Cloning and Characterization of a Novel Human Phosphodiesterase That Hydrolyzes Both cAMP and cGMP (PDE10A)*

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Kotomi Fujishige, Jun Kotera, Hideo Michibata, Keizo Yuasa, Shin-ichiro Takebayashi‡, Katsuzumi Okumura‡, and Kenji Omori§

From the Discovery Research Laboratory, Tanabe Seiyaku Co. Ltd., 2-50, Kawagishi-2-chome, Toda, Saitama 335-8505 and the Laboratory of Biological Chemistry, Faculty of Bioresources, Mie University, Mie 514-8507, Japan

Cyclic nucleotides cAMP and cGMP are well known as second messengers and regulate many functions in various tissues (1–4). Intracellular cAMP and cGMP concentrations are controlled via stimulation of adenyl and guanyl cyclases in response to extracellular signaling and their degradation by cyclic nucleotide phosphodiesterases (PDEs), respectively. Many kinds of PDEs are involved in the metabolism of cyclic nucleotides. Based on their amino acid sequence homology, biochemical properties, and inhibitor profiles, seven PDE families have been recognized in mammalian tissues (5, 6). PDE1 is Ca2+ calmodulin-dependent, hydrolyzing both cAMP and cGMP. Based on their amino acid sequence to those of other PDE families. Recombinant PDE10A transfected and expressed in COS-7 cells hydrolyzed cAMP and cGMP with Km values of 0.26 and 7.2 μM, respectively, and Vmax with cGMP was almost twice that with cAMP. Of the PDE inhibitors tested, diprydramole was most effective, with IC50 values of 1.2 and 0.45 μM for inhibition of cAMP and cGMP hydrolysis, respectively. cGMP inhibited hydrolysis of cAMP, and cAMP inhibited cGMP hydrolysis with IC50 values of 14 and 0.39 μM, respectively. Thus, PDE10A exhibited properties of a cAMP PDE and a cAMP-inhibited cGMP PDE. PDE10A transcripts were particularly abundant in the putamen and caudate nucleus regions of brain and in thyroid and testis, and in much lower amounts in other tissues. The PDE10A gene was located on chromosome 6q26 by fluorescent in situ hybridization analysis. PDE10A represents a new member of the PDE superfamily, exhibiting unique kinetic properties and inhibitor sensitivity.

PDE3 is cAMP-inhibited. PDE4 is cAMP-specific and rolipram-sensitive. PDE5 is cGMP-specific. PDE6 is a photoreceptor cGMP PDE. PDE7 is cAMP-specific and rolipram-insensitive. Very recently, cDNAs encoding two kinds of novel PDEs were isolated from humans and mice (7–11). One is cAMP-specific (PDE8), and the other is cGMP-specific (PDE9). PDE7 and the two latter PDEs are insensitive to the nonspecific PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX), cDNA cloning of these novel PDEs was done by an approach using bioinformatics. A search of data bases of expressed sequence tags (ESTs) was performed using parts of PDE sequences such as the catalytic domain. The approach was shown to be effective for the isolation of novel PDE cDNAs.

Genome sequencing projects are progressing in many organisms, providing us information of a variety of pathways involved in the cyclic nucleotide metabolism necessary to maintain life. In Caenorhachus elegans, which is a small soil nematode found in temperate regions, there are five pairs of autosomal chromosomes and a pair of sex chromosomes; that is, at least five distinct sequences show significant similarity with a catalytic domain of PDE5 based on the nucleotide sequence data base. Probably many PDE homologs still remain unidentified in humans, and they likely play distinct physiological roles. Characterization of these unidentified PDEs will be very important for understanding complex mechanisms in controlling cyclic nucleotide levels and for finding pharmacologically significant molecules.

In this work, we report the cloning of the full-length cDNA encoding a novel human phosphodiesterase family, PDE10A, using bioinformatics. The cloned cDNA was transfected into COS-7 cells, and enzymatic properties of the recombinant protein were investigated. The tissue-specific expression patterns of PDE10A transcripts were examined. Furthermore, we also demonstrate chromosomal localization of the human PDE10A gene.

