, trachea, and lung RNA protected 150 bases of this riboprobe suggesting that these tissues express an isoform structurally related to the 61-kDa CaM PDE.

Northern analysis of mRNA isolated from brain, heart, aorta, liver, kidney, lung, and trachea revealed that the cDNA hybridizes with a 3.8- and a 4.4-kb (kilobase) mRNA species. Interestingly, Northern blots of bovine cerebral cortex and heart mRNA probed under stringent conditions with antisense transcripts corresponding to either the 5' or 3'-untranslated sequence of the 61-kDa CaM PDE cDNA hybridized with only the 4.4-kb mRNA from both tissues. Since different, yet structurally similar CaM PDE isoforms are expressed in brain and in heart, this result, in addition to the RNase protection data, is consistent with the idea that the mRNAs encoding these two CaM PDE isoforms are products of an alternately spliced gene.

The calmodulin-stimulated cyclic nucleotide phosphodiesterases (CaM PDEs) constitute a growing family of structurally and kinetically distinct isoforms that catalyze the hydrolysis of cyclic nucleotides (1, 2). Presently, six CaM PDE isoforms have been identified (2). All of these isoforms appear to be composed of two identical subunits, possessing a catalytic domain, and regions involved in regulation of activity. In the presence of calcium, CaM binds to these subunits and increases hydrolytic activity several-fold. These enzymes differ, however, in their kinetic properties, subunit mobility on denaturing polyacrylamide gels, substrate specificity, and sensitivity to CaM-activation.

The complete amino acid sequence of the 61- and 63-kDa CaM PDE isoforms have been determined by directly sequencing the purified protein (3) or by deducing the sequence from the cDNA clone (4). A comparison of these sequences suggest that they are products of different albeit homologous genes. Partial amino acid sequence of the 59-kDa CaM PDE purified from bovine heart is nearly identical to the 61-kDa isoform except for the amino-terminal residues, implying that these two isoforms are products of an alternately spliced gene (5).

The location of two functionally distinct domains have been predicted by comparing the primary structure of the 61-kDa CaM PDE with other mammalian PDE sequences (3, 6), and have been experimentally supported using limited proteolysis techniques and synthetic peptides (3, 5, 7). An approximately 250-residue region located near the carboxyl terminus and conserved among mammalian PDE sequences is completely contained within a 36-kDa tryptic peptide fragment of the 61-kDa CaM PDE (3). The peptide is catalytically active and insensitive to CaM activation, supporting the idea that the conserved region defines a catalytic domain. In addition, a synthetic peptide corresponding to 15 residues of the 61-kDa CaM PDE amino terminus with a structural motif believed to define a CaM-binding region competitively inhibits CaM activation of the purified enzyme. Similar results have been observed for the 63- and 59-kDa isoforms (5). These data indicate that the amino terminus of the 61-kDa CaM PDE defines at least a portion of the CaM-binding domain.

Several studies imply that CaM PDEs may be activated by hormones in intact tissues and cells. For example, agonists that trigger increases in intracellular calcium in human astrocytoma cells (8), rat cardiomyocytes (9), thyroid tissue (10), and certain neurons (11, 12) blunt hormone-induced cyclic
nucleotide accumulation. This response does not appear to involve inhibition of adenyl cyclase or guanylyl cyclase, and in some cases, pretreatment with phosphodiesterase inhibitors blocks this inhibitory effect. In addition, CaM PDE activation has been observed, or at least suggested, in smooth muscle treated with various vasconstricting agents (13, 14). These examples further suggest that CaM PDEs may be an important component of calcium-dependent signal transduction pathways that modulate physiological responses mediated by cyclic nucleotides. It is of considerable interest, therefore, to determine the tissues or cell types that express CaM PDE isoforms.

The brain is a rich source of several CaM PDEs, including the 61-, 63-, and 75-kDa isoforms (15-17). The cellular and subcellular distribution of CaM PDEs have been determined histochemically (18) and immunohistochemically using antisera raised against a 61-kDa CaM PDE preparation (19, 20). These studies indicate that CaM PDEs are expressed in subsets of neurons in several different brain regions, including the cerebral cortex, hippocampus, caudate putamen, and cerebellum. However, the precise distribution of the different brain isoforms has not been determined using these techniques.

