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Cloning and Characterization of the cDNA Encoding the \(\alpha\)-Subunit of cGMP-Phosphodiesterase in Canine Retinal Rod Photoreceptor Cells

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Rod photoreceptor cyclic GMP-phosphodiesterase (cGMP-PDE, EC 3.1.4.17) is a key enzyme in the phototransduction cascade of the vertebrate retina. The enzyme is composed of \(\alpha\)- and beta-catalytic subunits, and two identical inhibitory gamma-subunits. Once the phototransduction cascade is initiated by the absorption of light by rhodopsin, activated cGMP-PDE rapidly hydrolyses cGMP, depletion of which shuts cGMP-gated cation channels in the plasma membrane. The consequent hyperpolarization of the photoreceptor outer segment represents a large signal amplification and generates the visual neural impulse. Aberrant function of cGMP-PDE is causally associated with retinal degenerative diseases in man and animals. Mutations in the genes for the \(\alpha\)- and beta-subunits of cGMP-PDE (PDEA and PDE6B, respectively) cause retinitis pigmentosa (OMIM entry) in some human families [6,8,9]. Defects in PDE6B also cause retinal degeneration in the rd mouse [3,11] and the rcd1 dog [5,13,14]. Rod-cone dysplasia 2 (rcd2), which affects the collie dog, also represents a defect of retinal cyclic GMP metabolism since retinal cGMP levels are significantly elevated and cGMP-PDE activity is deficient [16]; however, rcd2 is not caused by a defect in PDE6B[1]. Thus it is likely that the rcd2 locus codes for either another cGMP-PDE structural subunit [PDEA, or PDEG (the gene for the gamma-subunit of cGMP-PDE)], or for one of the other proteins in the phototransduction cascade that activates cGMP-PDE. Since the canine chromosomal locations of the rcd2 locus and the genes involved in phototransduction are unknown, none of these candidate genes can be ruled out based on their map location relative to the rcd2 locus.

In order to identify the mutation responsible for the rcd2 disorder, we have begun to examine the different candidate genes that code for the phototransduction cascade proteins in the dog. The cDNAs for the \(\alpha\)-subunit of cGMP-PDE (PDE\(\alpha\)) have been cloned and characterized from man [12], mouse [2], and cow [10,12]. However, no information is available in the literature on the canine PDE\(\alpha\) cDNA.

In this study we characterize the canine PDE\(\alpha\) cDNA from normal dog, present evidence for usage of alternate polyadenylation sites to generate the two different transcripts described in multiple species [2,7,12], and compare the deduced amino acid sequences for conservation through evolution.

RESULTS

Initially two different segments of canine PDE\(\alpha\) cDNA were obtained by reverse transcription (RT) and polymerase chain reaction (PCR) using total retinal RNA and consensus primer pairs (PDEA-1/PDEA-2 and PDEA-3/PDEA-4; Table 1) based on the coding regions of known PDE\(\alpha\) cDNA sequences from other species. These RT-PCR amplified fragments were cloned (1-PDEA and 2-PDEA) in pcRII vector (Invitrogen; San Diego, CA) and sequenced to confirm authenticity of the retina-specific PDE cDNA sequence. From the confirmed canine

![Figure 1. Strategy for cloning the canine PDE\(\alpha\) cDNA. The cDNA containing the coding sequence (shaded box), the untranslated regions at the 5'- and 3'-end (open boxes) and two sites where poly (A) are added, is shown as an insert in the vector pBK-CMV (Stratagene, La Jolla, CA) used for the construction of the library. Clones containing different overlapping regions of the cDNA (1-PDEA, 2-PDEA, 5'-PDEA, 3'-PDEA, PDEA-S and PDEA-L), the sizes of the cDNA fragments in the clones, and the primers (see Table 1 for sequence) used for generating those fragments are identified. The clones 1-PDEA and 2-PDEA were obtained by reverse transcription and polymerase chain reaction (RT-PCR) from canine retinal total RNA using the RT-PCR kit (Perkin-Elmer; Foster City, CA) as recommended by the manufacturer. Amplification of cDNA to obtain 1-PDEA and 2-PDEA was done for 30 cycles at 94 °C (1 min), 54 °C (2 min), 72 °C (2 min). Other clones (5'-PDEA, 3'-PDEA, PDEA-S and PDEA-L) were obtained by screening a canine retinal cDNA library. The PCR for screening the canine retinal cDNA library was done in 50 µl containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, 2.0 mM MgCl2, 10% DMSO, and 1.25 units Taq polymerase (Life Technologies; Grand Island, NY). The conditions for PCR amplification of each fragment were as follows: 5'-PDEA and 3'-PDEA were obtained by 30 cycles at 94 °C (1 min), 60 °C (1.5 min), 72 °C (2 min); PDEA-S and PDEA-L were obtained by 30 cycles at 94 °C (1 min), 58 °C (2 min), 72 °C (3 min). All reactions were concluded with a single step extension reaction at 72 °C for 10 min.