EXPERIMENTAL PROCEDURES

Materials—Restriction endonucleases and DNA-modifying enzymes were obtained from Takara Shuzo (Kyoto, Japan). [α-32P]dCTP, [3H]cAMP, [3H]cGMP, plasmid pSVL, and Hybrid N+ nylon membrane were from Amersham Pharmacia Biotech. The plasmid pBlueScript II SK(+) was a product of Stratagene. The GeneAmp RNA polymerase chain reaction (PCR) Core kit was a product of PE Applied Biosystems. Human fetal lung mRNA, human RNA Master Blot, and multiple tissue Northern blots were purchased from CLONTECH. IBMX, erythro-9-(2-hydroxy-3-nonyl)-adenine (EHNA), diprydramole, and zaprinast were from Sigma. SCH51866, milrinone, rolipram, and E4021 were synthesized at Tanabe Seiyaku Co. Ltd., Japan.

General Methods—The nucleotide sequence was determined by the dideoxy chain termination method using a Thermo Sequenase cycle sequencing kit (Amersham Pharmacia Biotech) and an infrared automated DNA sequencer LI-COR 4000L (LI-COR). Alternatively, an automated DNA sequencer ABI PRISM 310 and a BigDye terminator cycle sequencing reaction kit were also used (PE Applied Biosystems).
Nucleotide and amino acid sequence data were analyzed by the computer programs GENETYX (Software Development, Japan).

Isolation and Analysis of cDNA Sequences—The amino acid sequence including a catalytic domain (amino acid residues 500–875) of human PDE5A (12) was used as a query to search the database of ESTs with the Basic Local Alignment Search Tool (BLAST) (13). Many EST sequences showing homology to the query sequence were obtained by the search, and then we examined whether they might encode a known or a novel PDE by searching the GenBank database with BLAST. One EST clone (W04835) was demonstrated to possess a sequence that was part of a unique PDE.

Screening of cDNA Library—To isolate the DNA fragment encoding the W04835 sequence, PCR was performed. The first round of PCR (30 cycles) was performed with the primer set 1 (PDEX31: 5'-AAGCTGTCCTACCATAGC-3' and PDEX51: 5'-GGCTGCGGCCAGAGGGTG-3') and a human fetal lung cDNA library constructed in λgt11 (CLON-FIG. 1. Nucleotide and deduced amino acid sequences of the human PDE10A cDNA. The deduced amino acid sequences are shown in three-letter designation below the nucleotide sequence. The termination codon at the end of the ORF is represented by an asterisk. A putative cGMP binding sequence (upper) and a catalytic domain (lower) are boxed. The in-frame termination codon upstream of the initiation codon of the ORF is double-underlined. The primer sequences for reverse transcriptase-PCR are underlined. The boxed AATAAA sequence represents a putative polyadenylation signal.

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The reaction mixture (1/10) was used as a template for the second round of PCR (30 cycles) with the primer set 2 (PDEX32: 5'-ACTTTCAGAAGAGTGGCC-3' and PDEX52: 5'-TGCAGGTAACTGTTACTG-3'). PCR was carried out through 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 2 min. The PCR product was cloned into TA cloning vector pGEM-T easy (Promega), and then the nucleotide sequence was confirmed. This DNA fragment was radiolabeled using a 32P random primer labeling kit (Takara Shuzo) and then used as a probe for plaque hybridization. Library screening was performed according to the standard procedure. Plaques of the same cDNA library constructed in λgt11 were plated onto 18 plates at approximately 5 × 10⁴ plaques/plate, lifted using Hybond N+ membrane, and then screened by hybridization with the 32P-labeled probe in 6×SSC, 0.5% SDS, 50 mM sodium phosphate, pH 7.0, at 65 °C for 16 h. The filters were exposed to x-ray film at −70 °C for 1 day. Plaques that hybridized to the probe were purified by two rounds of replating and rescreening. One positive clone (HSPDE10-1) was isolated, and the inserted DNA (4.6 kb) of the clone was digested with EcoRI. The isolated fragment was subcloned into the EcoRI site of pBluescript II SK(−). Three kinds of resultant plasmids were determined for nucleotide sequence. The 2.8-kb SspI-NotI fragment of the HSPDE10-1 insert was also introduced into the SpeI (blunt-ended)-NotI sites of pBluescript II SK(−), producing pBlue-PDE10A. This plasmid was used as the probe for fluorescent in situ hybridization (FISH) as described below.