Recently, expression of mRNA encoding the 63-kDa CaM PDE has been determined using the isozyme-specific RNase protection assay (4). The transcript encoding this isoform is expressed in several different brain regions, but appears to be most concentrated in the basal ganglia. This study also suggests that a structurally related isoform is expressed in kidney papilla, adrenal medulla, and testis.

Other CaM PDE activities have been purified or at least detected in a number of different tissues such as heart (21), vascular smooth muscle (22-24), lung (25), T-lymphocytes (26), and testis (27). With the exception of the 63-kDa CaM PDE, the distribution and relative abundance of these different CaM PDE isoforms have not been determined. Here we present the nucleotide sequence of a cDNA representing one of possibly two bovine brain mRNAs encoding the 61-kDa CaM PDE. Using this cDNA, we examined the expression of the 61-kDa CaM PDE and structurally related isoforms among RNA isolated from a variety of bovine tissues. The potential involvement of CaM PDEs in physiological processes regulated by calcium-dependent signal transduction pathways is also discussed.

**Experimental Procedures**

**Materials**

[a-32P]dTCTP (3000 Ci/mmole), [a-32P]UTP (3000 Ci/mmole), [a-32S]dATP (1200 Ci/mmole), and NEN Sorb-20 nucleic acid purification cartridges were purchased from Du Pont-New England Nuclear. [2,2-3H]cAMP and [2,2-3H]cGMP were purchased from ICN Biochemicals. The poly(A) Quik (M, 1) mRNA purification kit and protocol based on established methods (29). Total RNA was poly(A)-selected using the poly(A) Quik purification kit and protocol based on established methods (29).

**Methods**

**RNA Isolation**—Total RNA was extracted from bovine tissues using the method of Chomczynski and Sacchi (28). In preparing total RNA from hippocampus, basal ganglia, and adrenal gland, tissue isolated from several different animals was pooled and processed. The yield of total RNA was consistently 1 mg/10 g of tissue. The RNA quality was assessed spectrophotometrically and by electrophoresing 10 μg of total RNA on a 1% agarose/6.7% formaldehyde gel. We stained the gel with ethidium bromide and visually inspected the staining intensity of the ribosomal RNA using established methods (29). The 28 S and 18 S ribosomal RNA bands from all of the tissues surveyed displayed an approximate two-to-one ratio of staining intensity, respectively, indicating that these preparations were not degraded (29). Additionally, the absorbance ratio of the RNA samples at 260 and 280 nm, and at 260 and 230 nm, exceeded a value of 1.8 indicating that the RNA was free of protein and phenol contamination, respectively (29).

**DNA Amplification**—A partial cDNA Encoding the 61-kDa CaM PDE—Degenerate sense and antisense oligonucleotides (23 bases) corresponding to the amino acid sequences Lyso-Met-Gly-Met-Lys-Lys-Lys-Lys and Met-Gln-Glu-Glu-Glu-Met-Aasn-Val, respectively, were synthesized using an Applied Biosystems DNA synthesizer (Model 381A). Oligonucleotides were phosphorylated using T4 polynucleotide kinase (29) and purified using NEN Sorb-20 columns according to the manufacturer's protocol. These oligomers, in combination with Taq DNA polymerase, were subsequently used for amplification of a partial cDNA encoding a portion of the 61-kDa CaM PDE in a manner similar to previously established methods (30). First strand cDNA was synthesized as described previously (31) using poly(A)-selected RNA isolated from bovine brain and avian myeloblastosis virus infected tissue transcription. Amplification was performed using a 94 °C for 1 min 94 °C for 1 min, 94 °C for 1 min, and 72 °C for 1 min. Using 25 μl of the resulting DNA product as a template, we used 30 consecutive DNA polymerization cycles (1 cycle = 94 °C for 1 min, followed by 52 °C for 1 min and then, 72 °C for 1 min) using a Hybaid thermal reactor (National Labnet Ltd.). The resulting DNA products were purified on a 1% low melting point agarose gel, and DNA bands of interest were excised with a razor blade and purified using the Geneclean kit (29).

