Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A

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We report here the cloning, expression, and characterization of human PDE11A1, a member of a distinct cyclic nucleotide phosphodiesterase (PDE) family. PDE11A exhibits ≤50% amino acid identity with the catalytic domains of all other PDEs, being most similar to PDE5, and has distinct biochemical properties. The human PDE11A1 cDNA isolated contains a complete open reading frame encoding a 490-amino acid enzyme with a predicted molecular mass of 55,786 Da. At the N terminus PDE11A1 has a single GAF domain homologous to that found in other signaling molecules, including PDE2, PDE5, PDE6, and PDE10, which constitutes a potential allosteric binding site for cGMP or another small ligand. Tissue distribution studies indicate that PDE11A mRNA occurs at highest levels in skeletal muscle, prostate, kidney, liver, pituitary, and salivary glands and testis. PDE11A is expressed as at least three major transcripts of ≈10.5, ≈8.5, and ≈6.0 kb, thus suggesting the existence of multiple subtypes. This possibility is further supported by the detection of three distinct proteins of ≈78, ≈65, and ≈56 kDa by Western blotting of human tissues for PDE11A isoforms. Recombinant human PDE11A1 hydrolyzes both cGMP and cAMP with K_m values of 0.52 μM and 1.04 μM, respectively, and similar V_{max} values. Therefore, PDE11A represents a dual-substrate PDE that may regulate both cGMP and cAMP under physiological conditions. PDE11A is sensitive to the nonselective PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX) as well as zaprinast and dipyridamole, inhibitors that are generally considered relatively specific for the cGMP-selective PDEs, with IC_{50} values of 49.8 μM, 12.0 μM, and 0.37 μM, respectively.

The second messengers cAMP and cGMP play pivotal regulatory roles in a wide variety of signal transduction pathways and in various tissues (1). For example, they mediate processes such as vision (2), olfaction (3), platelet aggregation (4), aldosterone synthesis (5), insulin secretion (6), T cell activation (7), and smooth muscle relaxation (8, 9). Intracellular levels of cAMP and cGMP are tightly controlled both by their rate of synthesis by adenylyl and guanylyl cyclases, respectively, in response to extracellular signals, and by their rate of hydrolysis by cyclic nucleotide phosphodiesterases (PDEs). PDEs form a superfamily of enzymes that catalyze the hydrolysis of 3′,5′-cyclic nucleotides to the corresponding nucleoside 5′-monophosphates. On the basis of their substrate specificities, kinetic properties, allosteric regulators, inhibitor sensitivities, and amino acid sequences, the known mammalian PDEs are subdivided into 10 major families, PDEs 1–10 (1, 10–16). Furthermore, each family and even members within a family also exhibit distinct tissue, cell, and subcellular expression patterns (1, 11–17) and hence are likely to participate in discrete signal transduction pathways and thus physiological and pathophysiological processes—e.g., penile erection (8, 9) and asthma (18). Therefore, PDEs are of both fundamental and pharmacological interest. To further increase our understanding of the biochemistry of PDEs and the physiological processes they modulate, we have sought to identify novel human PDEs. We report here the cloning and characterization of a human PDE gene family, which we denote as PDE11A in accordance with standardized nomenclature (10).

Materials and Methods

Expressed Sequence Tag (EST) Database Searching and Analysis of DNA Sequences. EST databases were searched by using the sequences of mammalian PDEs. Each EST identified with PDE homology was then used as a query to search GenBank to determine whether it was a known or potentially novel PDE. Homology searches were performed by using either BLAST (19) or Position-Specific Iterated BLAST (PSI-BLAST) (20), alignments were constructed by using CLUSTAL (21), and motif searches were carried out by using PROSITE (22). Other sequence analyses were conducted with either the Wisconsin GCG package (Genetics Computer Group, Madison, WI) or Vector NTI (InforMax, North Bethesda, MD). A search of the Incyte LifeSeq database (Incyte Pharmaceuticals, Palo Alto, CA) using human PDE5 (accession no. AJ004865) (23) as a query sequence led to the identification of Incyte clone 826776 as a potential novel human PDE.

DNA Sequencing and Sequence Analyses. Plasmid DNA was prepared by using miniprep or maxiprep kits (Qiagen, East Sussex, U.K.). Fluorescence-tagged dye terminator cycle sequencing (Perkin–Elmer) was performed on both strands and was followed by analysis on an ABI 373A DNA sequencer (Applied Biosystems).

DNA Probe Synthesis and cDNA Library Screening. DNA probes were labeled with [α-^{32}P]dCTP at 6,000 Ci/mmol (1 Ci = 37 GBq) by using a Megaprime kit (Amersham) and purified on Chromospin-30 columns (CLONTECH). The complete cDNA insert of Incyte clone 826776 (≈1 kb) was recovered as a *Sal*I/*Not*I restriction fragment and used to generate a probe for screening ≈1 × 10^6 plaque-forming units of a human skeletal muscle 5′-Stretch Plus λgt10 cDNA library (CLONTECH) by using standard procedures (24). This procedure led to the isolation of λ clone 1a.1, which encodes a complete open reading frame for human PDE11A1. cDNA inserts from positive clones were subcloned into pBluescript KS+ (Stratagene) for sequencing as described above.