Reverse Transcriptase-PCR Analysis—To detect the mRNA coding for PDE10A in human fetal lung mRNAs, the reverse transcriptase reaction was carried out using random hexamers at 42 °C for 60 min according to the manufacturer's instructions for the GeneAmp RNA PCR Core kit. The resultant cDNA and the primer sets (5'-CGCTGCTCTTCGCTCGCG3' and 5'-GGATCTGTAGGTGGGACAGCG-3') as a template. The reaction mixture (1/10) was used as a template for the second round of PCR (30 cycles) with the primer set 2 (PDEX32: 5'-ACCTTCTGAAGAGTGGCC-3' and PDEX52: 5'-TCGAGGTAACGGTITACG-3'). PCR was carried out through 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 2 min, and extension at 72 °C for 2 min. The PCR product was cloned into TA cloning vector pGEM-T easy (Promega), and then the nucleotide sequence was confirmed. This DNA fragment was radiolabeled using a 32P random primer labeling kit (Takara Shuzo) and then used as a probe for plaque hybridization. Library screening was performed according to the standard procedure. Plaques of the same cDNA library constructed in λgt11 were plated onto 18 plates at approximately 5 × 10⁴ plaques/plate, lifted using Hybond N+ membrane, and then screened by hybridization with the 32P-labeled probe in 6×SSC, 0.5% SDS, 50 mM sodium phosphate, pH 7.0, at 65 °C for 16 h. The filters were exposed to x-ray film at −70 °C for 1 day. Plaques that hybridized to the probe were purified by two rounds of replating and rescreening. One positive clone (HSPDE10-1) was isolated, and the inserted DNA (4.6 kb) of the clone was digested with EcoRI. The isolated fragment was subcloned into the EcoRI site of pBluescript II SK(−). Three kinds of resultant plasmids were determined for nucleotide sequence. The 2.8-kb SspI-NotI fragment of the HSPDE10-1 insert was also introduced into the SpeI (blunt-ended)-NotI sites of pBluescript II SK(−), producing pBlue-PDE10A. This plasmid was used as the probe for fluorescent in situ hybridization (FISH) as described below.
**Novel Human PDE (PDE10A)**

18441

for the first amplification and 5′-GGGATTCTAGGATGATGAA-
GAGGAATACG-3′ and 5′-AGCCGGTTCAGGGTACCGTCG-3′ for the second amplification) were used for PCR. 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 2 min. The amplified DNA fragments were cloned into pBluescript II SK+ (Stratagene). Clones having inserts of the correct size were sequenced directly. Sequence analysis was performed with the DNA Sequencer software (Hitachi).

**Sequence similarity of a catalytic domain and a putative cGMP binding sequence of human PDE10A to those of other PDE families**

The amino acid sequence of PDE10A was compared with those of other PDE families. The positions of a catalytic domain and a putative cGMP binding sequence are shown in parentheses. Two putative cGMP binding sequences were found in PDE5A, PDE6A, and PDE6B. The amino acid identities of each region to that of PDE10A are shown. In PDE3A and PDE3B, the best matched identity is also indicated. Δ means the deletion of the amino acid sequence.

| PDE family | Accession no. | Catalytic domain | cGMP binding sequence |
|------------|--------------|------------------|-----------------------|
| PDE1A | 100 (493–728) | 100 (243–445) | This work |
| PDE1B | 28 (192–428) | PS5750 |
| PDE1C | 25 (202–439) | Q14123 |
| PDE2A | 42 (632–871) | 38 (386–581) | Q00408 |
| PDE3A | 21 (728–1013) | Q13370 |
| PDE3B | 25 (728–1013,Δ(778–825)) | |
| PDE4A | 29 (408–654) | Q13370 |
| PDE4B | 28 (381–627) | Q07343 |
| PDE4C | 30 (363–609) | Q08493 |
| PDE4D | 29 (317–563) | Q19999 |
| PDE5A | 47 (588–728) | 35 (142–347) | D89094 |
| PDE6A | 40 (534–784) | 23 (50–254) | P16499 |
| PDE6B | 42 (533–783) | 18 (258–473) | |
| PDE7A | 29 (187–425) | Q13948 |
| PDE8A | 19 (415–674) | AF056490 |
| PDE8B | 22 (383–619) | AF079529 |
| PDE9B | 28 (288–925) | AF048857 |

