cDNAs encoding a novel phosphodiesterase, phosphodiesterase 11A (PDE11A), were isolated by a combination of reverse transcriptase-polymerase chain reaction using degenerate oligonucleotide primers and rapid amplification of cDNA ends. Their catalytic domain was identical to that of PDE11A1 (490 amino acids) reported during the course of this study. However, the cDNAs were isolated had N termini distinct from PDE11A1, indicating two novel N-terminal variants of PDE11A. PDE11A3 cDNA encoded a 684-amino acid protein including one complete and one incomplete GAF domain in the N-terminal region. PDE11A4 was composed of 934 amino acids including two complete GAF domains and shared 630 C-terminal amino acids with PDE11A3 but had a distinct N terminus containing the putative phosphorylation sites for cAMP- and cGMP-dependent protein kinases. PDE11A3 transcripts were specifically expressed in testis, whereas PDE11A4 transcripts were particularly abundant in prostate. Recombinant PDE11A4 expressed in COS-7 cells hydrolyzed cAMP and cGMP with $K_m$ values of 3.0 and 1.4 $\mu$M, respectively, and the $V_{\text{max}}$ value with cGMP was almost twice that with cAMP. Although PDE11A3 showed the same $K_m$ values as PDE11A4, the relative $V_{\text{max}}$ values of PDE11A3 were approximately one-sixth of those of PDE11A4. PDE11A4, but not PDE11A3, was phosphorylated by both cAMP- and cGMP-dependent protein kinases in vitro. Thus, the PDE11A1 gene undergoes tissue-specific alternative splicing that generates structurally and functionally distinct gene products.

Cyclic nucleotide phosphodiesterases (PDEs)$^1$ metabolize cAMP and cGMP, which are second messengers regulating many functions in various cells and tissues. Based on their amino acid sequence homology, biochemical properties, and inhibitor profiles, many kinds of PDEs have been identified in mammalian tissues (1–3). The PDE1 family is Ca$^{2+}$/calmodulin-dependent and hydrolyzes both cAMP and cGMP. PDE2 is stimulated by cGMP and hydrolyzes cAMP and cGMP, while PDE3 is cGMP-inhibited. The cAMP-specific and rolipram-sensitive PDEs belong to the PDE4 family. PDE5 is a cGMP-binding, cGMP-specific PDE. The photoreceptor cGMP PDEs are in the PDE6 family. PDE7 is cAMP-specific and rolipram-insensitive. PDE8 is a cAMP-specific PDE, and PDE9 is a cGMP-specific PDE (3–8). Recently, we revealed a new member of the PDE group, PDE10A, which hydrolyzes both cAMP and cGMP (9).

Some of these PDEs constitute subfamilies encoded by distinct genes. In each PDE family, alternative splice variants have been reported (1, 10, 11). In many cases, different gene products and alternative splice variants in each PDE family show different expression patterns in tissues and different subcellular localization (1, 12–22). PDEs encoded by alternatively spliced mRNAs have been reported to differ in their regulation by some kinases including cAMP-dependent protein kinase (cAK) and cGMP-dependent kinase (cGK) and associated proteins (19, 23). Thus, cyclic nucleotide levels are controlled by a complex system.

Each PDE is involved in controlling cyclic nucleotide levels and probably plays a distinct physiological role in different tissues and cells. The hydrolysis of cyclic nucleotides is multiplied by PDEs co-existing in the same cells. Therefore, the finding and characterization of novel PDEs could lead to better understanding of the complex regulatory mechanisms of cyclic nucleotide-mediated cellular functions. Novel PDEs may also be valuable as pharmacologically significant targets. cDNA cloning of PDEs, PDE9A, and PDE10A was done by an approach using bioinformatics (4–9). A search of data bases of expressed sequence tags was performed using parts of PDE sequences, such as the catalytic domain. The approach was shown to be effective, but not always successful, for the cDNA cloning of a novel PDE. Only the sequences submitted in the expressed sequence tag databases could be cloned by this procedure. To isolate novel PDE cDNAs, which have not yet appeared in the expressed sequence tag data bases, we employed an approach using PCR (polymerase chain reaction) with degenerate primers designed from a conserved sequence in the PDE catalytic domain and rapid amplification of cDNA ends (RACE) for the isolation of full-length cDNAs. Unique N-terminal splicing variants of human PDE11A were obtained, and their tissue-specific expression patterns were examined. The expression plasmids encoding two PDE11A variants were transfected into COS-7 cells, and the enzymatic properties of the recombinant proteins were investigated in detail.

**EXPERIMENTAL PROCEDURES**

*Materials*—Restriction endonucleases, DNA-modifying enzymes, 5'-Full RACE Core Set, and LA PCR$^\text{TM}$ Kit version 2.1 were obtained from...
Two Novel PDE11A Splice Variants

Takara Shuzo (Kyoto, Japan). [α-32P]dCTP, [γ-32P]ATP, [3H]GMP, hydriobond-N+ nylon membrane were from Amersham Pharmacia Biotech. The mammalian expression vector pcDNA/HisMax was purchased from Invitrogen. The GeneAmp RNA PCR Core kit was a product of PE Biosystems. SMART™ RACE CDNA amplification kit; Marathon-Ready cDNA (human prostate and hippocampus) was purchased from CLONTECH. Dipyridamole, 3-isobutyl-1-methylxanthine, erythro-9-(2-hydroxy-3-onyl)-adenine, and zaprinast were from Sigma. SCH515866, rolipram and E4021 were synthesized at Tanabe Seiyaku Co., Ltd., Japan.

Nucleotide Sequencing Analysis—The nucleotide sequence was determined by an automated DNA sequencer ABI PRISM™ 310 and a BigDye terminator cycle sequencing reaction kit (PE Biosystems). Nucleotide and amino acid sequence data were analyzed by the computer programs GENETYX (Software Development, Tokyo, Japan).