Northern Blotting. DNA probe was generated as described above, using a 428-bp PCR product (amino acids 121–263; Fig. 1*B*), which was amplified with the following primers: 5′-GAT-

Abbreviations: EST, expressed sequence tag; IBMX, 3-isobutyl-1-methylxanthine; PDE, cyclic nucleotide phosphodiesterase.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AJ251509).

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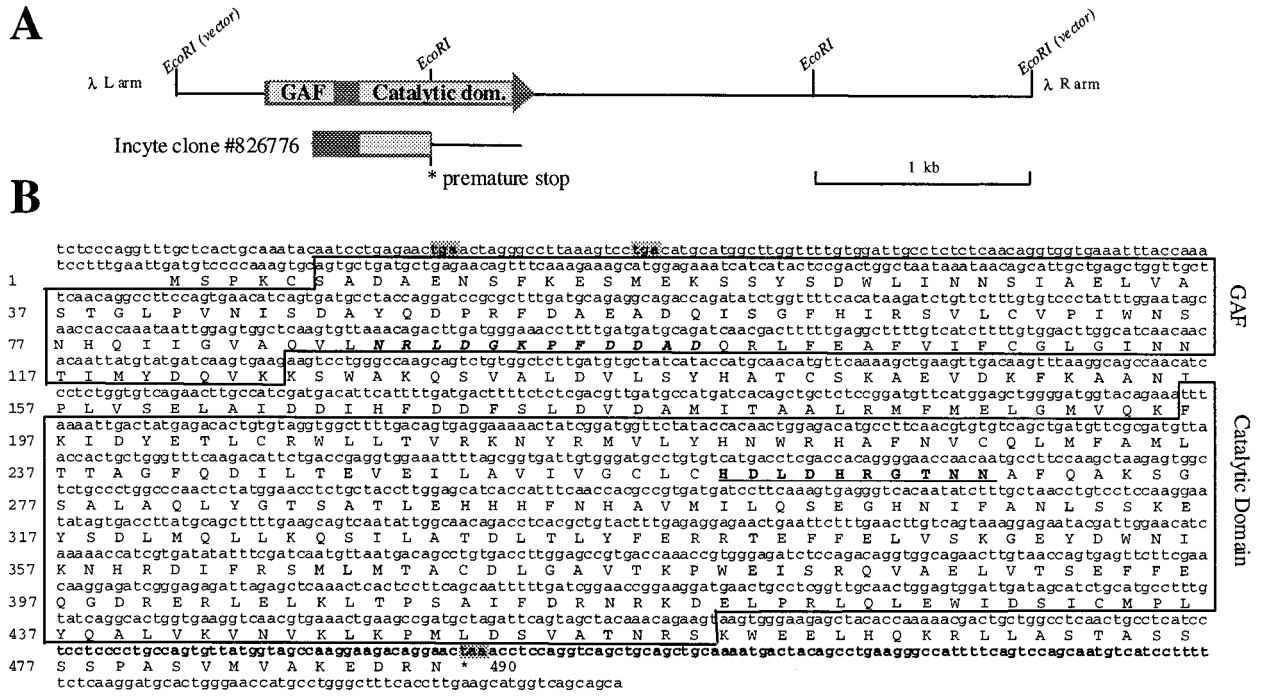

Fig. 1. (*A*) Schematic representation of Incyte clone 826776 and the λgt10 cDNA clone (1a.1) encoding full-length human PDE11A1. (*B*) Nucleotide and amino acid sequences of human PDE11A1. Boxed sequences indicate domains identified by sequence similarity. Amino acid residues in boldface italics (GAF domain) are thought to be important for cGMP binding, and those in boldface and underlined (catalytic domain) correspond to the PDE signature motif. In-frame stop codons are in boldface shaded text.

CAAGTGAAGAAGTCC-3′ and 5′-TCGAGGTCATGACACAGG-3′. Human Multiple Tissue Northern blots [2 μg of poly(A)$^+$ RNA per tissue] and RNA dot blots (CLONTECH) were prehybridized in ExpressHyb (CLONTECH) at 68°C for 1 hr, then hybridized (≈1 × 10^6 cpm of probe per ml of ExpressHyb) at 68°C overnight. Blots were washed in 2× SSPE/0.05% SDS at 50°C [four times, 15 min each; 1× SSPE (standard saline phosphate/EDTA) is 0.15 M NaCl/10 mM sodium phosphate, pH 7.4/1 mM EDTA] followed by 0.1× SSPE/0.1% SDS at 50°C for 1 hr, and then exposed to film for 2–7 days. Northern blots were also checked for equal loading of poly(A)$^+$ RNA in each lane by using a human β-actin cDNA probe by the same procedure (data not shown).