**FISH—FISH was performed as described previously (15). Chromoso-
me spreads were obtained from phytohemagglutinin-stimulated
blood lymphocytes of a healthy donor after thymidine synchro-
nization and bromodeoxyuridine incorporation by the method described previ-
ously (16). The cDNA clone pBlue-PDE10A was labeled with biotin-16-
UTP (Roche Biochemicals) by nick translation. After in situ ho-
rization, hybridized probe was detected with fluorescein isothiocyanate-
conjugated avidin (Roche Biochemicals). Chromosomes were counterstained with 0.2 µg/ml propidium iodide for R-banding. Fluor-
escence signals were imaged using a Zeiss Axioplan2 epifluorescence
microscope equipped with a cooled charge-coupled device camera, Pen-
taMax-1317K/1 (Princeton Instruments). Image acquisition was per-
nected on a Power Macintosh 9600/2000MP computer with software program IFLab (Signal Analysis). The images were then pseudocolored and merged using Adobe Photoshop 2.5J. Fluorescein isothiocyanate and propidium iodide images were shown in green and red, respectively. The merged image of fluorescein isothiocyanate and propidium iodide was directly printed in a gray scale by Fuji Pictography 3000 from a Macintosh computer.

**RESULTS**

cDNA Cloning of a Novel Human PDE—Using a bioinfor-
matic approach, the nucleotide sequence corresponding to

amino acid residues 500–587 of human PDE5A was used as a
query in searching the EST data base. A homologous sequence
(W04835) was found in the EST data base from a human fetal
lung cDNA library. This sequence contained the PDE motif
(HD)LIVMFYXHDXG(A/G)X(NQ)X(LIVMFY) and is found within the catalytic domains of PDEs. For cDNA library screening, we first isolated the DNA fragment having the W04835 sequence and a human fetal lung cDNA library as a template. Specifically amplified DNA fragments of approximately 400 base pairs were subcloned and sequenced. The DNA fragments were revealed to be PCR products containing the W04835 sequence amplified with primers PDEX31 plus PDEX51 or primers PDEX31 plus PDEX52. Using this DNA fragment as a probe, we screened a human fetal lung cDNA library to isolate a full-length cDNA. One clone giving a positive signal was obtained from the library. After mapping, three EcoRI fragments (1.5, 0.8, and 2.3 kb) of the in-
serted DNA were subcloned into pBluescript II SK+ and se-
quenced. The nucleotide sequence of the full-length cDNA (4576
base pairs) is shown in Fig. 1. An open reading frame (ORF) of 2340
nucleotides spanned from the first initiation codon ATG to the
termination codon TGA (nucleotides 251–258) and an in-frame
STOP codon was found in 3′ non-coding region of the cDNA (nucleotides 2730, 3239, 3304, 3408, and 4079).

Because only one positive clone was analyzed, we confirmed the
presence of the corresponding mRNA by reverse transcrip-
tase-PCR. cDNAs were synthesized from human fetal lung
mRNAs by reverse transcriptase reaction. Sense and antisense
primers were designed based on the 5′-untranslated region and 3′-
untranslated region sequences of the cDNA cloned here (see
“Experimental Procedures”). A DNA fragment of 2.4 kb was

- untranslated region and 3′-untranslated region sequences of the cDNA cloned here (see “Experimental Procedures”). A DNA fragment of 2.4 kb was
specifically amplified, and the length of the fragment agreed with that predicted (data not shown). The mapping and partial sequencing analysis of the PCR product demonstrated that the product corresponds to the ORF of the cDNA.