**Bovine Brain cDNA Library Screening**—Approximately 600,000 plaque-forming units (50,000 plaque-forming units on 12-150-mm NZY-agar plates) were screened as described previously (31) using the [32P]-labeled partial cDNA, pCAMPDE-1. Putative clones were plaque-purified and subcloned by in vivo excision using R408 M13 helper phage (32).

**Sequencing**—Templates for sequencing putative 61-kDa CaM PDE cDNAs were prepared by constructing a series of nested deletions as described previously (31). These templates were primed with oligonucleotides complementary to vector sequence flanking the insert DNA. In some cases, chemically synthesized oligonucleotides complementary to the insert cDNA were used to prime the template to attain overlapping sequences. Sequencing ladders were generated using [a-32P]dATP and either Sequenase or TaqTrack sequencing kits and
protocals adapted from the method of Sanger (33). Comparisons of the nucleotide sequence and the deduced amino acid sequence of the large open reading frame of the pCAMPDE-40 cDNA were performed using the FASTA and TFASTA programs provided with the Genetics Computer Group sequence analysis software package, version 7.0 (34).

**Synthesis of Radiolabeled Nucleic Acid Probes**—32P-Labeled DNA probes were synthesized according to the method of Feinberg and Vogelstein (35) using heat-denatured restriction endonuclease fragments of the pCAMPDE-40 cDNA printed with random hexamers and extended with Klenow fragment in the presence of [α-32P]dCTP. The 32P-labeled DNA products were purified from unincorporated [α-32P]dCTP with EtBr-stained agarose gel. The nucleotide sequence and deduced amino acid sequence of the large open reading frame of the pCAMPDE-40 cDNA were performed using an RNA transcription kit and protocol (Stratagene) based on established methods (29). The reaction mixture contained 40 mM Tris-HCl (pH 8.0), 4 mM MgCl2, 1 mM spermidine, 25 mM NaCl, 1 μg of template DNA, 0.4 mM each of ATP, GTP, and CTP, 1 μCi of 32P-labeled UTP (3000 Ci/mmol), 50 mM dithiothreitol, and 100 U of RNasin, and 10 units of either T3 or T7 RNA polymerase. The reaction mixture was supplemented with 4 μM UTP when preparing riboprobes for RNase protection assays. After a 30-min incubation at 37 °C, the DNA template was removed by digestion with RNase-free DNase I, and the reaction mixture was phenol/chloroform-extracted, ethanol-precipitated, and redissolved in 2 μg/ml RNase-free water (Stratagene). Typically, 30–50% incorporation was achieved using these reaction conditions.

**RNase Protection Assays**—RNase protection assays were carried out as described previously (31). Specifically, 20 μg of ethanol-precipitated total RNA was resuspended in a hybridization buffer containing 80% formamide, 40 mM PIPES buffer (pH 6.4), 0.4 M NaCl, 1 mM EDTA, and 32P-labeled antisense riboprobe (50,000 cpm/μl). The samples were heated at 85 °C for 5 min and then immediately transferred to a 45 °C water bath and incubated overnight. Following the hybridization, each sample was digested with 700 μl of an RNase mixture (10 mM Tris-HCl (pH 7.5), 500 mM NaCl, 5 mM EDTA, and 2 μg/ml RNase T1) at 30 °C for 60 min. The digestion was stopped by adding SDS (0.5%) and proteinase K (130 μg/ml) and incubating at 37 °C for 15 min. Each sample was phenol/chloroform-extracted and ethanol-precipitated after adding 10 μg of yeast tRNA to serve as a carrier. The RNA samples were resuspended in loading buffer (40% formamide, 1 mM EDTA (pH 8.0), 0.1% bromophenol blue, and 0.1% xylene cyanol), heated at 85 °C for 3 min, and electrophoresed on a 6% polyacrylamide, 7 M urea gel (29). The gels were dried and exposed to x-ray film for 16–24 h at −70 °C. The size of the protected riboprobe fragments was determined by running a sequencing ladder (pBluescript SK−) primed with either T3 or T7 promoter primers) adjacent to the samples. The data presented (see Fig. 4, B and C) is a representative sample of three different assays using RNA prepared from two or more isolations.