Subcloning of Full-Length Human PDE11A1. The open reading frame of human PDE11A1 was isolated by PCR from λ clone 1a.1 using a sense primer (5′-CCAAATCCCGGTCCGAGATGTCCCCAAAGTGCAGTGCTGATGC-3′) covering the initiation codon (underlined) and incorporating an *Rsr*II restriction site, and an antisense primer (5′-CGGGTACCTCGAGTTATTAGTTCCTGTCTTCCTTGGCTACC-3′) covering the termination codon (underlined) and incorporating a tandem stop codon and a unique *Xho*I restriction site. PCR was performed with the Expand High Fidelity PCR system (Boehringer Mannheim) with the following cycle parameters: 94°C/105 s, 1 cycle; 94°C/15 s, 65°C/30 s, 72°C/105 s, 20 cycles; and 72°C/5 min, 1 cycle. The PCR product was digested with *Rsr*II/*Xho*I and ligated into pFASTBAC baculovirus transfer vector (Life Technologies, Gaithersburg, MD), and the resulting construct (pFASTBAC-PDE11A1) was sequenced.

Expression and Purification of PDE11A1. pFASTBAC-PDE11A1 was used to produce recombinant baculoviral stocks by using the Bac-to-Bac system (Life Technologies) according to the manufacturer's protocol. Recombinant human PDE11A1 was expressed by infecting 3 × 10^7 *Sf*9 cells in 30 ml of Sf900 II serum-free medium (Life Technologies) with virus at a multiplicity of infection of 1. Cells were incubated at 27°C, harvested 48 hr after infection by centrifugation (10 min at 3,000 × *g*) and resuspended at 1 × 10^7 cells per ml in homogenization buffer [20 mM Hepes, pH 7.2/1 mM EDTA/20 mM sucrose/150 mM NaCl, one protease inhibitor tablet (Boehringer Mannheim) per 50 ml]. Cells were then disrupted by sonication and debris was removed by centrifugation (15 min at 12,000 × *g*) followed by filtration (0.2-μm filter). For purification, the clarified supernatant was dialyzed against 20 mM Hepes, pH 7.4/1 mM EDTA/150 mM NaCl at 4°C for 16 hr, and 2-ml aliquots were fractionated on a 1-ml Mono Q HR (5/5) column (Pharmacia Biotech) by eluting with a linear NaCl gradient up to 1 M over 65 ml. Fractions (2 ml) were collected and assayed for PDE activity (see below), and those containing high levels were pooled and stored in aliquots at −70°C; uninfected cells have negligible PDE activity (data not shown).

Western Blotting and PDE11A Antibody Generation. Human prostate cytosolic protein was prepared by using a method described previously (9) and human skeletal muscle protein was purchased (CLONTECH). Protein samples, 25 μg of tissue protein or 10 μg of partially purified recombinant PDE11A1, were separated by SDS/PAGE using a Tris-glycine mini-gel system (Novex, San Diego), and then either visualized by Coomassie blue staining or transferred to a poly(vinylidene difluoride) membrane (Novex) by using a mini-transblot system (Bio-Rad) for immunoblotting. For Western analysis, membranes were blocked with Tris-buffered saline (TBS) containing 5% (wt/vol) nonfat milk (TBS-NFM) for 2 hr and probed with rabbit anti-human PDE11A primary antibody diluted 1:1,000 in TBS-NFM for 1 hr. Excess antibody was removed by washing in TBS containing 0.1% Tween-20, and then horseradish peroxidase-conjugated anti-rabbit IgG antibody (Amersham) diluted 1:2,500 in TBS-

NFM added for 1 hr. After washing, proteins were detected with an enhanced chemiluminescence kit (ECL; Amersham).

Under contract with Avecia (formerly Zeneca CRB, Cheshire, U.K.), three anti-human PDE11A polyclonal antisera were raised in rabbits against the following peptides conjugated to keyhole limpet hemocyanin: EPH-2 (NNTIMYDQVKKSWAK), EPH-3 (SAIFDRNRKDELPRL), and EPH-4 (VATNRSKWEELHQKR), corresponding to amino acids 115–129, 410–424, and 454–468, respectively (Fig. 1*B*). Each antiserum was harvested 10 weeks after the initial inoculation and four biweekly booster injections, and then affinity-purified against the respective peptide. These antisera were shown not to crossreact with other closely related PDEs (data not shown).