PCR Amplification of Novel PDE cDNAs with Degenerate Primers—Two degenerate, oppositely oriented oligonucleotide PCR primers designed based on actual nucleic acid sequences deduced from the most probable codons for the amino acid sequences in two highly conserved catalytic domains of a variety of PDEs (Fig. 1A). First-strand cDNA was prepared from the human testis and hippocampus mRNAs according to the manufacturer’s instructions for the Gene Amp RNA PCR Core kit. Consecutive rounds of 5′-end-5′-end (nucleotides 45–468), Marathon-Ready 9′ and primer sets A (AP1 primer plus 5′-GCGCTTGCAGCCCAGGGC-3′) and primer sets B (AP1 primer plus 5′-TGTATCCAGTTAGCTTGTC-3′) were performed through 30 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The PCR products were cloned into the TA cloning vector pGEM-T Easy (Promega), and the nucleotide sequences were then determined and compared with those of PDEs previously reported.

5′- and 3′-RACE of the Novel PDE cDNA—5′-RACE was performed using 5′-Full RACE Core Set and Marathon-Ready™ cDNA (human prostate and testis). First PCR template was prepared from the human testis mRNA, a specific antisense primer (5′-CTGCTTCAAAGAAGCTG-3′) designed from the nucleotide sequence of clone t21, and 5′-Full RACE Core Set. PCR was carried out using the LA PCR Kit and two primer sets, 5′-ATGATCUTCCTAAGGTTAGGTG-3′ plus 5′-GTGAGCGAG-GTTGCCCTAGATGTTG-3′ for first amplification and 5′-GTCACATATACCTGCTAATCCGT-3′ plus 5′-CTTACGCTTGAAGGTCATTGCG-3′ for second amplification. The PCR products were cloned and determined for the nucleotide sequence as described above. Further 5′-upstream regions were obtained using Marathon-Ready™ cDNA (human prostate and testis). For the amplification of the 5′-region of PDE11A3 (nucleotides 45–468), Marathon-Ready™ cDNA (human testis) was used as a PCR template and primer sets A (AP1 primer plus 5′-GCGCTTGCAGCCCAGGGC-3′) and primer sets B (AP1 primer plus 5′-TGTATCCAGTTAGCTTGTC-3′) were used for first PCR and second PCR amplification, respectively. The PCR products were cloned into the TA cloning vector pGEM-T Easy (Promega), and the nucleotide sequences were then determined and compared with those of PDEs previously reported.

3′-RACE was performed using the SMART™ RACE cDNA Amplification kit with human thyroid mRNA according to the manufacturer’s instructions for the SMART™ RACE cDNA Amplification kit. PCR was performed with 3′-SMART cDNA, two primer sets (UPM primer plus 5′-ACACAATGCCTTCCAAGAGGTG-3′ for first PCR and UPM primer plus 5′-TCAAGCTTCTCATTGCTTCAAAGAAGCTG-3′ for second PCR), and Advantage 2 Polymerase Mix. Two splice variants of PDE11A1 were obtained as shown in Fig. 1B.

In all RACE, reaction cycles were as follows: 94 °C for 1 min; five cycles of 94 °C for 30 s, 72 °C for 3 min; five cycles of 94 °C for 30 s, 70 °C for 30 s, 72 °C for 3 min; and 25 cycles of 94 °C for 30 s, 68 °C for 30 s, 72 °C for 3 min. The PCR products were cloned into pGEM-T Easy and sequenced.

Reverse Transcriptase (RT)-PCR Analyses—for the determination of human prostate mRNAs and the primer set (the 5′-primer 5′-GGCGCTTGCAGCCCAGGGC-3′ and 3′-primer 5′-TCAAGCTTCTCATTGCTTCAAAGAAGCTG-3′ (covering nucleotides 1–2185 of PDE11A3 cDNA)) were used for PCR of PDE11A3. The cDNA synthesized from human prostate mRNAs and the primer set (the 5′-primer 5′-TGGCGCTTGACCATGGAGATCTGTTGAGGA-3′ and 3′-primer 5′-GCGCTTGCAGCCCAGGGC-3′ and 3′-primer 5′-TCAAGCTTCTCATTGCTTCAAAGAAGCTG-3′ (covering nucleotides 204–3164 of PDE11A4 cDNA)) were used for PCR of PDE11A4. PCR was carried out with conditions of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. The amplified DNA fragment was cloned into pGEM-T Easy. Six independent PCR clones of PDE11A3 or PDE11A4 were sequenced to verify the correct cDNA sequence. One of the clones, pGEM-PDE11A3F or pGEM-PDE11A4F, was used for further experiments.

Northern Blot and Dot Blot Analyses—Human MTE™ Array CLONTECH, Human Testis, Thyroid, Prostate, and Hippocampus cDNA Arrays (Clontech Laboratories, Palo Alto, Calif., USA), and E4021 were hybridized with a 32P-labeled DNA probe prepared from cDNA encoding a common region of the human PDE11A3 cDNAs. Hybridization was performed in 6× SSC, 0.5% SDS, 5× Denhardt’s solution, 100 mg/ml salmon sperm DNA, and the 32P-labeled probe at 55 °C for 2 h. All blots were washed finally in 6× SSC and 0.5% SDS at 55 °C for 15 min. The membranes were exposed to x-ray film at −80 °C for 3 days.

PCR and Southern Blot Analyses—To examine expression patterns of human PDE11A3 and PDE11A4 in transcripts in human tissues, PCR was performed using MTC panels (CLONTECH) as templates and Advantage 2 Polymerase Mix. The cDNA fragments encoding PDE11A3 (amino acid residues 21–272) were produced using the 5′-primer 5′-AAGGTGAATTCAACAGACCTGTTGCT-3′ and the 3′-primer 5′-GGTTGCTTCTCATTGCTTCAAAGAAGCTG-3′. PCR was performed through 32 or 27 cycles of denaturation at 95 °C for 30 s and extension at 68 °C for 1 min. The PCR products were subjected to 1.5% agarose gel electrophoresis, and the fractions were transferred onto Hybond-N+ nylon membrane. To confirm that PCR products were derived from human PDE11A1 transcripts, we detected both PCR products by Southern blot analysis using a 32P-labeled DNA probe prepared from an oligonucleotide (TGGGAATCGCCATATCTAACGCT) coding for a common region of the human PDE11A cDNAs. Hybridization was performed in 6× SSC, 0.5% SDS, 5× Denhardt’s solution, 100 mg/ml salmon sperm DNA, and the 32P-labeled probe at 55 °C for 2 h. All blots were washed finally in 6× SSC and 0.5% SDS at 55 °C for 15 min. The membranes were exposed to x-ray film at −80 °C for 1 day. All of the PCR reactions were performed under conditions in which each amplification did not reach saturation.