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The two full length canine rod PDEα cDNAs are 2988 and 3233 nucleotides long, including the poly (A) tail (Figure 3). We have previously reported the presence of two distinct PDEα mRNAs, 3.3 and 3.0 kb, expressed in equivalent amounts in canine retina by northern blot [7]. Thus the sizes of the canine PDEα cDNAs described here (GenBank accession number U52868) are in complete agreement with our observation from northern analysis [7]. We have not formally determined the transcription start site; however, the clone containing the most upstream 5'-noncoding region contains 139 nucleotides in the 5'-UTR followed by the ATG codon for initiation of translation. The stop codon (TAA) corresponds to positions 2723 through 2725 of the cDNA sequence. The 3'-UTRs of the 3.0 kb and 3.3 kb transcripts, represented by clones PDEα-S and PDEα-L, are 263 and 508 nucleotides respectively. A non-consensus putative polyadenylation signal (AATAAA) is present in the corresponding locations of the smaller (PDEα-S) and larger (PDEα-L) clones. In PDEα-S this first polyadenylation signal (Figure 3, nucleotides 2948 through 2953) is 15 nucleotides upstream of the poly (A) tail.

![Figure 2](image_url)

**Figure 2. Identity of PDEα-L clone as PDEα cDNA sequence.** (A) Relative size of the inserts in the PDEα-L and PDEα-S clones, the DNA fragments amplified by PCR, primers (see Table 1 for sequence) used, and the predicted sizes of the amplified fragments are shown. PCR condition for amplified fragments are as follows: fragments 1 and 2 were obtained by 30 cycles at 94 °C (1 min), 64 °C (1 min), 72 °C (2 min); fragment 3 was obtained by 30 cycles at 94 °C (1 min), 54 °C (1 min), 72 °C (1.5 min); and fragment 4 was obtained by 35 cycles at 94 °C (1 min), 58 °C (2 min), 72 °C (3.5 min). All reactions were concluded with a single step extension reaction at 72 °C for 5 min. (B) Agarose gel (0.8%) electrophoresis of PCR products from PDEα-S (S) and PDEα-L (L) clones. Numbers 1 through 4 identify DNA fragments amplified from regions of the clones represented in panel A. Lane M1 represents BstE II-digested lambda DNA markers (1 kb). Lane M2 represents Hae III-digested phi X 174 markers (1.5 kb). (C) Polyacrylamide gel (6%) electrophoresis of restriction enzyme digestion of PCR product (4 in panel A) from PDEα-S (S) and PDEα-L (L) clones. Numbers 1 through 4 identify DNA fragments amplified from regions of the clones represented in panel A. Lane M1 represents BstE II-digested lambda DNA markers (1 kb). Lane M2 represents Hae III-digested phi X 174 markers (1.5 kb). (D) Polyacrylamide gel (6%) electrophoresis of restriction enzyme digestion of PCR product (4 in panel A) from PDEα-S (S) and PDEα-L (L) clones. Numbers 1 through 4 identify DNA fragments amplified from regions of the clones represented in panel A. Lane M1 represents BstE II-digested lambda DNA markers (1 kb). Lane M2 represents Hae III-digested phi X 174 markers (1.5 kb).
In the larger clone (PDEA-L) there is a second polyadenylation signal (Figure 3, nucleotides 3187 through 3192) 23 nucleotides upstream of the poly(A) tail. The ORF of the canine sequence predicts a protein of 99.7 kDa containing 861 amino acids.

**DISCUSSION**

Comparison of the ORF nucleotides of the canine PDEα cDNA with that of other species shows that it shares similar nucleotide identity with the bovine (91.0%) and human (90.4%) sequences, and slightly lower identity with the mouse (86.5%) sequence. However, the similarity between the canine sequence and that of other species is higher than that of other species and that of other species is higher than that of other species.

The α cDNA sequence predicts a protein of 99.7 kDa containing 861 amino acids. The position of the nucleotides and the deduced amino acids are indicated on the right. The two extra amino acids that are inserted between the canine sequence and that of other species is higher than that of other species.