**Quantification of CaM PDE mRNA**—The relative abundance of CaM PDE mRNA among the different bovine tissues was quantitated by the autoradiographic paradigm described above, we amplified a single 612-bp cDNA

**RESULTS**

Cloning, Sequence, and Characterization of cDNAs Encoding the 61-kDa CaM PDE—Using the polymerase chain reaction paradigm described above, we amplified a single 612-bp cDNA
(designated pCAMPDE-1) from bovine brain first strand cDNA (Fig. 1). We found that the amino acid sequence deduced from the nucleotide sequence of this cDNA was identical to the amino acid sequence of the purified protein (3) in the region targeted for amplification. This cDNA was subsequently used as a probe to screen a bovine brain cDNA library.

Of the 42 putative clones from the initial library screening, one clone representing the entire coding sequence of the 61-kDa CaM PDE was identified. The nucleotide sequence and predicted amino acid sequence of the large open reading frame of this cDNA (designated pCAMPDE-40) is shown in Fig. 2. A methionine codon with flanking sequence typical of translational initiation sites (38) is located at position +1 (Fig. 2). Since it is immediately preceded by an in-frame stop codon (position -45), this codon seems to be the most likely translational initiation site. The large open reading frame of pCAMPDE-40 encodes a 530-residue polypeptide (calculated molecular mass 60,843 daltons) that is identical to the amino acid sequence of the 61-kDa CaM PDE determined by directly sequencing the purified enzyme (3). Several data bases (GenBank, release 70.0; GenPept, release 69.0; EMBL, release 29.0; and SwissProt, release 20.0) were searched for sequences similar to the nucleotide and predicted amino acid sequence of the 61-kDa CaM PDE (4), only PDE sequences were found to be homologous to the Drosophila dunce PDE, were significantly more similar (41%) to the 61-kDa CaM PDE than any other PDE sequences.

In order to determine if the pCAMPDE-40 cDNA encodes a functional PDE, we transfected COS cells with the cDNA using the expression vector pCDM8 and assayed cell extracts for CaM-stimulated PDE activity (Fig. 3). When compared to mock transfected cells, extracts of COS cells transfected with the pCAMPDE-40 cDNA contained significantly greater cAMP and cGMP hydrolytic activities in the presence of EGTA. Assay of pCAMPDE-40-transfected COS cell extracts in the presence of calcium and CaM resulted in a 5–10-fold stimulation of cAMP and cGMP hydrolysis. Little CaM-stimulated cyclic nucleotide hydrolysis was detected in the mock transfected cells.

Identification and Partial Characterization of Two Distinct 61-kDa CaM PDE mRNAs—Poly(A)-selected RNA from bovine tissues that contained CaM PDE transcript as determined by RNA protection assays were subjected to Northern blot analysis. A 32P-labeled riboprobe (Fig. 4A) from autoradiograms like those shown in Fig. 4, B and C. We assume that the percentage of mRNA in total RNA is similar among the tissues surveyed in this study. The 61-kDa CaM PDE transcript is most abundant in total RNA isolated from brain. However, the amount of a transcript structurally related to the 61-kDa CaM PDE were relatively high in RNA isolated from heart and moderately abundant in RNA isolated from aorta, kidney medulla, and kidney papilla. This transcript is also moderately abundant in trachea and lung RNA. Little CaM PDE mRNA was detected in liver and kidney cortex RNA, and no transcript was detected in adrenal cortex, adrenal medulla, spleen, and T-lymphocyte RNA.

In order to determine which mRNA species the pCAMPDE-40 cDNA represents, Northern blots of poly(A)-selected RNA isolated from cerebral cortex and heart were probed with 32P-labeled antisense transcripts corresponding to either a portion of the coding sequence, or the 5' or 3' untranslated sequence (Fig. 6). The riboprobe corresponding to the coding sequence hybridized with both mRNA species. However, the probes corresponding to the untranslated sequences hybridized only with the 4.4-kb transcript of both cerebral cortex and heart mRNA.