Construction of Expression Plasmids—To generate an expression plasmid of PDE11A3, PCR was performed using the 5′-primer 5′-GGATCCATGGCAGCCTCC-3′ and the 3′-primer 5′-TTCATCATTCTGAGTAACTG-3′ (covering amino acid residues 1–106 of PDE11A3), and the amplified DNA fragment was cloned into pGEM-T Easy, and then confirmed by sequencing. The SacI–EcoRI and EcoRI–SalI DNA fragments of pGM-PDE11A3F, and the BamHI–SacI DNA fragment of pGM-PDE11A3BM were subcloned into the BamHI and Xhol sites of pcDNA4/HisMax (pHis), resulting in pHis-PDE11A3. To generate a mammalian expression plasmid of human PDE11A4, PCR was performed using the 5′-primer 5′-GCGCTTGCAGCCCAGGGC-3′ and the 3′-primer 5′-GAGGTCACAATTTGAGATCTGTTGAGGA-3′. The amplified DNA fragment was cloned into pGEM-T Easy, resulting in pGEM-PDE11A4BM, and then confirmed by sequencing. The KpnI–SauI DNA fragment of pGM-PDE11A4f and the BamHI–KpnI DNA fragment of pGM-PDE11A4BM were subcloned into the BamHI and Xhol sites of the mamalian expression vector, pHis, resulting in pHis-PDE11A4.

Expression of Human PDE11A3 and PDE11A4 in COS-7 Cells—COS-7 cells were grown in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C with 5% CO2 and were serially passaged before reaching confluence. The expression plasmid pHis-PDE11A3 or pHis-PDE11A4 was transfected into COS-7 cells by LipofectAMINE PLUS (Life Technologies, Inc.), according to the manufacturer’s instructions. 24 hours after transfection, cells were washed with ice-cold phosphate-buffered saline and scraped in ice-cold homogenization buffer (40 mM Tris-HCl, pH 7.5, 15 mM benzamidine, 5 μM β-galactosidase, and 5 μM leupeptin). The homogenates were sonicated for 15 s (three times with 1-min intervals), and the homogenates were centrifuged at 100,000 × g for 1 h. The resultant supernatant was added to a plastic tube containing nickel nitrolotriacetate resin (QIA-GEN), equilibrated with the homogenization buffer, and incubated by rotation at 4 °C for 4 h. The nickel nitrolotriacetate resin was poured into a plastic column (0.8 × 5 cm) and allowed to drain. The packed
resin was washed with wash buffer (40 mM Tris-HCl, pH 7.5, 15 mM benzamidine, 200 mM NaCl, 5 mM imidazole, 5 µg/ml pepstatin A, and 5 µg/ml leupeptin), and the proteins were then eluted by elution buffer (40 mM Tris-HCl, pH 7.5, 15 mM benzamidine, 200 mM NaCl, 200 mM imidazole, 5 µg/ml pepstatin A, and 5 µg/ml leupeptin). After PDE assay, the PDE11A fractions were diluted with glycerol at a final concentration of 50% and stored at −25 °C until use.

**PDE and Protein Assays**—The PDE assay was performed by the radiolabeled nucleotide method as described previously (9). Relative $V_{\max}$ values were determined according to the methods of McPhee et al. (14). Relative concentrations of PDE11A proteins expressed in COS-7 cells were calculated by immunoblotting with anti-Xpress polyclonal antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), as we described previously (25). The membranes were incubated with ECL reagents at room temperature for 1 min and then exposed to x-ray film for 2–10 s, under conditions in which each exposure to x-ray film did not reach saturation. The resultant films were scanned by ARCUS II (Agfa-Gevaert), and quantitated using the Quantity One program (PDI, Inc.). The optical densities versus the amount of pHis-encoded protein were plotted to measure the relative concentrations of PDE11A proteins.
FIG. 2. Nucleotide and deduced amino acid sequences of the human PDE11A cDNA. A. DNA and amino acid sequences of human PDE11A4. The deduced amino acid sequences are shown in three-letter designations below the nucleotide sequence. The termination codon at the
Relative Vmax values were calculated from Lineweaver-Burk plots (26), using proteins that provided relatively equal enzymatic activity. The protein concentration of the cytosolic fractions of transfected COS-7 cells was determined by a protein assay kit (Bio-Rad) using bovine serum albumin as a standard.

In Vitro Kinase Assay—The full-length bovine cGK II cDNA was a gift from Dr. Thomas M. Lincoln (University of Alabama at Birmingham). The full-length human cGK Iβ cDNA and mouse cAK catalytic subunit α cDNA were obtained by standard PCR protocol as we described previously (25) and subcloned into pHis. To prepare truncated and constitutively active cGK I (cGK Iα) (27), the PstI–SalI DNA fragment of human cGK Iβ (covering amino acid residues 325–685) was subcloned into the PstI and XhoI sites of pHis. Site-directed mutagenesis was performed using the QuickChange™ site-directed mutagenesis kit (Stratagene) according to the protocol of the manufacturer. To introduce the desired mutations, the following primers were used: 5′-CTGACGCGGAGAGCTGTCGAAGAAAGCTGAGG-3′ plus 5′-CTTCTCTTGAGCAGCTCTCCGCAGAGG-3′ (PDE11A4 S117A); and 5′-CTTCTCCGGAAGGAGAAGCTCCTCCGGAGAAG-3′ plus 5′-GGTGGGGGCCAGGGAGACTCTGGTGTAAG-3′ (PDE11A4 S162A). In each case, the mutation was confirmed by DNA sequencing analysis.