Enzyme Kinetics and Inhibitor Studies. PDE activity was measured by a scintillation proximity assay (SPA)-based method. Assay samples were diluted as required and incubated at 30°C in 100 μl of assay buffer (20 mM Tris·HCl, pH 7.4/5 mM $MgCl_2$/1 mg of BSA per ml) containing the desired concentration of cGMP or cAMP substrate (3:1 ratio unlabeled to ^{3}H-labeled). All reactions, including buffer-only blanks, were conducted in triplicate in white, flat-bottomed Microfluor plates (Dynex Technologies, Chantilly, VA) and allowed to proceed for an incubation time giving <25% substrate turnover (empirically determined). Reactions were terminated by adding 50 μl of yttrium silicate SPA beads (Amersham) containing 3 mM of the unlabeled cyclic nucleotide. Each plate was then resealed and shaken for 20 min, after which the beads were allowed to settle for 30 min in the dark. Plates were read on a TopCount microtiter plate reader (Packard, Meriden, CT), and enzyme activities were calculated from the amount of radiolabeled product detected. K_m and V_{max} values were determined by measuring hydrolysis with a range of substrate concentrations (0.10–10 μM) and a fixed amount of dilute enzyme over a time course of 5–60 min. Initial rates were calculated at each substrate concentration and plotted against substrate concentration, from which kinetic parameters were determined by using the "Fit Curve" Microsoft Excel extension. The effect of PDE inhibitors was investigated by assaying a fixed amount of recombinant PDE11A1 in the presence of various inhibitor concentrations and low substrate, i.e., 0.17 μM cGMP ($\approx$⅓ K_m) such that $IC_{50} \cong K_i$. Reactions were initiated by adding a fixed amount of enzyme, incubated for 30 min at 30°C, and then terminated, and reaction products were counted as above. Radioactivity units were converted to percent activity of an uninhibited control (100%) and plotted against inhibitor concentration, and inhibitor IC_{50} values were obtained by using the "Fit Curve" Microsoft Excel extension.

Results

Cloning and Sequence Analysis of PDE11A. Bioinformatic searching of the Incyte database yielded one EST clone, Incyte clone 826776, encoding a potentially novel human PDE with closest homology (42%) to part of the catalytic domain of human PDE5. The complete nucleotide sequence of the cDNA insert of Incyte clone 826776 (973 bp) was determined on both strands, translation of which revealed an incomplete coding sequence of 208 amino acids most closely related to human PDE5 (23)—48% identity by PSI-BLAST (21) (data not shown). A CLUSTAL alignment (22) suggested that the clone was not only incomplete at the 5′ end but also encoded a C-terminal truncated PDE as compared with PDE5 and the "consensus" catalytic domain conserved across all mammalian PDE families (25) (Fig. 1*A*).

Tissue distribution studies (see below) indicated prostate and skeletal muscle to be sites of relatively high expression of the corresponding mRNA. Therefore, a human skeletal muscle cDNA library was screened, which led to the isolation of a λ cDNA clone containing a 3.9-kb cDNA insert comprising three *Eco*RI restriction fragments (Fig. 1*A*), which were subcloned and sequenced. Analysis of the consensus sequence enabled identification of a single large open reading frame encoding a 490-amino acid polypeptide with a predicted molecular mass of 55,786 Da (Fig. 1*B*). The presence of two in-frame stop codons upstream of the initiator methionine (Fig. 1*B*) indicates that the full N-terminal coding sequence is present. Database searches and alignments reveal that the encoded protein has not been described previously and is most similar to human PDE5, especially across the catalytic domain (Table 1)—the most highly conserved region across all PDE families (25). Furthermore, PROSITE analysis (22) indicates the presence of the PDE signature motif HDX_2HX_4N (26) (amino acids 260–269; Fig. 1*B*), hence supporting the conclusion that the encoded protein is a novel human PDE. The degree of similarity across the catalytic domain as compared with other human PDEs by using PSI-BLAST suggests that it is not a PDE5 subtype, since it shares only 50% amino acid identity (Table 1). Therefore, as indicated also by the biochemical data (see below), the encoded enzyme is a member of a distinct PDE family, hence we denote it as HSPDE11A1 in accordance with standardized nomenclature (10). The sequence alignments also revealed similarity to the noncatalytic cGMP-binding domains of PDE2, PDE5, PDE6, and PDE10 (16, 27, 28) upstream of the catalytic domain (see *Discussion*). The full-length PDE11A1 clone isolated not only has an intact N terminus but also has a different C terminus as compared with Incyte clone 826776. The C-terminal truncated nature of the latter, as compared with the catalytic domain of all other mammalian PDEs (Fig. 1*A*), suggests that it would not encode a catalytically active PDE and hence probably represents an inaccurately spliced mRNA or a cloning artifact.