The deduced amino acid sequence of the ORF was compared with those of human PDEs reported. From the sequence similarity, a putative cGMP binding sequence and a catalytic domain composed of the amino acid residues 243–445 and 493–728 were apparent, respectively. The cGMP binding sequence showed 18–35% identity with those of cGMP binding PDEs including PDE2A, PDE5A, and the photoreceptor PDEs (Fig. 2A and Table I). The catalytic domain was 42, 47, 40, and 42% identical to those of PDE2A, PDE5A, PDE6A, and PDE6B, respectively (Fig. 2B and Table I), whereas there was lower identity (19–30%) with other human PDEs. From the sequence analysis, it was concluded that the ORF encodes a novel PDE, designated PDE10A. According to the nomenclature of the 1994 American Society of Pharmacology and Experimental Therapeutics Conference (18), we denoted this gene as HSPDE10A1.

**Tissue Distribution of PDE10A Transcripts**—Dot blots of mRNA were examined using a 32P-labeled DNA probe corresponding to PDE10A (nucleotides 1509–2308) to show the distribution of the PDE10A transcripts (Fig. 3A). 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). PDE10A transcripts were particularly abundant in the putamen and caudate nucleus regions of brain and testis. Moderate expression was observed in the thyroid gland, pituitary gland, thalamus, and cerebellum. Northern blot analysis of multiple human tissues was performed with the same 32P-labeled probe (Fig. 3B). A band of approximately 10 kb was detected in many tissues in agreement with the dot blot analyses. In the testis and thyroid, an approximately 4.0-kb band was observed in addition to the transcripts of 10 kb.

**Expression of PDE10A in COS-7 Cells and Partial Purification of Phosphodiesterase**—To investigate the enzymatic properties of human PDE10A, expression studies were performed using COS-7 cells. The 2.8-kb fragment of the human PDE10A cDNA was subcloned into pBluescript II SK(+), producing pBlue-PDE10A. The insert DNA was then transferred into the expression vector pSVL, and the resultant plasmid pSVLPDE10A was introduced into COS-7 cells. Cell extracts were prepared from the transfected COS-7 cells and assayed for cyclic nucleotide hydrolysis using either 1 μM cGMP or 1 μM cAMP as substrates.
Novel Human PDE (PDE10A)

Mono Q-Sepharose chromatography of cytosolic extracts of transfected COS-7 cells. The pSVL-PDE10A expression plasmid was transfected into COS-7 cells by the LipofectAMINE reagent. Cytosolic extracts were prepared as described under “Experimental Procedures.” The extracts from transfected cells were loaded onto a Mono Q-Sepharose column. The column was washed with a buffer without NaCl, and the proteins were eluted with buffers containing 350 mM NaCl and then 800 mM NaCl. Each fraction was assayed for the activity of cAMP and cGMP hydrolysis using 1 μM cAMP or 1 μM cGMP as a substrate. Open and closed circles represent the activity of cAMP hydrolysis of the mock- and pSVL-PDE10A-transfected COS-7 extracts, respectively. The cGMP-hydrolytic activities of the mock- and pSVL-PDE10A-transfected COS-7 extracts are indicated by open and closed triangles, respectively.

FIG. 4. Mono Q-Sepharose chromatography of cytosolic extracts of transfected COS-7 cells. The pSVL-PDE10A expression plasmid was transfected into COS-7 cells by the LipofectAMINE reagent. Cytosolic extracts were prepared as described under “Experimental Procedures.” The extracts from transfected cells were loaded onto a Mono Q-Sepharose column. The column was washed with a buffer without NaCl, and the proteins were eluted with buffers containing 350 mM NaCl and then 800 mM NaCl. Each fraction was assayed for the activity of cAMP and cGMP hydrolysis using 1 μM cAMP or 1 μM cGMP as a substrate. Open and closed circles represent the activity of cAMP hydrolysis of the mock- and pSVL-PDE10A-transfected COS-7 extracts, respectively. The cGMP-hydrolytic activities of the mock- and pSVL-PDE10A-transfected COS-7 extracts are indicated by open and closed triangles, respectively.