The full-length cGK Iα, cGK Iβ, cAK, or truncated cGK I α cDNAs in the expression vector pHis were transiently expressed in COS-7 cells. 24 h after transfection, cells were washed with ice-cold phosphate-buffered saline twice and scraped in an ice-cold TNE buffer (10 mM Tris-HCl at pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mg/ml aprotinin, 10 mM leupeptin, and 1 mM dithiothreitol). Cell extracts were centrifuged at 16,000 × g for 15 min at 4 °C to remove cellular debris. The supernatants were incubated with anti-Xpress antibody and protein G-Sepharose overnight at 4 °C by rotation. The beads were washed three times with TNE buffer, and the immunoprecipitated samples were used for the in vitro kinase assay. Likewise, cell extracts of COS-7 cells transfected with either pHis–PDE11A3 or pHis–PDE11A4 were immunoprecipitated with anti-Xpress antibody. In vitro kinase assays were performed as described previously (25). Reactions were performed in the presence or absence of cGMP (5 μM final concentration). Phosphorylation by cAK was performed in the same buffer but in the presence of 5 μM TNE buffer (+ TNE buffer). The position of the amino acid sequence in common with PDE11A4 is shown by an arrow. The in-frame termination codon of human PDE11A4 is underlined. The in-frame termination codon upstream of the initiation codon of the ORF is double-underlined.
Six independent PCR clones were sequenced to verify the correct cDNA sequence for the full coding region. The C-terminal moiety of PDE11A4 (Met<sup>415</sup>-Asn<sup>934</sup>) was identical to the entire PDE11A1 sequence (Met<sup>1</sup>-Asn<sup>490</sup>.

The nucleotide sequence encoding the N-terminal region of PDE11A3 is shown in Fig. 2B. The presence of the transcripts coding for the PDE11A3 ORF was also confirmed by RT-PCR analysis, using the specific primers for the PDE11A3 sequence and human testis mRNA as a template. Specific PCR products of approximately 2.2 kb, which were in good agreement with the length of the predicted product, were obtained (data not shown). The amplified DNA fragment was cloned into pGEM-T Easy and sequenced to confirm the PDE11A3 sequence.

As shown in Fig. 2B, the C-terminal sequence (630 amino acids) of PDE11A3 (Asp<sup>56</sup>-Asn<sup>934</sup>) was identical to that of PDE11A4 (Asp<sup>503</sup>-Asn<sup>934</sup>), except for the unique N-terminal portion. The PDE11A3 protein was 194 amino acids longer than the PDE11A1 protein.

The deduced amino acid sequences of the ORFs were searched using SMART (Simple Modular Architecture Research Tool) (34), and compared with those of human PDEs reported. PDE11A4 was revealed to contain two complete GAF (cGMP binding and stimulated phosphodiesterases, Anabaena adenylyl cyclases, and Escherichia coli FhlA) domains (amino acid residues 217-380 and 402-568) (35) and a catalytic domain sequence of approximately 2.2 kb, which were in reasonable agreement with the molecular masses of 78,713 Da. As shown in Fig. 1B, the C-terminal sequence (630 amino acids) of PDE11A3 (Asp<sup>56</sup>-Asn<sup>934</sup>) was identical to that of PDE11A4 (Asp<sup>503</sup>-Asn<sup>934</sup>), except for the unique N-terminal portion. The PDE11A3 protein was 194 amino acids longer than the PDE11A1 protein.

The complete GAF domains of PDE11A4 showed 19-47% identity with those of PDEs including human PDE2A, PDE5A, and PDE6s, and one incomplete (amino acid residues 1-130) GAF domain. The complete GAF domains of PDE11A4 showed 19-47% identity with those of PDEs including human PDE2A, PDE5A, and PDE6s, and PDE11A4 (Fig. 3). The catalytic domain sequence of PDE11A4 showed high identity (42-51%) with those of PDEs having GAF domains (data not shown), and that of PDE11A4, but not PDE11A3, had typical phosphorylation sites (RRA<sup>117</sup>S and RKA<sup>162</sup>S) for cAK (RR<sup>X</sup>S) and cGK (RR<sup>X</sup>S/T), respectively, in the N-terminal region (36).

**Tissue Distribution of Human PDE11A Transcripts**—Dot blot analysis of human mRNA was performed using a <sup>32</sup>P-labeled PDE11A cDNA probe corresponding to the common region of PDE11A3 and PDE11A4 transcripts (Fig. 4A). The amounts of the mRNAs loaded were normalized using cDNAs for human ubiquitin and major histocompatibility complex class Ic as probes (described in the instructions from CLONTECH). PDE11A transcripts were particularly abundant in prostate. Moderate expression was observed in testis, salivary gland, pituitary gland, thyroid gland, and liver. Northern blot analysis of multiple human tissues was performed with the same <sup>32</sup>P-labeled probe (Fig. 4A). A band of approximately 3 kb was detected in testis, and a major band of approximately 6 kb and minor bands of 2 and 10 kb were observed in prostate.

In some cases, alternative splice variants in each PDE family show different expression patterns in tissues. The expression patterns of each PDE11A3 and PDE11A4 transcript in human tissues were examined by a combination of PCR and Southern blot analysis. To know the relative amounts of PDE11A3 and PDE11A4 transcripts, the efficiency of PCR amplification using specific primer sets for PDE11A3 and PDE11A4 was first examined as follows. PCR was performed using the same amounts of pHis-PDE11A3 and pHis-PDE11A4 DNAs as a template under the same conditions, revealing that amplification using the primer set for PDE11A3 was more efficient than that using the primer set for PDE11A4 (data not shown).

Considering the degree of efficiency and the condition that PCR did not reach saturation, PCR was carried out and Southern blot analysis was performed using an oligonucleotide probe from a common sequence. The ratio of the two PCR products amplified using specific primer sets and MTC panels as templates reflects the amounts of their transcripts. Interestingly, PDE11A3 transcripts were specific in testis, whereas PDE11A4 transcripts were particularly abundant in prostate (Fig. 5).

**Expression of Human PDE11A3 and PDE11A4 in COS-7 Cells**—To produce recombinant human PDE11A3 and PDE11A4 proteins, full-length cDNAs for these variants were subcloned into the N-terminal histidine tag mammalian expression vector pCMV6-AC (data not shown) and cloned into COS-7 cells. Cytosolic fractions were prepared from COS-7 cells transfected with an expression vector encoding histidine-tagged PDE11A3 or PDE11A4 (pHis-PDE11A3 or pHis-PDE11A4). The proteins were analyzed by immunoblotting using anti-Xpress polyclonal antibody, which reacts with histidine-tagged proteins. While no signal was observed in the mock-transfected cells, specific bands of approximately 78 and 100 kDa, which were in reasonable agreement with the molecular masses pre-
dicted for the histidine-tagged PDE11A3 and PDE11A4, were detected in cytosolic fractions of cells transfected with the expression plasmids pHis-PDE11A3 and pHis-PDE11A4, respectively (data not shown). The cytosolic fractions were assayed for cyclic nucleotide hydrolytic activities using either 1 mM cAMP or 1 mM cGMP. Both cytosolic fractions from COS-7 cells transfected with pHis-PDE11A3 or pHis-PDE11A4 exhibited ~20- and ~75-fold higher levels of cAMP and cGMP hydrolytic activities, respectively, than those from the mock-transfected COS-7 cells (data not shown).