Table 1. Amino acid sequence comparison of the catalytic domain of PDE11A to those of other PDEs

PDE family	Catalytic domain		Accession no.
	Identities	Similarities	
HSPDE5A	133/264 (50%)	191/264 (71%)	AJ004865
HSPDE6C	122/273 (44%)	175/273 (63%)	X94354
HSPDE6A	117/273 (42%)	175/273 (63%)	M26061
HSPDE10A	108/261 (41%)	167/261 (64%)	AF110507
HSPDE6B	114/273 (41%)	169/273 (61%)	X66142
HSPDE2A	107/264 (40%)	157/264 (58%)	U6773
HSPDE4D	86/271 (31%)	144/271 (52%)	U50157
HSPDE9A	80/263 (30%)	140/263 (52%)	AF048837
HSPDE1B	74/242 (30%)	127/242 (51%)	U5676
HSPDE7A	80/267 (29%)	136/267 (49%)	U67932
HSPDE8A	72/257 (28%)	119/257 (46%)	AF056490
HSPDE3B	74/289 (25%)	118/289 (40%)	D50640

Tissue Distribution of PDE11A. Northern blotting analysis was performed on poly(A)$^+$ RNA from a range of human tissues by using a 428-bp probe covering the N terminus of the catalytic domain and upstream 75 amino acids (amino acids 121–263; Fig. 1*B*). The data obtained indicate that PDE11A is expressed as at least three distinct, major transcripts (Fig. 2*A*). That is, a single major transcript of $\approx$8.5 kb in skeletal muscle and two major transcripts of $\approx$10.5 kb and $\approx$6.0 kb in prostate. Comparable data were obtained with a probe derived from Incyte clone 826776 (data not shown). No transcripts were detectable in bladder, colon, heart, small intestine, stomach, and uterus by Northern blotting. These data suggest the existence of multiple splice variants and hence PDE11A subtypes with distinct tissue expression patterns. A wider analysis by RNA dot-blot, although not as quantitative, confirmed the above findings in terms of detectable expression and, furthermore, indicated wider expres-

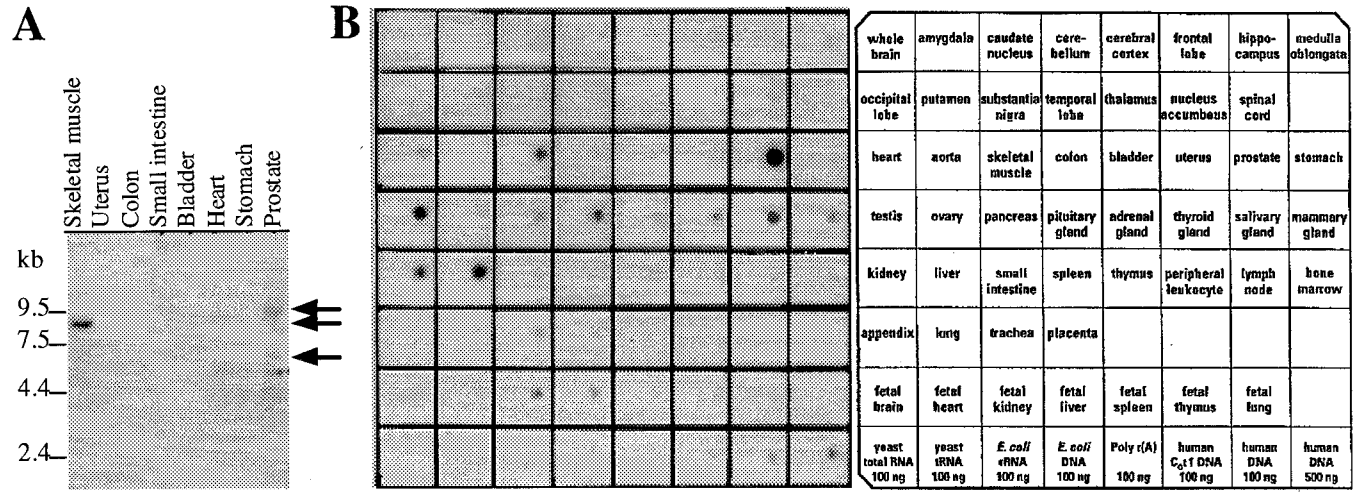

Fig. 2. Northern and RNA dot-blot analyses of PDE11A expression in human tissues. (*A*) Two micrograms of poly(A)$^+$ RNA per tissue (indicated above lane) was probed. Three distinct transcripts were detected: ≈10.5 and ≈6.0 (prostate) and ≈8.5 kb (skeletal muscle). (*B*) RNA dot-blot of multiple tissues showing data consistent with the Northern blots and expression in additional tissues.

sion in kidney (adult and fetal), liver (adult and fetal), pituitary and salivary glands, and testis (Fig. 2*B*). Lower levels were also detected in adrenal, mammary, and thyroid glands as well as pancreas, spinal cord, and trachea.

In addition to the mRNA distribution, the expression of PDE11A protein was examined by Western blotting in human tissue extracts by using three separate anti-PDE11A polyclonal antisera raised against peptides derived from the human PDE11A1 polypeptide sequence. The results show that human prostate contains a single protein of ≈56 kDa that comigrates with recombinant human PDE11A1 (Fig. 3) and is in close agreement with the predicted molecular weight of human PDE11A1. A similar sized protein is also observed in skeletal muscle; however, two additional protein bands of ≈65 kDa and ≈78 kDa are also observed (Fig. 3). Each antiserum gave a similar pattern of bands with both tissues and had been shown not to crossreact with other closely related PDEs (data not shown). These data confirm the existence of a protein corresponding to PDE11A1 in human tissue extracts and further support the existence of additional PDE11A subtypes with distinct tissue expression patterns.