FIG. 5. Kinetic analysis of partially purified human PDE10A. Lineweaver-Burk plots at concentrations of 0.1–2 μM cAMP (closed circles) and 0.2–16 μM cGMP (open circles) are shown. The human PDE10A was prepared from the cytosolic fractions of transfected COS-7 cells as described under “Experimental Procedures.” $K_v$ and $V_{max}$ values are the means of triplicate assays ± S.D. A plot typical of three independent experiments is shown.

cAMP as a substrate. Most PDE activities were observed in the cytosolic fractions, with barely detectable levels in particulate fractions (data not shown). Although COS-7 cells possess endogenous cAMP PDE, the activity of cAMP hydrolysis in the cell extracts was increased 7-fold by transfection of pSVL-PDE10A compared with that of the mock-transfected COS-7 cells (data not shown). Mono Q-Sepharose chromatography using a stepwise gradient of NaCl was carried out to separate the pSVL-PDE10A-derived PDE from endogenous PDE of the COS-7 cells (Fig. 4). The activity of cAMP hydrolysis in the mock-transfected COS-7 cells was eluted as an apparent single peak at a high NaCl concentration (800 mM). The activity of cGMP hydrolysis was too low to detect. By contrast, the cytosolic extract of the pSVL-PDE10A-transfected COS-7 cells yielded two peaks showing the activity of cAMP hydrolysis, which were eluted at the intermediate and high NaCl concentrations (350 and 800 mM, respectively). The peak in 350 mM NaCl fractions, which specifically appeared in the pSVL-PDE10A-transfected COS-7 extracts, also exhibited the activity of cGMP hydrolysis. Therefore, we concluded that the PDE activity in the first peak corresponded to the recombinant PDE10A.

Kinetic properties of the human PDE10A enzyme—$K_v$ values were derived from Lineweaver-Burk plots (19) of activity with cGMP or cAMP as a substrate for the partially purified recombinant PDE10A as described above. Assays measuring the activities of cAMP and cGMP hydrolysis were performed at substrate concentrations of 0.1–2 μM for cAMP and 0.2–16 μM for cGMP, respectively. As shown in Fig. 5, the $K_v$ values of the human PDE10A for cAMP was 0.26 ± 0.05 μM, and was significantly lower than that for cGMP (7.2 ± 0.6 μM). $V_{max}$ values of the human PDE10A for cAMP and cGMP hydrolysis were 0.63 ± 0.12 and 1.4 ± 0.08 pmol/min/μg of the partially purified recombinant protein, respectively. These analyses reveal that the affinity for cGMP was about 11-fold lower than that for cAMP. The activity of cAMP hydrolysis was not activated by the addition of cGMP unlike PDE2 but rather was inhibited at a high cGMP concentration ($IC_{50} = 14$ μM) (Fig. 6).

The effects of various PDE inhibitors on PDE10A activity were examined using the partially purified PDE10A (Table II). The nonspecific PDE inhibitor IBMX inhibited PDE10A ($IC_{50}$

| Inhibitor | $IC_{50}$ values for PDE10A | cAMP | cGMP |
|----------|----------------------------|------|------|
| IBMX     | 17                        | 11   |      |
| Vinpocetine | 77                      | 73   |      |
| EHNA     | >100                      | >100 |      |
| Milrinone | >100                      | >100 |      |
| Rollipram | >100                     | >100 |      |
| Zaprinast | 22                        | 14   |      |
| Dipyridamole | 1.2                    | 0.45 |      |
| SCH51866 | 3.6                       | 3.3  |      |
| E4021    | 7.2                       | 4.2  |      |
| cAMP     | ND                        | 0.39 |      |
| cGMP     | 14                        | ND   |      |

$^a$ ND, not determined.

Effect of cGMP on cAMP hydrolysis of PDE10A. Enzyme concentration and incubation time were optimized to give about 10% cAMP hydrolysis in the absence of cGMP. The cAMP concentration used was 0.3 μM. All assays are triplicate. The result shown is representative of two separate determinations.

Enzyme concentration and incubation time were optimized to give about 10% hydrolisis of cAMP and cGMP in the absence of inhibitors. Partially purified PDE10A produced in COS-7 cells was used for the assay. The concentrations of cAMP and cGMP were 0.3 and 7 μM, respectively. $IC_{50}$ values were calculated by linear regression. Data are the means of at least triplicate assays. The experiments were performed at least twice with different transfections.