**Kinetic Properties of Human PDE11A Enzyme**—To determine \( K_m \) and \( V_{max} \) values, the histidine-tagged PDE11A3 and PDE11A4 proteins were partially purified by using a nickel affinity column. The eluate prepared from PDE11A-expressing cells, but not from mock-transfected cells, exhibited cAMP and cGMP PDE activities. The relative concentrations of the partially purified histidine-tagged PDE11A3 and PDE11A4 proteins were measured by immunoblotting (Fig. 6B). The \( K_m \) values of PDE11A3 and PDE11A4 were derived from Lineweaver-Burk plots (26) of activities using cGMP or cAMP as substrate (0.1–10 mM) for the partially purified histidine-tagged PDE11A3 and PDE11A4 proteins. The \( K_m \) values of the human PDE11A4 for cAMP and cGMP were 3.0 ± 0.26 and 1.4 ± 0.06 mM, respectively. \( V_{max} \) values of PDE11A4 for cAMP and cGMP hydrolysis were 270 ± 28 and 120 ± 4.7 pmol/min/μg with the partially purified recombinant protein, respectively. As shown in Table I, both cAMP and cGMP \( K_m \) values of PDE11A3 were almost the same as those of PDE11A4 (cAMP and cGMP \( K_m \) values of PDE11A3 were 3.0 ± 0.28 and 1.5 ± 0.07 mM, respectively). Relative \( V_{max} \) values were calculated to compare the \( V_{max} \) of PDE11A3 with that of PDE11A4. The \( V_{max} \) values of PDE11A3 relative to PDE11A4 (i.e., \( V_{max} = 1.0 \)) were 0.16 ± 0.01 for cAMP and 0.17 ± 0.03 for cGMP. However, the \( V_{max} \) ratio (cAMP/cGMP) of PDE11A4 (2.2 ± 0.40) was very similar.

**Fig. 4. Analysis of expression of human PDE11A transcripts in various tissues by dot blot and Northern blot.** Hybridization was carried out with a \(^{32}\)P-labeled fragment of human PDE11A cDNA under the conditions described under "Experimental Procedures." A, dot blot of mRNAs from various human tissues obtained from CLONTECH was hybridized with the \(^{32}\)P-labeled probe. RNA sources are shown in the diagram. B, Northern blot analysis of mRNAs from several human tissues. The PDE11A transcripts were detected using the same \(^{32}\)P-labeled probe. The sizes (in kb) and positions of mRNA size markers are shown on the left.

**Fig. 5. Detection of two spliced transcripts of human PDE11A3 and PDE11A4 by PCR.** PCR was performed using MTC panels (CLONTECH) as templates. PCR amplification was carried out through 32 cycles for human PDE11A3 (756 base pairs) and 27 cycles for human PDE11A4 (756 base pairs), under conditions in which PCR amplification did not reach saturation. The PCR products of PDE11As were subjected to Southern blot analysis using the \(^{32}\)P-labeled DNA probe to detect both PCR products. As a control, PCR was performed using 0.01 ng/ml pHis-PDE11A3 or pHis-PDE11A4 plasmid DNA. The same results have been obtained with two separate PCR analyses.
The inhibitory effects of PDE inhibitors on PDE11A3 activity were 2–3-fold more potent than those on PDE11A4.

The inhibitory effects of cGMP on cAMP hydrolysis and vice versa were also examined using the partially purified PDE11A3 and PDE11A4. cAMP hydrolysis was measured in the presence of 3.5 μM cAMP and a range of cGMP concentrations of 0.01–100 μM. The reverse was also performed in the presence of 1.3 μM cGMP and a range of cAMP concentrations of 0.01–100 μM. Neither cGMP nor cAMP stimulated hydrolytic activity (data not shown). cAMP and cGMP inhibited the activities of cGMP and cAMP hydrolysis of PDE11A4 with IC$_{50}$ values of 9.0 ± 0.38 and 3.1 ± 0.16 μM, respectively (Table II).

**Other Characteristics of PDE11A4: Phosphorylation by cAK and cGK and Cyclic Nucleotide Binding**—Some PDEs have been reported to be regulated by phosphorylation by kinases including cAK and cGK (1). Human PDE11A4, but not PDE11A3, also contains typical phosphorylation sites (RRA$^{117}S$ and RKA$^{162}S$) of both cAK (RRXS) and cGK (RRXS/S/T) in its N terminus (36). To determine whether PDE11A4 is phosphorylated by cAK, cGK, or both, an in vitro kinase assay was performed using the recombinant histidine-tagged PDE11A4 protein. PDE11A1, but not PDE11A3, was phosphorylated by cAK catalytic subunit α (Fig. 7A), and its phosphorylation was almost completely inhibited by cAK inhibitor peptide (Fig. 7B). Phosphorylation of PDE11A4 by cGK I was examined using a truncated form of cGK Iα, cGK Iα (amino acid residues 322–685 of human cGK Iα), because the molecular sizes of autophosphorylated cGK I subunits are similar to that of PDE11A3. cGK Iα as well as cAK phosphorylated PDE11A4 but not PDE11A3, although the phosphorylation levels of PDE11A4 by cGK Iα were lower than those by cAK. In addition, both full-length cGK Iα and cGK Iα also phosphorylated PDE11A4 in a cGMP-dependent manner. The phosphorylation of potential residues Ser$^{117}$ and Ser$^{162}$ by cAK and/or cGK was further examined using site-directed mutagenesis. The mutant PDE11A4 S117A/S162A carrying double substitutions of Ser$^{117}$ and Ser$^{162}$ with Ala, showed significant reduction in cAK- and cGK-mediated $^{33}$P incorporation compared with wild type PDE11A4 (Fig. 7C).