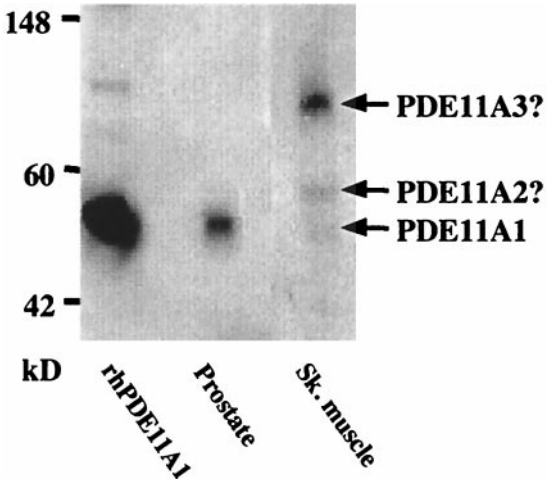

Fig. 3. Western blot analysis of PDE11A expression in human tissues. Twenty-five micrograms total protein per tissue (indicated below each lane) was immunoblotted along with recombinant human PDE11A1 (rhPDE11A1). Three distinct proteins were detected of ≈78, ≈65 (putative PDE11A2 and -A3), and ≈56 kDa, the latter corresponding to PDE11A1, with each of three separate antisera to human PDE11A.

Expression of PDE11A1 and Characterization of PDE11A1 Activity. The ability of the human PDE11A1 cDNA to yield a functionally active protein of the predicted size was examined by expressing FLAG-tagged and untagged cDNA constructs in *Sf*9 insect cells using baculovirus vectors. Both expression constructs, tagged and untagged, resulted in the production of an ≈56-kDa polypeptide as expected, which could be detected by both Coomassie blue staining and immunoblotting (data not shown). The following data relate to the partially purified, untagged recombinant human PDE11A1 (full length)—similar data were obtained with the tagged enzyme, and mock-infected (control) *Sf*9 cells had negligible hydrolytic activity for either cAMP or cGMP (data not shown). By using a range of substrate concentrations (0.10–10 μM) and subsequently taking those data points that were in the linear part of the reaction, it was shown that both cAMP and cGMP are substrates for PDE11A1 with K_m values of 1.04 ± 0.23 μM and 0.52 ± 0.34 μM, respectively (Fig. 4; mean ± SEM of five separate experiments). These data also show that the maximal rate of turnover of substrate (V_{max}) is approximately equal for both these cyclic nucleotides, i.e., 3.6 and 3.9 pmol/min per μg of partially purified enzyme, respectively, indicating similar specific activities despite the ≈2-fold higher affinity for cGMP as compared with cAMP. These data confirm that PDE11A1 is a *bona fide* PDE capable of hydrolyzing both cAMP and cGMP at physiologically relevant concentrations.

The sensitivity of recombinant human PDE11A1 to a range of standard nonselective and selective PDE inhibitors was examined with cGMP as a substrate at ⅓ K_m concentration—i.e., so IC_{50} equates to K_i (Table 2). PDE11A1 was insensitive to both milrinone and rolipram, which are selective for PDE3 and PDE4, respectively, up to 100 μM. The nonselective PDE inhibitor IBMX inhibited PDE11A1 with an IC_{50} of 49.8 μM, which is within the range observed for other PDEs except the recently identified IBMX-insensitive PDEs, PDE8A, PDE8B, and PDE9 (11–15). The so-called cGMP-specific PDE inhibitor zaprinast inhibited PDE11A1 with moderate potency (IC_{50} = 12.0 μM) at a concentration 9- and 80-fold higher than that considered specific for PDE5 and PDE6, respectively. Whereas PDE11A1

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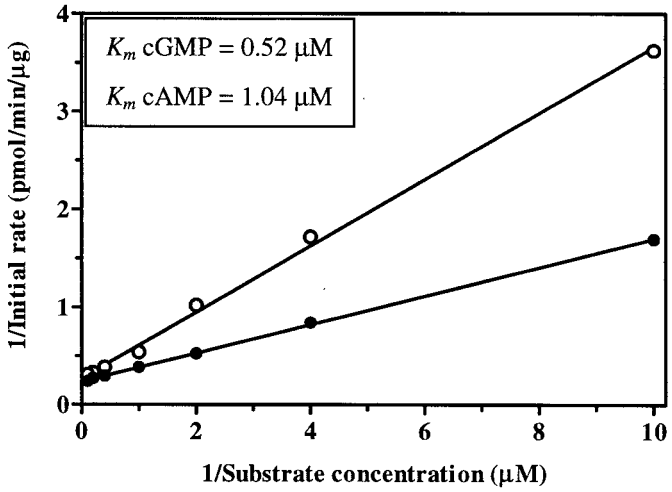

Fig. 4. Lineweaver–Burk plots for human PDE11A1-catalyzed cGMP and cAMP hydrolysis. Initial linear rates of cAMP (○) or cGMP (●) hydrolysis were determined and used to calculate K_m and V_{max} values from five separate data sets.

was inhibited potently by dipyridamole (IC_{50} = 370 nM) at concentrations similar to those previously considered specific for PDE5 and PDE6—i.e., 0.4- and 1.0-fold, respectively. These kinetic and inhibitor sensitivity data further support the assignment of PDE11A as a distinct PDE family.