Enzyme $K_v$ and $V_{max}$ values were derived from Lineweaver-Burk plots (19) of activity with cGMP or cAMP as a substrate for the partially purified recombinant PDE10A as described above. Assays measuring the activities of cAMP and cGMP hydrolysis were performed at substrate concentrations of 0.1–2 μM for cAMP and 0.2–16 μM for cGMP, respectively. As shown in Fig. 5, the $K_v$ values of the human PDE10A for cAMP was 0.26 ± 0.05 μM, and was significantly lower than that for cGMP (7.2 ± 0.6 μM). $V_{max}$ values of the human PDE10A for cAMP and cGMP hydrolysis were 0.63 ± 0.12 and 1.4 ± 0.08 pmol/min/μg of the partially purified recombinant protein, respectively. These analyses reveal that the affinity for cGMP was about 11-fold lower than that for cAMP. The activity of cAMP hydrolysis was not activated by the addition of cGMP unlike PDE2 but rather was inhibited at a high cGMP concentration ($IC_{50} = 14$ μM) (Fig. 6).

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= 17 μM for cAMP, 11 μM for cGMP). EHNA, milrinone, and rolipram, which are PDE2, PDE4, and PDE3 inhibitors, respectively, were inactive up to 100 μM. The PDE1 inhibitor vinpocetine showed weak inhibitory effect on PDE10A (IC_{50} = 77 μM for cAMP and 73 μM for cGMP). Compounds that inhibit PDE5 showed inhibitory effects on PDE10A. Zaprinast demonstrated moderate inhibition (IC_{50} = 14–22 μM). E4021, a more potent PDE5 inhibitor (20), and SCH51866, a PDE1 and PDE5 inhibitor (21), inhibited PDE10A with IC_{50} values of 4.2–7.2 μM and 3.3–3.6 μM, respectively. Dipyridamole was the most effective antagonist for PDE10A with IC_{50} values of 1.2 μM for cAMP and 0.45 μM for cGMP among PDE inhibitors tested. The inhibitory effect of these compounds was observed on the PDE10A activities of cAMP and cGMP hydrolysis. Interestingly, cAMP and cGMP inhibited the activities of cGMP and cAMP hydrolysis, respectively (IC_{50} = 0.39 and 14 μM), suggesting that PDE10A activity may be controlled by intracellular cyclic nucleotide levels of both cAMP and cGMP. cAMP especially inhibits the activity of cGMP hydrolysis at low concentrations. Therefore, PDE10A exhibited properties of a cAMP PDE and a cAMP-inhibited cGMP PDE.

**Chromosomal Localization**—To localize the human PDE10A gene (HSPDE10A1) to a specific chromosome, colorimetric FISH analysis was performed. The plasmid pBlue-PDE10A containing 2.8-kb human PDE10A cDNA was used for a probe. In situ hybridization localized the plasmid pBlue-PDE10A to chromosomal region 6q26 as shown in Fig. 7, without further cross-hybridization to other chromosomal locations.

**DISCUSSION**

A full-length cDNA, which was isolated by an approach using bioinformatics, encoded a novel PDE containing a catalytic domain in the carboxyl-terminal portion and a putative cGMP binding sequence in the amino-terminal portion of the molecule. The primary structure of PDE10A was more analogous to those of cGMP-binding PDEs such as PDE2, PDE5, and PDE6s. A putative cGMP binding sequence containing the conserved amino acid residues among many cGMP-binding proteins (22, 23) was single in PDE10A like PDE2A but not doublet as found in PDE5A and PDE6s. However, an incomplete cGMP binding sequence was also found (amino acid residues 65–203). It lacked some conserved amino acids (22, 23) which exist on the carboxyl-terminal side of a typical cGMP binding sequence. From the sequence analysis, PDE10A was categorized as a cGMP-binding PDE. The gene product encoded by the cDNA demonstrated the activity of not only cGMP but also cAMP hydrolysis when expressed in COS-7 cells. The affinity for cAMP was about 11-fold higher than that for cGMP. V_{max} with cGMP was about twice as fast as that with cAMP. Interestingly, cAMP potently antagonized cGMP hydrolysis with IC_{50} value of 0.39 μM, whereas cAMP hydrolysis was inhibited by cGMP at high concentration (IC_{50} = 14 μM). In PDE3, which is known as a cGMP-inhibited cAMP PDE, cAMP hydrolysis is antagonized by cGMP at a low concentration (IC_{50} = 0.2 μM) (24). In PDE10A, the concentration of cAMP necessary to inhibit the activity of cGMP hydrolysis was as low as that of cGMP on PDE3 inhibition. These observations indicated that PDE10A is a novel cAMP and cAMP-inhibited cGMP PDE.