Cyclic nucleotide binding activity was also examined using cytosolic fractions from COS-7 cells transfected with PHis-PDE11A4. The expression level of the histidine-tagged PDE11A4 protein in the transfected COS-7 cells was shown to be equal to that of the histidine-tagged PDE5A1 used as a positive control by immunoblot analysis using anti-Xpress antibody. B, Lineweaver-Burk plots at concentrations of 0.1–10 μM cAMP (closed circles) and cGMP (open circles) are shown. Partially purified PDE11A4 was prepared as described above. $K_m$ and $V_{max}$ values are the means of triplicate assays ± S.D. A plot typical of three independent experiments is shown.

to that of PDE11A3 (2.4 ± 0.37).

The effects of various PDE inhibitors on PDE11A3 and PDE11A4 activities were examined using the partially purified proteins described above (Table II). The nonspecific PDE inhibitor, 3-isobutyl-1-methylxanthine, showed a weak inhibitory effect on PDE11A4 (IC$_{50}$ values were 65 ± 13 μM for cAMP and 81 ± 16 μM for cGMP). Vinpocetine, erythro-9-(2-hydroxy-3-nonyl)-adenine, milrinone, and rolipram, which are PDE1, PDE2, PDE3, and PDE4 inhibitors, respectively, were inactive up to 100 μM. Compounds that inhibit PDE5 showed inhibitory effects on PDE11A4. Zaprinast demonstrated moderate inhibition (IC$_{50}$ = 26 ± 6.8 μM for cAMP and 33 ± 5.3 μM for cGMP). SCH51866, a PDE1 and PDE5 inhibitor (37), inhibited PDE11A4 with IC$_{50}$ values of 22 ± 1.8 μM for cAMP and 25 ± 5.8 μM for cGMP. E4021, a more potent PDE5 inhibitor (38), showed IC$_{50}$ values of 1.8 ± 0.33 μM for cAMP and 1.8 ± 0.25 μM for cGMP. Among the PDE inhibitors tested, dipyridamole was the most effective antagonist for PDE11A4, with IC$_{50}$ values of 0.82 ± 0.28 μM for cAMP and 0.72 ± 0.08 μM for cGMP. The inhibitory effects of PDE inhibitors on PDE11A3 activity were 2–3-fold more potent than those on PDE11A4.

**DISCUSSION**

Two kinds of full-length cDNAs of PDE11A were isolated by an approach using PCR with degenerate primers designed from highly conserved regions in catalytic domains of PDE families and RACE. The application of this strategy to cloning a novel PDE cDNA has already been reported (39), but by selecting the sequences for designing the PCR primer sets, it was also effective to isolate cDNA encoding a novel class of PDE. The two clones obtained included a catalytic domain identical to PDE11A1, which was quite recently isolated from a human skeletal muscle cDNA library during the course of this study (30), but they had two distinct and unique N termini, indicating two novel N-terminal splice variants of PDE11A. PDE11A1 was composed of 490 amino acids, whereas PDE11A3 and PDE11A4 were 784 and 934 amino acids, respectively. It is
Inhibitory effect of the various PDE inhibitors on human PDE11A variants

Partially purified PDE11A3 and PDE11A4 produced in COS-7 cells were used for the assay. The concentrations of cAMP and cGMP used were 3.5 and 1.3 μM, respectively. IC_{50} values were calculated by linear regression. Data are the means of three separate determinations ± S.D. All assays were performed in duplicate. IBMX, 3-isobutyl-1-methylxanthine; EHNA, erythro-9-(2-hydroxy-3-nonyl)adenine; ND, not determined.

| Inhibitor     | IC_{50} values for PDE11A3 | IC_{50} values for PDE11A4 |
|---------------|----------------------------|----------------------------|
|               | cAMP                      | cGMP                      | cAMP                      | cGMP                      |
|               | μM                        | μM                        | μM                        | μM                        |
| IBMX          | 30 ± 3.9                  | 38 ± 3.5                  | 65 ± 13                   | 81 ± 16                   |
| Vinpocetine   | 49 ± 9.2                  | 68 ± 4.0                  | >100                      | >100                      |
| EHNA          | >100                      | >100                      | >100                      | >100                      |
| Milrinone     | >100                      | >100                      | >100                      | >100                      |
| Rolipram      | >100                      | >100                      | >100                      | >100                      |
| Zaprinast     | 18 ± 10                   | 11 ± 3.6                  | 26 ± 6.8                  | 33 ± 5.3                 |
| Dipyridamole  | 0.36 ± 0.11               | 0.34 ± 0.09               | 0.82 ± 0.28               | 0.72 ± 0.08               |
| SCH51666     | 11 ± 4.8                  | 8.6 ± 2.7                 | 22 ± 1.8                  | 25 ± 5.8                 |
| E4021         | 0.88 ± 0.13               | 0.66 ± 0.19               | 1.8 ± 0.33                | 1.8 ± 0.25               |
| cAMP          | ND                        | 8.2 ± 0.43                | ND                        | 9.0 ± 0.38               |
| cGMP          | 5.1 ± 0.12                | 3.1 ± 0.16                | 16 ND                     |

Intriguing that two novel PDE11A variants have distinct N termini from PDE11A1. A search using SMART revealed that PDE11A4 contains two complete GAF domains (amino acid residues 217–380; E = 7.1 × 10^{-20}) and amino acid residues 402–568, E = 3.6 × 10^{-25}, whereas PDE11A3 has one complete (amino acid residues 152–318, E = 3.6 × 10^{-25}) and one incomplete GAF domain (amino acid residues 1–130, E = 7.4 × 10^{-7}). On the other hand, PDE11A1 has an incomplete GAF domain, which lacks the N-terminal part of the GAF consensus sequence (E = 2.0 × 10^{-6}). Alterations of the calmodulin-binding domain and upstream conserved regions have been reported in N-terminal variants of PDE1 and PDE4. However, no report has described the alteration of the GAF domain in splice variants of PDEs containing the GAF domain, although many splice variants of PDEs containing the GAF domain have been reported (10, 11, 40–43). Thus, PDE11A1 constitutes a unique family distinct from other PDEs including the GAF sequence.

In PDE5A, the motif N(K/R)X_{3}FX_{3}D in the GAF domain has been known to be necessary for the support of cGMP binding (44, 45). PDE11A4 also contains all of four residues of this motif in both GAF domains. However, unexpectedly, the cGMP binding activity of PDE11A4, which was expressed as a histidine-tagged protein in COS-7 cells, was much lower than that of PDE5A1 under the conditions used in this study (see “Experimental Procedures”). No significant cAMP binding activity was observed under those conditions. Although PDE2A, PDE5A, and PDE6s proteins show apparent cGMP binding function, that of PDE10A has been reported to be insignificant (3, 9). As shown in the case of PDE11A4, it is likely that cGMP binding is not the function of the GAF domain in all cases. For example, E. coli FhlA, a transcriptional regulatory protein, is known to bind formate within the N terminus, which contains two GAF domains (46). Further study will elucidate the function of the GAF domain in PDE11A variants, including PDE11A1 and PDE11A3.