![Fig. 1. Strategy for cloning cDNAs encoding the 61-kDa CaM PDE.](image-url)
**CaM PDE cDNA Cloning and Expression**

The 61-kDa CaM PDE is a member of a growing family of PDE isozymes that are activated by calcium and CaM. At least six different CaM PDEs have been identified, and three of these, the 61-, 63-, and 75-kDa CaM PDEs, are concentrated in brain tissue (2). We have cloned a cDNA from a bovine brain library encoding a polypeptide that is identical to the sequence of the 61-kDa CaM PDE as determined directly from the purified protein. A high level of sequence identity was observed between the 61- and 63-kDa CaM PDEs, and a high degree of conservation was noted for CaM-binding regions of the 61- and 75-kDa PDEs. The 61-kDa CaM PDE mRNA is widely expressed in brain. This result contrasts markedly with the distribution of mRNA encoding the 63-kDa CaM PDE, which appears to be most abundant in the basal ganglia (4). Our results agree generally, with previous experiments that examined immunohistochemically, the distribution of CaM PDE antigen in the brain using antibodies raised against a purified 61-kDa CaM PDE preparation (19). These investigators detected CaM PDE immunoreactivity in the somata and dendrites of cortical and hippocampal pyramidal neurons. The CaM PDE isozyme may extend to the spinal cord and, perhaps, the peripheral nervous system.

Total RNA isolated from heart, aorta, lung, trachea, and kidney completely protected the riboprobe corresponding to a segment of the catalytic domain. However, these RNA samples only protected ~150 bases of the CaM-binding domain.
CaM PDE cDNA Cloning and Expression

Among the RNA shown by RNase protection analysis to contain CaM PDE transcript, we determined the size of CaM PDE mRNA by Northern analysis. The pCAMPDE-40 cDNA hybridized with a 3.8-kb mRNA and a 4.4-kb mRNA from RNA isolated from all of the tissues surveyed. We also observed that probes corresponding to the 5'- and 3'-untranslated sequence of the pCAMPDE-40 cDNA hybridized with only the 4.4-kb mRNA from both cerebral cortex and heart RNA. This result indicates that the pCAMPDE-40 cDNA represents the 4.4-kb mRNA species. Presently, we are not certain whether the 3.8-kb mRNA detected in these tissues encodes a CaM PDE that is identical to the isozyme encoded by the 4.4-kb mRNA or a different isoform. We are currently pursuing this question. It is unlikely that the 4.4-kb CaM PDE mRNA encodes only the 61-kDa isoform since this species would have been easily detected in heart RNA by RNase protection analysis. Additionally, the results described above suggest that the 5'-untranslated sequence of the cerebral cortex and heart transcripts, which encode different CaM PDE isoforms, must be very similar. Transcripts derived from an alternately spliced gene often share the same first exon (39). This result is therefore consistent with the idea that the CaM PDE mRNA detected in brain, heart, and probably the other tissues are products of an alternately spliced gene.

Potential Involvement of CaM PDEs in Regulating Cyclic Nucleotide Metabolism—In cells that express a CaM PDE isozyme, a likely consequence of CaM PDE activation triggered by calcium-dependent signal transduction pathways is to modulate cAMP and cGMP accumulation, and thus, to antagonize physiological processes regulated by these second messengers. Several examples demonstrating the involvement of a CaM PDE activity in blunting hormone-induced cyclic nucleotide accumulation in tissues or intact cells have been reported. For example, in the neuronal cell line 1321N1, CaM PDE activation has been implicated as the mechanism by which muscarinic receptor agonists inhibit cAMP formation. How CaM PDE activation affects neuron function is not precisely known. However, CaM PDE activity and antigen have been detected near the membrane of dendritic projections of certain neurons, suggesting that these isozymes may play a role in postsynaptic neurotransmission (18, 20). Moreover, CaM PDEs may play a pivotal role in the opposing actions of calcium and cyclic nucleotides on ion currents in certain neurons (40). More recently, glutamate has been shown to suppress cGMP-mediated ion conductance in retinal depolarizing bipolar cells by a mechanism involving PDE activation in a manner similar to the phototransduction cascade in rod and cone cells (12). One might predict that glutamate-induced mobilization of intracellular calcium would activate a CaM PDE, thus negatively modulating channel activity by lowering intracellular cGMP.

In cardiac myocytes, PDE activation has been implicated as the mechanism by which stimulation of α1 adrenergic receptors decreases intracellular cAMP (9). Since stimulation of these receptors increases phosphoinositide turnover and intracellular calcium (41, 42), it seems likely that activation of a CaM PDE is involved in this response. Whether a CaM PDE is localized in the cardiomyocyte or nonmyocyte cell is a topic of some controversy, however (43).