**Figure 3.** The nucleotide sequence of PDEα cDNA from canine retina (GenBank accession number U52868), and the deduced amino acid sequence. The position of the nucleotides and the deduced amino acids are indicated on the right. The two extra amino acids that are inserted into the human and mouse sequences are shown in bold italics. The two extra amino acids that are inserted into the human and mouse sequences are shown in bold italics. The two extra amino acids that are inserted into the human and mouse sequences are shown in bold italics.

**GAC AAT GAG CAG ATG CAG AAA ATC AGG ACA AGA GAG GTG GTA 1564**

Asp Asn Glu Glu Glu Lys Leu Lys Leu Thr Arg Gly Val Tyr 1544

**GGA GAG CCG TGG TAC GAG GAA GAG GAC CTC ATG GAT 1569**

Gly Lys Glu Pro Try Glu Cys Glu Glu Glu Ala Arg Arg 1509

**GTC GAT GAG AAG AGA TAT ATT AAT TAC GTG AGA 1574**

Leu Glu Gly Leu Pro Asp Ala Lys Tyr Glu Ile Leu Tyr Asn 1505

**TTG TTC ACC AGC TAC ATG CAC TGG CCA GCT TCG CAG 1579**

TTC CAC TAC AGG TCC CCT CCC ACA AAG GAT GTG AAA 1969

**AAC TGA AGT GAT GAA CTT CTG GAA GAG AAC CTG AAT 1584**

Pro Arg Asp Glu Glu Val Leu Glu Glu Asp Val Ile Thr Arg 1904

**Glu Cys Ile Gly Met Tyr Tyr Gly Leu Lys Val Arg Lys Phe 1589**

AAC GAC CCC AGC ACC ACC AAC TAC TGC GAC TTC AAG 2315

**His Ile Pro Glu Glu Ala Leu Val Arg Phe Met Tyr Ser Leu Leu 1594**

His Arg Glu Glu Glu ACC ATG CCT TTC TGT CCA GCA GAA CAG 2519

**AAT GCC CAG ACC ATC CCT ACC TCT TAC ATG AGG 439**

Glu Gly Leu Glu Glu Arg Gln Pro Arg Gln Glu Ile Pro Leu 3233

**TTGATGGTCTTTTTTCTTTTTTTAA 3217**

Figure 3. The nucleotide sequence of PDEα cDNA from canine retina (GenBank accession number U52868), and the deduced amino acid sequence. The position of the nucleotides and the deduced amino acids are indicated on the right. The two extra amino acids that are inserted into the human and mouse sequences are shown in bold italics. The two extra amino acids that are inserted into the human and mouse sequences are shown in bold italics.
sites. The same mechanism could well account for the observed different transcript sizes within other species. The canine sequence does not contain the canonical polyadenylation signal (AATAAA); instead a similar sequence motif (ATTAAA) was identified in both appropriate locations (Figure 3). This alternative motif has been demonstrated to serve as a surrogate polyadenylation signal with ~80% efficiency [15]. A non-consensus polyadenylation signal (AATACA) has also been reported to be present in human PDE\(\alpha\) cDNA [12].

It is noteworthy that while PDE\(\alpha\) cDNA clones from 3 other species contain an ORF capable of coding for a polypeptide of 859 amino acids, the canine PDE\(\alpha\) polypeptide is predicted to contain 861 amino acids. With respect to the human and mouse sequences, the two extra amino acids are located as the 849th (Ala) and 850th (Gly) residues (Figure 3). Similar to the comparison made between human and bovine PDE\(\alpha\) sequences [12], we noted that the differences in amino acid sequence among the four species (human, mouse, bovine, and canine) are clustered in the 225 N-terminal and 45 C-terminal residues (data not shown). The conserved region includes the domain present in several eukaryotic cyclic nucleotide phosphodiesterases [4]. Alignment of amino acid sequences of canine PDE\(\alpha\) and PDE\(\beta\) shows a 72% overall identity, and the domains that are most dissimilar are at the N-terminus (first 50 residues) and C-terminus (last 30 residues).

The data presented here have been used to identify the possible mechanism for the presence of two transcripts for PDE\(\alpha\) in different species, and to compare nucleotide/amino acid identity among rod-specific PDE\(\alpha\) sequences of these species. Characterization of the wild type canine PDE\(\alpha\) cDNA sequence will allow us to detect PDE\(\alpha\) mutations in dogs affected with rcd2 or other inherited retinal degenerations. Because collie dogs affected with rcd2 have elevated retinal cGMP levels secondary to low PDE activity, we are currently investigating the possibility of a PDE\(\alpha\) mutation in this disease.

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