Discussion

The ≈3.9-kb cDNA identified and characterized here encodes another human PDE gene family—i.e., PDE11A. It comprises a full-length open reading frame of 490 amino acid residues, as indicated by the presence of two in-frame stop codons upstream of the initiator methionine (Fig. 1*B*), with a predicted molecular mass of 55,786 Da. However, the first methionine encountered exhibits a poor match to the consensus Kozak sequence, i.e., ACCATGG (29), whereas the second methionine (amino acid 17; Fig. 1*B*) has a much closer match. Therefore, the actual translated protein might be only 474 amino acids, although Western blotting data suggest that the native PDE11A1 protein is likely to be 490 aa (≈56 kDa)—assuming no major posttranslational modifications (data not shown).

The assignment as a distinct PDE family is supported by the degree of homology at the C terminus to the conserved PDE catalytic domain of all other mammalian PDEs (25), which includes the PDE signature motif HDX_2HX_4N (26). Previous studies have led to the identification of 10 distinct mammalian PDE gene families (1, 10–16), sequence analysis of which reveals at least 70% amino acid sequence identity between catalytic domains within the same gene family but only 30–50% identity across PDE gene families (data not shown). The catalytic domain of human PDE11A (264 amino acids; Fig. 1*B*) is most closely related to human PDE5 (23) with 50% amino acid identity, decreasing thereafter with PDEs 6, 10, and 2 (44–40%), followed by PDEs 4, 9, 1, 7, 8, and 3 (31–25%) (Table 2), hence is consistent with the conclusion that the encoded protein is a novel human PDE family member. This conclusion is substantiated by an analysis of the N-terminal region, which encodes the so-called regulatory domain [different for each PDE family (1, 10)], because a homologous but distinct putative noncatalytic cGMP-binding domain exists similar to that in PDEs 2, 5, 6, and 10 (16, 27, 28). In PDE5 the N-terminal cGMP-binding domain spans ≈380 amino acid residues and comprises tandem repeats of the conserved sequence motif $N(K/R)X_nFX_3DE$ (28), the NKX_nD motif having been shown by mutagenesis to be important for cGMP binding (30). In PDE5 cGMP binding is proposed to modulate enzyme activation by means of phosphorylation of the enzyme (31, 32). The analogous site in PDE2 functions as an allosteric activator of cAMP and probably cGMP hydrolysis (33), whereas in PDE6 cGMP binding is thought to regulate the interaction between the catalytic and inhibitory γ-subunits and transducin (34, 35). In PDE11A1 the homologous domain has a deletion as compared with PDE5 that coincides with the respective tandem NKX_nD motifs such that the result is a single putative cGMP-binding NKX_nD motif (amino acids 88–99, Fig. 1*B*). Therefore, PDE11A is distinct from PDE5 and other cGMP-binding PDEs in this region but could potentially bind cGMP at this putative allosteric site. However, kinetic studies *in vitro* examining the effect of cGMP on cAMP hydrolysis and *vice versa* suggest that neither cyclic nucleotide has an allosteric role to play in modulating substrate hydrolysis catalyzed by PDE11A1 (data not shown). In addition to occurring in the cGMP-binding PDEs, it has been observed that these conserved repeats occur in a variety of signaling proteins from eubacterial, archaeal, and eukaryotic organisms, and have thus been termed GAF (cGMP-binding and stimulated PDEs, *Anabaena* adenylyl cyclases, and *Escherichia coli* FhlA) domains (36). It is suggested that GAF domains function as structurally conserved binding sites for small molecules (36)—e.g., cGMP in PDEs 2, 5, and 6 and formic acid in *E. coli* FhlA, a member of the NtrC family of transcription factors (37). Therefore, it is conceivable that the GAF domain in PDE11A1 (Fig. 1*B*) binds a different small molecule, especially since there is only a single GAF domain in PDE11 as compared with PDEs 2, 5, 6, and 10, which have two GAF domains at their N termini and, at least for PDE5, occupancy at both cGMP-binding sites is required to elicit an allosteric effect (38).