The differences in the biochemical characteristics of the PDE11A variants are intriguing. The first difference concerns enzymatic characteristics. The catalytic domain of PDE11As was the most homologous to that of PDE5A in the PDE families, and vice versa. In addition, the structure of PDE11A4, including two complete GAF domains, was very similar to that of PDE5A. Interestingly, although PDE5A is highly specific for cGMP, both PDE11A variants demonstrated hydrolytic activity not only for cGMP but also for cAMP when expressed in COS-7 cells, indicating that PDE11As resemble PDE2A and PDE10A. However, PDE11As were activated by neither cAMP nor cGMP, and the K_{m} values of PDE11A for cAMP and cGMP were almost the same, being distinguishable from PDE2A and PDE10A. These characteristics supported the position that PDE11A is a distinct family from other PDEs containing the GAF domain. Patterns of the inhibitory effects of PDE inhibitors used on PDE11A3 and PDE11A4 activities were similar to those of PDE11A. Dipyridamole was the most effective against these PDE11A variants. We found differences in the relative V_{max} and in the sensitivity to PDE inhibitors of PDE11A3 and PDE11A4, suggesting that the N-terminal region of PDE11A affects the conformation of the protein, leading to the change of enzymatic profile. Similar effects of N-terminal splicing variability have been demonstrated for some PDEs including PDE1A, PDE1C, PDE4A, PDE4B, and PDE7A (15, 16, 18, 20, 47). For example, rat PDE4A isoforms have been reported to exhibit 2–5-fold differences in their V_{max} values and in their sensitivity to the PDE4-specific inhibitor, rolipram (16). Distinct N termini derived from alternative splicing may provide PDE11A variants with different enzymatic profiles.

The second difference lies in the presence of phosphorylation sites. PDE11A4, but not PDE11A3, contains typical phosphorylation sites (RRA^{11}S and RKA^{16}S), allowing both cAK (RRXS) and cGK (RKX(S)T) in the N terminus (36). In vitro kinase assays suggested that PDE11A4, but not PDE11A3, is a good substrate for both cAK and cGK, although the phosphorylation by cGK was weaker than that by cAK. The double mutant (PDE11A4 S117A/S162A) was still phosphorylated by both cAK and cGK, at a lower level, indicating that additional phosphorylation sites may be present in PDE11A4. However, its additional phosphorylation sites would be located in the N terminus of PDE11A4, because no phosphorylation of PDE11A3 by cAK and cGK I was observed. Modifications of PDE activity by phosphorylation have been reported in some PDEs. For example, PDE1A is phosphorylated by cAK, and thus its affinity for calmodulin is reduced (48). PDE3 and PDE4 are actually phosphorylated by cAK and activated by agents that increase cAMP levels in intact cells and by cAK in vitro (49–51). Binding of cGMP to noncatalytic binding sites in the regulatory domain of PDE5A enhances the phosphorylation of PDE5A by cGK (52, 53). Further work is needed to determine whether phosphorylation of PDE11A4 occurs in vivo and also to
determine what effect is brought out by the phosphorylation of PDE11A4.

The third difference involves tissue expression patterns. In many cases, alternative splice variants in each PDE family show different expression patterns in tissues and different subcellular localization (1, 12–20). Human PDE11A transcripts were highly expressed in prostate and moderately in testis. PCR and Southern blot analyses demonstrated that PDE11A variants also showed different tissue expression patterns, although it is not yet known whether there is a difference in subcellular localization. PDE11A3 transcripts were specifically expressed in testis, whereas PDE11A4 transcripts were strongly expressed in prostate. Testis-specific expression of PDE11A3 variants implies the production of PDE11A3 transcripts under the control of a testis-specific promoter. The genomic origin of PDE11A variants is interesting from the view of the formation of multiple variants and their specific regulation.

In regard to the physiological function of PDE11A, the following factors should be considered. Many PDEs have been reported to exist in the testis. Several works have focused on the cAMP-signaling pathway during sperm differentiation (54–58), whereas cGMP has been shown to control the Ca^{2+} entry into sperm through a cyclic nucleotide-gated channel, suggesting that the cGMP signaling pathway may be involved in sperm motility (59, 60). In prostate, PDEs have been little studied, but several reports have described the physiological roles of cAMP and cGMP. The elevation of intracellular cAMP in human prostate cancer cells has been demonstrated to induce neuroendocrine differentiation (61–63). The PDE inhibitors, 3-isobutyl-1-methylxanthine and papaverine, also initiate morphologic differentiation in human prostate cancer cells and inhibit the proliferation and invasive potential of the cells (61, 62). Furthermore, withdrawal of the agents that increase cAMP causes rapid loss of the neuroendocrine phenotype, indicating that chronic cAMP signaling is required to block the proliferation of prostate tumor cells and to induce neuroendocrine differentiation (63). On the other hand, nitric oxide, which produces cGMP via the activation of soluble guanylyl cyclase, has been shown to play a role in the regulation of the contractile function of smooth muscle cells and the growth of several types of cells. In prostate, nitric oxide has been reported to function as a mediator of prostate smooth muscle activity (64). In addition, a recent study has demonstrated that both nitric oxide donors and cGMP analogs exert antiproliferative actions in human prostatic smooth muscle cells (65). These reports suggest that the involvement of a cAMP and cGMP PDE, PDE11A, in controlling prostate or testis functions is plausible. The precise localization of PDE11A in prostate and testis and further analysis will clarify the physiological roles of PDE11A.

In conclusion, we revealed the structure and tissue-specific expression patterns of transcripts of a novel human PDE, PDE11A. Presently, the physiological role of this enzyme remains unknown. Analysis of tissue distribution in detail by means of in situ hybridization and immunohistochemical analyses will be informative in revealing the role of this enzyme. Pharmacological analysis using selective inhibitors for this en-
zyme will elucidate new physiological functions of cAMP or cGMP in prostate and testis.