There is biochemical and pharmacological evidence suggesting that activation of a CaM PDE may augment contraction of vascular and nonvascular smooth muscle by lowering intracellular cAMP or cGMP (13, 14, 22, 44). Based on the RNase protection pattern generated with RNA isolated from aorta, trachea, and lung, it seems likely that a CaM PDE isoform, possibly the 59-kDa CaM PDE, may be expressed in the smooth muscle beds of these tissues.

Our results indicate that mRNA encoding a PDE isoform

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**Fig. 3. Expression of 61-kDa CaM PDE activity in COS cells.** COS cells were transfected with the pCAMPDE-40 cDNA ligated into the expression vector, pCDM8. Extracts of transfected COS cells were assayed for PDE activity using either cAMP or cGMP (2 μM) in the presence (cross-hatched columns) or absence (solid columns) of calcium and CaM.
Fig. 4. Distribution of mRNA encoding the 61-kDa CaM PDE or structurally related isoforms in bovine tissues. A, 32P-labeled antisense riboprobes corresponding to a portion of either the CaM-binding domain (riboprobe I; AccI (+6) through SetI (+243)) or catalytic domain (riboprobe II; Tth111 I (+819) through HincII (+1232)) of the CaM PDE were constructed from restriction endonuclease cleavage fragments of the pCAMPDE-40 cDNA. The large box delineates the coding sequence. RNase protection assays were carried out using these riboprobes and total RNA (20 µg) isolated from either cerebral (cerebral cortex, basal (bas.) ganglia, hippocampus, cerebellum, medulla, and spinal cord (spinal cord)), heart, aorta, adrenal (adr.) cortex, adrenal medulla (A) or cerebral cortex, liver, kidney (kid.) cortex, kidney medulla, kidney papilla, trachea, lung, spleen, or T-lymphocytes (T-lymph.) (B). The size of the protected riboprobes in bases (bp) are shown on the left. The roman numerals on the lower right side of each panel indicates the riboprobe used in each experiment. D, the relative amount of CaM PDE mRNA expressed in each tissue was determined by densitometrically quantitating the 410-base band representing RNase-protected riboprobe I and either the 234-base band or the 154-base band representing either fully or partially protected riboprobe I, from autoradiograms like those shown in Fig. 4, B and C (see “Methods”). The relative amount of RNA in each tissue (x axis) is expressed as a percentage of cerebral cortex RNA (the mean, ± S.E.) generated by integrating the optical density over the entire band area from three separate experiments.

structurally related to the 61-kDa CaM PDE is abundant in the inner medullary regions of the kidney. The CaM PDE may be concentrated in the collecting duct or thick ascending limb, since these nephron segments are highly concentrated in the inner medullary region of the kidney. Although the role that CaM PDEs play in renal function are not known, several studies have alluded to the possibility that they may be a component of signal transduction processes that blunt water reabsorption in response to the antidiuretic hormone, arginine-vasopressin in the collecting duct (45). Further studies designed to determine the precise cellular distribution of CaM PDE isozymes need to be determined since the kidney, as well as tissues like lung, trachea, and heart, are composed of several different cell types.

In conclusion, we have isolated a cDNA representing a mRNA species encoding the 61-kDa CaM PDE. Our results indicate that the 61-kDa CaM PDE is widely distributed in the brain. A structurally related isoform, possibly the 59-kDa CaM PDE, is also expressed in heart, aorta, trachea, lung, and the inner medullary region of the kidney. We detected little or no CaM PDE transcript in adrenal gland, kidney cortex, spleen, and T-lymphocytes, indicating that these CaM PDE isozymes are expressed in a tissue-specific manner. Northern analysis revealed that two mRNAs encoding the 61-kDa CaM PDE, or isoforms structurally related to the 61-kDa isoform, are expressed in these tissues. Moreover, the 5'-untranslated sequence of cerebral cortical and heart mRNA encoding different, yet structurally related CaM PDEs appear to be identical based on the hybridization stringency of our Northern blot analyses. These data together suggest that the isoforms expressed in cerebral cortex and heart, and perhaps aorta, kidney, trachea, and lung, may be products of an alternately spliced gene.

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