Partially purified full-length recombinant human PDE11A1 is capable of hydrolyzing both cGMP and cAMP with K_m values of 0.52 μM and 1.04 μM, respectively, and with approximately equal maximal turnover rates (V_{max}) (Fig. 4). This versatility is in marked contrast to its most closely related PDE, PDE5, which is very specific for cGMP (28, 31) with a K_m of 5.6 μM (bovine) (31) and 6.1 μM (human recombinant) (39). Therefore, there are clearly some key amino acid differences within the respective catalytic sites, despite significant identity (50%) and similarity (71%) (Table 1), which in PDE5 confer specificity for cGMP. Furthermore, unlike several other PDEs with dual-substrate specificity—i.e., PDEs 2, 3, and 10 (16, 40, 41)—PDE11 exhibits very similar K_m and V_{max} values. Consequently, like PDE1 (1), PDE11A appears to be a genuine dual-substrate PDE rather than a PDE that is capable of hydrolyzing both cyclic nucleotides *in vitro*, but under physiological conditions more likely to hydrolyze one and be modulated by the other with the lower K_m and V_{max}. These data confirm that PDE11A1 is a *bona fide* PDE capable of hydrolyzing both cAMP and cGMP at physiologically relevant concentrations and hence regulating both cAMP and cGMP pathways *in vivo*, and further support its assignment as a distinct PDE family.

In addition to the amino acid sequence and kinetic data, the sensitivity of PDE11A1 to standard PDE inhibitors also supports

Table 2. Potency of various PDE inhibitors on human PDE11A1

Inhibitor	Selective for PDE type	IC_{50}, μM	PDE11A1 IC_{50}, μM
IBMX	Nonselective	2–59	49.8
Milrinone	PDE3	1.3	>100
Rolipram	PDE4	2.0	>100
Zaprinast	PDE1/5/6	6.9/0.76/0.15	12.0
Dipyridamole	PDE5/6/9/10	0.9/0.38/4.5/1.1	0.37

its assignment as a (pharmacologically) distinct PDE family (Table 2). PDE11A is sensitive, like most other PDE families, to the nonselective inhibitor IBMX with an IC_{50} of 49.8 μM, but insensitive to the PDE3- and PDE4-selective inhibitors milrinone and rolipram. Zaprinast, an inhibitor of PDEs 1, 5, and 6, does inhibit PDE11A, but with 2-, 9-, and 80-fold lower potency, respectively, hence supporting the conclusion that PDE11A, although closely related to PDEs 5 and 6, is distinct. On the other hand, dipyridamole inhibits PDE11A with potency approximately equal to that for PDE5 and PDE6. However, other recent data (14, 16) clearly indicate that dipyridamole should not be considered specific because it can also potently inhibit PDE9 and PDE10, the rank order of relative potencies being 1 (PDE11): 1 (PDE6): 2.4 (PDE5): 3.0 (PDE10): 12.2 (PDE9). It will be of interest to examine other inhibitors to determine which of their structural features can confer selectivity for these closely related cGMP-hydrolyzing PDEs.

PDE11A appears to be expressed as at least three distinct major transcripts (Fig. 2*A*) of ≈10.5, ≈8.5, and ≈6.0 kb, thus suggesting the existence of multiple splice variants and hence PDE11A subtypes. This observation, along with the authenticity of PDE11A1, is confirmed by Western blotting of tissue lysates with multiple anti-human PDE11A antisera (Fig. 3), whereby three distinct protein bands were detected—i.e., ≈78, ≈65, and ≈56 kDa, the latter being the same size as that predicted from the PDE11A1 sequence. In the tissues examined, human prostate exhibits two major transcripts (≈10.5 and ≈6.0 kb) but only one major protein band (≈56 kDa; PDE11A1) whereas skeletal muscle expresses a single major transcript (≈8.5 kb) but all three protein bands (PDE11A1 and putative -A2 and -A3, the latter being the most abundant)—in fact, Incyte clone 826776 originated from a prostate cDNA library and full-length PDE11A1 was cloned from skeletal muscle. These data indicate the existence of multiple PDE11A splice variants (subtypes) with distinct tissue expression patterns. No transcripts were detectable in bladder, colon, heart, small intestine, stomach, and uterus by either Northern blotting or RNA dot-blotting; however, the latter revealed wider expression in kidney (adult and fetal), liver (adult and fetal), pituitary and salivary glands, and testis (Fig. 2*B*). Lower levels were also detected in adrenal, mammary, and thyroid glands as well as pancreas, spinal cord, and trachea. The precise localization of PDE11A in these tissues and potential physiological roles require further investigation.

Conclusions

Human PDE11A1 is a 490-amino acid, ≈56-kDa enzyme that represents an additional PDE gene family, PDE11A. It is most closely related to human PDE5, with 50% amino acid identity across the catalytic domain, and contains a single GAF domain at the N terminus similar to but distinct from the tandem GAF domains found in PDEs 2, 5, 6, and 10. PDE11A hydrolyzes both cGMP and cAMP with approximately equal specific activities and has a 2-fold lower K_m value for cGMP—i.e., 0.52 μM vs. 1.04 μM—and thus is a dual-substrate PDE that may regulate both cGMP and cAMP under physiological conditions. A corresponding protein is expressed in human tissues, and there is evidence for additional subtypes that exhibit distinct tissue expression patterns.

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