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REFERENCES

1. Beavo, J. A. (1995) Physiol. Rev. 75, 725–748
2. Burns, F., Zhao, A. Z., and Beavo J. A. (1996) Adv. Pharmacol. 36, 29–48
3. Soderling, S. H., and Beavo, J. A. (2000) Curr. Opin. Cell Biol. 12, 174–179
4. Soderling, S. H., Bayuga, S. J., and Beavo, J. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5964–5968
5. Fisher, D. A., Smith, J. F., Pillar, J. S., St. Denis, S. H., and Cheng, J. B. (1998) Biochem. Biophys. Res. Commun. 246, 570–577
6. Sutoh, K., Bayuga, S. J., and Beavo, J. A. (1998) J. Biol. Chem. 273, 15553–15558
7. Fisher, D. A., Smith, J. F., Pillar, J. S., St. Denis, S. H., and Cheng, J. B. (1998) J. Biol. Chem. 273, 15559–15564
8. Hayashi, M., Matsuoka, K., Ohashi, H., Tsunoda, M., Murase, A., Kawara Y., and Tanaka, T. (1998) Biochem. Biophys. Res. Commun. 250, 751–756
9. Fujishige, K., Koteria, J., Michibata, H., Yusa, K., Takebayashi, S., Okumura, K., and Omori, K. (1999) J. Biol. Chem. 274, 18483–18488
10. Koteria, J., Fujishige, K., Akatsu, H., Imai, Y., Yanaka, N., and Omori, K. (1999) J. Biol. Chem. 273, 26882–26890
11. Koteria, J., Fujishige, K., Yusa, K., and Omori, K. (1999) Biochem. Biophys. Res. Commun. 261, 551–557
12. Yan, C., Bentley, J. K., Sonnenburg, W. K., and Beavo, J. A. (1994) J. Neurosci. 14, 973–984
13. Reinhardt, R. R., Chiu, E., Zhou, J., Taira, M., Murata, T., Manganelli, V. C., and Bond, C. A. (1995) J. Clin. Invest. 95, 1528–1538
14. Han, P., Zhu, X., and Michaeli, T. (1997) J. Biol. Chem. 272, 16152–16157
15. Degerman, E., Belfrage, P., and Manganiello, V. C. (1997) J. Biol. Chem. 272, 6823–6826
16. Han, P., Zhu, X., and Michaeli, T. (1997) J. Biol. Chem. 272, 16152–16157
17. Rust, S. L., Shepherd, S., Reifsnyder, D. H., Moore, T. A., Lerea, K. M., and Beavo, J. A. (1999) J. Biol. Chem. 274, 10553–10558
18. Sette, C., Iona, S., and Conti, M. (1994) J. Biol. Chem. 269, 9245–9252
19. Sette, C., and Conti, M. (1996) J. Biol. Chem. 271, 16526–16534
20. Thomas, M. K., Francis, S. H., and Corbin, J. D. (1990) J. Biol. Chem. 265, 14971–14975
21. Turko, I. V., Francis, S. H., and Corbin, J. D. (1998) J. Biol. Chem. 273, 505–510
22. Macphee, C. H., Reddsnyder, D. H., Moore, T. A., Lerea, K. M, and Beavo, J. A. (1988) J. Biol. Chem. 263, 6875–6880
23. Salanova, M., Chun, S.-Y., Iona, S., Puri, C., Stefanini, M., and Conti, M. (1994) Biochemistry 33, 8948–8954
24. Macphee, C. H., Reddsnyder, D. H., Moore, T. A., Lerea, K. M. and Beavo, J. A. (1988) J. Biol. Chem. 263, 10553–10558
25. Sette, C., Iona, S., and Conti, M. (1994) J. Biol. Chem. 269, 9245–9252
26. Sette, C., and Conti, M. (1996) J. Biol. Chem. 271, 16526–16534
27. Thomas, M. K., Francis, S. H., and Corbin, J. D. (1990) J. Biol. Chem. 265, 14971–14975
28. Turko, I. V., Francis, S. H., and Corbin, J. D. (1998) Biochem. J. 329, 505–510
29. Nandel, F., Monaco, L., Foulquier, N. S., Masquiler, D., LeMeur, M. Heinriksen, K., Dierich, A., Parvinen, M., and Sassone-Corsi, P. (1996) Nature 380, 159–162
30. Blendy, J. A., Kaestner, K. H., Weinhauer, G. F., Nieschlag, E., and Schütz, G. (1996) Nature 380, 162–165
31. Gauert-Courteille, C., Salanova, M., and Conti, M. (1998) Endocrinology 139, 2588–2599
32. Salanova, M., Chun, S.-Y., Iona, S., Puri, C., Stefanini, M., and Conti, M. (1999) Endocrinology 140, 2297–2306
33. Vijayaraghavan, S., Gnolli, S. A., Davey, M. P., and Carr, D. W. (1997) J. Biol. Chem. 272, 4747–4752
34. Weyand, I., Lehmann, M., Frings, S., Weber, J., Altenhofen, W., Hatt, H., and Kaupp, U. B. (1994) Nature 368, 859–863
35. Wiesauer, B., Weiner, J., Middendorff, R., Hagen, V., Kaupp, U. B., and Weyand, I. (1996) J. Cell Biol. 134, 473–484
36. Bang, Y.-J., Persia, F., Pang, W.-G., Kang, W. K., Sarti, O., Whitesell, L., Ha, M. J., Tsokos, M., Sheahan, M. D., Nguyen, P., Nikolinski, W. T., Myers, C. E., and Trepel, J. B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 5330–5334
37. Goto, T., Matsuhashi, H., Kasuya, H., Hosaka, Y., Kitamura, T., Kawabe, K., Hida, A., Ohta, Y., Sixin, Z., and Takeda, K. (1999) Int. J. Urol. 6, 314–319
38. Cox, M. E., Deeb, P. D., Lakhani, S., and Parsons, S. J. (1999) Cancer Res. 59, 3921–3930
39. Takeda, M., Tang, R., Shiapura, E., Burnett, A. L., and Lepor, H. (1995) Urology 45, 440–446
40. Gub, J.-H., Hwang, T.-L., Ko, F.-N., Chueh, S.-C., Lai, M.-K., and Teng, C.-M. (1998) Mol. Pharmacol. 53, 467–474

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