PDE10A lacked the phosphorylation site of cAMP or cGMP-dependent kinase, which is found in PDE5A (23, 25). In PDE5, when cGMP binding sites are occupied by a substrate, cAMP and cGMP-dependent protein kinases can then phosphorylate a specific serine residue of PDE5 (5, 25, 26), which may lead to the regulation of PDE5 activity. In PDE10A, instead of the phosphorylation site by cAMP- and cGMP-dependent protein kinases, putative protein kinase C phosphorylation sites (amino acid residues 158–160, 363–365, 504–506, and 650–652) were observed, suggesting regulation of PDE10A activity by protein kinase C. Although we investigated the cGMP binding activities in cytosolic extracts from transfected COS-7 cells, unexpectedly, cGMP binding activities were not found under the conditions described elsewhere (data not shown) (27). Further study will elucidate the regulation of PDE10A.

A nonspecific PDE inhibitor, IBMX, showed moderate inhibition of PDE10A. The catalytic domain of PDE10A showed the highest sequence similarity with that of PDE5A (47%) and PDE2A (42%). PDE5 inhibitors (zaprinast, E4021, SCH51866, and dipyridamole) were more effective antagonists for PDE10A activity than were other PDE inhibitors. Despite the sequence similarity, a PDE2 inhibitor (EHNA) was not an inhibitor of PDE10A. Among PDE5 inhibitors, dipyridamole, which showed inhibition against PDE2 and PDE4 (28), was the most effective against PDE10A. These findings are interesting and informative and could be useful in developing a PDE10A-specific inhibitor.

In the testis, highly abundant short transcripts of 4.0 kb were observed in addition to 10-kb transcripts, which are found in several other tissues. Five putative polyadenylation sites were found in the 3’-noncoding region. The short transcripts in the testis were suspected to be transcripts truncated at these sites. The localization of PDE10A transcripts in the brain is of interest. Dopamine receptors D_{1} and D_{2} are expressed prominently in the putamen and caudate nucleus regions. Signals from D_{1} and D_{2} receptors are transmitted via cAMP: D_{1} stimulates the adenyl cyclase, and D_{2} inhibits the enzyme. PDE1B1 expressed in these regions is suggested to play important roles for regulating these signals by controlling cAMP levels (29, 30). Specific expression of PDE10A transcripts in the same regions described above also suggest that PDE10A may be involved in controlling cAMP levels there. Because PDE10A is a cAMP PDE and cAMP-inhibited cGMP PDE, cGMP hydrolysis could be regulated by intracellular cAMP levels in the cells where PDE10A is localized; that is, cAMP-mediated signals may control the function of cGMP. In the putamen and caudate nucleus, cAMP generated by dopaminergic stimulation possibly leads to an increase in cGMP, which likely plays a role in neuronal signal transduction. The precise localization of
PDE10A and further analysis are needed to clarify this hypothesis.

The human PDE10A gene, HSPDE10A1, was mapped to chromosome 6q26. The genome data base OMIM was searched for disease markers or genes on chromosome 6. Many loci have been identified in 6q26. One locus of interest, juvenile Parkinson’s disease, has been identified in 6q25.2-q27 (31). Juvenile parkinsonism is defined as particular parkinson manifestations including bradykinesia, rigidity, and tremor with onset before 40 years of age. Very recently, the gene responsible for autosomal recessive juvenile parkinsonism has been identified, and the gene product was named Parkin (32). PDE10A is expressed in the putamen and caudate nucleus regions that have dopamine receptors and are related to juvenile parkinsonism. Thus, the possibility of genetic linkage of the PDE10A gene with this disease is intriguing.

In conclusion, we revealed the structure and tissue-specific expression patterns of the transcripts of a novel human PDE, PDE10A. Presently the physiological role of this enzyme remains unknown. Analysis of tissue distribution in detail such as in situ hybridization analysis and immunohistochemical approach will be informative in revealing a role of this enzyme. Pharmacological analysis using selective inhibitors for this enzyme will elucidate a new physiological function of cAMP or cGMP in specific tissues or cells.

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